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Evaluation of clinical, laboratory and treatment modalities of pituitary adenomas: Single center experience

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Abstract

Pituitary adenomas are a group of disease with broad different clinical characteristics and complications. We aimed to present the data of patients being followed in a single center and discuss the pituitary adenomas based on the literature. Two hundred and twenty patients followed at Department of Endocrinology and Metabolism, Medical School of Ondokuz Mayis University, were included into study. Clinical characteristics, laboratory findings and treatment modalities were examined retrospectively. 59% of patients were female and 41% were male. Mean age during the diagnosis was 43.7 years. Pituitary macroadenomas were 62% of all adenomas and 73% of pituitary adenomas were functional. Among the functional adenomas, the most frequently seen types were prolactin-secreting adenoma and growth-hormone secreting adenoma. Treatment options were surgical treatment (67%), medical treatment (20%) and radiotherapy (7%). Post-operative complications were developed in 27 (13.2%) of patients. In patients with non-functional adenoma, the cure after surgery was detected as 19.6% and the remission after the surgery was detected as 8.9%. In this study, the characteristics of the pituitary adenomas were found similar to the literature in general. The early diagnosis of the disease has a significant importance in terms of treatment and the response to the treatment.

Keywords: acromegaly, Cushing disease, nonfunctional adenomas, pituitary adenoma, prolactinoma, TSHoma

1. Introduction

Pituitary adenomas are the benign tumors of the anterior pituitary gland and the most common cause of sellar masses up to 10 percent of all intracranial neoplasms (1). Autopsy and radiological studies revealed that the expected prevalence may be as high as 20% (2). Population studies from different countries have estimated that the prevalence of pituitary adenomas is 75.7–115.5 cases per 100,000 of population (3-5). Adenomas can originate from any type of cell of the pituitary and may produce hypersecretion of the hormones. Decreased secretion of hormones due to compression of pituitary cells may also occur. Sellar masses can present with abnormalities related to hypo or hypersecretion of pituitary hormones, or as an incidental finding on radiological examination performed for different indications. Most of these tumors are incidentalomas without clinical significance.

Pituitary adenomas have an important role in endocrinological practice. The aim of this study is to collect the data of the pituitary adenoma cases to reveal our own results based on the literature and discuss them.

2. Materials and methods

This single-centered retrospective study was carried out in patients with pituitary adenomas followed at Department of Endocrinology and Metabolism, Medical School of Ondokuz Mayis University. Two hundred and twenty patients followed between June 2004 – October 2014 were enrolled in the study. The study protocol was approved by the Local Ethics Committee. Sociodemographic data of all participants regarding age, sex, marital status and level of education were recorded. Age at diagnosis, adenoma type, adenoma size, application complaints, levels of preliminary pituitary hormones, radiological examination method used in diagnosis, types of treatment, mode of operation, postoperative complications, medical treatment, radiotherapy, remission, cure and recurrence rates, follow-up frequency were examined. For prolactinoma, measurement of serum prolactin; a level above the upper limit of normal was used for the diagnosis after excluding medication use, renal failure, hypothyroidism, and parasellar tumors. Acromegaly was diagnosed with the measurement of serum insulin like growth factor-1 (IGF-1) and nadir growth hormone (GH) after 75 gr oral glucose tolerance test (OGTT). GH equal to or greater than 1 µg/L after an OGTT, in conjunction with clinical suspicion and high IGF-I levels adjusted for age and sex matched references were the criteria for the diagnosis of acromegaly. The diagnosis of Cushing disease was

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established based on clinical features of hypercortisolism coupled with the failure to suppress plasma cortisol at 8 a.m. to a level below 1.8 μg per deciliter after the administration of 1 mg of dexamethasone at 11 p.m. or elevated 24-hour urinary free cortisol levels. Inferior petrosal sinus sampling was done if the adenoma size was < 6 mm. A central-to-peripheral plasma ACTH gradient of ≥2.0 before CRH administration, or ≥3.0 after CRH, verified the diagnosis of a pituitary source of ACTH. TSH secreting adenoma was diagnosed with elevated or inappropriately suppressed thyrotropin levels with normal or elevated thyroid hormone levels. Differential diagnosis was made with high molar ratio of serum alpha subunit to TSH. Adenomas with no hormonal production was accepted as nonfunctional adenoma. Symptoms were evaluated separately in each group of adenomas. They were not classified as symptoms related with mass effect or hormonal hyperfunction. Panhypopituitarism, central diabetes insipidus, rhinorrhea, meningitis, central, hypothyroidism, deep vein thrombosis was evaluated as postoperative complications.

2.1. Statistical analysis

SPSS 15.0 statistical package program was used for statistical evaluation of the data. Categorical variables were defined as % ratio. The findings were evaluated with the student T test. Chi-square test was used to compare categorical variables. $p<0.05$ was regarded as significant.

3. Results

Of the 220 cases included in the study, 129 (59%) were female and 91 (41%) were male. The age at the time of diagnosis varied from 18 to 75 years, with an average age of 43.7 years. The average age was 40.9 years in female and 47.7 years in men ($p<0.05$). Pituitary microadenoma was detected in 38% of the patients and pituitary macroadenoma in 62% of the patients. When evaluated clinically, 73% of pituitary adenomas were functional and 27% were found nonfunctional. The average age was 41.9 years for functional adenomas and 48.8 years for nonfunctional adenomas ($p<0.05$) (Table 1). In functional adenomas, microadenoma rate was 45% and macroadenoma rate was 55%, these rates were 18.6% and 81.4%, respectively for nonfunctional adenomas ($p <0.05$).

Among functional adenomas, 29.5% were prolactin-secreting adenoma, 29% of GH-secreting adenoma, 12.7% of ACTH producing adenoma and 1.8% TSH-secreting adenoma. When adenoma type and adenoma size were compared; 28.6% were ACTH-secreting adenomas, 81.2% of GH-secreting adenomas, 40% of PRL-secreting adenomas and 81.4% of non-functional adenomas were macroadenoma (Table 2).

The patients were evaluated according to the symptoms at the time of admission. Symptoms were present in 90% of patients. The most common symptom was headache (21%). Enlargement in hands-foots and visual impairment were the following complaints. When the cases were evaluated in terms of radiological diagnosis, the first used diagnostic imaging was pituitary MRI in 196 cases (89%). It was followed by brain MR (7.3%).

Table 1. Basic characteristics of pituitary adenomas

	N	*%*	p
Male	91	41.4	< 0.05
Female	129	58.6	
	Mean	**SD**	**p**
Age (total)	43.7	10.9	
Male	47.7	12.7	< 0.05
Female	40.9	9.1	
	N	*%*	p
Microadenoma	84	38	< 0.05
Macroadenoma	136	62	
Functional	160	73	< 0.05
Nonfunctional	60	27	

The results were examined in terms of treatment. Surgical treatment was applied to 64.7% of the patients. Forty-two of the patients who did not undergo surgery received medical treatment, only one patient received gam knife after medical treatment and one patient received gam knife treatment alone because of surgery risk. Eighteen patients were followed without any treatment. Treatment information of sixteen patients could not be reached. It has been determined that 92.3% of surgically operated cases were operated transsphenoidally and 7.6% were operated transcranially.

It was determined that postoperative complications were developed in 27 cases (13.2%). Mortality due to postoperative complication was not detected in any of the cases. The average age of those who developed postoperative complications was 46 years and 43 years for those who did not develop complications ($p>0.05$). In terms of postoperative complications; panhypopituitarism in eight cases (29.7%), partial diabetes insipidus in five cases (18.5%), rhinorrhea and meningitis in three cases (11.1%), hypothyroidism in three cases (11.1%), rhinorrhea only in two cases (7.4%), ophthalmoplegia and meningitis in one case (3.7%), acute renal failure in one case (3.7%), hypothyroidism and diabetes insipidus in one case (3.7%), rhinorrhea and pneumocephalus in 1 case (3.7%), deep vein thrombosis in one case (3.7%) and epistaxis in one case (3.7%) were detected. The postoperative complication rates were 15.4%, 12%, 9.5% and 18.9% for ACTH secreting adenomas, GH secreting adenomas, PRL secreting adenomas and non-functional adenomas, respectively. There was no statistically significant difference between adenoma types and complication rates ($p> 0.05$). In term of compliance for patients for postoperative follow-up, 58 (26.3%) patients came to control once a year, 42 (19%) twice a year, 24 (10%) four times a year and one patient came every month. While it was observed that 125 cases followed up regularly, it was determined that others did not come controls regularly.

3.1. Non-functioning adenoma

Nonfunctioning adenomas were account for 27% of all cases.

47.5% were female and 52.5% were male. The average age of diagnosis was 48 years. In 89.8% of cases with nonfunctional adenomas, symptoms were detected at the time of diagnosis. The most common symptom was headache (35.1%). Other symptoms were impaired vision (22.5%), loss of libido (4.8%) and nonspecific symptoms (27.4%). Pituitary insufficiency was detected in laboratory examination in 32% of patients. Surgical treatment was applied to 39 of 59 (69.6%) patients. Hormone replacement therapy was applied in 13 (33.3%) patients due to pituitary insufficiency developed after surgery.

Table 2. Characteristics of subtypes of pituitary adenomas

		Results (n/%)				
		PRL-secreting adenoma	GH-secreting adenoma	ACTH secreting adenoma	TSH-secreting adenoma	Non-functional adenoma
Adenoma type	**n/%**	65 (29.5)	65 (29)	28 (12.7)	3 (1.8)	59 (27)
Sex	**Female**	40 (61.5)	37 (57)	23 (82.1)	1 (33.3)	28 (47.5)
	Male	25 (38.5)	28 (43)	5 (17.9)	2 (66.7)	31 (52.5)
Average age of diagnosis	**Year**	40	45	37	52	48
Adenoma size	**Microadenoma**	39 (60)	12 (18.8)	20 (71.4)	1 (33.3)	11 (18.6)
	Macroadenoma	26 (40)	53 (81.2)	8 (28.6)	2 (66.7)	48 (81.4)
Symptoms	**Present**	54 (83)	60 (92.2)	28 (100)	2 (66.7)	53 (89.8)
	Non present	11 (17)	5 (7.8)	0 (0)	1 (33.3)	6 (10.2)
Surgery	**n/%**	7 (1)	60 (92.3)	21 (84)	2 (66.6)	39 (66.1)
Postoperative complication	**%**	9.5	12	15.4	0	18.9

3.2. Prolactin secreting adenomas

Prolactin-secreting adenomas were account for 29.5% of all cases. 61.5% of the cases were female. The average age of diagnosis was 40 years. 60% of adenomas were microadenoma. Prolactin levels were detected >200 ng/ml in 67.8% of macroprolactinoma cases, <200 ng / in 73% of microprolactinoma cases. At the time of diagnosis, symptoms were detected in 83% of cases with prolactinoma. The most common symptom was galactorrhea (36%). Other symptoms were menstrual irregularity (16.4%), loss of libido (13.9%), headache (11.3%), visual impairment (7.3%) and nonspecific symptoms (15.1%). Surgical treatment was applied to 24 (36.9%) of the 65 cases. Postoperative medical treatment was continued in 17 of 24 patients who underwent surgical treatment. Of the 65 cases followed for prolactinoma, 47 were treated with cabergoline. Remission was achieved in 76% cases with only bromocriptine therapy, in 3% cases with only cabergoline therapy, in 6% cases with surgical therapy alone and in 9% cases with surgery + medical treatment in microprolactinomas. 4% of microprolactinomas were found to be unstable despite all treatment modalities. In macroprolactinoma cases remission rates were 24% with cabergoline alone, 36% with surgery and cabergoline, 8% with surgical treatment alone. Remainders were not in remission despite any surgical and/or medical treatment.

3.3. Growth hormone secreting adenomas

Growth hormone secreting adenomas were found to be 29% of all cases. 57% of the cases were female. The average age of diagnosis was 45 years. 79% of GH secreting adenomas were macroadenomas. At the time of diagnosis, 92.2% of the cases were symptomatic. While the most common symptom was enlargement of hands and feet (68.7%), other symptoms were headache (18.7%), impaired vision (7.8%), enlargement of tongue and snoring (1.8%), hoarseness (1.5%) and loss of libido (1.5%), respectively. Surgical treatment was applied 55 (90%) patients. Five patients did not receive surgical treatment. One patient was followed up without treatment. Treatment information was not available for four patients. 61.4% of patients who underwent surgical treatment were treated with medical treatment in the postoperative period. In addition to medical treatment, gam knife was applied to six and radiotherapy was applied to two of these patients. In medical treatment, octreotide was applied to 33 patients. Pegvisomant, cabergoline or pegvisomant + cabergoline were added plus to octreotide in eight patients.

3.4. ACTH secreting adenomas

ACTH secreting adenomas account for 12.7% of all cases. 82.1% of the cases were female. The average age at the time of diagnosis was 37 years. 71.4% of adenomas were microadenoma. 100% cases had symptoms at the time of diagnosis. The most seen symptom was weight gain (26%). Other common symptoms were easy bruising (18%), edema in the body (18%), hirsutism (12%), galactorrhea (6%), headache (6%), infertility (4%), visual impairment (2%) and unregulated diabetes mellitus (2%). There were nonspecific symptoms in 6% of cases. Surgery was applied for 21 (84%) patients. Recurrence was developed in 20% of cases after surgery. Post-surgical cure was detected in 60% of cases. In 20% of cases remission could not be achieved after surgery due to residual tumor. Of these patients metirapon treatment was applied to 1 patient.

3.5. TSH secreting adenoma

Only three (1.8%) patients had TSH secreting adenoma. Two patients were male and had macroadenomas. Female patient

had microadenoma and followed without treatment. Surgery was applied to two patients with macroadenomas. Octreotide treatment was given both of two patients after surgery due to un remission.

4. Discussion

In our case series, prolactin-releasing adenomas, GH secreting adenomas and non-functional adenomas (NFPAs) were the most seen adenomas. ACTH secreting adenoma and TSH secreting adenoma were the following adenomas. In a report from England, the distribution of each pituitary adenoma subtype was 57% for prolactinoma, 28% for nonfunctioning adenoma, 11% for acromegaly, 2% for corticotrophin adenoma and 2% for unknown functional status (6). Prolactinomas are the most common functional adenomas, accounting for up to 60% of all pituitary adenomas. Microadenomas are more frequent than macroadenomas and a net predominance is observed in women aged 25–44 years compared to men, while this difference disappears after menopause (7). In accordance with the literature, 61.5% of the cases of our prolactinoma series was female, the average age of diagnosis was 40 years and 60% of adenomas were microadenoma. For prolactinomas, serum prolactin levels generally parallel with tumor size and prolactin level of more than 250 ng/mL is usually diagnostic for a macroprolactinoma (8). Our findings support these basic characteristics of prolactinoma. We measured prolactin levels >200 ng/mL in 67.8% of macroprolactinoma cases, <200 ng/mL in 73% of microprolactinoma cases. In treatment dopamine agonist therapy is recommended to lower prolactin levels, to decrease tumor size and to restore gonadal function for symptomatic prolactin-secreting microadenomas or macroadenomas (9). Cabergoline is the first option among dopamine agonists because it has higher efficacy in normalizing prolactin levels and higher frequency of pituitary tumor shrinkage. In the medically treated patients, normalization of prolactin level was achieved in 71% of the patients and total or partial degree of tumour shrinkage in 80% of the patients (10). In our study, remission was achieved in 76% of microprolactinoma and 24% of macroprolactinoma after dopamin agonist therapy.

Non-functioning pituitary adenomas do not cause a hormonal hypersecretion. The prevalence of NFPAs is 7–41.3 per 100,000 of population and they account for 14–54% of pituitary adenomas (11). They usually found incidentally on brain imaging performed for any unrelated indication. Clinically nonfunctioning macroadenomas account for about 80% of all pituitary macroadenomas (12). This can be explained with that clinically nonfunctioning adenomas are silent at the stage of microadenomas and only become clinically evident at the stage of macroadenomas. We also found 81.4% of nonfunctional adenomas as macroadenoma in our study. Most symptoms are related with mass effect, such as headaches, visual field defects and hypopituitarism. Laboratory evaluation for hypopituitarism should be performed for all NFPAs. The overall prevalence of partial hypopituitarism in patients with NFPAs ranges from 37% to 85% (13, 14). In our study, pituitary insufficiency was detected in 32% of patients in laboratory examination. Therefore, it should be kept in mind that approximately 1/3 patients may have insufficiency to require hormone replacement therapy among NFPAs.

Growth hormone secreting adenomas cause acromegaly due to increased growth hormone and insulin-like growth factor-1 (IGF-1) secretion. Prevalence of pituitary adenomas is 8.6/100.000 for somatotroph adenomas (6). Approximately 70% of patients with acromegaly have an invasive macroadenoma at diagnosis. We also found macroadenomas in 79% of GH secreting adenomas in our cases. Excess of GH and IGF-1 leads to multiple characteristic changes in the patient's appearance, skeletal deformities, and metabolic disorders. These changes include altered facial appearance, frontal-skull bossing, prognathism, enlarged extremities, increased shoe or ring size, carpal tunnel syndrome, hyperhidrosis, and coarse oily skin. Symptoms were present at the time of diagnosis in 92.2% of our cases and the most common symptom was enlargement in extremities (68.7%). The main treatment goals for acromegaly are resecting the pituitary adenoma and suppressing growth hormone and IGF-1 hypersecretion. The usual first-line treatment is surgery, if remission cannot be achieved, medical treatment with somatostatin analogues, dopamin agonists and pegvisomat and radiotherapy are available for second line treatments. Control of growth hormone secretion and IGF-1 levels was achieved in 73% of patients with microadenomas and 61% of patients with macroadenomas who underwent surgical resection (15, 16). Surgical treatment was applied 90% of our GH secreting adenomas. 61.4% of patients who underwent surgical treatment were treated with medical treatment in the postoperative period. In the literature, it is seen that hyperprolactinemia is present in 30% of patients with pituitary acromegaly due to pituitary stalk compression or cosecretion of prolactin from somatotroph adenomas with growth hormone (17). In our study, prolactin elevation was also detected in 20% of cases with GH secreting adenoma.

Corticotropin-secreting adenomas cause Cushing disease. They account for up to 15% of pituitary tumors (18). Adenomas are typically microadenoma and female to male ratio is 5-10/1. Compatible with the literature, ACTH secreting adenomas in our study was account for 12.7% of all cases, 82.1% of the cases were female and 71.4% of adenomas were microadenoma. Selective transsphenoidal adenomectomy is recommended as initial therapy. Remission achieved in approximately 75% of patients and recurrence seen in approximately 10% of patients (19). The second line treatment options are radical pituitary surgery, radiotherapy, stereotactic radiosurgery, medical therapy, and bilateral adrenalectomy. Medical therapy may improve clinical and biochemical outcomes but results of the studies about medical

therapy of Cushing disease are often inconsistent (20). In our study, post-surgical cure was detected in 60% of ACTH-secreting adenoma cases and medical treatment was given as metirapon only one patient.

Thyrotropin-secreting tumors are the least frequent type of pituitary adenomas compromising for approximately 1% of adenomas (21). Goiter, thyroid nodules, and hyperthyroidism are the manifestations in patients with TSH-secreting adenomas. Treatment of thyrotropinoma generally involves a transsphenoidal operation. Sanno et al. reported remission rate as 62.5% with surgery and 87.5% with combination therapy in 16 thyrotropin pituitary adenoma cases (22). We had only three TSHoma cases and surgery applied to two of them with macroadenoma. No remission was revealed and medical therapy with octreotide was given two of them.

The biological and morphological features of pituitary adenomas show variability. It is important to evaluate pituitary adenomas with clear biochemical imaging, and clinical phenotypes and define tumor characteristics and endocrine syndromes for individualized treatment or follow-up procedures.

Conflict of interest

There is no conflict of interest to declare.

Acknowledgments

None to declare.

Ethical Approval

The study was approved by the Ethics Committee of Ondokuz Mayıs University (date: 22.08.2013, No. 2013/392). The study was conducted in accordance with the principles of the Declaration of Helsinki.

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The dilemma of cesarean myomectomy: Is it safe or not?

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Abstract

To evaluate the safety and effectiveness of myomectomy during cesarean section. The data of fifty-four pregnant who underwent cesarean myomectomy and twenty-six pregnant with uterine leiomyoma who had cesarean section without myomectomy between the years of 2017 and 2020 in our tertiary clinic were examined retrospectively. There was no significant difference in terms of maternal age, weeks of gestation, gravida, parity, use of additional uterotonics, type of leiomyoma, size of leiomyoma, cesarean indications, blood transfusion requirement, postoperative fever, preoperative hemoglobin (g/dl), change in hemoglobin (g/dl), preoperative hematocrit (%), change in hematocrit (%), length of hospital stay between the two groups ($p>0.05$). While no significant difference was observed according to the location of the leiomyomas between the anterior, fundal and posterior location between the two groups, cervical leiomyomas were significantly higher in the CS group ($p<0.05$). This study shows that cesarean myomectomy is a safe procedure in selected cases. It also offers the advantage of avoiding a second operation in patients.

Keywords: cesarean, leiomyoma, pregnancy, uterine myomectomy

1. Introduction

Leiomyomas or uterine fibroids are benign monoclonal tumors of smooth muscle taking origin in the myometrium. They are the most common benign tumors in women of reproductive age and especially in the 30's. Although the etiology is largely unknown, they are known to be estrogen and progesterone dependent tumors (1). The reported prevalence of uterine leiomyomas in pregnancy is between 1.6 and 10.7% (2). In the future, it is expected that the incidence of leiomyomas during pregnancy will increase in association with delay in childbearing (3).

The first trimester is the ideal time to measure and identify the leiomyomas in pregnancy. Enlarging uterus size and growing of the fetus may prevent visualization of them in later gestational weeks (4). Considering that the risk for obstetric complications increases with the growth of leiomyomas, they should be followed up regularly with ultrasonographic imaging determining the number, size and location of them. The leiomyomas are usually asymptomatic during pregnancy. On the contrary, they may present with some symptoms in 10–30% of patients. The most common symptoms are pain due to red degeneration, miscarriage, bleeding, preterm labor, preterm premature rupture of membranes, placental abruption, increased leiomyoma size, abnormal presentation, congested labor, and postpartum hemorrhage (5). About 5–21% of pregnant women with leiomyoma are hospitalized during pregnancy for pain control, which is often associated with large leiomyoma (>5 cm), posterior leiomyoma or red degeneration (4, 6). Red degeneration is a pregnancy-specific condition and occurs more frequently in the first trimester and onset of pregnancy when leiomyoma grow faster. The pain is thought to be due to necrotic infarction (due to rapid growth and tissue anoxia), the change in the blood flow of the growing uterus, and the release of prostaglandins from cellular damage (7). Uterine leiomyoma may negatively affect the implantation, placentation, and ongoing pregnancy through mechanical disruption of the endometrial cavity, impaired endometrial vascularization, and endometrial inflammation. Therefore, leiomyomas are associated with miscarriage, intrauterine growth restriction, intrauterine fetal death, preterm labor, placental abruption, and postpartum hemorrhage (8).

Due to the marked increase in uterine blood flow during pregnancy, obstetricians usually hesitate to perform cesarean myomectomy (CM). Even if some authors recommend only cesarean section (CS), most authors recommend that CM can be performed in selected cases by experienced surgeons. Indications and contraindications for CM are still not clearly defined and still it is a controversial issue. Since the size of the uterus increases during pregnancy (the uterus/tumor ratio is higher than the non-pregnant) leiomyomas can be removed with a relatively small incision and can be sutured more easily by dint of increased elasticity and reduced fragility of the uterus (9). Considering the incidence of uterine leiomyomas in pregnancy continues to increase worldwide like CS birth rates, we aimed to share the outcome of our CM experiences to contribute to the literature in this regard.

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2. Materials and methods

This is a retrospective study of patients with leiomyoma who had a cesarean delivery in our Obstetrics and Gynecology Department between the years of 2017 - 2020. Patients with leiomyomas detected during pregnancy follow-up were included in the study. We excluded patients with multiple pregnancy, placental adhesion anomalies, preeclampsia, uterine hypotonia, uterine atony, multiple leiomyomas, leiomyomas located close to the great vessels, and known congenital or acquired coagulopathies which are contraindications for CM (10-12). Pregnant women with leiomyoma who had only CS but not myomectomy was chosen as the control group. This study was approved by the ethics committee and the study was conducted in accordance with the Helsinki Declaration.

Data were collected from inpatient file records, operation, and discharge notes. Patients' age, gravida, parity, cesarean indications, gestational weeks, leiomyoma location, leiomyoma size and leiomyoma types detected during cesarean were recorded. In addition, pre-operative, and post-operative 24th hour hemoglobin (Hb) values, differences between Hb values, pre-operative, and post-operative 24th hour hematocrit (Htc) values, differences between Htc values, additional uterotonic requirement, blood transfusion requirement, duration of hospital stay, postoperative fever (temperature greater than or equal to 38°C) and histopathological examination results of myomectomy materials were examined. The key point to be considered in the surgical technique is the protection of the leiomyoma pseudo-capsule. This method allows the maintenance of healthy myometrial tissue around the leiomyomas and myometrial healing after myomectomy. Many neurotransmitters and neuropeptides necessary for myometrial physiology have been found in the leiomyoma pseudo-capsule (13). A linear incision was made on the leiomyoma with the help of electrocautery or scalpel and was removed with the sparing of the leiomyoma pseudo-capsule. The remaining myometrial cavity and serosa were closed ensuring adequate tissue tension with the number of 2/0 or 0 vicryl sutures. In cases where the endometrial cavity was opened during myomectomy, the endometrium was sutured. The remaining uterine serosa is sutured in a running baseball fashion. The measurement of leiomyomas was based on the largest diameter measured in histopathological examinations.

Table 1. Demographic and preoperative characteristic for all patients

		Min-Max			Median	Mean ± SD/n-%		
Maternal age (years)		21.0	-	42.0	34.0	33.3	±	4.2
Gestational age (week)		28.0	-	40.5	38.4	38.0	±	2.4
Gravida (n)	Primigravid					49		61.3%
	Multigravid					31		38.8%
Parity (n)	Nulliparity					61		76.3%
	Multiparity					19		23.8%
Use of additional uterotonics	(+)					14		17.5%
	(-)					66		82.5%
Location of myoma	Anterior					32		40.0%
	Fundal					25		31.3%
	Posterior					20		25.0%
	Cervical					3		3.8%
Type of myoma	Subserosal					41		51.3%
	Submucosal					7		8.8%
	Intramural					32		40.0%
Size of myoma	≤5 cm					37		46.3%
	>5 cm					43		53.8%
Cesarean indications	Previous uterine surgery					29		36.3%
	Cephalopelvic disproportion					20		25.0%
	Non-progressive labor					8		10.0%
	Abnormal presentation					10		12.5%
	Fetal Distress					13		16.3%
Blood transfusion requirement	(-)					79		98.8%
	(+)					1		1.3%
Postoperative fever	(-)					79		98.8%
	(+)					1		1.3%
Myomectomy	(+)					54		67.5%
	(-)					26		32.5%
Preoperative Hb (g/dL)		8.9	-	13.9	12.0	12.0	±	1.0
Change in Hb (g/dL)		0.10	-	4.30	1.30	1.39	±	0.91
Preoperative Htc (%)		26.8	-	41.5	36.0	35.6	±	2.6
Change in Htc (%)		0.1	-	12.4	3.7	4.2	±	2.7
Length of hospital stay (day)		2.0	-	7.0	2.0	2.1	±	0.6

Average, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured by the Kolmogorov -Smirnov test. Independent sample t-test, Kruskal – Wallis and Mann - Whitney U test was used in the analysis of quantitative independent data. In the analysis of qualitative independent data, the chi-square test, Fischer test was used when chi-square test conditions were not met. SPSS 26.0 program was used in the analysis.

3. Results

Eighty pregnant women between the ages of 21-42 were included in the study. The mean gestational week of the patients was 38.0 ± 2.4 (Table 1). While 54 patients were in the CM group, 26 were in the CS group. Table 2 summarizes comparisons of clinical characteristics in women with cesarean myomectomy and with only cesarean section, revealing no significant differences concerning maternal age, gestational age, gravida, parity, cesarean indication distributions and hospital stay of the two groups (p>0.05). In the CM group, the indications for cesarean delivery were 33.3% previous uterine surgery, 25.9% cephalopelvic disproportion, 16.7% fetal distress, 13% abnormal presentation and 11.1% non-progressive labor. While no significant difference was observed according to the location of the leiomyomas between the anterior, fundal, and posterior location between the two groups, cervical leiomyomas were significantly higher in the CS group (p<0.05). In terms of leiomyoma types, while 53.8% of intramural leiomyoma was observed in the CS group 59.3% of subserous leiomyoma was observed most frequently in the CM group (p>0.05). The additional uterotonic need in the CM group was 20.1%, while the need in the CS group was 11.5% (p>0.05) (Table 2). While 61.1% of leiomyomas in the CM group were >5 cm, in the CS group only 38.5% were observed greater than 5 cm (p: 0.057). In the CM group, transfusion requirement and postoperative fever were observed in 1 patient while no need for transfusion required, and postoperative fever seen in the CS group (p>0.05) (Table 2). Preoperative Hb, Hb change, preoperative Htc and Htc change did not differ significantly between the groups (p>0.05). In the CM group, Hb change, Htc change, additional uterotonic dose need, transfusion requirement, postoperative fever and duration of hospital stay have no relation with the location, size and types of the leiomyomas (p>0.05) (Table 3-5).

Table 2. Comparisons of clinical characteristics and outcomes across women with and without cesarean myomectomy

		Cesarean myomectomy				Cesarean				p	
		Mean ± SD/n-%			Median	Mean ± SD/n-%			Median		
Maternal age (years)		33.3	±	4.3	34.0	33.3	±	4.0	34.0	0.992	m
Gestational age (week)		37.8	±	2.8	38.5	38.3	±	1.2	38.4	0.777	m
Gravida (n)	Primigravid	35		64.8%		14		53.8%		0.346	x^2
	Multigravid	19		35.2%		12		46.2%			
Parity (n)	Nulliparity	42		77.8%		19		73.1%		0.644	x^2
	Multiparity	12		22.2%		7		26.9%			
Use of additional uterotonics	(+)	11		20.4%		3		11.5%		0.330	x^2
	(-)	43		79.6%		23		88.5%			
Location of myoma	Anterior	25		46.3%		7		26.9%		0.157	x^2
	Fundal	18		33.3%		7		26.9%		0.747	x^2
	Posterior	11		20.4%		9		34.6%		0.270	x^2
	Cervical	0		0.0%		3		11.5%		***0.031***	x^2
Type of myoma	Subserosal	32		59.3%		9		34.6%		0.118	x^2
	Submucosal	4		7.4%		3		11.5%			
	Intramural	18		33.3%		14		53.8%			
Size of myoma	≤5 cm	21		38.9%		16		61.5%		0.057	x^2
	>5 cm	33		61.1%		10		38.5%			
Cesarean indications	Previous uterine surgery	18		33.3%		11		42.3%		0.952	x^2
	Cephalopelvic disproportion	14		25.9%		6		23.1%			
	Non-progressive labor	6		11.1%		2		7.7%			
	Abnormal presentation	7		13.0%		3		11.5%			
	Fetal Distress	9		16.7%		4		15.4%			
Frequency of blood transfusion	(-)	53		98.1%		26		100%		1.000	x^2
	(+)	1		1.9%		0		0.0%			
Postoperative fever	(-)	53		98.1%		26		100%		1.000	x^2
	(+)	1		1.9%		0		0.0%			
Preoperative Hb (g/dL)		11.9	±	1.0	11.9	12.1	±	1.1	12.1	0.573	t
Change in Hb (g/dL)		1.40	±	0.94	1.25	1.38	±	0.87	1.35	0.914	m
Preoperative Htc (%)		35.6	±	2.6	35.5	35.8	±	2.7	36.5	0.739	t
Change in Htc (%)		4.3	±	2.8	3.7	3.9	±	2.6	3.6	0.674	m
Length of hospital stay (day)		2.1	±	0.7	2.0	2.0	±	0.2	2.0	0.532	m

t, t test; m , Mann-Whitney U Test; X^2 ,Chi square test (Fischer test)

Table 3. Comparison of outcomes in women with cm concerning localization of leiomyoma

		Anterior				Fundal				Posterior				p	
		Mean ± SD/n-%			Median	Mean ± SD/n-%			Median	Mean ± SD/n-%			Median		
Preoperative Hb (g/dL)		11.9	±	1.2	12.0	12.1	±	0.9	11.9	11.6	±	0.8	11.3	0.313	K
Change in Hb (g/dL)		1.37	±	0.94	1.10	1.45	±	1.02	1.30	1.39	±	0.88	1.40	0.913	K
Preoperative Htc (%)		35.5	±	3.1	36.0	35.9	±	2.3	35.4	35.2	±	2.2	34.5	0.674	K
Change in Htc (%)		4.0	±	2.8	3.5	4.5	±	2.9	4.0	4.6	±	2.7	4.5	0.684	K
Use of additional uterotonics	(+)	4		16.0%		6		33.3%		1		9.1%		p>0.05	X^2
	(-)	21		84.0%		12		66.7%		10		90.9%			
Blood transfusion requirement	(-)	25		100%		17		94.4%		11		100.0%		p>0.05	X^2
	(+)	0		0.0%		1		5.6%		0		0.0%			
Postoperative fever	(-)	25		100%		17		94.4%		11		100.0%		p>0.05	X^2
	(+)	0		0.0%		1		5.6%		0		0.0%			
Length of hospital stay		2.2	±	1.0	2.0	2.1	±	0.3	2.0	2.0	±	0.0	2.0	0.550	K

^{K}, Kruskal-Wallis; X^2, Chi-square test (Fischer test)

Table 4. Comparison of outcomes in women with CM concerning type of leiomyoma

		Subserosal				Submucosal				Intramural				p	
		Mean ± SD/n-%			Median	Mean ± SD/n-%			Median	Mean ± SD/n-%			Median		
Preoperative Hb (g/dL)		11.9	±	1.0	11.8	12.7	±	0.8	12.7	11.8	±	1.1	11.5	0.277	K
Change in Hb (g/dL)		1.54	±	1.04	1.35	1.18	±	0.39	1.30	1.21	±	0.82	0.90	0.534	K
Preoperative Htc (%)		35.5	±	2.6	35.3	36.8	±	2.2	36.9	35.4	±	2.9	35.7	0.537	K
Change in Htc (%)		4.6	±	3.1	3.8	3.5	±	1.1	3.4	3.9	±	2.3	3.4	0.708	K
Use of additional uterotonics	(+)	6		18.8%		2		50.0%		3		16.7%		p>0.05	X^2
	(-)	26		81.3%		2		50.0%		15		83.3%			
Blood transfusion requirement	(-)	31		96.9%		4		100.0%		18		100.0%		p>0.05	X^2
	(+)	1		3.1%		0		0.0%		0		0.0%			
Postoperative fever	(-)	31		96.9%		4		100.0%		18		100.0%		p>0.05	X^2
	(+)	1		3.1%		0		0.0%		0		0.0%			
Length of hospital stay		2.2	±	0.9	2.0	2.0	±	0.0	2.0	2.1	±	0.2	2.0	0.741	K

^{K} Kruskal-wallis / X^2 Chi-square test (Fischer test)

Table 5. Comparison of outcomes in women with CM concerning size of leiomyoma

		Size of myoma ≤5 cm				Size of myoma > 5 cm				p	
		Mean ± SD/n-%			Median	Mean ± SD/n-%			Median		
Preoperative Hb (g/dL)		12.0	±	1.0	11.8	11.9	±	1.1	12.0	0.831	m
Change in Hb (g/dL)		1.31	±	0.80	1.10	1.45	±	1.02	1.30	0.789	m
Preoperative Htc (%)		35.5	±	2.3	35.3	35.6	±	2.9	36.0	0.467	m
Change in Htc (%)		3.9	±	2.6	3.1	4.5	±	2.9	3.9	0.394	m
Use of additional uterotonics	(+)	4		19.0%		7		21.2%		0.847	x^2
	(-)	17		81.0%		26		78.8%			
Frequency of blood transfusion	(-)	21		100.0%		32		97.0%		1.000	x^2
	(+)	0		0.0%		1		3.0%			
Postoperative fever	(-)	21		100.0%		32		97.0%		1.000	x^2
	(+)	0		0.0%		1		3.0%			
Length of hospital stay (day)		2.0	±	0.2	2.0	2.2	±	0.9	2.0	0.545	m

^{m} Mann-whitney u test / X^2 Chi-square test (Fischer test)

4. Discussion

As the primary outcome, we evaluated the difference between preoperative and postoperative Hb levels and the difference between preoperative and postoperative Htc levels. Besides, blood transfusion requirement, prolonged hospitalization, postoperative fever and peripartum hysterectomy requirement were evaluated as the secondary outcomes. Intraoperative hemorrhage has been reported to be the most frequent complication of CM's (14). There are many factors that affect the amount of hemorrhage during operation. Many preventative techniques such as vasopressin, ergometrine, oxytocin, misoprostol, uterine tourniquet and bilateral uterine artery ligation can be used to reduce bleeding before myomectomy operation (15). Apart from these, other factors affecting the amount of bleeding are the surgeon's experience, selection of the correct indication and the lack of standard surgical technique for the operation. Considering all these factors, some studies have shown that performing myomectomy during cesarean did not negatively affect the change in Hb (14, 16-19), while some studies have shown that it increased the change of Hb (20, 21). Although none of the additional methods were used in any of the cases in our study, we found the hemoglobin decrease similarly to the control group. We achieve this situation with the right patient choice and the right dose application of additional uterotonics when it was necessary. Uterine leiomyomas are classified based on location: sub serosal (distorting the external contour of the uterus, >50% of the leiomyoma must project outside the myometrium), intramural (within the myometrium, distorting neither the external contour nor cavity) and submucosal (>50% of the leiomyomas mass projects into the uterine cavity covered by endometrium and distorting the cavity)

(22). While no significant difference was observed according to the location of the leiomyomas between the anterior, fundal, and posterior location between the two groups, cervical leiomyomas were significantly higher in the CS ($p<0.05$). In the literature, most of the surgeons preferred to excise often subserous leiomyomas and corpus located leiomyomas like us (12, 19).

Another factor that is effective in deciding to perform myomectomy during the cesarean section is the size of leiomyomas. Although 61.3% of our CM group consisted of >5 cm leiomyomas, the postoperative Hb reduction compared to <5 cm leiomyomas were similar. It was also showed in several studies that leiomyoma size did not have a negative effect on postoperative hemoglobin decrease (18, 19, 23). After cesarean delivery, the routine dose of oxytocin was applied in all cases but the additional uterotonic drugs were applied only in atonic cases with excessive bleeding. Dedes et al. found that additional uterotonic need was higher but not statistically significant in the CM group than CS group as in our study (p: 0.33) (16). Although cesarean myomectomies have been associated with heavy bleeding, higher need for reoperation, hysterectomy, arterial embolization, arterial ligation, and ileus, none of these risks was encountered in our patients (12). Since it was a retrospective study, we were unable to reach operating times completely and therefore we could not include this parameter in our study. Many studies in the literature showed that performing myomectomy during cesarean increased the duration of surgery significantly (16,19,24,25). Only 1 of our 54 CM patients required blood transfusion ($p > 0.05$). As stated in a meta-analysis, the need for blood transfusion did not increase in CMs (24). Although our study shows that myomectomy during cesarean did not prolong hospital stay there were different results in the literature. In some of these studies, while the duration of hospitalization in CM patients was longer (17, 26, 27), on the contrary some studies suggested that there was no significant difference in the duration of hospitalization (18, 23, 28). Consistent with the literature, we did not find any evidence of CM causing postoperative fever in patients (19, 24). The risk of malignancy for uterine leiomyomas is extremely low and is estimated to be 1/400 (29). In our study, all myomectomy samples were examined histopathologic ally and all of them were confirmed as benign leiomyoma.

There are some limitations in our study. It was a retrospective study with a small sample size. We also didn't have the long-term follow-up data of patients and could not emphasize its impact on future pregnancies. The results of our study show that cesarean myomectomy had no adverse effect on morbidity and mortality in the intrapartum or in the early postpartum period. For this reason, we suggest that cesarean myomectomy can be performed safely in selected cases by obstetricians in order not to do another surgery and burden its additional cost.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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Research Article

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Evaluating the histopathological and mechanical effects of a new forceps design: comparison of hemispheric bipolar forceps tip with usual bipolar tip on fresh cadaver cattle brain model

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Abstract

Without electrocautery, many modern surgical interventions are practically impossible. In neurosurgery, bipolar cautery forceps has been evolved to not only be an auxiliary, but as a principal instrument wielded by the dominant hand of the surgeon to navigate through the most delicate tissue that there is. The purpose of this study is to introduce our original bipolar forceps designed exclusively for microneurosurgical interventions and compare its feasibility with a standard bipolar forceps tip. This study has been conducted on two fresh cadaveric cow brains under the operating microscope. The coagulative and ablative effects of the hemispheric bipolar forceps tip (HBFT) have been histologically compared with those of the standard bipolar forceps tip (SBFT). Likewise, their efficacies as a dissection instruments have been compared via performing dissections from the parietal surface down to the corpus callosum. HBFT proved less traumatic to the uninvolved brain tissue during dissection. Also, histological analyses have revealed that ablative effects of the HBFT are more confined to the bleeding point, more effectively sparing the uninvolved brain tissue. Results of this experimental study suggest that HBFT is a better instrument to be used in microneurosurgical interventions, along with other surgical disciplines where selective diathermy is critical.

Keywords: brain surgery, coagulation, hemispheric tip bipolar forceps, microsurgery

1. Introduction

Upon its introduction in 1940 by Greenwood, bipolar diathermy forceps revolutionized neurosurgical operations by not only providing a safe means for bleeding control, but also for navigating through the brain substance as well. Unlike unipolar cautery in which electricity courses from the entry point to the lead attached to a remote region of the patient; a lesser but more intense electric current passing between the tiny forceps tips carbonizes the vessels and stops the bleeding while minimally affecting the sensitive bystander neural tissue (1-5).

Microneurosurgery takes place in a tight spot. The luxury of changing instruments at whim is hardly afforded and the surgeons eventually came to rely on the tool in their dominant hand for practically everything, bleeding control, microdissection and even tumour ablation and removal. There are many types of bipolar forceps tips in use, including those used by the authors in their routine practice; yet none of them is specially designed for neurosurgery (1). It has been noted that although a neurosurgeon does much more than coagulation by these instruments, they are hardly any different than those used only for coagulation by other disciplines. Consequently, a bipolar forceps tip which is better suited to work as a multi tool inside arguably the most valuable tissue on earth has been designed.

A prototype with two subtypes has been constructed by a local medical instrument manufacturer and its efficacy has been compared with that of a SBFT on a cadaveric cattle brain model.

2. Materials and methods

No living creature was used in this study.

2.1. Instrument design

In the new design, features which better accommodate the conditions surrounding micro neurosurgery have been sought, including limited electrodessication, atraumatic dissection and efficient but selective tissue ablation. Unlike SBFT, the new design features a forceps the tips of which do not taper down to converge as a pointy end, but rather two concave hemispheres with bevelled edges which complement each other (model a). Another version (model b) includes not a smooth bevelled but toothed rim designated for handling denser masses (Fig. 1). HBFT's has been manufactured by a private medical instrument manufacturer company out of medical grade pure silver for the body and medical high quality silicone coating on the handles. And, Medtronic (USA) leads which fit standard Valley lab ™ Medtronic (USA) electrocautery device sockets. The material properties of the HBFT are like those of the SBFT (Bahadır

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Electrosurgical Instruments, Turkey) used as the counterpart in this study.

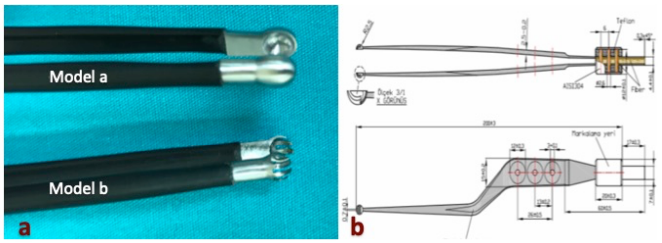

Fig. 1. Design of Hemispheric Bipolar Forceps Tips (HBFT). A: Smooth edged (top) and toothed (bottom) HBFT designs. B: Technical diagram

2.2. Experimental design

Three fresh cadaveric cattle brains have been used to assess the effectiveness of both types of HBFTs and electrical damage inflicted, dissection efficacy, and ablative efficacy have been compared with those yielded by SBFT. Parameters which are taken into consideration are as follows:

- With each instrument, cranial surfaces and pia maters of each brain have been cauterized five times with 15 watts power setting. Under the operating microscope, mechanical effects have been assessed.
- On each brain, cortical splits were done on both parietal lobes with a no. 15 scalpel blade and dissections were carried out with both instruments. During dissections, mechanical damage inflicted on the brain tissue (tears, punctures) was observed under the operating microscope.
- Within the subcortical parenchymal tissue, ten 15-watt coagulations were done on each brain and afterwards, cauterized specimens were harvested for histological analyses.
- A parenchymal tumour model was created using moderately boiled egg yolk in each brain's subcortical space. Egg yolk maintained the consistency of a mass while lacing the surrounding tissues, mimicking the surgical behaviour of a glial tumour. Afterwards they were cauterized away using both instruments. The effectiveness of tumour ablation and each tool's collateral damage was compared.

3. Results

When pia coagulation was mechanically evaluated, it has been observed that HBFT produces a homogenous, superficial coagulation zone while SBFT produces a deeper desiccation with a carbonized central puncture on all cauterizations (Fig. 2).

During cortical dissection, it has been observed that when the tips are closed the HBFT probes through the brain substance without allowing the tissue to get caught on any pointy end. When released to separate tissue, convex outer surfaces gently push the tissues apart without inflicting any tears. When the same feat is attempted by SBFT, it has been noted that despite best efforts, it is highly likely to inflict punctures and tears along the way. When closed, SBFT's needle like tip was highly likely to cut through tissue rather than dissecting it, and when separated the tips inflicted tears while pushing tissues apart, even with care was taken to avoid it (Fig. 3).

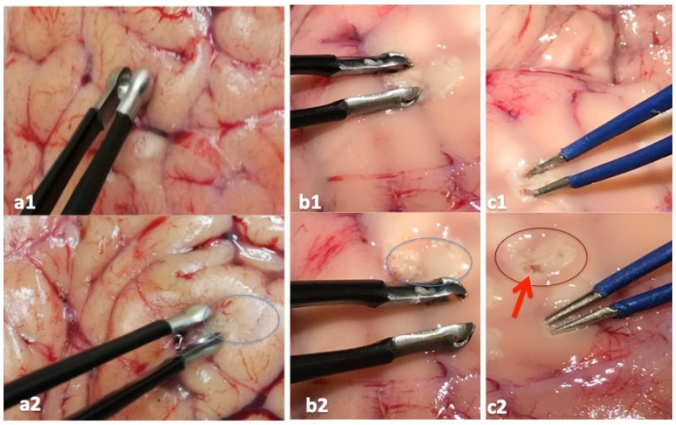

Fig. 2. a1-2: pia coagulation with HBFT (model a); b1-b2: pia coagulation with HBFT (model b); c:1-2 homogenous superficial coagulation zone without puncture with SBFT; red arrow: pucture on pia

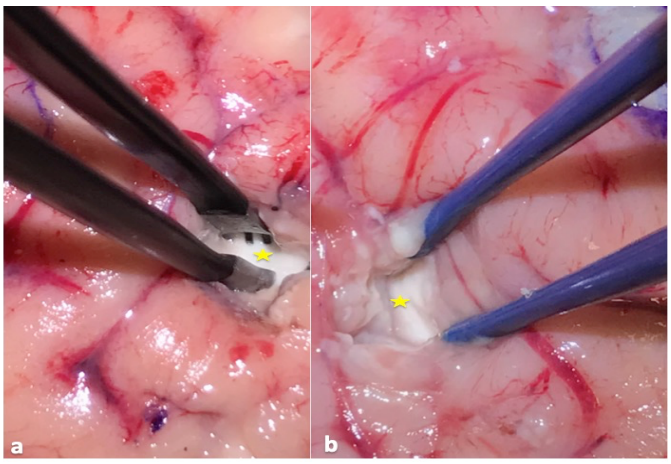

Fig. 3. a: cortical dissection with HBFT without inflicting any tears; b: cortical dissection with SBFT inflicting tears yellow star: corpus callosum

Both types of HBFT have proved as a highly effective tool for tumour ablation as well. Its scoop-like shape harvested the tumour while superficially cauterizing it away from the healthy tissue. The result was an evenly cauterized cavity, which is totally cleared of the tumour. This function could not be achieved by SBFT. Instead, it repeatedly chipped away on the tumour model without removing it (Fig. 4).

Histopathological evaluations further demonstrated the difference between the collateral damages associated with cautery use. The coagulation depth and the severity of desiccation were remarkably higher with the SBFT while the coagulation zone of the HBFT was subtler, more even and superficial (Fig. 5).

In tumour ablation, HBFT demonstrated a superficially desiccated parenchymal surface. The histological data pertaining to coagulation and tumour ablation of both tips has been summarized in Table 1.

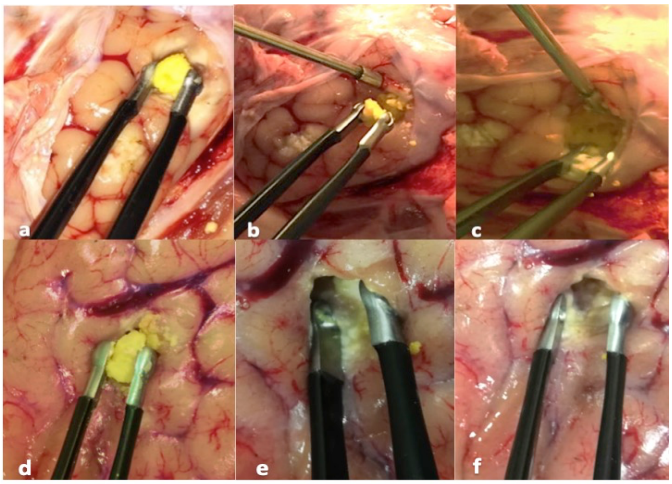

Fig. 4. A parenchymal tumour model was created using moderately boiled egg yolk. a-b-c tumour ablation with HBFT like tumour forceps (model a); d-e-f tumour ablation with HBFT like tumour forceps (model b)

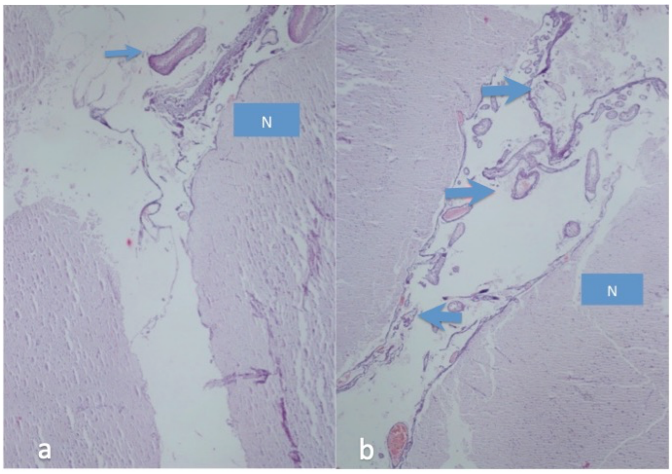

Fig. 5. Histopathological evaluations difference between the collateral damages associated with cautery use. a: The coagulation depth and the severity of desiccation were lower with the HBFT; blue arrow: shows cauterized veins only on the surface area; N: neuroglial tissue; b: The coagulation depth and the severity of desiccation were higher with the SBFT; blue arrows: show cauterized veins on the surface and deep area; N: neuroglial tissue

Table 1. The histological data pertaining to coagulation and tumour ablation of both SBFT and HBFT (HBFT model a and b)

SBHT Parenchymal surface coagulation depth	HBFT Parenchymal surface coagulation depth	SBHT Tumour ablation coagulation depth	HBFT Tumour ablation coagulation depth
5.3 mm	3.1 mm	6.2 mm	4 mm
5.3 mm	3.2 mm	6.2 mm	4.1 mm
6 mm	3.2 mm	6.4 mm	4.2 mm
6.4 mm	3.4 mm	6.4 mm	4.2 mm
7 mm	4 mm	6.9 mm	4.4 mm

4. Discussion

For many surgeons, bipolar forceps is an instrument that is used to zap bleeders, most of the time wielded by the assistant. For a neurosurgeon bipolar forceps is a multi-tool glued to his/her dominant hand until the job is done. It is effectiveness as a coagulation tool is of cardinal importance, as a haematoma as small as a few millimetres can have deleterious repercussions (3, 4). However, in neurosurgical practice it goes beyond that; since neurosurgeons can need the bipolar practically anytime, they tend to hold on to it throughout the surgery and use it for navigating through the brain substance. Therefore, a bipolar cautery forceps designed for such special circumstances must be more effective and less traumatic in dissecting brain tissue, minimally subject the surrounding tissues to electrical damage while effectively stopping the bleeding, and effectively bite away any tissue to be ablated while, again, minimally effecting the tissues other than those the surgeon wants to get rid of.

Since its use in neurosurgery has been introduced by Greenwood in 1940, bipolar cautery tips remained pretty much the same. Other than the popularization of bayonet design by Yaşargil, bipolar tips are hardly customized for carrying out these delicate tasks. Rather, neurosurgeons made do with the designs, which are available for general use.

As practitioners of neurosurgery, we believe that a bipolar forceps tip to be used exclusively in neurosurgery is much needed and developed a design which can overcome the shortcomings of the instruments readily available.

Classical pointed bipolar forceps tip is designed to cauterize small vessels and it effectively delivers. However, in neurosurgery not only tiny vessels, but also little sinusoid venules are also encountered, and their bleeders are very troublesome to control with the SBFT, if not at all impossible. HBFT has been shown to create a surface coagulation rather than a point coagulation, which is broader but more superficial, as would be desired in neurosurgical procedures.

We tried out the new design and compared it with a tip available for clinical use in a cadaveric model. While using a cadaveric, instead of live model can be regarded as a weakness of our study design, we believed that we needed a large brain to mimic the challenges encountered in actual brain surgery. It is our belief that researchers tend to use live specimens not necessarily because it is absolutely required but to increase the manuscript's chances of being accepted. Since we are using a clinical counterpart to assess efficacy, we concluded that sacrificing live subjects with a brain size comparable with humans was ethically and practically redundant.

Since neurosurgeons prefer to keep the bipolar readily available, using it for dissection of brain substance is commonplace. However, since it has not been manufactured to be used in this manner, it does not provide effective dissection and exposure with its slim body. Moreover, due to its sharp pointy end, it can easily inflict trauma to the surrounding tissues in all but steadiest of hands. HBFT has a smooth, blunt tip when it is closed, and it follows the natural cleavage of the brain substance. When the tip is opened, it gently pushes the tissues apart thanks to the wide and convex

outer surface. In our experiment, it has been noted as a faster and safer means of cortical dissection.

When the tumour is accessed, the daunting task of fully removing it without bleeding out the patient stands. Therefore, the surgeon must switch between the tumour forceps and bipolar cautery tip, taking turns ablating the tumour and stopping the bleeding. This course of action is distracting, frustrating and time consuming; all of which are very undesirable during a brain surgery. In this situation HBFT also doubles as a tumour forceps. It cuts away the tumour, stops bleeding before it starts, control a bleeder if present, and leave a superficially desiccated cavity, ablating any remnant tumour substance along the way. In our experiment, both types of HBFT's were similarly atraumatic.

We believe that we came up with a design which can enhance the practice, speed, and success of micro neurosurgical procedures. The HBFT has been designed, as a response to the needs of neurosurgeons and requirements of neurosurgery and it is likely to be emulated by other surgical disciplines as well. The patent application is made and as of now pending.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Research Article

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Does recurrent pregnancy loss have an inflammatory background?

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Abstract

Although several pathophysiological mechanisms are defined in etiology recurrent pregnancy loss, still causes of half of the cases haven't revealed yet. It is reported that inflammatory processes take place in the etiology of the disease. In our study, we aimed to reveal the relationship between recurrent pregnancy loss with white blood cell count (WBC), C-reactive protein (CRP) and ferritin levels. We included our study 90 pregnant women having recurrent miscarriage history and 101 pregnant women without recurrent miscarriages, 191 patients in total. Maternal and gestational age, height, weight, body mass index (BMI), gravidity, parity, abortion and living children count and WBC, CRP and ferritin levels of these pregnant were evaluated retrospectively. According to outcomes, while the age ($p = 0.01$; $p<0.05$), gravidity ($p = 0.00$; $p<0.01$) and abortion counts ($p = 0.004$; $p<0.01$) of the study group were found significantly to be higher than that of the control group, weight measurement of them was significantly lower than that of the control group ($p = 0.04$; $p <0.05$). Height and BMI measurements, parity and living children counts of the groups showed no statistically significant difference ($p>0.05$). While WBC levels of the study group was found to be lower ($p=0.045$, $p<0,05$) than that of control group, there was no significant difference regarding ferritin and CRP levels ($p> 0.05$). In our study, WBC, CRP and ferritin parameters did not indicate the inflammatory background in recurrent pregnancy loss. We think that further prospective randomized controlled studies are required regarding these parameters.

Keywords: C-reactive protein, ferritin, recurrent pregnancy loss, white blood cell count

1. Introduction

Clinically diagnosed miscarriage accounts for 15 to 25% of all pregnancies and most of them occur under 10 weeks due to chromosomal numerical abnormality (1). The Practice Committee of the American Society for Reproductive Medicine (ASRM) defines recurrent pregnancy loss as two or more clinical miscarriage (2). Less than 5% of women experience two consecutive pregnancy losses while 1% of them experience three consecutive pregnancy losses (1). Those with miscarriage are more likely to have miscarriage again (3). Many pathological mechanisms such as uterine anomalies, endocrine and metabolic problems, genetic anomalies, acquired or congenital thrombophilia and autoimmune diseases have been defined in the etiology of recurrent pregnancy loss. Unfortunately, the underlying pathological mechanism cannot be revealed in half of the patients (4).

An inflammatory microenvironment is needed for successful embryo implantation (5). However, when the inflammatory response is more than necessary, it causes pregnancy complications such as recurrent pregnancy loss, preeclampsia and preterm birth (6). In recurrent pregnancy loss, the immune response is handled in two ways: immune suppression and immune tolerance. Antigens expressed in fetal or placental tissues stimulate the alloimmune response. In contrast, T helper 1 and T helper 2 cells play an important role in immune tolerance and rejection response. The dominance of T helper 2 cells is important in the continuation of normal pregnancy (4). However, if T helper 1 dominance exists and there is an increase in the number and cytotoxicity of Natural Killer (NK) cells responsible for the relative excess of proinflammatory cytokines such as Tumor necrosing factor (TNF) α, Interleukin (IL) 1, 6 in blood and endometrium and reconstruction of vessels and trophoblasts, we may encounter adverse pregnancy outcomes such as recurrent pregnancy loss (7-9).

Ferritin is a protein that stores iron and is actually not involved in transport, and it is widely used to determine the iron status in the body. In inflammatory processes, serum ferritin levels increase due to ferritin release from the destructed cells. As a result of this mechanism, the high ferritin value is actually an indicator of an inflammatory process (10).

C- Reactive protein is a marker released from other cells such as hepatocytes and trophoblasts, indicating low-grade chronic inflammatory response (11). CRP level may increase

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in inflammatory conditions, cancer, asthma, diabetes, cardiovascular diseases as well as in adverse pregnancy outcomes (12-15). Although it increases slightly in the first four weeks of pregnancy compared to normal (16), it is found at higher levels in cases such as preeclampsia, preterm labor, intrauterine growth retardation (15, 17, 18).

Based on this information, in our study, we aimed to investigate the role of ferritin, CRP and White Blood cell Count (WBC), which are some of the inflammatory markers that can be detected easily and cheaply in blood, in the etiology of recurrent pregnancy loss.

2. Materials and methods

191 patients who applied to Samsun Gynecology and Obstetrics Hospital and Health Sciences University Samsun Training and Research Hospital Gynecology and Obstetrics Department between December 2016 and January 2020 were included in the study. Ninety patients who had 5-17 weeks gestational aged (median nine weeks) pregnancy and an intact gestational sac within the heart beats of the fetus could not be obtained by transvaginal ultrasonography and had two consecutive pregnancy loss between 7-10 weeks, included in the recurrent pregnancy loss group. Those with uterine abnormality, chromosome abnormality, thyroid dysfunction, toxoplasma, rubella, cytomegalovirus and herpes virus infection, diabetes, hypertension and autoimmune disease were excluded from the study. 101 women with a live, healthy pregnancy that gestational age matched (5-19 weeks median nine weeks) with the study group and having no miscarriage history were included in the control group. The study was planned as a retrospective cross-sectional study. Maternal and gestational age, height, weight, body mass index (BMI), gravidity, parity, abortion and living children count, ferritin, CRP and WBC levels of participants were obtained from previous hospital records and evaluated.

The ethical committee approval of Health Sciences University Samsun Training and Research Hospital Medical Specialization Training Board, dated 27/05/2020 and No GOKA 2020/7/28 was obtained for conducting the research. In addition, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. Sample size was determined for α: 0.05 and ß: 0.80 by a biostatistics specialist in 19 Mayıs University. NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used when evaluating the study data. The suitability of quantitative data for normal distribution was tested by Kolmogorov-Smirnov, Shapiro-Wilk test and graphical evaluations. Student's t-test was used for comparing two groups of normally distributed quantitative data, and Mann Whitney U test was used for two-group comparisons of non-normally distributed data. Significance was considered at least $p<0.05$.

3. Results

The total age of 191 cases varies between 17 and 44, with an average of 29.03±0.87 years. Weight measurements of the cases ranged from 41 to 110 kg, with an average of 69.20±1.18 kg; their height varied between 1.45 and 1.92 m, with an average of 1.59 ± 0.09 m; BMI measurements ranged from 17.07 to 40.18 kg/m^2, with an average of 25.55 ± 4.17 kg/m^2. The gestational weeks of the cases ranged from 5 to 19 weeks, with an average of 9.52 ± 0.42 weeks. Gravidity counts range from 1 to 10, and the median was three pregnancies; the parity counts ranged from 0 to 6, the median was 1; the number of abortions ranged from 0 to 5, the median was 2 and the number of living children ranged from 0 to 5, the median was 1.

As shown in Table 1, while the ages ($p = 0.01$; $p <0.05$), gravidity ($p = 0.00$; $p <0.01$) and abortion counts ($p = 0.004$; $p <0.01$) of the study group were found significantly to be higher than that of the control group, weight measurement of them was significantly lower than that of the control group ($p = 0.04$; $p <0.05$). Height and BMI measurements, parity and living children counts of the groups showed no statistically significant difference ($p>0.05$). While WBC levels of the study group was found to be lower ($p=0.045$, $p<0.05$) than that of control group, there was no statistically significant difference between groups regarding ferritin and CRP levels ($p> 0.05$) is presented in Table 2.

4. Discussion

In almost half of cases of recurrent pregnancy loss, the underlying cause cannot be fully revealed despite all the examinations (19). For most of the cases in this category, autoimmune responses such as antiphospholipid antibodies, antinuclear antibodies, antithyroglobulin antibodies, the formation of antimicrosomal antibodies, and cellular immunological causes, including increased NK cell count and cytotoxicity and increased T helper 1/T helper 2 ratio were blamed (20).

According to the results of our study, there is no statistically significant difference in CRP levels between patients with recurrent pregnancy loss and the control group. In a prospective study by Sarı et al. (21), CRP and Growth Differentiation Factor 15 values were compared between healthy control group (including 45 pregnants) and repetitive pregnancy loss group (including 45 patients) with the same demographic characteristics. These two values were found statistically significantly higher in the recurrent pregnancy loss group. In another study, serum CRP levels and CRP gene polymorphisms were compared between the groups in a retrospective case control study including 275 recurrent pregnancy loss and 290 healthy control groups conducted by Ahmed et al. (22).

Table 1. Evaluation of demographic features by groups

		Total (n=191)	Recurrent pregnancy Loss (n=90)	Live pregnancy (n=101)	p
Age (years)	Min-Max (median)	17-44 (29)	17-44 (31)	19-42 (27)	**[a]0.01***
	mean±SD	29.03±0.87	30.26±1.37	27.93±1.07	
Weight (kg)	Min-Max (median)	41-110 (68)	41-96 (65)	45-110 (70)	**[a]0.04***
	mean±SD	69.20±1.18	67.20±2.97	70.99±2.57	
Height (m)	Min-Max (median)	1.45-1.92 (1.43)	1.5-1.8 (1.6)	1.4-1.9 (1.6)	**[a]0.187**
	mean±SD	1.59±0.09	1.60±0.05	1.59±0.10	
BMI (kg/m^2)	Min-Max (median)	17.07-40.18 (25.11)	17.06-36.13 (25.3)	18.72-40.18 (25.2)	**[a]0.11**
	mean±SD	25.55±4.17	25.80±4.27	24.40±4.07	
Gravidity	Min-Max (median)	1-10 (3)	2-8 (3)	1-10 (2)	**[b]0.000****
	mean±SD	2.99±1.73	3.53±1.33	2.50±1.90	
Parity	Min-Max (median)	0-6 (1)	0-3 (1)	0-6 (1)	**[b]0.13**
	mean±SD	0.95±1.14	0.83±1.83	1.05±0.22	
Abortion	Min-Max (median)	1-5 (1)	2-8 (3)	0-0 (0)	**[b]0.004****
	mean±SD	1.41±1.40	3.53±1.33	0	
Living children count	Min-Max (median)	0-5 (1)	0-5 (1)	0-5 (1)	**[b]0.917**
	mean±SD	0.98±1.08	1.03±1.16	0.97±1.06	

[a]; Student t Test, [b]; Mann Whitney U Test, **; p<0.01, *; p<0.05, BMI; Body Mass Index, Min; Minimum, Max; Maximum, SD; Standard deviation

Table 2. Evaluation of laboratory findings by groups

		Total (n=191)	Recurrent Pregnancy Loss (n=90)	Live pregnancy (n=101)	p
Ferritin (µg/L)	Min-Max (median)	3.9-162.7 (24.7)	4.1-147.2 (22.7)	3.9-162.7 (26.2)	**[b]0.340**
	mean±SD	34.10±29.12	30.83±26.09	35.36±30.17	
WBC (x10^3/µL)	Min-Max (median)	1.2-16.5 (8.9)	3.6-14.5 (8.8)	1.2-16.5 (9)	**[a]0.045***
	mean±SD	9.06±2.25	8.62±2.17	9.21±2.26	
CRP (mg/L)	Min-Max (median)	0.2-57.1 (5.5)	0.3-57.1 (4.8)	0.2-33.2 (5.7)	**[b]0.633**
	mean±SD	6.89±6.92	7.90±9.96	6.52±5.41	

[a]; Student t Test, [b]; Mann Whitney U Test, **; p<0.01, *; p<0.05, BMI; Body Mass Index, Min; Minimum, Max; Maximum, SD; Standard deviation

According to the results of this study, serum CRP values were found to be statistically significantly higher in patients with recurrent pregnancy loss compared to the control group, and it was demonstrated that this elevation was observed in those carrying the rs2794520 T allele. In addition, they stated that some CRP gene variants increase the risk of recurrent pregnancy loss without causing an increase in CRP levels. In a recent retrospective study by Weghofer et al. (23), preconceptional CRP values and genetic examination results of conception material of 100 infertile patients with missed abortion were evaluated. CRP values of individuals with euploid material were higher than those with aneuploid material. This finding has been interpreted that inflammatory process takes place in the etiology of euploid pregnancy loss more than those with aneuploid one. In our study, we consider that serum CRP values might be affected by inequality of such factors as socioeconomic status, dietary carbohydrate intake and smoking (24) between groups as a result of retrospective design of the study. Also possible existence of CRP gene polymorphism (22) in our study population might have influence on present results. Complete blood count parameters such as WBC change in parallel with the increase in T helper 1 and granulocyte count and decrease in T helper 2 and monocyte count throughout pregnancy (25). Macrophages and monocytes stimulate extravillous trophoblast invasion, spiral artery forming and the onset of delivery. However, dysregulation in these cells can lead to complications such as preeclampsia and preterm labor (26). Polymorphonuclear leukocytes stimulate tissue remodeling and angiogenesis at the site of infection and secrete defensin. When this infection occurs in decidua, it triggers endometritis, which causes recurrent pregnancy loss (27). In the study conducted by Baş et al. (28) including 325 women who had miscarriage and 245 given term birth, the whole blood parameters at the 6th gestational week were evaluated. They found that women having miscarriage had higher inflammatory markers such as neutrophil count, lymphocyte count and neutrophil lymphocyte ratio. As a result, they stated that these parameters could be used to predict the possibility of miscarriage in a pregnant woman. In addition, a retrospective study comprised of 120 patients with recurrent pregnancy loss and 120 healthy pregnants conducted by Yılmaz et al., inter-groups comparison was performed in terms of complete blood count parameters. While no statistically significant difference was found between the groups in terms of hemoglobin, platelet, Mean Corpuscular Volume (MCV) and WBC values, they found Mean Platelet Volume (MPV) values significantly higher in the recurrent

pregnancy loss group (29). Unlike both studies, we found WBC values to be lower in the study group. WBC value alone may not be a strong parameter in demonstrating inflammation in recurrent pregnancy loss. Moreover, although there are studies in the literature demonstrating intense inflammatory reaction and cell activation in the decidua of patients with recurrent or sporadic miscarriage (30, 31), the reflection of this inflammatory reaction in peripheral blood could not be seen in our study population.

Ferritin is used as an acute phase reactant showing inflammation (32, 33). In the literature, we have not found any studies demonstrating the relation between ferritin levels and recurrent pregnancy loss. In a cross-sectional study conducted by Gou et al. (34), 20 non-pregnant women, 27 pregnants at first trimester, 38 pregnants at second trimester and 36 were evaluated in terms of iron parameters. Ferritin levels of patients with spontaneous miscarriage were found to be statistically significantly higher than that of other groups. However, no statistically significant difference was found between recurrent pregnancy loss and control group in the results of our study.

The limitations of the study are being single center based and could not being controlled some parameters such iron replacement, dietary carbohydrate intake and socioeconomic status that could affect the outcomes of the study as a result of retrospective design. However, this is the first study evaluating the ferritin as an acute phase reactant in recurrent pregnancy loss.

Recurrent pregnancy loss is a condition that deeply wounds women and their families after every miscarriage. Unfortunately, although the etiology of only half of the disease is known, immune disorders are responsible for the vast majority of the unknown part. As a result of our study, WBC, CRP and ferritin parameters did not indicate the inflammatory etiology of recurrent pregnancy loss. Besides, we think that further prospective randomized controlled studies are required regarding these parameters.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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Investigating Adipokine levels in the sera of patients with myocardial infarction in a 6-month follow up

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Abstract

Adipokines are peptides that regulate endothelial function, inflammation, blood pressure, and hemostasis. We measured Leptin, Adiponectin, Resistin and Adipsin in a cohort of 36 STEMI patients. Serum levels of Adipokines were measured in three time-points by a multiplex assay. A significant difference in Adipsin concentration between day-5 (T5) and day-180 (T180) post-MI was observed. Resistin levels decreased significantly from day 0 (T0) to T5 and T180. Adiponectin/Resistin ratio increased from T0 to T180. Leptin at T0 and T5 were higher in non-smokers. Adipsin at T0 was inversely correlated with heart rate, respiratory rate and pulse rate. Adiponectin/Resistin ratio at T180 negatively correlated with respiratory-rate. Adiponectin/Resistin ratio at T180 was higher in patients with grade-1 atrioventricular (AV) block. Anteroseptal hypokinesia (AH) correlated with Resistin at T0 and Adipsin at T5 while Leptin at T0 and T5 correlated with AH. Adiponectin/Resistin ratio at T180 was; however, lower in patients with AH. A decreasing trend in Resistin and its T0 association with AH plus correlation of Leptin at T0 and T5 with AH show the effect of Adipokines on mechanical complications after MI. We suggest that Adipokine networks have both beneficial and harmful effects and may be new cardiac biomarkers and/or drug targets.

Keywords: adipokine, biomarker, inflammation, myocardial infarction

1. Introduction

The rate of cardiovascular diseases in middle- and low-income countries is growing rapidly (1). One of the major cardiovascular pathologies occurs in myocardial infarction (MI) which is the most detrimental atherosclerosis related complication (2). Different risk factors such as stress, hypertension, physical inactivity, obesity, diabetes mellitus and cigarette smoking lead to ischemia, reperfusion injury and MI (3). A complex cascade of inflammatory events results in progression of atherosclerosis, plaque rupture, and emboli in MI. Lymphocyte infiltration is found in MI patients who die immediately, in short (four weeks), or long (four months) time after coronary thrombosis (2).

Adipose tissue, consisted of adipocytes, fibroblasts, lymphocytes, macrophages and other cells, is an active tissue which expresses a number of biologically active molecules named Adipokines (4). It is now well received that obesity, insulin resistance, type 2 diabetes, high blood pressure and cardiovascular (CV) system are highly influenced by the action of Adipokines (5-8). Adipokines are peptides that are produced by Adipocytes, endothelial and immune cells, fibroblasts, and other cells which regulate endothelial function, inflammation, blood pressure, hemostasis, adipogenesis, immune cell migration, adipocyte metabolism and function (9, 4, 10).

Leptin was the first discovered Adipokine in 1994. Administration of Leptin has cardio-protective effects e.g., reduces the extent of myocardial infarction (MI) and protects against reperfusion damage (11). Nevertheless, most studies consider Leptin as a hazardous Adipokine in cardiovascular system that is associated with atherosclerosis, hypertension and the metabolic syndrome. Also, Leptin affects blood pressure, insulin resistance, platelet aggregation and has pro-inflammatory effects (11). Higher levels of Leptin are associated with myocardial infarction (MI) and stroke, independent of traditional risk factors or obesity (11, 12).

Resistin, another Adipokine, is a 12.5-kDa cysteine-rich polypeptide, and a member of the FIZZ (found in inflammatory zones) family of proteins (13). There is a relationship between Resistin and classic mediators of inflammation, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) (14). In fact, Resistin has been shown to have pleiotropic functions in metabolism and physiology with roles in inflammation (15), endothelial dysfunction (16) cardiomyocyte function (17), and cholesterol metabolism (18). In vitro experiments have shown that Resistin activates endothelial cells to upregulate the expression of adhesion molecules and inflammatory cytokines, induces proliferation and migration of smooth muscle cells, and accelerates

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transformation of foam cells (19). Therefore, Resistin may contribute to the atherosclerotic process by stimulating multiple pro-atherosclerotic pathways (20). Increased serum Resistin levels are shown to be associated with coronary artery disease (CAD) and the risk of cardiovascular death (21).

Adiponectin, also named as ACRP30 and AdipoQ, is an adipose tissue-derived Adipokine (21, 22). Many Adipokines are positively regulated by adiposity, but adiponectin levels in plasma are negatively regulated by accumulation of body fat (23). Clinical studies implicate hypo-adiponectinemia in the pathogenesis of CAD. Adiponectin plays a protective role in the development of insulin resistance, hypertension and cardiovascular disease (CVD) (24).

Another Adipokine, Adipsin, is mainly expressed in adipocytes and is involved in the activation of the alternative pathway of complement with the acyl-stimulating protein as the final component. The positive correlation between Adipsin levels and body mass suggests the role of Adipsin in the increase of fat mass through acyl-stimulating protein synthesis, increase of differentiation of pre-adipocytes, and synthesis of triglycerides (25). Adipsin level is reduced in murine models of obesity but it either increases or remains unchanged in obese human subjects (26). Despite a large body of data on many Adipokines, the information on the Adipsin and its correlation with MI is scarce. In this study, we measured the serum levels of Leptin, Resistin, Adiponectin, and Adipsin in a cohort of patients with MI in a 6-month follow up and investigated their association with the risk factors and clinical criteria of patients.

2. Materials and methods

2.1. Study population

In this study, a total of 50 individuals with STEMI who developed a first coronary event were recruited. All patients with chest pain complaints, increase in TnT levels and ST elevation in anterolateral and anteroseptal leads, if aged <75 years, were eligible for inclusion in the study. Myocardial infarction was diagnosed based on the presenting electrocardiogram (ECG) in combination with serial TnT measurements. Selective coronary angiography in the hospital course was used to further confirm the diagnosis. Patients with Chronic Renal Failure (CRF), autoimmune diseases, and cardiogenic shock were excluded from the study. All patients were monitored for six months. After six months echocardiography was done for all the patients for evaluation of LV systolic function. LV ejection fraction was assessed by echocardiography performed by a single blinded expert operator. Follow-up end points were defined as a new ACS (e.g. cardiac ischemia and AMI) or a repeat coronary revascularization (PCI and CABG) after the initial event, which were combined as nonfatal events. The fatal events comprised all cases of all-cause mortality. Otherwise, follow-up ended at the date of withdrawal from the study or at six months after entry.

For each patient, demographic and clinical information including age, gender, and history of hypertension (Systolic blood pressure > 150 mmHg and Diastolic blood pressure > 90 mmHg), hyperlypidemia (Total Cholesterol >240 mg/dL or LDL- Cholesterol >160 mg/dL or Triglycerides >200 mg/dL), diabetes mellitus, obesity and BMI, and smoking were obtained and recorded. This datasheet as well as their laboratory data were used for statistical analysis. A resting heart rate between 60 and 100 beats per minute was considered normal. Higher than 100 beats per minute (tachycardia) and lower than 60 beats per minute (bradycardia) were considered not normal. Respiratory rate was recorded as the number of breaths taken per minute. The resting respiration rate of 12 to 20 breaths per minute was considered normal. A respiration rate under 12 or over 25 breaths per minute while resting was considered not normal. The sera were collected at three time points as: 1) On admission (T0) 2) 5th day of hospitalization (T5) 3) 180 days after MI (T180). Of 50 original individuals who consented to enter the study only 36 remained available for the last (T180) sampling. Therefore, the levels of Adipokines were measured in the sera of 36 STEMI patients (24 men and 12 women) in the T0 and T5. In T180, three patients had been expired and therefore, the number of cases decreased to 33 at this time point. All demographic, general and clinical information of the patients were recorded at the time of sampling by collaborating cardiologist.

2.2. Serum separation

Blood samples were drawn from patients and aliquots of serum were collected from each individual at the three time points. The sera were frozen in -40˚C until tested.

2.3. Adipokine multiplex assay

Circulating levels of serum Adiponectin, Resistin, Adipsin, Leptin were determined in three time-points with a commercially available multiplex bioassay (LEGENDplex™ Human Metabolic Panel 1 (4-plex)). The assay was performed according to the manufacturer's specifications. The samples were tested using a FACS Calibur Flow Cytometer (BD) instrument system.

2.4. Serum separation

Blood samples were drawn from patients and aliquots of serum were collected from each individual at the three time points. The sera were frozen in -40˚C until tested.

2.5. Adipokine multiplex assay

Circulating levels of serum Adiponectin, Resistin, Adipsin, Leptin were determined in three time-points with a commercially available multiplex bioassay (LEGENDplex™ Human Metabolic Panel 1 (4-plex)). The assay was performed according to the manufacturer's specifications. The samples were tested using a FACS Calibur Flow Cytometer (BD) instrument system.

Table 1. Adipokine levels based on demographical features of AMI patients in each time point

Subjects		No (%)	Adiponectin Mean (SD) (ng/ml ×10^3)			Adipsin Mean (SD) (pg/ml×10^3)			Resistin Mean (SD) (pg/ml ×10^3)			Leptin Mean (SD) (pg/ml ×10^3)		
			T0	T5	T180	T0	T5	T180	T0	T5	T180	T0	T5	T180
No.		36 (100)												
Age (mean ± SD)		58.8±8.6	9.5 (2.5)	1.1 (4.6)	10.9 (2.8)	6.4 (2.6)	1.08 (2.8)	7.8 (4.4)	2.2 (1.5)	1.99 (1.7)	1.1 (8.5)	3.77 (4.4)	3.4 (3.1)	5.8 (3.8)
	#P		0.54	0.12	0.48	0.27	**0.007**	0.24	0.43	0.16	0.46	0.60	0.84	0.84
Gender (M/F)		24/12	9.8 (2.9)/1.0 (2.9)	1.09 (3.7) /1.1 (4.6)	12.2 (9.1) /8.6 (30.7)	8.2 (3.7) /5.2 (2.3)	1.1 (6.8) /9.2 (7.6)	8.8 (6.9) /4.8 (2.4)	2.3 (2.9)/2.5 (1.8)	1.5 (1.18) /2.2 (1.9)	1.09 (9.3) /3.8 (4.4)	3.8 (4.4) /7.04 (6.8)	2.8 (2.8) /7.02 (6.02)	5 (4.2) /5.4 (4.6)
	P		0.6	0.75	0.25	**0.01**	0.14	**0.03**	0.39	0.26	0.95	0.14	**0.03**	0.86
BMI	18.5-24.9	9 (25)	9.6 (3.1)	8.8 (2.7)	12.2 (7.3)	7.6 (2.5)	8.2 (2.17)	7.99 (3.3)	3.4 (4.5)	1.7 (1.3)	0.9 (9.05)	6.2 (6.4)	3.1 (3.8)	3.7 (1.7)
	25-30	10 (27.8)	1.07 (2.2)	1.4 (5.2)	10.3 (3.5)	7.8 (4.9)	1.2 ()7.08	7.3 (4.8)	1.96 (1.7)	2.1 (1.9)	1.02 (6.6)	3.7 (2.5)	5.2 (4.6)	5.07 (4.7)
	>30	1 (2.78)	8.4	1.38	5.5	3.6	1.38	3.7	2.8	1.6	0.5	1.7	5.4	5.1
	Missing	16 (44.4)	9.8 (2.9)	1.04 (2.0)	1.1 (10.3)	6.9 (2.3)	1.04 (8.99)	7.8 (8.1)	2.05 (1.2)	1.6 (1.38)	1.15 (9.77)	4.1 (5.6)	4.08 (5.1)	5.9 (5.1)
	P		0.44	0.06	0.44	0.39	0.17	0.37	0.59	0.79	0.39	0.26	0.43	0.74

Correlation P value between age and Adipokines levels at each time point

2.6. Statistical analysis

Mann-Whitney U test was used for comparing clinical and biochemical variables between groups. To assess differences between in-group variables, Chi-Square test was used. Spearman's correlation coefficients were estimated to determine associations between Adipokine concentrations and anthropometric measurements and biochemical variables. All statistical assessments were considered significant as P-value < 0.05. All analyses were performed using SPSS version 16 (version 9.2; SAS Institute, Cary, NC).

3. Results

The demographical and clinical features of MI patients and Adipokine levels in the groups are shown in tables 1 and 2.

3.1. Concentration of serum Adipokines in the studied time points

There were no significant differences in serum Adiponectin and Leptin concentrations between the three time points in MI patients (P= 0.3 and P= 0.3, respectively; Figure 1). However, a significant difference in serum Adipsin concentration between day 5 (T5) and day 180 (T180) post-MI was observed (P= 0.02, Fig. 1). In addition, there was a significant difference in serum Resistin levels between day 0 (T0) and day 180 (T180) as well as day 5 (T5) and day 180 (T180) (P= 0.0001 and P= 0.009, respectively, Fig. 1). Interestingly, the Adiponectin/Resistin ratio increased from T0 to T180 and the difference between T180 and T0 was significant (P=0.002).

3.2. Concentration of Adipokines in the studied time points based on gender

The serum Resistin concentration at T0 was significantly higher than T180 among women and men (P= 0.004, and P= 0.02, respectively). A trend of decrease in Resistin level in both genders was observed from T0 to T180 as well (Figs. 2A and 2B). Adipsin T180 levels were higher in men than women (P=0.03, Figure 2C), while T5 Leptin levels were higher in women than men (P=0.03, Fig. 2C). No other significant difference was observed between Adipokine levels based on gender.

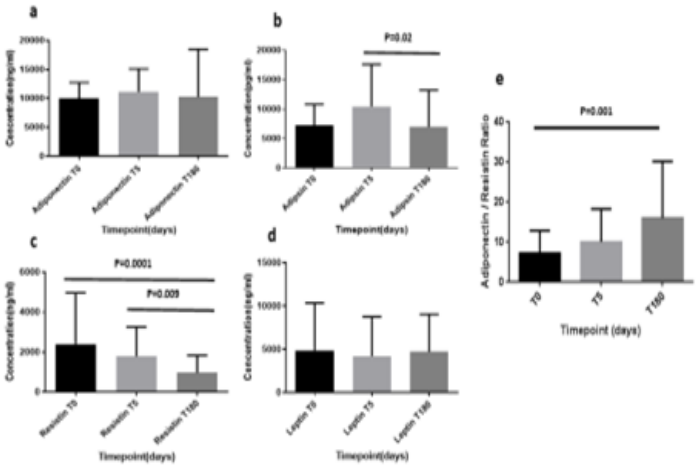

Fig. 1. Serum Adipokine levels in patients with MI at the time of admission to the hospital (T0), five days after admission (T5) and 180 days after MI (180). (a) Adiponectin levels (b) Adipsin levels, (c) Resistin levels, and (d) Leptin levels as well as Adiponectin to Resistin ratio (e) are shown

Table 2. Adipokine levels based on clinical histories of AMI patients in each time point

Subjects		No (%)	Adiponectin Mean (SD) (ng/ml ×10^3)			Adipsin Mean (SD) (pg/ml×10^3)			Resistin Mean (SD) (pg/ml ×10^3)			Leptin Mean (SD) (pg/ml ×10^3)		
			T0	T5	T180	T0	T5	T180	T0	T5	T180	T0	T5	T180
DM														
	Pos	9 (25)	10.1 (2.3)	12.9 (4.9)	10.3 (3.6)	6.6 (3.4)	14.1 (11.8)	7.8 (4.1)	2 (1.5)	2.1 (1.6)	1.5 (1.3)	3.1 (3.1)	3.8 (4.9)	5.02 (4.7)
	Neg	27 (75)	9.95 (2.8)	1.05 (3.5)	1.13 (8.6)	7.4 (3.66)	9.1 (4.3)	7.5 (6.6)	2.5 (2.8)	1.6 (1.4)	0.97 (0.7)	5.4 (5.9)	4.3 (4.5)	5.1 (4.3)
	P value		0.91	0.12	0.69	0.66	0.77	0.60	0.94	0.31	0.34	0.19	0.59	0.98
HTN														
	Pos	20 (55.6)	10.1 (2.2)	11.5 (4.4)	10.5 (5.5)	6.5 (2.9)	9.7 (2.7)	6.5 (3.8)	2.8 (3.2)	2.0 (1.6)	1.3 (1.0)	4.5 (5.3)	3.6 (3.0)	6.6 (4.9)
	Neg	16 (44.4)	9.8 (3.2)	10.5 (3.5)	12 (10.3)	8.1 (4.1)	11.3 (7.1)	8.8 (8.03)	1.8 (1.1)	1.5 (1.1)	0.7 (0.4)	5.2 (5.9)	4.9 (5.9)	3.3 (2.6)
	P value		0.88	0.60	0.92	0.24	0.42	0.53	0.47	0.51	0.25	0.88	0.88	**0.04**
HLP														
	Pos	9 (25)	9.2 (2.2)	12.4 (3.3)	12.8 (13.3)	8.07 (3.2)	15.0 (9.6)	9.3 (10.2)	1.7 (1.4)	1.9 (1.6)	0.8 (0.4)	4.7 (5.1)	4.6 (3.8)	6.4 (4.3)
	Neg	27 (75)	10.2 (2.8)	10.6 (4.1)	10.5 (4.9)	6.9 (3.7)	8.9 (5.4)	6.9 (3.7)	2.6 (2.8)	1.7 (1.4)	1.1 (0.9)	4.9 (5.7)	4.1 (4.8)	4.6 (4.2)
	P value		0.33	0.10	0.61	0.36	0.06	0.95	0.21	0.64	0.85	0.80	0.29	0.19
Smoking														
	Yes	22 (61.1)	10.6 (2.7)	11.9 (4.3)	12.7 (9.2)	7.6 (3.1)	11.1 (8.2)	8.7 (6.9)	2.4 (3.1)	1.5 (1.2)	1.2 (0.9)	3.1 (4.1)	2.7 (3.0)	5.7 (4.9)
	No	14 (38.9)	9.0 (2.4)	9.8 (3.0)	8.1 (2.9)	6.6 (4.2)	9.4 (5.2)	5.4 (3.4)	2.3 (1.4)	2.2 (1.8)	0.7 (0.3)	7.5 (6.3)	6.5 (5.6)	4.0 (2.4)
	P value		0.09	0.17	**0.04**	0.15	0.73	0.06	0.25	0.19	0.37	**0.01**	**0.01**	0.66
VD														
	CAD	6 (16.7)	12.1 (2.3)	11.7 (3.8)	10.1 (2.9)	7.9 (4.2)	12.6 (11.3)	5.5 (3.1)	1.8 (1.4)	1.1 (0.3)	0.7 (2.3)	4.5 (7.5)	4.4 (4.3)	6.2 (3.8)
	No VD	30 (83.3)	9.6 (2.6)	10.9 (4.1)	11.4 (8.7)	7.1 (3.5)	10.0 (6.2)	8.1 (6.5)	2.5 (2.7)	1.9 (1.5)	1.1 (9.1)	4.9 (5.1)	4.1 (4.6)	4.9 (4.4)
	P value		0.07	0.53	0.76	0.51	0.89	0.30	0.28	0.29	0.51	0.36	0.83	0.20
HR														
	Normal (60-100 bpm)	24 (66.7)	9.7 (2.9)	10.6 (3.8)	11.6 (9.1)	8.01 (3.7)	11.0 (7.4)	8.3 (6.8)	2.6 (2.9)	1.7 (1.4)	0.9 (0.7)	5.1 (5.8)	4.5 (5.1)	4.5 (4.0)
	Abnormal (ELSE)	12 (33.3)	10.5 (2.1)	12.1 (4.3)	9.9 (3.1)	5.6 (2.7)	9.2 (6.6)	5.5 (2.9)	1.9 (1.4)	1.9 (1.5)	1.2 (1.1)	4.3 (4.7)	3.5 (3.3)	6.6 (4.8)
	P value		0.43	0.19	0.86	0.06	0.46	0.27	0.73	0.48	0.65	0.81	0.91	0.25
SBP														
	Normal (<150 mmHg)	19 (53)	9.9 (2.9)	10.8 (303)	11.6 (10)	6.4 (3.1)	9.9 (7.2)	8.8 (7.8)	2.7 (3.2)	2 (1.6)	8.5 (8.3)	5.8 (6.7)	3.8 (4.2)	3.9 (2.5)
	Abnormal (ELSE)	17 (47)	10.1 (2.5)	11.1 (4.7)	10.8 (5.6)	8.2 (3.9)	11.0 (7.3)	6.5 (3.9)	2.0 (1.5)	1.5 (1.3)	1.3 (0.8)	3.9 (3.6)	4.6 (5.0)	6.3 (5.3)
	P value		0.66	1.0	0.71	0.21	0.51	0.49	0.66	0.14	**0.02**	0.75	0.49	0.22
RR														
	Normal (12-20 bpm)	16 (44.4)	9.9 (2.3)	11.1 (4.2)	10.8 (6.4)	5.6 (2.5)	8.8 (6.6)	6.03 (4.2)	2.5 (1.6)	1.7 (1.4)	1.1 (8.6)	6.07 (6.3)	6.1 (5.9)	4.5 (4.2)
	Abnormal (ELSE)	20 (55.6)	10 (3.02)	11.1 (3.9)	11.3 (9.01)	8.5 (3.8)	11.1 (7.4)	8.6 (7.02)	2.3 (3.1)	1.8 (1.5)	1.01 (8.5)	3.9 (4.6)	2.6 (2.1)	5.5 (4.3)
	P value		0.79	0.96	0.89	**0.03**	0.09	0.15	0.19	0.84	0.65	0.42	0.24	0.43
Total		36 (100)												

3.3. Concentration of Adipokines in the studied time points based on smoking status

Serum Adiponectin concentration at all three time-points was higher in smokers compared with non-smokers, but only at T180 the difference reached the significant level (P=0.04, Fig. 3A). The levels of Adipsin were also non-significantly higher in smokers than non-smokers (Fig. 3B). Serum Resistin levels varied over time and was only non-significantly higher in non-smokers at T5. Leptin levels were higher in non-smokers than smokers, but the difference reached a significantly higher level only at T0 and T5 (P=0.01 and P=0.01, respectively, Figs. 3C and D).

3.4. Correlation of Adipokine levels with risk factors and clinical and demographical characteristics of patients

In bivariate correlation analyses, serum Adipsin concentration at T0 was found to be significantly and inversely correlated with heart rate, respiratory rate and pulse rate (P=0.01, r=-0.39,

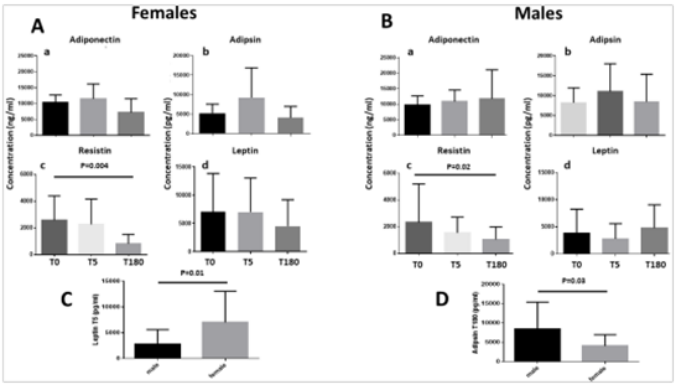

Fig. 2. Concentration of adipokines in the studies time points based on gender. Adiponectin (a), Adipsin (b), Resistin (c) and Leptin (d) in Females (A) and (B) Males. Differences in the Leptin T5 (C) and Adipsin T180 (D) between genders were statistically significant (P<0.05)

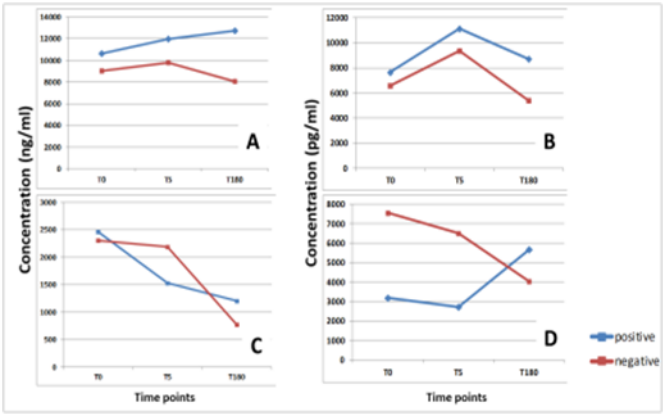

Fig. 3. Concentration of adipokines in the studies time points based on smoking status. Trends of changes in (A) Adiponectin, (B) Adipsin, (C) Resistin, and (D) Leptin in sera of patients

P=0.01, r=-0.40 and r=-0.35, p=0.03, respectively; Figs. 4A, 4B and 4C). Adipsin T5 levels were significantly and inversely correlated with age (r=-0.44, P=0.007, Fig. 4D). In addition, serum Leptin concentration at T5 and serum Resistin concentration at T180 were lower in those patients who had atrioventricular (AV) block (P=0.04 and P=0.05 respectively; Fig.5 A, B). Anteroseptal Hypokinesia correlated with serum Resistin level at T0 (P=0.02) and serum Adipsin level at T5 (P=0.02) while Leptin at T0 and T5 correlated with Anterolateral Hypokinesia (P=0.03 and P=0.004, respectively; Figs. 5 C-F).

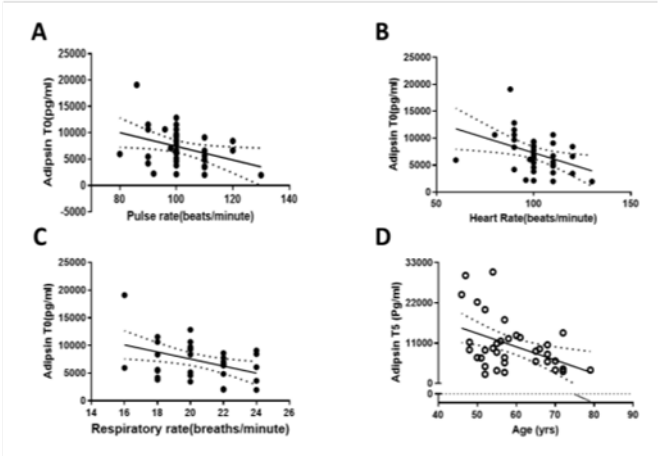

Fig. 4. Correlation of serum Adipsin concentration at T0 with heart rate, respiratory rate and pulse rate as well as Adipsin T5 with age in patients

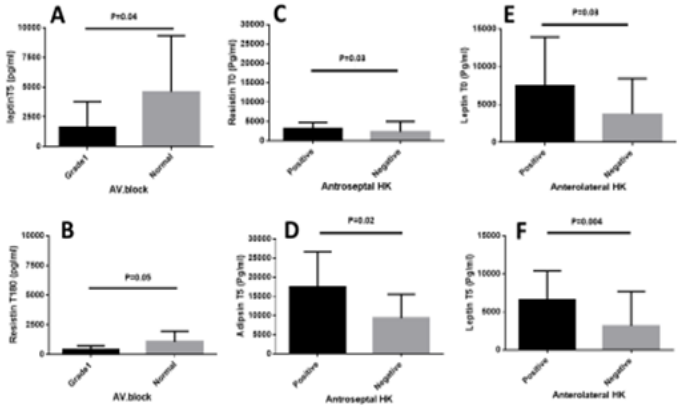

Fig. 5. Comparison of adipokine levels between patients. (A, B) Leptin levels at T5 and Resistin levels at T180 in patients with Atrioventricular (AV) block as compared to patients without block, C) Resistin T0 levels, D) Adipsin T5 levels, E) Leptin T0 levels and F) Leptin T5 levels in patients with Anteroseptal hypokinesia and those without

A significant negative correlation was observed between Adiponectin/Resistin ratio at T180 and Respiratory rate (r=-0.35, P= 0.048, Fig. 6A). Moreover, Adiponectin/Resistin ratio at T180 was significantly higher in patients with AV block grade-1 compared to those without AV block (P=0.025, Fig. 6B). Adiponectin/Resistin ratio at T180 was, however, lower in patients with Anterolateral Hypokinesia (P=0.024, Fig. 6C). Moreover, systolic blood pressure was found to be negatively correlated with serum Resistin (P=0.04, r= -0.34) and positively with serum Leptin concentrations at T180 (P=0.01, r=0.40, Fig. 7).

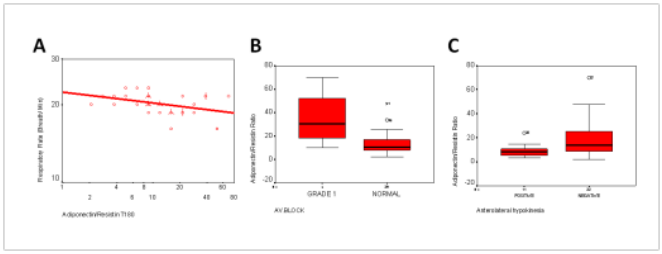

Fig. 6. Correlation between Adiponectin/Resistin ratio (at T180) and Respiratory rate (A), Atrioventricular (AV) block (B), and Anterolateral Hypokinesia (C) in patients with MI

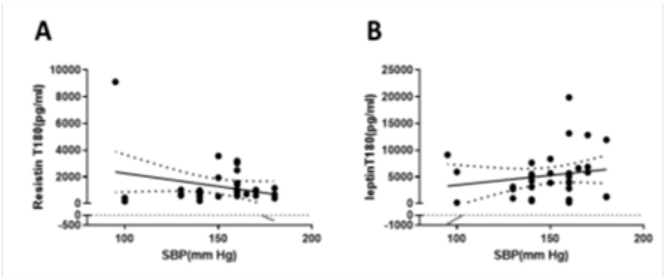

Fig. 7. Correlation of serum Resistin and Leptin concentrations at T180 with systolic blood pressure in patients

3.5. Comparison of Adipokine levels between patients based on their Acetylsalicylic Acid (ASA) and Statin treatments

We also compared the Adipokine levels between patients who had received ASA and/or Statin therapy in each time point. As shown in Table 3, there was no significant difference in the level of Adipokines between patients who received ASA and those who did not in any time point. For Statin, the only significant difference was observed in Resistin levels where those patients who had received Statin therapy had lower Resistin levels at the time of admission to the hospital (T0) compared to those who were not on Statin therapy (P=0.014) (Table 4).

Table 3. Adipokine levels in each time point based on ASA therapy

				ASA		
	Time point			T0	T5	T180
Adipokines	Adiponectin	P. value		0.074	0.077	0.432
		Mean ± SD (ng/ml ×10^3)	Pos. (N= 14)	11.1±2.3	12.08±3.6	13.1±11.4
			Neg. (N= 22)	9.2±2.7	10±4.1	10±5.1
	Adipsin	P. value		0.745	0.465	0.210
		Mean ± SD (pg/ml×10^3)	Pos. (N= 14)	7.3±3.5	9.8±7.9	10.2±8.8
			Neg. (N= 22)	7.1±3.6	10.8±6.7	6.1±3.1
	Leptin	P. value		0.236	0.626	0.389
		Mean ± SD (pg/ml×10^3)	Pos. (N= 14)	4.3±6.1	3.06±2.4	5.4±3.1
			Neg. (N= 22)	5.2±5.1	4.9±5.4	4.9±4.9
	Resistin	P. value		0.390	0.795	0.837
		Mean ±SD (pg/ml×10^3)	Pos. (N= 14)	2.5±3.6	1.9±1.5	1.2±1.1
			Neg. (N= 22)	2.3±1.6	1.7±1.4	0.9±0.6

Table 4. Adipokine levels in each time point based on Statin therapy

				STATIN		
	Time point			T0	T5	T180
Adipokines	Adiponectin	P. value		0.192	0.680	0.315
		Mean ±SD (ng/ml ×10^3)	Pos. (N= 5)	1.8±3.9	9.6±3.4	17.5±17.1
			Neg. (N= 31)	9.8±2.5	11.3±4.09	10.03±4.8
	Adipsin	P. value		0.200	0.086	0.050
		Mean ±SD (pg/ml×10^3)	Pos. (N= 5)	5.5±3.5	5.9±3.2	14.1±11.8
			Neg. (N= 31)	9.8±2.5	11.3±4.09	10.03±4.8
	Leptin	P. value		0.647	0.567	0.380
		Mean ±SD (pg/ml×10^3)	Pos. (N= 5)	3.9±4.7	2.5±1.8	4.5±4.1
			Neg. (N= 31)	5.03±5.6	4.4±4.8	4.8±4.3
	Resistin	P.value		**0.016***	0.423	0.379
		Mean ±SD (pg/ml×10^3)	Pos. (N= 5)	1.04±0.1	1.3±1.08	1.4±1.3
			Neg. (N= 31)	2.6±2.6	1.8±1.5	0.9±0.7

4. Discussion

It is shown that Adult cardiomyocytes produce Adipokines to protect the damaged cardiomyocytes (27). In our study, the serum concentrations of Adiponectin, Adipsin, Resistin and Leptin were measured in patients with MI at three different time points: during the first 12 hours of hospitalization (T0), five days after hospitalization (T5), and after six months of MI (T180). Our results indicated that Adipokines concentrations show various trends over time. Comparison of serum Adiponectin and Leptin concentrations at the three time points showed no significant changes over time. Resistin decreased significantly over the six-month follow-up and Adipsin levels fluctuated between time points. A previous study has shown that Resistin accelerates mechanisms involved in production of inflammatory cytokines (28, 29). Resistin increases p38 MAPK phosphorylation and NF-kappa B expression, however, anti-inflammatory treatments after MI, such as Aspirin, may decrease the effect of Resistin (28, 29). In our hands, however, there was no difference in the Resistin levels between those who were treated with Aspirin and those who were not in any of the time points. For Statin, however, we found that those patients who had received Statin therapy before MI had lower Resistin levels at the time of admission to the hospital (T0) compared to those who did not. This difference, however, should be interpreted with caution as the number of cases in the Statin treated group was very low (n=5). In accordance with studies in polycystic ovary syndrome (30) and acute coronary syndrome (31) we observed an increasing trend in Adiponectin to Resistin ratio after six months.

Resistin decrease started earlier in men compared to women after incidence of MI (Fig. 2). Previously, it was shown that Resistin is higher in women with diabetes than men with diabetes (32). A previous study on a small group of obese healthy individuals, has shown a higher level of Adipokines, including Resistin in females than males (33). Of note, Resistin is not associated with menstruation and does not change significantly during this period (34, 35).

In our study, there was a significant inverse relationship between smoking and serum Leptin concentration at earlier time points. This finding is not new and is in accordance with several previous studies (36, 37). However, there are contradictory reports that state otherwise in individuals with MI and atherosclerosis (38, 39). Knowing that after the first myocardial infarction, smokers have a shorter life expectancy because they may suffer from stroke at a younger age (40), and that smoking independently predicts major cardiovascular events, heart failure, and mortality (41), our results on the correlation of serum Leptin levels five days after MI as well as serum Adiponectin levels six months after MI with smoking may be important.

Interestingly, higher Adipsin levels at T0 were associated with decreased heart rate, decreased pulse rate, and decreased respiratory rate. A correlation between Adipsin and heart failure is reported (42), and Adipsin is suggested as one of the single predictors of heart failure (43). A recent study on a cohort of 370 patients with atherosclerosis showed that patients with higher Adipsin levels had a 4.2 fold increase in all-cause death and 2.4 fold increase in rehospitalisation (44). Interestingly, four of the five patients who developed MI during their course of follow-up and died because of MI had Adipsin levels higher than 400 ng/ml (44).

We also found that Leptin T5 and Resistin T180 were associated with atrioventricular block which is in accordance with previous studies showing the significance of these Adipokines in Atrial Fibrillation associated disorders (45-47).

We also found that Leptin levels were higher in patients with Anterolateral Hypokinesia in T0 and T5. Previous studies have shown that BMI correlates with left ventricular diastolic dysfunction (48), however, it decreases global hypokinesia of the heart (49). Bearing in mind that in addition to being an obesity related Adipokine, Leptin is also an inflammatory cytokine which contributes to other pathways and various diseases, the mechanistic involvement and significance of Leptin in Anterolateral Hypokinesia remains to be investigated (8, 10). Our results also showed that Anteroseptal Hypokinesia was associated with Resistin T0 and Adipsin T5 levels. These finding may be related to the cross-talk between heart adipose tissue and heart myocytes through these Adipokines similar to what is seen for Adiponectin (50). The lower ratios of Adiponectin/Resistin at T180 in patients with Anterolateral Hypokinesia may indicate a beneficial effect of Adiponectin on myocytes as previously suggested (50). Interestingly, the Adiponectin/Resistin ratio at T180 negatively correlated with respiratory rate at the time of admission, indicating the negative correlation of this index with MI prognosis.

Our results indicate that like other cytokines, Adipokines play their role in a network and may have both beneficial and harmful effect which is balanced by other Adipokines. Finding their interaction and their mechanism of action may pave the way for finding new cardiac biomarkers and/or drug targets.

Conflict of interest

The author(s) declare no conflicts of interest.

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Comparison of the efficacy of PSI, CURB-65, CALL and BCRSS in predicting prognosis and mortality in COVID-19 patients

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Abstract

This study aimed to determine whether the PSI, CURB-65, CALL and BCRSS had any superiority over each other as a prognostic determinant in patients with COVID-19. This prospective cohort study included patients over 18 years of age that presented to the emergency department between May 12 and August 12, 2020 and had a positive COVID-19 polymerase chain reaction (PCR) test. The PSI, CURB-65, CALL and BCRS scores were calculated. SPSS version 22 was used for all statistical analyses. A total of 213 patients with a positive COVID-19 PCR result were included in the study. The total 30-day mortality rate was determined as 14.08%. PSI, CURB-65, CALL and BCRSS had a statistically significant relationship with mortality ($p<0.001$). The best parameter in predicting mortality was determined as PSI (area under the curve: 0.900; 95% CI: 0.972-0.828). A positive correlation was found between each scoring system, both with the length of hospital stay (PSI, CURB-65, CALL and BCRSS: $r=0.696$, $p=0$; $r=0.621$, $p=0$; $r=0.75$, $p=0$; and $r=0.666$, $p=0$, respectively). Scoring systems, which include comorbidity, vital signs as well as laboratory, imaging findings, will be more effective than other scoring systems in determining the mortality in patients with covid-19.

Keywords: BCRSS, CALL, COVID-19, CURB-65, PSI

1. Introduction

A previously unidentified viral pneumonia was detected in Wuhan in December 2019 and named SARS-CoV-2 or COVID-19. It rapidly spread to other countries within a few months and was soon declared a pandemic. In Turkey, the first cases were seen in the March 11, 2020, and the number of cases reached a peak level toward the end of April and started to decrease in late May. An increasing trend started to be seen again as of 15 June, and the increase of 1,000-1,500 cases per day continued until August 30. A daily increase of 100-500 cases was observed from August 30 to December 10. The number of cases has been showing a decreasing trend since December 10 (1). The Pneumonia Severity Index (PSI), CURB-65 (cased on the presence or absence of the following criteria: new confusion, urea >7 mmol/l, respiratory rate > 30/min, systolic blood pressure <90 mm Hg or diastolic blood pressure ≤60 mm Hg, and age ≥65 years) and CALL (comorbidity, age (years), lymphocyte, lactate dehydrogenase) scoring systems as predictors of prognosis and mortality in patients with pneumonia (2-4). It remains unclear whether these scoring systems can also be determinant in the prognosis and mortality of patients with COVID-19. The Brescia-COVID Respiratory Severity Scale/Algorithm (BCRSS) has been previously used in COVID-19 cases but there are only a limited number of studies evaluating its efficacy (5).

Like the situation in other parts of the world, after the COVID-19 pandemic reached Turkey, we started to apply current algorithms based on frequently encountered symptoms and clinical findings to predict the prognosis of these patients. However, patients' outcomes sometimes differ from what is expected. Therefore, we planned to conduct a comprehensive study on whether pneumonia scoring systems or different severity indicators were superior to each other in predicting prognosis in patients with COVID-19 and share the results with the literature. This study aimed to show whether PSI, CURB-65, CALL and BCRSS had any superiority over each other as prognosis determinants in COVID-19 cases.

2. Materials and methods

2.1. Study design

This prospective cohort study was conducted in the emergency department of Ümraniye Training and Research Hospital, which includes a COVID outpatient clinic. This emergency department is a well-equipped clinic serving an average of 500,000 patients every year with green, yellow and red zones and a resuscitation unit.

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2.2. Patient population

The study included patients over 18 years of age, who presented to the emergency department with different symptoms between May 12 and August 12, 2020 and had a positive COVID-19 polymerase chain reaction (PCR) test. Patients without COVID-19 PCR results and those concurrently diagnosed with other diseases in addition to COVID-19 were not included in the study.

2.3. Data collection

The presenting symptoms, history, vital signs, examination findings, and laboratory test results of the patients were recorded. The patients with and without computed tomography findings were grouped. At the time of presented, the patients were categorized according to the algorithm of the World Health Organization and the Turkish Ministry of Health (1, 6). Contact history was questioned. According to the outcome, the patients were divided into three groups as those that were discharged, those that were hospitalized, and those that were admitted to the intensive care unit (ICU). The length of hospital stay, and 30-day mortality were recorded. According to the clinical findings, the PSI, CURB-65, CALL and BCRSS values were obtained and compared in terms of their efficacy in predicting prognosis and mortality. The primary outcome of this study was the comparison of the efficacy of the PSI, CURB-65, CALL and BCRSS in predicting mortality in patients with COVID-19, and the secondary outcome was to determine the superiority of these scoring systems over each in terms of the length of hospital stay.

2.4. Statistical analyses

Statistical analysis was performed using SPSS version 22.0. The conformance of variables to normal distribution was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov). The chi-square test was conducted to evaluate the relationship between categorical data. The Mann-Whiney U test was used to compare non-parametric numerical data between two groups. If there were more than two groups, the Kruskal-Wallis test was used to compare non-parametric numerical data. We also formed a characteristic curve (ROC) for 30-day mortality and obtained the area under the curve (AUC) for individual variables. Spearman's correlation analysis was undertaken to determine the relationship between the PSI, CURB-65, CALL and BCRSS systems and length of hospital stay. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. A p value of <0.05 was accepted as statistically significant.

2.5. Ethics

For the study, ethical approval was obtained from the local clinical research ethics committee of our hospital (date: April 28, 2020, number: B.10.1.TKH.4.34.H.GP.0.01/123). Patients with a sufficient level of consciousness and the relatives of those who did not have sufficient consciousness were invited to participate in the study. An informed consent form was signed by the patients or their relatives who accepted to participate in the study.

3. Results

The study included a total of 213 patients (53.1% male) with a positive COVID-19 PCR result. The mean age mean was 46.54 ± 19.52 years. It was observed that increasing age increased 30-day mortality ($p<0.001$). Total 30-day mortality rate was determined as 14.08%. Of the patients that died, 56.7% were male. Within the first 24 hours, 29.01% of the patients were admitted to inpatient services and 15.02% to ICU. On first physical examination, a statistically significant relationship was found between the respiratory rate per minute and oxygen saturation at the time of the first measurement and mortality ($p<0.001$). A high respiratory rate and a low saturation value increased mortality. When comorbidities were examined, the mortality rate was higher among the patients with hypertension, congestive heart failure (CHF) ($p<0.001$), chronic obstructive pulmonary disease (COPD) ($p=0.001$), chronic kidney disease (CKD) and coronary artery disease (CAD) ($p<0.001$). In addition, the presence of confusion, cough, and weakness ($p<0.001$), shortness of breath ($p = 0.008$), myalgia ($p = 0.024$) and sore throat ($p<0.005$) increased mortality. Mortality was also higher in the patients with a history of contact ($p<0.001$). It was observed that prolonged hospital stay was associated with increased mortality ($p<0.001$). Increased CURB-65 and PSI values indicated higher mortality ($p<0.001$) (Table.1). The relationships between demographic data, symptoms, physical examination findings and mortality are shown in Table 1.

We divided the BCRSS and CALL scores into classes using the special grouping method for these systems and determined that as the BCRSS level or CALL class increased, there was an increase in mortality at a statistically significant level ($p <0.001$) (Table 2).

The Spearman correlation test was performed between PSI, CURB-65, CALL and BCRSS and age, saturation value, and length of hospital stay. Saturation was negatively correlated with CURB-65, CALL, and BCRSS. The scores increased as the saturation value decreased, and age increased. Other data were positively correlated with each other. Each scoring system was correlated with each other, as well as with the length of hospital stay and age (correlation between the length of hospital stay and PSI, CURB-65, CALL and BCRSS: $r=0.696$, $p=0$; $r=0.621$, $p=0$; $r=0.75$, $p=0$; and $r=0.666$, $p=0$, respectively) (Table 3). We conducted the receiver operating characteristic (ROC) curve analysis of the relationship between the four scoring systems with mortality within 30 days of presentation to the emergency to calculate the cut-off values of these scoring systems in predicting mortality.

Table 1. Relationship of demographic characteristics, symptoms, physical examination findings and scoring systems with mortality

	Total	Survivor	Non-survivor	P value
Age (mean±SD)	46.54±19.52 (18-95)	42.55±16.87 (18-95)	70.87±16.95 (22-92)	<0.001
Gender (n, %)				0.669
Female	100 (46.9%)	87 (47.5%)	13 (43.3%)	
Male	113 (53.1%)	96 (52.5%)	17 (56.7%)	
Systolic TA (mean±SD)	121.53±14.61(62-188)	121.44±10.8(90-164)	122.10±28.75(62-188)	0.888
Diastolic TA (mean±SD)	73.71±10.08 (34-118)	74.32±8.79 (45-118)	70.03±15.56 (34-102)	0.056
Respiratory rate (mean±SD	19.33±5.35 (12-40)	18.24±3.78 (12-40)	25.97±8.18 (15-40)	<0.001
Fever (mean±SD)	36.5±0.37 (36-39)	36.5±0.38 (36-39)	36.5±0.29 (36-38)	0.82
Saturation (mean±SD	95.23±6.97 (50-100)	96.86±3.65 (71-100)	85.30±12.37 (50-99)	<0.001
Length of hospitalstay (days)	3.98±6.8 (0-35)	2.98±5.49 (0-33)	10.07±10.24 (0-35)	<0.001
Comorbidities (n, %)				
Hypertension	49 (23%)	30 (16.4%)	19 (63.3%)	<0.001
Diabetesmellitus	32 (15%)	25 (13.7%)	7 (23.3%)	0.169
COPD	7 (3.3 %)	3 (1.6%)	4 (13.3%)	0.001
CCF	9 (4.2%)	3 (1.6%)	6 (20%)	<0.001
CKD	9 (4.2%)	5 (2.7%)	4 (13.3%)	0.029
CAD	11 (5.2%)	6 (3.3%)	5 (16.7%)	0.009
Contacthistory (n,%)	115 (54%)	108 (59%)	7 (23.3%)	<0.001
Symptoms (n,%)				
Fever	69 (32.4%)	63 (34.4%)	6 (20%)	0.118
Confusion	21 (9.9%)	7 (3.8%)	14 (46.7%)	<0.001
Shortness of breath	69 (32.4%)	53 (29%)	16 (53.3%)	0.008
Cough	114 (53.5%)	107 (58.5%)	7 (23.3%)	<0.001
Myalgia	66 (31%)	62 (33.9%)	4 (13.3%)	0.024
Sorethroat	27 (12.7%)	27 (14.8%)	0	0.024
Inabilitytotaste	13 (6.1%)	12 (6.6%)	1 (3.3%)	0.494
Inabilitytosmell	11 (5.2%)	10 (5.5%)	1 (3.3%)	0.625
Weakness	75 (35.2%)	51 (27.9%)	24 (80%)	<0.001
SBP<90 mmhg (n%)	4 (1.9%)	0	4 (13.3%)	<0.001
DBP<60 mmhg(n%)	12 (5.6%)	8 (4.4%)	4 (13.3)	0.122
Respiratory rate >30/min (n%)	10 (4.7%)	3 (1.6%)	7 (23.3)	<0.001
CT finding (n%)	116 (54.5%)	90 (49.2%)	26 (86.7%)	<0.001
CURB-65 (mean±SD	1±1.07 (0-5)	0.74±0.75 (0-3)	2.6±1.35 (0-5)	<0.001
BCRSS (mean±SD	0.49±1.03 (0-4)	0.23±0.65 (0-4)	2.07±1.48 (0-4)	
CALL (mean±SD	5.97±2.72 (0-13)	5.36±2.14 (0-12)	9.7±2.91 (4-13)	
PSI (mean±SD	58.05±45.93 (8-253)	45.67±28.93 (8-147)	133.57±57.46 (22-253)	<0.001

COPD: chronic obstructive pulmonary disease, CCF: congestive cardiac failure, CKD: chronic kidney disease, CAD:coronary artery disease, SBP: systolic blood pressure, DBP: diastolic blood pressure

Table 4 presents the cut-off, sensitivity, specificity, 95% confidence interval and area under the curve (AUC) values. The cut-off values of each scoring system were statistically significant in predicting mortality, and the AUC values were at a good level. The best parameter in predicting mortality was determined to be PSI with an AUC value of 0.900 (95% CI: 0.972-0.828). The length of hospital stay was a relatively poor determinant of mortality, with an AUC value of 0.790 (95% CI: 0.874-0.706). The remaining parameters had similar efficacy in predicting mortality (Table 4) (Fig.1).

Table 2. Relationship of BCRSS and CALL with mortality

	Total	Survivor	Non-survivor
BCRSS			
Level 0	161 (75.6%)	155 (84.7%)	6 (20%)
Level 1	24 (11.3%)	18 (9.8%)	6 (20.0%)
Level 2	12 (5.6%)	7 (3.8%)	5 (16.7%)
Level 3	7 (3.3%)	1 (0.5%)	6 (20.0%)
Level 4	9 (4.2%)	2 (1.1%)	7 (23.3%)
CALL			
Class 0	143 (67.5%)	138 (75.8%)	5 (16.7%)
Class 1	38 (17.9%)	32 (17.6%)	6 (20.0%)
Class 2	31 (14.6%)	12 (6.6%)	19 (63.3%)

Table 3. Correlations between the investigated parameters

		Age	Saturation	LOHS (day)	CURB-65	BCRSS	CALL	PSI
Age	p	.	0	0	0	0	0	0
	r		-0.565	0.658	0.646	0.445	0.715	0.911
Saturation	p	0		0	0	0	0	0
	r	-0.565		-0.65	-0.61	-0.619	-0.606	-0.63
LOHS (days)	p	0	0		0	0	0	0
	r	0.658	-0.65		0.621	0.666	0.75	0.696
CURB-65	p	0	0	0		0	0	0
	r	0.646	-0.61	0.621		0.574	0.68	0.722
BCRSS	p	0	0	0	0		0	0
	r	0.445	-0.619	0.666	0.574		0.619	0.581
CALL	p	0	0	0	0			0
	r	0.715	-0.606	0.75	0.68	0.619		0.734
PSI	p	0	0	0	0		0	
	r	0.911	-0.63	0.696	0.722	0.581	0.734	

LOHS; length of hospital stay

Table 4.Receiver operating characteristic analysis of CURB-65, BCRSS, CALL, and PSI forthe prediction of 30-day mortality

	Cut-off value	Sensitivity	Specificity	95% CI	AUC	p
Age	>67	76.7	91.8	0.948-0.798	0.874	0.000
Respiratory rate	19	83.3	72.68	0.916-0.731	0.823	0.000
Saturation	<94	73.3	94	0.939-0.761	0.850	0.000
Length of hospital stay (days)	>0	90	63.9	0.874-0.706	0.790	0.000
CURB-65	>1	83.3	86.9	0.957-0.762	0.859	0.000
BCRSS	>0.50	80	84.7	0.944-0.764	0.854	0.000
CALL	8.5	80	85.2	0.947-0.772	0.860	0.000
PSI	>101	80	85.2	0.972-0.828	0.900	0.000

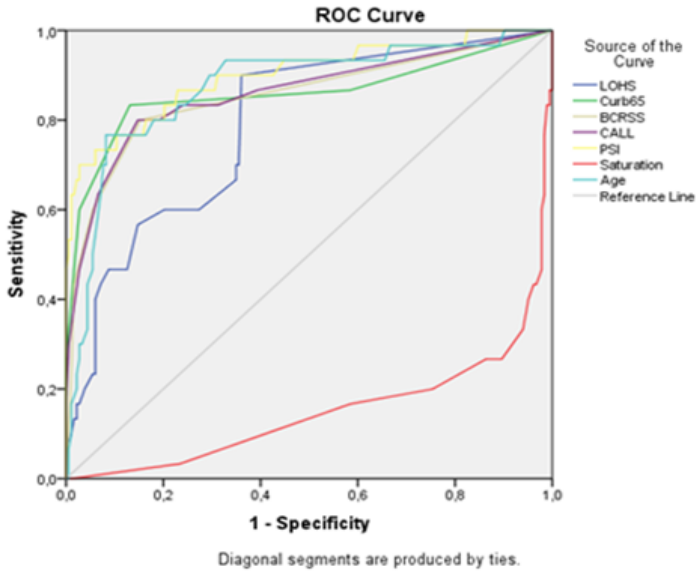

Fig.1. ROC analysis of PSI, CURB-65, BCRSS, CALL, length of hospital stays, age, and saturation in the prediction of mortality

4. Discussion

In our study, a statistically significant relationship was found between the PSI, CURB-65, CALL and BCRSS scores and mortality in patients withCOVID-19, and it was determined that PSI was superior to the other scoring systems in predicting mortality in this patient group. Although the literature contains studies comparing different classifications, to the best of our knowledge, our study is the first to compare PSI, Curb-65, CALL and BCRSS in the prediction of prognosis in patients with COVID-19.

In studies comparing scoring systems in COVID-19 patients, the effects of comorbidity and symptoms on mortality were also evaluated. Kamran et al. suggested that comorbidity was not area under the curve: (p = 0.565) for mortality (7) while another study evaluating 52 intensive care patients with COVID-19 reported a relationship between advanced age and comorbidities and mortality (8). Zhang et al., examined 80 patients and found a significant relationship between cardiac diseases and hypertension and mortality (9) whereas Zhu et al. retrospectively observed that among 181 patients with COVID-19, comorbidities were not predictive of mortality (10). In the same study, it was

revealed that the symptoms of cough, shortness of breath and diarrhea were statistically significantly associated with mortality (10). In a study conducted in Wuhan, Zhou et al. determined the most common symptoms as fever and cough in 191 patients and noted that mortality was higher in patients with diabetes mellitus and coronary artery disease (11). In our study, while our patients most frequently presented with a cough, mortality was significantly higher in those with the comorbidities of CHF, HT, COPD, CKF and CAD. Confusion, cough ($p < 0.001$), myalgia and sore throat ($p < 0.024$) were found to be statistically significant in determining mortality.

In the prediction of prognosis and mortality using scoring systems, not only symptoms and comorbidities but also examination findings and laboratory test results can contribute to this evaluation. In a study examining 419 patients with community-acquired pneumonia, it was emphasized that PSI better defined a low risk of death while CURB-65 was able to show a high risk of death but did not have any prediction about comorbidities (12). In patients diagnosed with COVID-19, different scoring systems have been used, and their superiority in predicting mortality differed. For example, in a prospective study evaluating 249 patients with COVID-19, Garcia-Clemente et al. found that PSI and CURB-65 were determinants of mortality (13). In another study comparing laboratory parameters, as well as scoring systems, there was a statistically significant relationship between the CURB-65, PSI and COVID-19 severity scores and length of hospital stay, and the authors noted that CURB-65 and PSI outperformed the COVID-19 severity score in predicting mortality, but they were not superior to each other (10). In a retrospective classification study conducted by Fan et al. in COVID-19 cases, it was stated that CURB-65 and PSI did not have significant advantages over each other in predicting mortality (14). In our study, a statistically significant relationship was found between PSI and CURB-65 and mortality, and PSI was superior to CURB-65 in this evaluation. Both PSI and CURB-65 were positively correlated with the length of hospital stay.

In addition to the previously used scoring systems, different classification systems have been introduced to evaluate prognosis and predict mortality in COVID-19. BCRSS was developed by the Italian Society of Infectious and Tropical Diseases to determine patients that should be given tocilizumab treatment. This score was applied to the patients with a high risk of COVID-19 and those with a positive COVID-19 PCR result. Accordingly, it was determined that BCRSS should be ≥ 3(5, 15). In a study showing that tocilizumab treatment could prevent acute lung injury in patients with COVID-19, BCRSS was used as a guide to show the necessity and efficacy of treatment. In that study, it was determined that the coexistence of BCRSS-guided treatment and a low comorbidity rate resulted in reduced mortality (16). And it was also reported that BCRSS ensured that this treatment was effective in the early period (17). However, there are very few studies comparing BCRSS with other scoring systems. In a retrospective study in which 313 patients with COVID-19 were examined, it was found that CURB-65 had higher ability to determine in-hospital mortality compared to BCRSS while the latter was a better predictor of intensive care requirement (18). In contrast, in our study, both BCRSS and CURB-65 showed a positive correlation with the length of hospital stay, and neither was superior to the other in predicting mortality.

The CALL scoring system involves the evaluation of comorbidities, age, lymphocyte, and lactate dehydrogenase, which have been emphasized to play a role in COVID-19 since the emergence of the disease, and studies have been conducted to investigate the effect of these parameters on mortality. In a study examining 252 patients with COVID-19, it was shown that CALL was a reliable model for predicting mortality and determining the progression of the disease (7). In another study, Grifoni et al. similarly determined that CALL was a reliable model for predicting mortality in patients with COVID-19 but noted that it did not show disease progression at a sufficient level (19). In the current study, CALL was positively correlated with the length of hospital stay and presented as a reliable model for predicting mortality.

In our study, the four scoring systems were positively correlated with each other and with the length of hospital stay. However, the length of hospital stay was relatively weaker in predicting mortality compared to the scoring systems evaluated in our study. This can be explained by the rapid progression of the disease with the presence of comorbidities and increasing age. However, despite its weak predictive ability, the length of hospital stay was still statistically significantly correlated with mortality. Therefore, PSI, CURB-65, BCRSS and CALL also showing a positive correlation with the length of hospital further confirm that these scoring systems are determinants of both mortality and progression in patients with COVID-19.

This study was conducted in a single center with patients that had a positive PCR test. Although this test is accepted worldwide, some of our patients with two or three negative PCR test results, who were followed up for symptoms, were determined to have CT findings compatible with COVID-19. This patient group was not included in the study because their PCR test was negative.

Scoring systems are especially important in predicting the progression of COVID-19. Scoring systems, which include comorbidity and vital signs as well as laboratory and imaging findings, will be more effective than many scoring systems in determining the prognosis and mortality in patients with COVID-19. The comparative data of scoring systems will contribute to the literature in terms of taking the

necessary precautions, intervening in a timely manner, and making follow-up decisions in the hospital. There is a need for meta-analyses using different scoring systems and comparing the data obtained from different countries. This will assist in determining a common algorithm and achieve reduced mortality and length of hospital stay.

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Relationship between the national institutes health stroke scale score and bispectral index in patients with acute ischemic stroke

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Abstract

This study aimed to investigate the relationship between the bispectral index and the National Institutes of Health Stroke Scale (NIHSS) score in patients admitted to the emergency department with a first-time acute ischemic stroke. Methods: This prospective, observational study was conducted with patients admitted to our clinic with acute ischemic stroke symptoms. Patients with known cranial pathologies, such as space-occupying lesions, those with a history of clinically significant cerebrovascular events or sedative drug administration, and those with altered consciousness due to metabolic causes were excluded from the study. The National Institutes Health Stroke Scale scores were recorded by the clinician. Cerebral arterial territories were assessed on DWI and CT. The relationship between the NIHSS score and bispectral index was evaluated. Results: Forty-three patients were included in the study. The mean bispectral index of the cases was 84.23 ± 9.50. There was no significant correlation between the bispectral index values and the NIHSS score ($p<0.05$). Conclusion: In our study, the bispectral index values were decreased due to ischemic stroke. The results should be reevaluated studies conducted with larger series to reveal the relationship between infarcted territories, NIHSS score, bispectral index, and the GCS score.

Keywords: consciousness, consciousness monitors, ischemic stroke, physical examination, stroke

1. Introduction

Stroke refers to a sudden onset of focal neurological syndrome due to cerebrovascular disease. The World Health Organization defines stroke as rapidly developed clinical signs of focal or global disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than of vascular origin (1).

The National Institutes Health Stroke Scale (NIHHS) is an instrument that examines neurological functions and offers an idea about the long-term prognosis in patients that have had an ischemic stroke (2). In this patient group, there are also scales for a clinical evaluation, such as the Glasgow Coma Scale (GCS) and electronic devices for monitoring consciousness, such as the bispectral Index (3). The bispectral index equipment is based on an algorithm incorporating the Fourier analysis that compiles the pattern of electrical activity in the frontal region. It also shows the electroencephalography (EEG) line, suppression rate, and electromyographic response. It measures cortical inputs and guides anesthetic depth control since it is expected to show lower cortical activation in stroke patients. Furthermore, BIS values can be indirectly altered through cerebral hypoperfusion (4).

This study investigated the relationship between the NIHSS score and bispectral index in patients admitted to the emergency department with a first-time acute ischemic stroke. We aimed to reveal the relationship between BIS and stroke as the main outcome and the relationship between BIS and infarcted territories as the secondary outcome.

2. Materials and methods

2.1. Study design

This study was conducted prospectively after receiving the approval of the ethics committee of University of Health Sciences Fatih Sultan Mehmet Training and Research Hospital. Adult patients consecutively admitted to the emergency medicine clinic of this hospital with acute ischemic stroke symptoms were evaluated prospectively according to the inclusion criteria.

2.2. Population

University of Health Sciences Fatih Sultan Mehmet Training and Research Hospital is a tertiary hospital with 140,000 emergency admissions a year. All patients who presented to our clinic with acute ischemic stroke symptoms between February 1, 2014, and June 1, 2014 were included in the study. We excluded those with known space-occupying lesions, those with a history of clinically significant

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cerebrovascular events, sedative drug administration or a hemorrhagic stroke, and those with altered consciousness due to metabolic causes. The patients with a severe stroke (GCS eight or less) were intubated with sedative drugs in line with the rapid sequence intubation (RSI) protocol. In severe stroke cases, we did not have enough time to seek patient consent for the study and BIS monitoring before RSI; therefore, these patients were also excluded.

2.3. Data collection and measurement

From the ischemic stroke patients, the following clinical data were collected: 1) demographics and medical histories, 2) initial vital signs at the emergency department (pulse and mean arterial pressure), 3) presence of early ischemic changes (EIC) on CT, 4) laboratory findings, 5) time from the onset of symptoms to hospital care, 6) infarcted territories, and 7) bispectral index, GCS and NIHSS scores (Table 1). The GCS and NIHSS scores were recorded by the clinician. The cerebral arterial territories were assessed on DWI and CT by a neuroradiologist blinded to the clinical histories of the patients. These territories were defined as middle cerebral artery (MCA) territory, posterior cerebral artery (PCA) territory, distal branches of MCA territory, anterior cerebral artery (ACA) territory, posterior inferior cerebellar artery (PICA) territory, and multiple arterial territories. The cerebral arterial territories were grouped as MCA, distal branches of MCA and other arterial territories.

Table 1. National Institutes of Health Strokes Scale (NIHSS) scoring

1a. Level of consciousness	
Alert	0
Not alert but arousable by minor stimulation	1
Not alert but requires repeated stimulation	2
Responds only with reflex motor or unresponsive	3
1b. LOC Questions (What is the month? What is your age?)	
Answers both questions correctly	0
Answers one question correctly	1
Answers neither question correctly	2
1c. LOC Commands (open and close the eyes and then to grip and release hand)	
Performs both tasks correctly	0
Performs one task correctly	1
Performs neither task correctly	2
2. Best Gaze	
Normal	0
Partial gaze palsy	1
Forced deviation	2
3. Visual	
No visual loss	0
Partial hemianopia	1
Complete hemianopia	2
Bilateral hemianopia	3
4. Facial Palsy	
Normal symmetrical movements	0
Minor paralysis	1
Partial paralysis	2
Complete paralysis	3
5. Motor Arm (5a. Left Arm 5b. Right Arm)	
No drift	0
Drift	1
Some effort against gravity	2
No effort against gravity	3
No movement	4
6. Motor Leg (6a. Left Leg 6b. Right Leg)	
No drift	0
Drift	1
Some effort against gravity	2
No effort against gravity	3
No movement	4
7. Limb Ataxia	
Absent	0
Present in one limb	1
Present in two limbs	2
8. Sensory	
Normal; no sensory loss	0
Mild-to-moderate sensory loss	1
Severe to total sensory loss	2
9. Best Language	
No aphasia	0
Mild-to-moderate aphasia	1
Severe aphasia	2
Mute, global aphasia	3
10. Dysarthria	
Normal	0
Mild-to-moderate dysarthria	1
Severe dysarthria	2
11. Extinction and Inattention (formerly Neglect)	
No abnormality	0
Visual, tactile, auditory, spatial, or personal inattention	1
Profound hemi-inattention or extinction to more than one modality	2

Score	
0	No stroke
1-4	Minor stroke
5-15	Moderate stroke
16-20	Moderate to severe stroke
21-42	Severe stroke

Bispectral index BIS monitoring was performed with the COVIDIEN complete monitoring system (PN / 185-0151, U.S.A) with the patient in the supine position. If the patient's condition was unstable or urgent sedative medication was required, the measurement was undertaken before the administration of medication if possible. The bispectral index monitors recorded the data for 15 minutes, and the mean value was calculated.

2.4. Statistical analysis

The Shapiro-Wilk test was used to determine normality. Descriptive statistical methods (mean, standard deviation, and frequency) were used to analyze the study data. The relationship between the bispectral index and NIHSS scores was evaluated using Spearman's correlation test. For the analysis of multiple groups, one-way analysis of variance or the Kruskal-Wallis test was used depending on the normality of data. Statistical analyses were performed using SPSS v. 20.0 (SPSS Inc., Chicago, IL, USA) in Windows, and $p<0.05$ was considered statistically significant.

3. Results

A total of 96 patients with acute ischemic stroke symptoms were admitted to our clinic between February 1, 2014, and June 1, 2014. After applying the exclusion criteria, the sample consisted of 43 patients (Fig. 1). The mean age of the included patients was 72.79 ± 11.59 years. The time from the onset of symptoms to hospital care ranged from 1 to 24 hours,

with a mean of 5.07 ± 3.67 hours. The initial vital signs and comorbidities of the patients are shown in Table 1, and their laboratory data in Table 2. Twenty patients (46.5%) had EIC on CT whereas 23 (53.5%) had no EIC on CT. The mean GCS scores ranged from 9 to 15 with a mean score of 14.12 ± 1.73. Bispectral index ranged from 61 to 98, with a mean of 84.23 ± 9.50, as shown in the box plot graph presented in Fig. 2. The NIHSS scores ranged from 0 to 20, and the mean was calculated as 7.86 ± 5.72. The box plot of bispectral index value for NIHSS score is shown in Fig. 3.

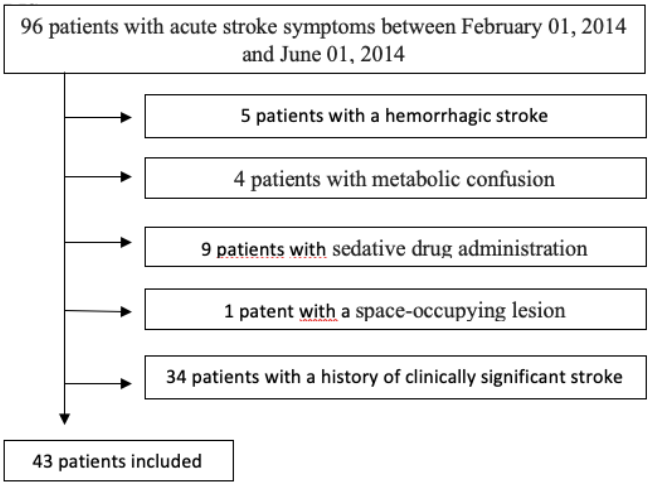

Fig.1. A flowdiagram of the study population

Table 2. Initial vital signs and comorbidities

Initialvitals	**Mean ± Standard deviation (Min-Max)**
Pulse (min)	81.40 ± 13.73 (40-112)
Mean arterial pressure	114.14 ± 17.16 (80-146.7)
Comorbidities	**n (%)**
Diabetes mellitus	17 (39.5%)
Hypertension	35 (81.4%)
Coroner artery disease	8 (18.6%)
Chronic renal failure	1 (2.3%)
Malignancy	1 (2.3%)
Atrial fibrillation	13 (30.2)
Laboratory parameters	**Mean ± Standard deviation (Min-Max)**
Leukocytes (K/uL)	9.80 ± 3.77 (4.70-21.30)
Hemoglobin (gr/dL)	13.10 ± 1.88 (7.40-16.70)
Platelets (K/uL)	247.60 ± 100.78 (141-730)
Mean platelet volume (fL)	8.14 ± 1.10 (6.6-11.8)
Troponin I (ng/ml)	0.09 ± 0.29 (0-1.74)
Glucose (mg/dL)	158.67 ± 90.91 (77-506)

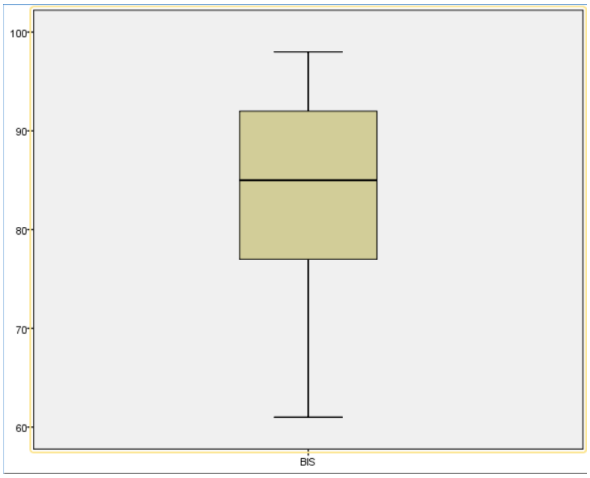

Fig. 2. The box plot of bispectral index value

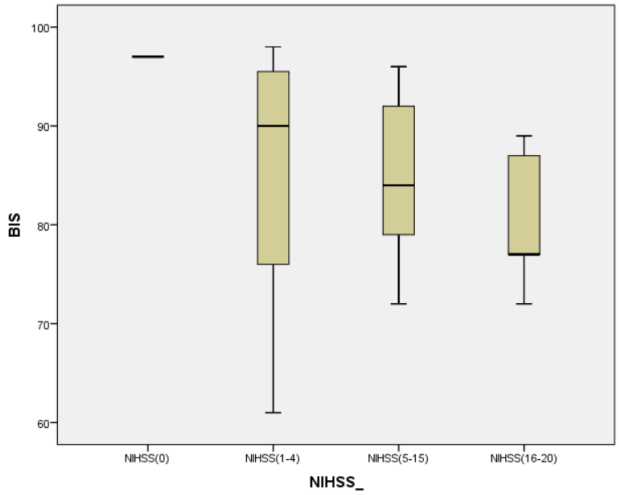

Fig. 3. The box plot of bispectral index value for NIHSS score. The NIHSS scores were categorized in 0, 1-4, 5-15, 16-20, and 21-42

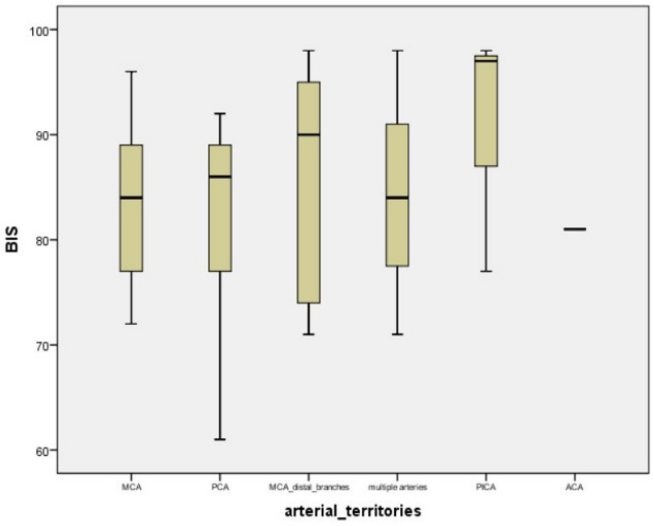

Fig.4. The box plot of bispectral index value for infarcted territories. BIS: Bispectral index; MCA: middle cerebral artery territory; PCA: posterior cerebral artery territory, MCA distal branches: distal branches of middle cerebral artery territory; multiple arteries: multiple arterial territories; PICA: posterior inferior cerebellar artery territory; ACA: anterior cerebral artery territory

The frequencies of infarcted territories were 44.2% (19 patients) for MCA territory, 11.6% (5 patients) for PCA territory, 27.9% (12 patents) for the distal branches of MCA territory, 2.3% (1 patient) for ACA territory, 7% (3 patients) for multiple arterial territories, and 7% (3 patients) for PICA territory. The box plot of bispectral index value for each territory is shown in Figure 4. There was no statistically significant relationship between the bispectral index and NIHSS scores (Spearman's correlation test, r = -0.274 p = 0.075). There was no statistically significant relationship between the grouped infarcted territories and the bispectral index and NIHSS scores (Table 3).

Table 3. Relationship between infarcted territories and NIHSS and BIS

	MCA territory	Distal branches of MCA territory	Other arterial territories	p
NIHSS	12 (2-20)	4.5 (2-12)	3 (0-16)	**0.015***
BIS	83.05 ±7.52	86.08 ± 10.65	84.25 ±11.51	**0.698**

Kruskal-Wallis test, One-wayanalysis of variance

4. Discussion

In this study, we investigated the relationship between the NIHSS score and bispectral index value in patients admitted to the emergency department with an acute ischemic stroke, and we did not determine a relationship between the two. This may be because NIHSS assesses the level of 11 clinical conditions, namely sensory deficit, coordination, language, speech, motor performance of the extremities, gaze, visual fields, facial weakness, hemi-inattention, and consciousness, of which only one is related to consciousness while the remaining ten focuses on motor, sensory and cerebellar examination (5). On the other hand, bispectral index correlates with the clinical measures of recall with high accuracy and reproducibility, sedation, and hypnosis (6). It was designed as valuable monitor of the level of sedation and loss of consciousness for sedative hypnotic drugs (7).

A second explanation for the lack of a relationship between the NIHSS score and bispectral index may be that the patients included in our study represented moderate and mild stroke cases and those with a severe stroke were excluded. The lowest bispectral index value of the patients included in the study was 61. We consider that in severe cases of stroke, lower bispectral index values may be observed. We did not determine extremely low values due to medical sedation and emergency vital interventions, such as RSI, which are required in patients with severe stroke indications and affected consciousness.

Although BIS is traditionally not designed to detect brain injuries, such as ischemic events, some previous reports attempted to address this subject, which largely remains controversial. Küsken et al. evaluated bispectral index in patients with head trauma and showed that these values were lower in patients with CT pathologies than in those without such findings (8).

In the literature, bispectral index changes due to cerebral hypoperfusion and ischemic stroke have been reported in patients undergoing preoperative bispectral index monitoring in the form of case reports (9-13). Morimoto et al. reported a case, in which the bispectral index value decreased during the arteriovenous shunt operation, which they attributed to possible cerebral hypoperfusion (9). Welsby et al. reported a patient with an unexpected bispectral index decrease during the coronary by-pass graft operation and a postoperative stroke sign. They suggested that this decrease might be due to stroke (10). In another case study, Leggat et al. observed that the bispectral index monitor recorded an acute change caused by an intraoperative stroke during the replacement of an aortic valve (11). Villacorta et al. reported another case of intraoperative stroke during the replacement of an aortic valve, which showed an unexplained, sustained fall in the bispectral index value (12). Nevertheless, Deogaonkar et al. presenting three cases, demonstrated the inability of the bispectral index monitor to detect cerebral ischemia (13).

In our study, the mean bispectral index value of the cases was 84.23 ± 9.50. All known probabilities that could affect the bispectral index values were excluded from the sample. It is possible to say that this general decrease in the bispectral index values of our patients was due to the stroke.

The ascending reticular activating system, located in the hypothalamus and brainstem, is primary responsible for consciousness (14). The cognition centers of brain are located in the prefrontal cortex, to which blood supply is provided by ACA and MCA while the branches of ACA, PCA and basilar arteries supply blood to the ascending reticular activating system (15). Therefore, multiple arterial territories are involved in consciousness, which may be the reason why we did not find any statistical difference between the grouped infarcted territories and bispectral index and NIHSS scores.

The most important limitation of this study was the exclusion of severe stroke patients. Secondly, the changes in bispectral index during the follow-up of patients could not be recorded due to early hospitalization. Therefore, we were not able to evaluate the relationship between the NIHSS scores and cognitive functions and bispectral index at the time of discharge from hospital. Finally, the number of patients with the same infarcted territories was not sufficient to investigate their relationship with bispectral index.

In this study, bispectral index decreased due to ischemic stroke, but there was no significant relationship between the bispectral index and NIHSS scores. The results should be reevaluated in studies conducted with larger series to reveal the relationship between infarcted territories, NIHHS score, bispectral index, and GCS score.

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Research Article

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Can we prevent falls in older individuals?

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Abstract

Falls are a major public health problem globally. Each year, 2.8 million elderly people (defined as those aged ≥65) are treated in emergency departments for fall injuries. A questionnaire containing 15 questions, prepared by researchers, was provided to patients who visited the emergency department due to falls and were aged ≥65. Patients who had fallen on the ground were included in the study group and were categorized using the following criteria: age, gender, education, socioeconomic status, life spots, falling location, chronic illnesses, previous falling episodes, cause of falls, medications used, hospital procedures undergone and the outcome of those procedures. A questionnaire was given to 159 patients that visited our emergency department as a result off all. When those patients that did not respond to the questionnaires, those who had high falls, and those who did not remember their traumas were excluded, there were 119 remaining. There were 83 female participants (69.7%) and 79.87 (SD, 7.98) were participants in the study. In our study, 21 (17.6%) patients were identified as having had previous falls. Age, gender, education status, living environment, number of drugs used, diseases and p values were compared according to old operative emoticons: 0.434, 0.855, 0.607, 0.502, 0.778, 0.324and 0.384, and there are no significant statistical differences between them. Despite the challenges mentioned above, educating people about the benefits of healthy ageing, and taking preventive measures will likely help to reduce negative outcomes in the future.

Keywords: accidental falls, aged, emergency service, healthy aging

1. Introduction

Falls are a major public health problem globally. Approximately 28%–35% of people aged >65 fall each year, increasing to 32%–42% for those with >70 years of age (1). Each year, 2.8 million elderly people (defined as those aged ≥65) are treated in emergency departments for fall injuries (2). Falls lead to 20%–30% of mild to severe injuries and are the underlying cause of 10%–15% of all emergency department visits. More than 50% of injury-related hospitalizations relate to elderly people (1). The major underlying causes for fall-related hospital admissions are hip fractures, traumatic brain injuries and upper limb injuries (1). According to data published in 2015 by the Centers for Disease Control and Prevention (CDC) in the United States (US), falls are the leading cause of fatal and non-fatal injuries among elderly people (2). The financial cost off all-related injuries are substantial. The average cost to the health system per fall injury in Finland and Australia is $3,611 and $1,049 respectively, regarding elderly people. Direct medical costs totalled $616.5 million for fatal injuries and $30.3 billion for non-fatal injuries in 2012, rising to $637.5 million and $31.3 billion, respectively, in 2015 (3). In 2004, the economic burden of injury in Canada was estimated at $19.8 billion, and the direct costs associated with falls among elderly people in Canada were estimated at over $2 billion. The cost of falls for Canadian seniors (per capita) was 3.7 times greater than that of individuals between the ages of 25 and 64 (4).

2. Materials and methods

2.1. Study groups

A questionnaire containing 15 questions, prepared by researchers, was provided to patients who visited the emergency department due to falls and were aged ≥65. Patients that responded to their questionnaire inappropriately or had fallen from a great height, and therefore, did not remember their traumas were separated from the study group. Patients who had fallen on the ground were included in the study group and were categorized using the following criteria: Age, gender, education, socioeconomic status, life spots, fall location, chronic illnesses, previous falling episodes, cause of falls, medications used, hospital procedures undergone and the outcome of those procedures. In the descriptive study, participants' demographics and responses to the scale questionnaire were objectively analysed. The clinical research protocol of this study was reviewed and approved by the Non-Interventional Clinical Studies Institutional Review Board of Katip Çelebi University Izmir, Turkey (Ethical Committee Number: 8, 21.01.2016).

2.3. Statistical analysis

The Statistical Package for the Social Sciences 20.0 program was used for statistical analysis of the data. In the data analysis, both explanatory and generic statistical methods were used to obtain the results. The demographic

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characteristics of the participants were analysed, and frequency distributions were established. The t-test was used to examine the relationship between the two groups of variables. A p value of <0.05 was considered statistically significant for all outcomes.

3. Results

A questionnaire was given to 159 patients that visited our emergency department because of all. When those patients that did not respond to the questionnaires, those who had high falls, and those who did not remember their traumas were excluded, there were 119 remaining. There were 83 female participants (69.7%) and 79.87 (SD, 7.98) were participants in the study. The demographic data of participants are shown in Table 1. A total of 91 participants (76.5%) were determined to have fallen at home. Detailed examinations according to fall location are given in Table 2. Only 21 (17.6%) of the participating patients indicated that they had fallen before, and only one of these had received medical support.

Table 1. Demographic data of participants

	Male	Female	Total
Study population	36 (30.3%)	83 (69.7%)	119 (100)
Age mean (SD)	76.97 **(**7.83**)**	81.13 **(**7.73**)**	79.87 **(**7.96**)**
Who lives with			
Alone	6	23	29 (24.4)
With his wife	24	16	40 (33.6)
With crowded family	6	43	49 (41.2)
With caregiver	0	1	1 (0.8)
Educational status			
Illiterate	7	64	71 (59.7)
Literate	12	4	16 (13.4)
Elementary school	16	15	30 (25.2)
Middle school	1	0	1 (0.8)
High school	1	0	1 (0.8)

SD, standard deviation

Table 2. Evaluation according to fall places

Fall from floor	Male	Female	Total (%)
Dwelling	21	70	91 (75.8)
Street	11	3	14 (12.5)
Garden	1	8	9 (7.5)
Mosque	1	0	1 (0.8)
Workplace	2	2	4 (3.3)
Dwelling fall	Male	Female	Total (%)
Toilet	2	14	16 (17.5)
Bathroom	0	7	7 (0.7))
Room	19	48	67 (73.6)
Kitchen	0	1	1 (0.1)

Information on the hospital process for each of the participants is given in Table 3. Our participants: 51 patients (42.5%) 1-3, 19 patients (15.8%) 4–5 and 29 patients (24.2%) using six or more drugs. Nineteen patients (15.8%) did not use any medication, and three patients (2.5%) used irregular or unknown drugs. One of the 19 medication-free patients had suffered vision loss, one had dementia and two had previously been operated on due to suffering a femur fracture. There were 14 patients with no diagnosed diseases or drug use.

Table 3. Information about the hospital process

Imagination	**Participant (%)**
Yes	117 (98.3)
No	2 (1.7)
Consultation	
Yes	85 (71.4)
No	34 (28.6)
Diagnosis	
Hip fracture	54 (45.4)
Soft tissue injury	38 (31.9)
Other*	18 (15.1)
Radius fracture	6 (5)
Pelvic fracture	3 (2.5)
Hospital outcome	
Admission	61 (51.3)
Discharge	56 (47.1)
Treatment rejection	2 (1.7)

Table 4. History of previous fall

Sex	**Previous fall**		**Total (%)**
	Yes (%)	**No (%)**	
Male	6 (5.0)	30 (25.2)	36 (30.3)
Female	15 (12.6)	68 (57.1)	83 (69.7)
Age			
65-69	4 (3.3)	14 (11.7)	18 (15.1)
70-79	4 (3.3)	29 (24.3)	33 (27.7)
80-89	8 (6.7)	47 (39.4)	55 (46.2)
90 and above	5 (4.2)	8 (6.7)	13 (10.9)
Educational status			
Illiterate	12 (10.0)	59 (49.5)	71 (59.6)
Literate	2 (1.6)	14 (11.7)	16 (13.4)
Elementary school	5 (4.2)	25 21.0)	30 (25.2)
Middle school	1 (0.8)	0	1 (0.8)
High school	1 (0.8)	0	1 (0.8)
Who lives with			
Alone	5 (4.2)	24 (20.1)	29 (24.3)
With his wife	9 (7.5)	31 (26.0)	40 (33.6)
With crowded family	7 (5.8)	42 (35.2)	49 (41.1)
With caregiver	0	1 (0.8)	1 (0.8)
Number of drug			
1-3	9 (7.5)	43 (36.1)	52 (43.6)
4-5	3 (2.5)	16 (13.4)	19 (15.9)
6 and above	5 (4.2)	24 (20.1)	29 (24.3)
No	4 (3.3)	15 (12.6)	19 (15.9)
Chronic disease			
Osteoporosis	6 (5.0)	15 (12.6)	21 (17.6)
Gonarthrosis	2 (1.6)	15 (12.6)	17 (14.2)
Osteoarthritis	0	1 (0.8)	1 (0.8)
Defect of vision	0	5 (4.2)	5 (4.2)
Arrhythmia	1 (0.8)	8 (6.7)	9 (7.5)
Epilepsy	1 (0.8)	2 (1.6)	3 (2.5)
Vertigo	1 (0.8)	9 (7.5)	10 (8.4)
Ht	10 (8.4)	29 (24.3)	39 (32.7)
SVD	2 (1.6)	9 (7.5)	11 (9.2)
DM	8 (6.7)	18 (15.1)	26 (21.8)
CAD	0	6 (5.0)	6 (5.0)
Anemia	1 (0.8)	7 (5.8)	8 (6.7)
Hypothyroidism	1 (0.8)	1 (0.8)	2 (1.6)
Cancer	0	1 (0.8)	1 (0.8)
CHF	0	3 (2.5)	3 (2.5)
Alzheimer	0	14 (11.7)	14 (11.7)
Previous operation			
Yes	3 (2.5)	8 (6.7)	11 (9.3)
No	18 (15.1)	90 (75.6)	108 (90.7)

In our study, 21 (17.6%) patients were identified as having had previous falls. The number of illnesses and drug use of these patients are given in Table 4. Age, gender, education status, living environment, number of drugs used, diseases and p values were compared according to old operative emoticons: 0.434, 0.855, 0.607, 0.502, 0.778, 0.324and 0.384, and there are no significant statistical differences between them (Tables 5 and 6).

Table 5. Comparison of previous fall history in terms of drug use, disease, and previous operation

Previous fall	Drug use	N	%	T	M	SD	t	p
Yes	1-3	9	42.9	21	2.19	1.209	.283	**.778**
	4-5	3	14.3					
	6 and above	5	23.8					
	0	4	19.0					
No	1-3	43	43.9	98	2.11	1.139		
	4-5	16	16.3					
	6 and above	24	24.5					
	0	15	15.3					
	Disease							
	No	12	57.1					
Yes	Osteoporosis. gonartrosis. Artritis	7	33.3					
	Visually	0	0.0	21	.6667	1.064		
	Arrhythmia	1	4.8					
	Epilepsy	1	4.8					
	Vertigo	0	0.0				.990	**.324**
	No	54	55.1					
	Osteoporosis. gonartrosis. Artritis	22	22.4	98	1.0102	1.509		
No	Visually	5	5.1					
	Arrhythmia	8	8.2					
	Epilepsy	2	2.0					
	Vertigo	7	7.1					
	Previous operation							
Yes	Yes	3	14.3	21	1.86	.359		
	No	18	85.7				.874	**.384**
No	Yes	8	8.2	98	1.92	.275		
	No	90	91.8					

Table 6. Comparison of previous fall history in terms of age, sex, education and living space

Previous fall		N	%	T	M	SD	t	z
	Age							
Yes	69 and under	4	19	21	2.66	1.06	.785	**.434**
	70-79	4	19					
	80-89	8	38.1					
	90 and above	5	23.8					
No	69 and under	14	14.3	98	2.50	.84		
	70-79	29	29.6					
	80-89	47	48					
	90 and above	8	8.2					
	Sex							
Yes	Male	6	28.6	21	1.71	.463	.183	**.855**
	Female	15	71.4					
No	Male	30	30.6	98	1.69	.465		
	Female	68	69.4					
	Education							
Yes	Illiterate	12	57.1	21	1.76	.944	.516	**.607**
	Literate	2	9.5					
	Elementary school	7	33.3					
	Middle school	0	0.0					
	High school	0	0.0					
No	Illiterate	59	60.2	98	1.65	.863		
	Literate	14	14.3					
	Elementary school	25	25.5					
	Middle school	0	0.0					
	High school	0	0.0					
	Living space							
Yes	Alone	5	23.8	21	2.43	1.207	.673	**.502**
	With his wife	9	42.9					
	With caregiver	0	0.0					
	With crowded family	7	33.3					
	Nursing home	0	0.0					
No	Alone	24	24.5	98	2.63	1.271		
	With his wife	31	31.6					
	With caregiver	0	0.0					
	With crowded family	43	43.9					
	Nursing home	0	0.0					

4. Discussion

Ninety-one of our participants (75.8%) were at home when they suffered their fall. In the study entitled Seniors' Falls in Canada: Second Report, it was reported that 50% of falls by individuals aged 65 or over occurred in the home and 17% took place in a residential institution. Rao states that one half to two thirds of falls occur in or around the patient's home (5). Masud et al. stated that 65% of women and 44% of men fell in their place of residence (6). Cambell et al. stated that falls are most common in the house and in oftenly used rooms (7). According to this study, it is evident that, of all the rooms in the house, the bedroom has the lowest frequency of falls, and the frequency of falls in bathrooms and toilets are also low. We found that falls at home often occur in the living room, as this is where the patients spent most of their time. Even if we accept that all falls in bathrooms and toilets occur on wet ground, it is evident from this study that the elderly mostly do not fall on wet ground, as the occurrence of these types of falls is much less than in other rooms frequently used. The environmental factors contributing to falls, as stated by Rubenstein et al. (8) included wet floors, bad lighting, and inconvenient bed height; however, these factors do not feature highly on the list of reasons for falls in our study. Rubenstein et al. also stated that the three most common causes off all in elderly patients were: accidents and environment-related factors; gait and balance disorders; and weakness, dizziness, and vertigo. Our studies found the above to be true.

In previous studies, having a history of falls was a risk factor for falling in the elderly. In the study by Rubenstein et al., it was reported that having a history of falls increased the future risk of falling three-fold. A study by Lord et al. showed that 67.7% of 341 participants did not fall during follow-up, and 39.3% had fallen at least once (9). In research by Talarska et al., 506 individuals were studied. Of these, 131 people were considered to tend to fall, according to set criteria, and it was found that 98 of those had already fallen at least once. The remaining 375 individuals were considered not to tend to fall, and it was found that 79 of those had previously fallen. In that study, therefore, 177 of the 506 participants had previously fallen, which is approximately 34% (10). In our study, however, only 21 of the participants (17.6%) had previously fallen; therefore, having a history of falls was not a serious risk factor in our experience.

Tinetti et al. concluded in their study that a multiple-risk-factor intervention strategy resulted in a significant reduction in the risk of falling among elderly persons in the community (11). In their study, patients with risk factors were evaluated; however, our study includes patients with no risk factors. In addition, our study has shown that, despite uncontrolled studies reporting the potential effects of intervention strategies on falls, preventive measures have not proven to be effective in controlled trials to date. These negative results were either too high or too low for a risk of falling because they were not intensive enough. Alternatively, falls among elderly persons may not be preventable (11). Prevention strategies should center around education, training, creating safe environments, prioritising fall-related research and establishing effective policies to reduce risk. As falling risk factors of elderly individuals; (three times), muscle weakness (four times), history of falls and walking-balance disorders (three times), use of assistive devices, vision problems, arthritis, decrease in daily activities, depression, cognitive disorders and over 80 years of age, colleagues pointed out that the balance and gait deficits in their collections in 2010 increased modestly (12). A systematic review found no evidence that refer to vision correction in older people being effective in reducing the numbers of people falling (13). The exercise program was not significantly reduced (14). In the present study, it was found that psychotropic medication significantly reduced the risk of falling, but the medication withdrawal was slow and difficult for the patient. Although some evidence supports the use of home environment assessment and intervention as a strategy to reduce falls, there are mixed reports (15). As regards the use of specific education for the elderly as a preventive measure, there is little evidence of any benefit; however, Duckham et al. did not affect bone mineral density in their randomized controlled trials (16). In a study conducted by Nilsonet al.in Sweden, (17) younger elderly (aged 65–79) were found to have a lower rate of fall-related injuries compared to the previous decade, whereas there was an increase in such incidents in the elderly (aged 80 or older), especially in males. Their study shows that elderly seniors in Sweden are increasingly hospitalized with fewer serious injuries, although more research is needed to fully understand the reasons for the differences between the sexes and different age groups. The change in nature of injuries is important to understand and to include in the future planning of health care and fall prevention.

The greatest challenge to developing countries regarding the prevention of falls in the elderly is the lack of relevant epidemiological data. In developed countries, the greatest challenge is the lack of information on the effectiveness of fall-prevention strategies. In a study in Canada, the issue is summarized as follows: "The research literature on risk factors for falls and on best practices in falls prevention reveals several research gaps (18). There is a lack of knowledge around the efficacy of falls prevention practices for subpopulations of Canadian seniors". In addition, there are several strategies that have been taken to reduce the risk of falling-related injuries among the elderly, but racial, socioeconomic and population disparities have created a knowledge gap and may limit generalizability (19).

In a 2012 report by the World Health Organization (WHO), risk factors for falling and falling injuries of elderly individuals were evaluated under four main headings: biological, socioeconomic, environmental, and behavioral. Biological risk factors, such as age, gender, race, chronic illnesses and decreases in physical cognition and affective

capacity seem to be unchangeable. In underdeveloped or developing countries, it is difficult to improve socioeconomic risk factors as they require material resources that may not be available. When we look at the environmental risk factors, we do not see the environmental factors mostly in the foreground because we deal only with ground depletions relative to our work. According to this report and our study results, the main factors that can be improved are the behavioral risk factors. These potential improvements include increased physical activity, healthy nutrition, proper and controlled regulation of medical treatments, and avoiding actions that could cause a fall. The preventive activities listed by the WHO include the following:

- Screening the living environment for risks;
- Clinical interventions to identify risk factors;
- Treatment of low blood pressure;
- Vitamin D and calcium supplementation;
- Treatment of correctable visual impairment;
- Home assessment and environment modification;
- Prescription of assistive devices to address physical and sensory impairments; and
- Muscle strengthening and balance retraining, incorporating fall prevention education and tai chi-type exercises or dynamic balance and strength training.

These items are discussed in terms of the socio-economic structures of countries, attitudes and behaviors of elderly individuals and their living environments. In Turkey, the greatest obstacles to the successful prevention of falls in the elderly are the lack of available care centers, the individual's desire to stay in their home, and the lack of financial resources to purchase the required equipment. These are perhaps the most common problems in all developing countries. There are also other obstacles, resulting from regional differences.

In conclusion, according to data from the CDC, there were 46 million people aged 65 or older in the US in 2014, with 29 million falls in that year. The elderly population is predicted to increase by 62% to 74 million by 2030, with a 68% increase in falls to 49 million. These data show that, despite all the preventive actions taken, it is not possible to prevent falls in elderly people (21). Despite the challenges mentioned above, educating people about the benefits of healthy ageing, and taking preventive measures will likely help to reduce negative outcomes in the future.

Conflict of interest

The authors declare no conflict of interest.

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The investigation survey of inguinal hernia operation techniques preferred by pediatric surgeons in Turkey

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Abstract

In inguinal hernia, standard procedure has been considered open repair for a long time, the number of pediatric surgeons who prefer laparoscopic techniques is increasing day by day. In this study, it was aimed to determine how pediatric surgeons in our country approach patients with inguinal hernia and manage the treatment process. Controversial issues on inguinal hernia repair were identified and a questionnaire was prepared to reveal the current situation. The questionnaire was delivered to members of Turkish Association of Pediatric Surgery on the official website of the association. The survey was directed to 420 people, 92 people returned with the answers to the questionnaire. Thirty-six of the surgeons prefer the laparoscopic method for inguinal hernia repair. The reason for choosing open surgery was questioned, the most frequent answer was to be more experienced and to have more cumulative knowledge on open repair method. According to survey, the greatest advantage of laparoscopy was stated to be the better evaluation of the contralateral inguinal canal (n=16, %44.4). Twelve of surgeons who performed laparoscopic treatment in the selection of patients stated that gender is important and prefer laparoscopic surgery for female patients. Thirty percent (n = 11) of the participants stated that they prefer laparoscopy in patients between 3 months and 13 years old, while the rest prefer laparoscopy at all ages. In conclusion thirty nine percent of surgeons in Turkey prefer laparoscopic repair. Open repair is still preferred in our country. There is still no consensus on perfect method and patient selection yet.

Keywords: hernia, inguinal, laparoscopy, surveys and questionnaires

1. Introduction

Inguinal hernia (IH) is one of the most common surgical pathologies treated by pediatric surgeons (1). Surgical treatment is required in children since IH can result in incarceration, strangulation, and intestinal ischemia (1). For years, standard surgical technique in IH for children has been considered the traditional open repair because of its low complication and recurrence rates (2). However, some studies reported that the results of laparoscopic surgery are comparable in terms of complications and recurrence (3). A guideline regarding which operation technique to prefer has not been established yet (4).

The reported advantages of laparoscopic hernia repair are better exposure, more accurate evaluation of the contralateral inguinal canal, minimal dissection, better cosmetic outcome. Recurrence and complication rates are found to be comparable and close to open repair (5). Especially after defining the PIRS method, laparoscopic IH repair is becoming more and more popular among pediatric surgeons (6).

Although both methods have advantages and disadvantages, there is no consensus regarding which method should be preferred yet (7). In this study, it was aimed to determine how pediatric surgeons in our country approach their patients with IH and manage the treatment process.

2. Materials and methods

After the literature review, controversial issues between laparoscopic repair and open repair in IH repair were identified and to clarify the situation, a questionnaire was prepared to reveal the current situation. The questionnaire was delivered to members of Turkish Association of Pediatric Surgery (420 pediatric surgeons) on the official website of the association. 92 surgeons participated in the study and the responses were evaluated. Survey outputs were evaluated with Microsoft Excel. Ethical approval for this study was obtained from Ankara University Faculty of Medicine Ethical Committee (İ3-183-20).

3. Results

Of the 92 respondents, 86 were pediatric surgeons and six were pediatric urologists. Forty percent of the participants (n=37) have more than 16 years of surgical experience. 80% of the participants (n=74) are performing minimally invasive surgery (MIS). Among the participants, 39% (n=36) of them

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prefer the laparoscopic method for IH repair. This number accounts for about half of those who are performing laparoscopic surgery.

For surgeons who prefer the open method, the most important reason why they do not prefer laparoscopy is that they think laparoscopic treatment is not superior. (78%, n=44). In a multi-answer question about why they prefer open surgery, the most common answer was to have more experience and to have more cumulative information about open surgery. The most common answer to the question about the disadvantages of the laparoscopic method was that open surgery is an extra abdominal procedure, whereas laparoscopy requires intraabdominal intervention.

66% (n=24) of surgeons who preferred laparoscopic surgery had been using laparoscopic repair in their clinical practice for 0-5 years. The most preferred laparoscopic method was percutaneous internal ring suturization (PIRS) (77% (n=28)). According to survey, the greatest advantage of laparoscopy was the better evaluation of the contralateral inguinal canal (n=16, 44.4%). Twelve of surgeons (33%) who performed laparoscopic treatment in the selection of patients stated that gender is important and prefer laparoscopic surgery for female patients. Thirty percent (n=11) of the participants stated that they prefer laparoscopy in patients between three months and 13 years old, while the rest prefer laparoscopy at all ages. The responds revealed that the vast majority do not need additional ports (70%, n=25). The most frequent reason for those who need an additional port was for peritoneal manipulation.

Surgeons who prefer laparoscopic approach stated that in 39% (n=14) of their surgeries the ventilation was provided by laryngeal mask and 61% (n=22) was provided by endotracheal intubation in anesthesical management. Spinal anesthesia was not preferred by any of the participants. Many of the participants 80% (n=29) preferred non-absorbable suture material (nylon) and polyethylene terephthalate suture (ethibond) was most frequently preferred six and 23, respectively. Hydrodissection was preferred by 12 of the surgeons (33%). Nineteen of the surgeons (52%) prefer redo laparoscopic method for treatment in patients who completed their laparoscopic first operation and subsequently developed recurrence. Most of surgeons (n=24, 66%)) repair the contralateral patent processus vaginal detected during surgery. Detailed information on applied questionnaire and answers were presented in Table 1.

Table 1. Detailed information on applied questionnaire; the questions and answers with the percentages

Questions	Answers and Percentages (%)
Specialty	
Pediatric Surgeon	86 (%93.48)
Pediatric Urologist	6 (%6.52)
Experience (duration)	
0-5 years	17 (%18.47)
6-10 years	16 (%17.40)
11-15 years	22 (%23.91)
>16 years	37 (%40.22)
Institutional Information	
University Hospital	33 (%35.86)
Training and Research Hospital	32 (%34.78)
Private Hospital	16 (%17.39)
Public Hospital	11 (%11.95)
Practical Use of Minimally Invasive Surgery	
In Use	74 (%80.43)
Not In Use	18 (%19.56)
Experience of Minimal Invasive Surgery (if available)	
0-5 years	22 (%29.73)
6-10 years	26 (%35.13)
11-15 years	18 (%24.32)
>16 years	8 (%10.81)
Performing diagnostic contralateral laparoscopy in open IH repair	
Yes	23 (%25)
No	69 (%75)
If Performing. Contralateral Laparoscopy Experience (duration)	
0-5 years	10 (%43.47)
6-10 years	7 (%30.43)
11-15 years	3 (%13.05)
>16 years	3 (%13.05)
Practical Use of Laparoscopic IH Repair	
In Use	36 (%39.13)
Not In Use	56 (%60.86)
Reasons for Not Using Laparoscopic IH Repair	
Equipment Shortage	5 (%8.92)
Lack of Experience	7 (%12.5)
Open Surgery Preferance	44 (%78.57)
Reasons for Open Surgery Preference *	
Lack of Laparoscopic Experience	12 (%10.15)
Equipment Shortage	5 (%4.1)
Extra abdominal Placement	33 (%28.9)
Enable to evaluate the inguinal canal and funiculus spermaticus by feeling the tissue	28 (%24.5)
Experience and Cumulative Knowledge	36 (%32)
Limitations of Laparoscopic Repair *	
Peritoneum Invasion	37 (%39.4)
Cord and its elements are more likely to be damaged	27 (%28.7)
Lack of Experience	7 (%7.5)
Equipment Shortage	5 (%5.3)
Causes related to anesthesia and operating room conditions	18 (%19.1)
Laparoscopic IH Repair Experience (duration)	
0-5 years	24 (%66.66)
6-10 years	10 (%27.77)
11-15 years	1 (%2.77)
>16 years	1 (%2.77)
Number of Patients Undergoing Laparoscopic IH Repair	
0-25 patients	20 (%55.55)
25-50 patients	5 (%13.88)
50-100 patients	2 (%5.55)
>100 patients	9 (%25)
The Method Used in Laparoscopic IH Repair	
Hernia Repair with 3 Ports	5 (%13.88)
Single Port Hernia Repair	2 (%5.55)
PIRS (Percutaneous Internal Ring Suturization)	28 (%77.77)
Burnia Technique (Cauterization of the Circumference of the Hernia Sac)	1 (%3.6)

Repair with Patch	0 (%0)
Laparoscopic IH Repair Preferred Situations *	
Emergent Situations	16 (%11.6)
Elective Situations	32 (%23)
Female Patients	27 (%19.5)
Male Patients	9 (%6.5)
Based on family's preference	19 (%13.6)
Age based	11 (%7.9)
Based on equipment	13 (%9.3)
For resident training purposes	12 (%8.6)
The Biggest Advantage of Laparoscopic IH Repair Over Open Surgery	
Being minimally invasive surgery	7 (%19.44)
Not being touched of cord and elements	9 (%25)
Comfortable viewing of the contralateral side	16 (%44.44)
Cosmetic superiority	4 (%11.11)
Disadvantages of Open IH Repair	
Cord and its elements exposed to tactile trauma	13 (%36.11)
Failure to control the contralateral side all the time	19 (%52.77)
Cosmetic reasons	1 (%2.77)
Surgical difficulty in some patient groups	3 (%8.33)
Is patient gender a criterion to choose laparoscopic repair?	
Yes. it is.	12 (%33.3)
No. it is not.	24 (%66.6)
If yes, what gender to choose?	
Female	12 (%100)
Male	0 (%0)
Preference of laparoscopic IH repair age group	
0-3 months	0 (%0)
3 months – 13 years	11 (%30.55)
13-18 years	0 (%0)
All of them	25 (%69.44)
Additional trocar requirement other than camera trocar in IH repair with laparoscopic PIRS technique	
Yes	7 (%19.44)
No	25 (%69.44)
Sometimes	4 (%11.1)
If yes. the reason of using additional trocar	
Peritoneum Manipulation	6 (%85.71)
Inguinal Canal Circumference Cauterization	1 (%14.28)
Anesthesia technique used in laparoscopic IH surgery	
Laryngeal Mask	14 (%38.88)
Intubation	22 (%61.11)
Spinal anesthesia	0 (%0)
Suture material used in laparoscopic IH repair	
Nylon (non-absorbent monofilament suture)	6 (%16.66)
Ethibond (non-absorbent polyethyleneterephthalate suture)	23 (%63.88)
Vicryl (absorbable polyglycolicacid suture)	5 (%13.88)
PDS (absorbable polydioxone suture)	2 (%5.55)
Hydrodissection Application	
Yes	12 (%33.33)
No	24 (%66.66)
Surgical preference in patients with recurrency after laparoscopic repair	
Open repair	17 (%47.22)
Laparoscopic repair	19 (%52.77)
The complication that the surgeons experience or are afraid of laparoscopic IH operation *	
Bleeding and hematoma	23 (%34.5)
Cord injury	14 (%20.9)
Abdominal organ injury	8 (%11.9)
İleus	3 (%4.5)
Recurrence	18 (%26.8)
Anesthetic-related complication	1 (%1.4)
Laparoscopic repair of PPV detected on the contralateral side during laparoscopic repair	
Yes	24 (%66.6)
No	12 (%33.3)

IH: Inguinal Hernia
Questions marked with "*" have multiple answers

4. Discussion

The first minimally invasive intervention in IH in children was described by Gans et al. in 1971; it was the examination of the contralateral inguinal canal from the hernia sac (8). With the minimally invasive approach and the development of instruments, laparoscopic IH repair has started to be applied more frequently in children (8). Although there are different surgical techniques described, this frequency has increased gradually with the definition of transumbilical single-port methods (9).

When the survey results were taken into consideration, Turkey seems to be more common in open surgery as the preferred method. In their survey study Zani et al. concluded that many surgeons preferred open method to laparoscopic method, but the number of surgeons who preferred laparoscopic method has been increased in recent years (8). In USA, 13% of IH cases in children were performed with laparoscopic methods between 2009 and 2014 (10). Between 2010 and 2016, the rate of IHs performed laparoscopically was 37% (11). When the cases performed between 2005 and 2017 were evaluated in the cohort study conducted by Nakashima et al. (3), it was observed that more than 50% of the cases performed in the last two years were completed laparoscopically. As a result of our study, it has been observed that most surgeons who prefer laparoscopy have recently adopted this method. These data may indicate that percentage of those who will prefer laparoscopic surgery may increase. Although the open method is preferred more frequently, the rate of surgeons who prefer laparoscopy is higher in Turkey comparing other survey and cohort studies (3, 10, 11).

In the other survey study by Zani et al. (8); surgeons who prefer the open method defined the most important advantages as low recurrence probability, less risk of injury of an abdominal organ, testicular vascular structures or spermatic cord and shorter surgery time. On the other hand, surgeons who prefer laparoscopic repair methods defined the advantages as the low possibility of occurrence of contralateral metachronous hernia, better cosmetic results, easier technique, lower risk of injury of testicular vessel and cord structures, and less postoperative pain (8). The results appear to be like our study. The most important advantage that was stated by the respondents was better evaluation of the contralateral side (44.4%). Other advantages mentioned were safety of the cord and its elements (25%), minimally invasive procedure (19.44%) and better cosmetic results (11.11%), respectively. In a meta-analysis including three randomized controlled trials and four observational studies (a total of

1543 laparoscopic approaches, 657 open approaches), laparoscopic repair has similar results with open repair for these concerns (12). The same meta-analysis shows that laparoscopy has better results in terms of metachron contralateral hernia and cosmetics (12). A randomized controlled trial evaluating laparoscopic repair, shows shorter surgery and discharge time, lower recurrence rate, lower testicular complication rate (5). There are systemic reviews showing that laparoscopy is superior to the open method in terms of less early complications and shorter anesthesia and operation time, especially for bilateral cases (13). On the other hand, it was seen that extraperitoneal repair methods are faster in unilateral cases (4).

In our study, lack of experience was observed as another reason stated by the participants for not choosing laparoscopy (n=7, 12%). In a national survey study in Denmark, most surgeons preferred open method for children under 12, while one in 7 was using both open and laparoscopic methods depending on the situation (14). In patients between 13-18 years old, it was seen that while two-thirds of surgeons prefer only open repair, 6% prefer only laparoscopic repair, and the rest were using both methods (14). Considering the reasons of the surgeons for not preferring the laparoscopic method, the most common reason is seen as the lack of experience (14). It has been shown that the experience of the surgeon is important in laparoscopy and the learning process may be longer in laparoscopy against open repair (15). It has been shown that learning process for advanced laparoscopic techniques is faster for surgeons who specialized laparoscopy (16). Surgeon's experience on performing laparoscopic procedures for other pathologies is important for the process. On the other hand, the number of cases that a surgery resident needed to complete without supervision was found to be much higher (16). The survey study conducted by Zani et al. (8) and another study of Bertozzi et. al. (17) stated that number of cases required to perform laparoscopic inguinal hernia repair safely without supervision was 20 cases. However, the treatment of transumbilical single-port laparoscopic IH (PIRS) has been found to be much easier to learn and apply due to no need for classical three-port laparoscopy maneuvers (18). As a result, learning process and competent practical use of the PIRS method may be faster than other techniques.

The participants preferred both open and laparoscopic surgery stated that the risk of damaging vas deferens and testicular vascular structures is one of the reasons for not choosing other methods. Although information showing how future fertility is affected after inguinal hernia surgery in children is not sufficient, it may be predicted that complications may arise due to interventions on vasal and spermatic vessels during surgery (19). In the open method, these structures are exposed to surgical manipulation and trauma. In the repair done with a single port, the cord and surrounding structures can be removed from the peritoneum by hydrodissection method, and the operation can be completed with a single safe hole (18). With this simple method, a safe and truly minimally invasive repair can be done by protecting the cord and surrounding tissues from manipulation and trauma (18). One third of the participants in this study use the hydrodissection method.

In our study, it was found that 66% (n = 24) of the surgeons preferring laparoscopic approach if the metachronous hernias were detected and the metachronous hernias were repaired in the same session. Although the probability of contralateral metachronous IH is 4-32%, there is no consensus among pediatric surgeons regarding the approach for checking metachronous contralateral inguinal hernia (20). Transinguinal laparoscopic evaluation appears to be effective in detecting contralateral hernia (21). Transinguinal laparoscopy on the contralateral non-hernia side is highly effective for preventing an unnecessary operation that may damage the cord and surrounding structures and the risk of re-anesthesia that may arise in the event of a subsequent contralateral metachron inguinal hernia (21). There are publications showing that contralateral PPV was detected in 38% of patients with unilateral IH (20, 21). Considering the current risks of contralateral exploration, since not every PPV is herniated, the application of this method is controversial (1).

Among the surgeons who perform laparoscopic surgery, 44% of them prefer laparoscopic treatment in emergency situations (such as incarceration). In incarcerated hernia repair, the edema of the operation area makes the operation far more difficult in the open approach (22). This disadvantage is eliminated in laparoscopy since the edema does not cause such problem. One of the advantages of laparoscopy in incarcerated cases is that it facilitates the reduction of herniated structures with the created pneumoperitoneum (23). At the same time, the condition of the incarcerated organs may be evaluated (24). Although surgeons mostly did not prefer laparoscopy for incarceration in our study, Nah et al. described the advantages of laparoscopic approach as less recurrence of incarceration and a decreased rate of potential complications such as vascular injury (25).

Laparoscopy in recurrent cases is also a matter of debate among surgeons. In this survey study, almost half of the surgeons who preferred laparoscopic approach, preferred open method as the treatment of recurrences after laparoscopic repair. On the other hand, there are studies supporting laparoscopic repair, especially in male patients, for recurrences after open repair (9). In the open approach, there may be an increased risk of damaging vas deferens and testicular vascular structures especially for recurrent cases (26). It was stated that laparoscopic approach reduced the risk of open re-surgery (26).

In the present study, it is seen that endotracheal intubation

is applied more frequently in routine anesthesiology practice for laparoscopic procedures. Extraperitoneal and intraperitoneal methods can be preferred for hernia repair, but extraperitoneal method is preferred more frequently by pediatric surgeons due to its effectiveness and easy application (27). When the groups of patients who underwent laparoscopic IH repair using laryngeal mask airway (LMA) and endotracheal tube were compared, duration of anesthesia time was shorter in the LMA group (28). The fast and reliable PIRS method makes it safe to perform anesthesia with LMA (28). There are studies showing that the use of laryngeal mask is associated with less bronchospasm, laryngospasm, cough, and edema (28). Increasing experience and shortening the operation time will allow LMA to be used in anesthesia, thereby reducing the anesthetic complications of patients and the comorbidities that may occur.

While selecting the suture material to be used in the operation, the number of participants who preferred the non-absorbable material was found to be higher in our study. There are very few studies comparing absorbable and non-absorbable suture materials in IH repair. In a study of 300 cases, no significant difference was found between the two materials in terms of recurrence (29). Other studies have shown that the rate of recurrence was lower when non-absorbable suture material was used (30). However, previously published studies have shown that better results can be obtained with more surgical experience by using absorbable suture material (31).

In conclusion, thirty nine percent of surgeons in Turkey prefer laparoscopic repair. Open repair is still the preferred method in our country. There is no consensus about method and patient selection yet. As the level of scientific evidence increases and the method becomes more frequent, we predict that laparoscopic inguinal hernia repair will be preferred more frequently than today.

Conflict of interest

There is no conflict of interest between authors.

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Relationship of inhaled ipratropium and inhaled salbutamol with pupil dilation: A prospective observational study

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Abstract

This prospective randomized study aimed to compare the effects of nebulizer drugs on unilateral or bilateral dilation of the pupil that may develop due to mask incompatibility or patient incompatibility in patients receiving inhaled therapy. Following the approval of the local ethics committee, this study was carried out prospectively and observationally in the period from October 1, 2018 to April 1, 2019 in an emergency clinic with an annual capacity of 600,000 patients in a tertiary education and research hospital. The patients were divided into three groups: those given salbutamol alone (200 mcg), those given ipratropium alone (250 mcg/2ml), and those receiving both salbutamol and ipratropium. The pupil diameters of the patients included in the study were measured twice using a pupilometer device at hours 0 and 2 of treatment. Ninety-one patients that received inhaler treatment in the emergency department were included in the study. The process of treatments given to patients with indications was not interrupted. Ipratropium and salbutamol (49.5%) were used together in 45 patients, ipratropium alone in 38 (41.7%), and salbutamol alone in eight (8.8%). When the absolute delta ratios were compared, a statistically significant difference was observed between the patients given salbutamol and those given ipratropium, and between the patients that received salbutamol and those given salbutamol and ipratropium ($p=0.001$, 95% CI: 1.36-6.11 and $p<0.001$, 95% CI: 1.51- 6.19, respectively). Salbutamol caused statistically significantly greater pupil diameter changes than the other drugs. Although neurological diagnoses are considered in patients with anisocoria, it should be kept in mind that anisocoria may also be due to the current or the treatment being given.

Keywords: anisocoria, ipratropium, nebulizer treatment, pupil dilatation, salbutamol

1. Introduction

Ipratropium and salbutamol are two agents recommended by the current Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease (GOLD) guidelines for the treatment of chronic obstructive pulmonary disease (COPD) (1). These active substances are usually given to the patient by inhalation (2). For this treatment to be effective, the mask through which the inhaler is applied must fit the face snugly. As a result of an ill-fitting mask, anisocoria cases have been reported in them literature (3, 4, 5). This situation forces clinicians to make a differential diagnosis on a newly developing neurological pathology and may lead to unnecessary use of resources, as well as unnecessary radiation exposure and/or invasive procedures.

Anisocoria is typically defined as a difference of more than 1 mm between the diameters of the two pupils, characterized by an abnormally small unilateral pupil in the dark indicating sympathetic damage and an abnormally large unilateral pupil in light suggesting parasympathetic damage (6, 7).

Although the development of anisocoria because of the application of inhaler beta agonist or anticholinergic treatment given with an ill-fitting mask is a well-defined clinical entity in case reports, to the best of our knowledge, no related research paper has been published in the literature to date. This prospective randomized study aimed to compare the effects of nebulizer drugs on unilateral or bilateral dilation of the pupil that may develop due to mask incompatibility or patient incompatibility in patients receiving inhaled therapy.

2. Materials and methods

Following the approval of the local ethics committee, this study was carried out prospectively and observationally in the period from October 1, 2018, to April 1, 2019 in an emergency clinic with an annual capacity of 600,000 patients in a tertiary education and research hospital. Consent was obtained from the patients included in the study and attention was paid to patient privacy.

Patients who presented to the clinic and were recommended inhaled treatment by clinicians to be administered by nurses were asked to participate in the study. Patients with cataracts, any pupil pathology, sympathomimetic drug use, and a history of acute or previous central nervous system pathology were excluded. The

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researchers did not interfere with the practice of nurses and clinicians performing the treatment. The demographic data (age, gender), comorbidities (hypertension, diabetes mellitus, coronary artery disease, congestive heart failure, chronic kidney disease, active malignancy) and drug groups were recorded. The patients were divided into three groups: those given salbutamol alone (200 mcg), those given ipratropium alone (250 mcg), and those receiving both salbutamol and ipratropium. Since salbutamol reaches a peak level in 15-30 minutes and continues its effect until 4-6 hours and ipratropium reaches a peak level for 1-2 hours and continues its effect for 6-8 hours, the reference time interval is taken as the 2nd hour. The pupil diameters of the patients included in the study were measured twice using a pupilometer device at hours 0th and 2nd of treatment. The right and left pupil diameter measurements of the patient groups were undertaken separately. The location of the patients and the amount of light in the room were not changed during the treatment. The examinations, follow-up and treatments of the patients were performed in an environment at 22±3 °C exposed to the same light dose without any change in the location. This partly allowed to obtain pupil diameter changes dependent on the drug. The difference between the diameters of the right and left pupils before and two hours after treatment was recorded as absolute delta. The relationship between the absolute delta values of the groups was statistically evaluated.

Statistical analysis was performed using SPSS version 26. The suitability of variables to normal distribution was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Normally distributed continuous data were expressed using mean and standard deviation values, while the data without normal distribution were obtained as median and interquartile ranges. Categorical data were indicated by frequency and percentages. The comparison between the groups was undertaken using Student's t-test for the independent continuous data with normal distribution, paired t-test for dependent continuous data, the Mann-Whitney U test for the independent continuous data without normal distribution, and the Wilcoxon test for the dependent continuous data without normal distribution. One-way analysis of variance (ANOVA) was conducted to compare multiple continuous data. The statistical significance level was accepted as $p<0.05$.

3. Results

A total of 99 patients received inhaler treatment in our emergency department from October 1, 2018 and April 1, 2019. Two of these patients were excluded because they had anisocoria before treatment, and six were excluded due to cataracts. Thus, 91 patients were evaluated in the study. Fifty-one (56%) patients were male and 40 (44%) were female. The process of treatments given to patients with indications was not interrupted. Ipratropium and salbutamol were used in 45 (49.5%) patients, ipratropium alone in 38 (41.7%), and salbutamol alone in eight (8.8%) (Table 1).

The right and left pupil diameters of the patients were compared before and two hours after treatment, and the data were analyzed with the Wilcoxon test. Accordingly, no statistically significant difference was found in the group that was given salbutamol alone (p=0.499 and p=0.528, respectively) and in the group given ipratropium alone (p=0.482 and p=0.383, respectively). In the group receiving both salbutamol and ipratropium, there was no statistically significant difference for the right pupils (p=0.464) while a statistically significant difference was observed for the left pupils (p=0.011) (Table 2).

Table 1. Descriptive characteristics of the patients (n = 91)

Age (median)	67	(IQR 55-80)
Gender(total)	91	(100%)
Male	51	(56%)
Female	40	(44%)
Patients (total)	91	(100%)
Patients given salbutamol alone	8	(8.8%)
Patients given ipratropium alone	38	(41.7%)
Patients given salbutamol + ipratropium	45	(49.5%)
Comorbidities	n (%)	
Hypertension	39	(42.85%)
Diabetes mellitus	23	(25.2%)
Coronary artery disease	23	(25.2%)
Congestive heart failure	19	(20.8%)
Chronic kidney disease	3	(3.3%)
Active malignancy	5	(5.5%)

IQR, interquartile range

Table 2. Analysis of the right and left pupil diameter changes in patients receiving salbutamol alone, ipratropium alone, and combined therapy

Therapy	Right pupil diameter Change	Left pupil diameter Change
Salbutamol alone	0.093 mm (±0.125)	−0.128 mm (±0.128)
p value	P = 0.499	p = 0.528
Ipratropium alone	0.005 mm (-0.03) - (0.06)	−0.01mm (-0.032) - (0.052)
p value	p = 0.482	P = 0.383
Salbutamol + ipratropium	0.01 mm (-0.045) - (0.08)	−0.02mm (-0.01) - (0.075)
p value	P = 0.464	p = 0.011

IQR, interquartile range

According to the Mann-Whitney U test, there was no statistically significant difference in the changes in the right and left pupil diameters between the salbutamol alone and ipratropium alone groups (p=0.212 and p=0.582, respectively), between the salbutamol alone and salbutamol + ipratropium groups (p=0.209, p=0.921, respectively), and between the ipratropium alone and salbutamol + ipratropium groups (p=0.898 and p=0.318, respectively). The mean pupil diameter was comparatively evaluated before and two hours after treatment using the paired t-test. According to the results, the differences in the mean pupil diameter between

the two measurement times were not statistically significant in any of the three groups (p = 0.683, 95% confidence interval (CI): (-0.779) -(1.147) for salbutamol alone; p=0.528, 95% CI: (-0.259) -(0.153) for ipratropium alone; and p = 0.159, 95% CI: (-0.046) -(0.272) for salbutamol + ipratropium) (Table 3).The mean absolute delta values were 0.550 mm (±0.146) for the patients given salbutamol alone, 0.175 mm (±0.039) for those receiving ipratropium alone, and 0.164 mm (± 0.032) for those in the salbutamol + ipratropium group. The relationship between these variables was compared using one-way ANOVA, and no statistically significant difference was found in the post-hoc analysis between the patients those given ipratropium alone and those given salbutamol + ipratropium (p>0.001). A statistically significant difference was found in the post-hoc analysis between the patients given salbutamol alone and those given ipratropium alone (Table 4), as well as between those given salbutamol alone and those given salbutamol + ipratropium (Table 5) (p = 0.001, 95% CI: 1.36- 6.11 and p <0.001, 95% CI: 1.51- 6.19, respectively). Thus, no drug produced a statistically significant difference between the mean pupil diameters measured before and after treatment, but when the total pupil diameter change caused by the drugs was compared numerically, the pupil diameter of the group receiving salbutamol alone was significantly higher than the other drug groups; thus, salbutamol led to statistically significant changes in the pupil diameter.

Table 3. Analysis of the mean pupil diameter changes in patients receiving salbutamol alone, ipratropium alone, and combined therapy

Therapy	Mean pupil diameter at hour 0	Mean pupil diameter at hour 2	p value
Salbutamol	3.182 mm (±0.058 mm)	3.165 mm (±0.086 mm)	0.683 95% CI: (-0.797)- (1.147)
Ipratropium	3.259 mm (±0.034 mm)	3.265 mm (±0.035 mm)	0.528 95% CI: (-0.259)- (0.135)
Salbutamol + İpratropium	3.232 mm (±0.030 mm)	3.221 mm (±0.030 mm)	0.159 95% CI: (-0.046)- (0.272)

Table 4. Comparison of the pupil diameter absolute delta values of patients receiving salbutamol alone and ipratropium alone

Drug	Absolute delta value	p value
Salbutamol	0.550 mm (±0.146)	p = 0.001 95% CI: 1.36-6.11
Ipratropium	0.175 mm (±0.039)	

CI, confidence interval

Table 5. Comparison of the pupil diameter absolute delta values of patients receiving salbutamol alone and salbutamol + ipratropium

Drug	Absolute delta value	p value
Salbutamol	0.550 mm (±0.146)	p < 0.001 95% CI: 1.51-6.19
Salbutamol + ipratropium	0.164 mm (±0.032)	

CI, confidence interval

4. Discussion

In this study, while no drug treatment produced a statistically significant difference in terms of the mean pupil diameter before and after treatment, salbutamol alone was found to lead to statistically significantly greater change in the total pupil diameter (delta) compared to the other drug groups. The fact that salbutamol caused more pupil dilation than ipratropium and combined treatment was important in terms of revealing that salbutamol may also be related to anisocoria cases that are often associated with ipratropium. In this study, we aimed to compare the effects of nebulizer drugs on unilateral or bilateral pupil dilatation due to mask incompatibility or patient incompatibility in patients receiving inhaled therapy. There are few studies related to drugs that cause anisocoria associated with pathologies other than central nervous system pathology, and there are many case reports. Apraclonidine may rarely cause anisocoria by acting on alpha receptors (8). Cocaine can cause anisocoria by inhibiting noradrenaline re-uptake and affecting only the sympathetic nervous system (9). Kuhn et al. detected dilatation in the right pupil diameter 30 minutes after the application of 2% concentration of isoflurane they used during induction in a girl they operated due to hiatal hernia (10). Similarly, in a case report reported by Akhlaghi, unilateral anisocoria occurring 30 minutes after the use of pofol, an anesthesia induction agent was reported in a patient undergoing surgery for an open extremity fracture (11).

To date, many case reports of unilateral mydriasis due to nebulized ipratropium have been reported (12-14). In a previous study, unilateral mydriasis developed in one patient who received salbutamol + ipratropium due to mask leakage after thoracic operation, and it was stated that in addition to unilateral pupil dilatations due to ipratropium, mydriasis could develop because of salbutamol (15). In another study, unilateral mydriasis was reported in a seven-month-old infant receiving combined therapy (16). In our study, it was found that inhaler agents cause mydriasis, and no statistically significant relationship was found in the analysis of the effect of single and combined forms on mean pupil diameter change.

A female patient receiving salbutamol and ipratropium was reported to develop acute glaucoma lasting four days in both eyes (17). Similar acute glaucoma cases related to combined therapy have been described (18, 19). In a previous study, intraocular pressures measured at the second hour of salbutamol, ipratropium, and combined nebulizer therapy were compared, and in patients with narrow angle glaucoma, intraocular pressure was found to be statistically significantly increased after combined therapy (20). Similarly, in our study, when compared in terms of the effect of salbutamol, ipratropium, and their combined forms on total pupil diameter, it was found that the salbutamol group caused statistically significantly more changes in pupil diameter compared to other drug groups. In a study conducted in rats, it

was observed that inhaled ipratropium did not cause a statistically significant change in pupil dilatation (21). Our study is the first to show that salbutamol may also cause pupil dilation in inhaled therapy, like ipratropium. In this study, a limited number of cases were investigated due to several factors, such as patient non-compliance, the inability to use nebulizers at the appropriate time due to the patients' need for continuous positive airway pressure and follow-up being undertaken without changing the patient location in the emergency department. Although neurological diagnoses are considered in patients with anisocoria, it should be kept in mind that anisocoria may also be due to the current or the treatment being given. We think that our study may be a guide for clinicians in terms of delaying immediate application to examinations aimed at excluding central events unrelated to the examination, especially in patients receiving inhaler therapy, in the case of anisocoria that is not detected at the first examination, may be due to a mask that is not fully fitted to the face.

Conflict of interest

No conflict of interest between authors.

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The efficacy of the boric acid-based maintenance therapy in preventing recurrent vulvovaginal candidiasis

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Abstract

Current gold-standard treatment of recurrent vulvovaginal candidiasis (RVVC) is mainly based on maintenance with fluconazole. Moderate to high recurrence rates at long-term use and secondary fluconazole resistance emerge as reasons to seek for new topical maintenance regimens. In this study, it is aimed to assess the efficacy and safety of boric acid-based treatment approach to treat clinical RVVC. In this retrospective study, patients who were diagnosed with RVVC received a treatment package for six months that consist of induction with boric acid vaginal suppositories 600 mg daily for 14 nights followed by maintenance for five nights starting with every fifth day of the menstrual cycles; a vaginal estriol-lactobacilli combination; and several rigorous life-style changes. The success was defined as the absence of symptomatic recurrence during the follow-up. Success rate at the first year was found to be 94.8% in a total of 173 patients. Mild, reversible side effects were observed in five patients (2.9%). Boric acid, along with a vaginal estriol-lactobacilli combination and lifestyle changes can be a safe and effective alternative in lieu of potent systemic antifungal drugs as a first-line treatment for the patients referred with RVVC.

Keywords: boric acid, lifestyle changes, recurrent vulvovaginal candidiasis, vaginal flora

1. Introduction

Recurrent vulvovaginal candidiasis (RVVC) is a challenging morbidity both for patients and clinicians. It substantially impairs the quality of life and sexual function due to a variety of symptoms that include vaginal discharge, vulvar itch, soreness, and dyspareunia (1).

Global annual prevalence of RVVC was estimated as 3871 per 100,000 patients and the 25–34-year age group had the highest prevalence with 9% (2). RVVC is very frequent that almost one-tenth of patients report four or more episodes in their lifetime. The probability of multiple recurrences was found to be 10% for patients aged 25 years and increased to 25% at the age of 50 years (3). Almost 5% of patients of reproductive age after an initial episode of VVC will progress to recurrent disease (4).

Candida albicans is also responsible for most infections in patients with RVVC as well as acute sporadic VVCs. Of the non-albicans Candida species, *Candida glabrata* is the most frequently isolated species from the vagina in symptomatic and asymptomatic patients (5).

The cause of RVVC is thought to be multifactorial and the mechanism behind the resistance to antifungal medications is not well known (6). Genetic susceptibility reduced mannose-binding lectin and host exaggerated inflammatory response are suggested theories of recurrence, however, a tool to control the host mucosal reaction still does not exist (5). The current therapeutical approach mainly aims to reduce and subsequently suppress the vaginal fungal load with several regimens, particularly with azoles, but not all Candida strains are azole-sensitive such as *Candida glabrata*.

Current gold-standard treatment of RVVC includes maintenance with fluconazole once weekly regimens that often needs to be continued for several years. The recurrence rate at one year with fluconazole following the maintenance regimen varies between 57-77% (7, 8). On the other hand, widespread use of fluconazole for either acute sporadic or recurrent cases caused an emerging trend of secondary fluconazole resistance (9).

The initial drug of choice in cases of azole-resistant non-albicans Candida species is vaginal boric acid 600 mg daily for 14 days (10). Vaginal boric acid was proposed to be effective with rapid relief of symptoms and culture negativity after two weeks of use in treating RVVC and further combination with other regimens were suggested where it alone fails (5, 11-13).

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Considering the pharmaceutical and economic burden of the long-term use of fluconazole and the success of boric acid in azole-resistant cases, we hypothesized that boric acid may be a successful tool to be used primarily in treating RVVC. It was aimed to assess the efficacy and safety of boric acid-based treatment approach including a package of behavioral changes to treat clinical RVVC in a pragmatic trial.

2. Materials and methods

The study presented retrospective analysis of prospectively collected data yielded at a tertiary health care centre from June 2017, through July 2019.

Recurrent VVC was defined as four or more symptomatic episodes over a 12-month period that shows resolution between the episodes. Progress with the antifungal treatment and exacerbation with the use of systemic antibiotics are also agreed to be relevant to RVVC. At least one of those episodes was confirmed by microscopy with the presence of blastospores, pseudohyphae and neutrophils during cytology. Patients included to the trial were aged over 18 years to 50 years old, not in menopause, did not have systemic illnesses and diagnosed with RVVC. Patients who had any other sexually transmitted disease, malignity, gynaecological anatomic disorder, any morbidity known to cause susceptibility to candidiasis including diabetes mellitus, pregnancy, patients currently on any related medications such as antibiotics or corticosteroids and who had used antifungal medication in the week before entry were excluded prior to the treatment. The study was conducted in according to the Helsinki Declaration, 2008 (http://www.wma.net/en/30publications/10policies/b3/index.html). The local institutional administration board approved the study (No:2020/42). Preliminary results have been presented in 17th National Gynecology and Obstetrics Congress, Antalya, Turkey.

All patients underwent a detailed urogynecological examination by the first author. Patients with mixed infection, urinary incontinence or symptomatic female genital prolapse over POP-Q grade II were excluded after the enrollment and received proper treatment. As discussed later, authors had the belief that urogynecological problems may substantially disturb the vulvovaginal flora.

Patients who met the entry criteria received a treatment package for six months that consist of three main components (Fig. 1):

(1) Induction therapy was provided with boric acid vaginal suppositories 600 mg daily for 14 nights followed by maintenance for five nights starting with every fifth day of the menstrual cycles considering that menstrual bleeding should significantly decrease or stop by that time. If mucosal irritation occurs, the dose was reduced to 300 mg daily.

(2) A vaginal estriol-lactobacilli combination (Gynoflor®, Abdi Ibrahim Ilac Pazarlama A.S., Medinova Ltd., Switzerland) following five-day boric acid administration to treat disrupted vaginal microflora that contains at least 1×108 colony-forming units of live Lactobacillus acidophilus, 0.03 mg estriol and approximately 600 mg lactose. This regimen was recommended to be repeated if patient had used any antibiotics for any reason after she was enrolled to the study.

(3) Several rigorous lifestyle and behavioral changes are seen. Couple was recommended to wash their external genitalia with warm water and mild or unscented soap prior to coitus. Patients was strained from vaginal washes, wipes, and douche with water and/ or soap, local irritants, oral sex and use of vaginal objects including tampon or vibrators. Patients was recommended to wear baggily and cotton clothes, to consume more yoghurt and kefir, reduce or quit smoking. Patients was suggested to avoid vaginal sex until their symptoms improved.

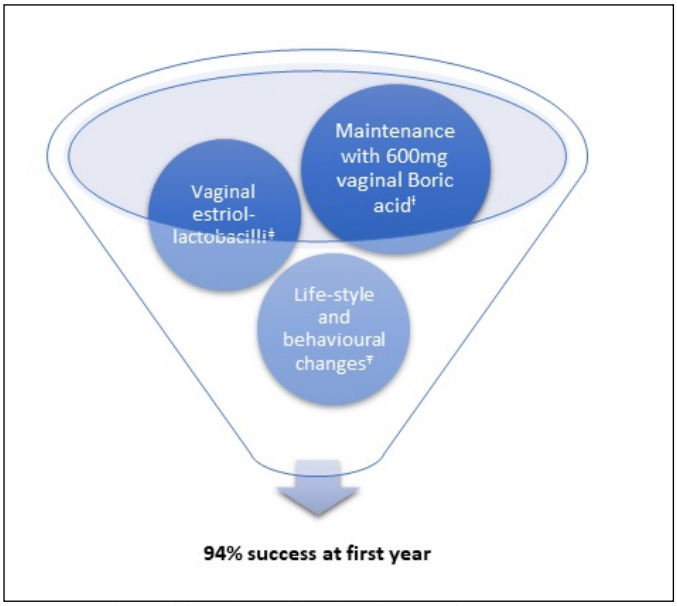

Fig. 1. Boric acid-based combination therapy for RCCV

Patients were required to return to the gynaecological outpatient clinic at their first, third and sixth months and the first year of the boric acid-based maintenance therapy for RVVC. The success was defined as the absence of symptomatic recurrence as stated by the patient during the follow-up. If recurrence occurs, fluconazole 150 mg orally every 72 hours in a total of three doses was suggested to ensure clinical remission. When oral therapy needs to be avoided 600 mg of intravaginal fluconazole administered on day one and four as an alternative. Therapy outcome was assessed with descriptive statistics.

3. Results

A total of 221 patients with RVVC have included. Seventeen and twenty-five patients were excluded before and the after the enrollment, respectively. Before the enrollment, nine patients due to recent use of different or unknown antifungal medication, six for premalignant cervical lesion and two for uncontrolled diabetes mellitus were excluded. After the

enrollment, patients with significant urogynecological symptoms were excluded and scheduled to midurethral mini sling in 13 patients, laparoscopic lateral suspension with mesh in five, anterior and posterior colporrhaphy in three, trans-obturator tape in two and combination of those in two patients. Six patients were lost to follow-up. In the end, a total of 173 patients were included to the descriptive analysis.

The mean age of the patients was 31.5 ± 4.4 years (Min = 24, max = 48). The median parity was two with an interquartile range of one (Min = 0, max = 5).

Induction therapy was successful in all cases. Overall success rate at the first year was found to be 94.8% out of 173 patients that was treated with boric acid-based maintenance therapy for RVVC.

Out of nine patients who had recurrence VVC episodes, one was at her first month, two were at third month, two were at sixth month, one was at ninth month and three were at her 12th month of the treatment (Fig. 2). Patient who had the earliest recurrence at the first month during the maintenance therapy was observed to have intrauterine contraception device. Five of the recurrent cases (55.5%) were observed during the maintenance therapy and the rest (44.5%) was observed after the maintenance therapy that lasted for six months.

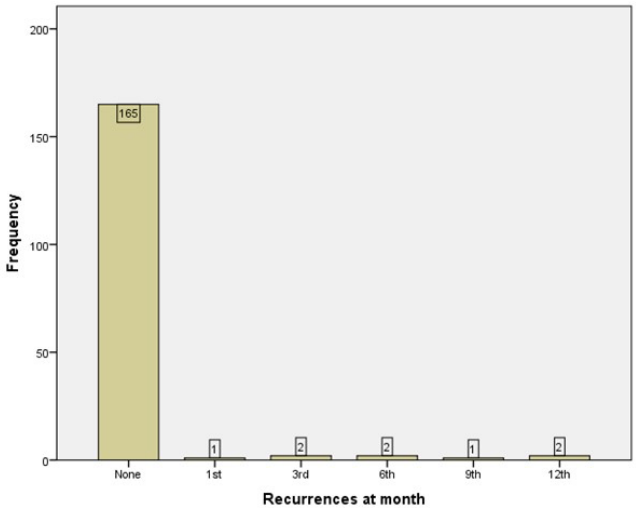

Fig. 2. Recurrences after maintenance therapy with boric acid

Mild or moderate side effects were observed in five patients (2.9%). Local burning sensation and erythema were observed in four and one of patients, respectively. Only the patient with erythema quitted the boric acid-based maintenance treatment and changed to fluconazole 150 mg orally once a week for six months. She was recurrence free at the at first year follow-up. The boric acid dose was reduced to 300 mg daily in the four patients with mild local burning sensation and none of them had recurrence.

4. Discussion

The findings of this study may promote the clinical use of boric acid in lieu of potent antifungal drugs as a first-line treatment for the patients referred with RVVC. The overall success rate was almost 95% at the first year with six months use of boric acid-based approach including supporting the vaginal microflora and the lifestyle and behavior changes. The side effect of the regimen was observed to be mild and low with 3% rate.

The current most recommended treatment of RVVC is known as suboptimal. The most accepted regimen to treat azole sensitive RVVC is the suppression with fluconazole, however, it is thought that almost half of the patients will have another recurrence episode within 3-4 months following the cessation of fluconazole (5). In a recent review of the current guideline recommendations, the regimens suggested by the most guidelines were found to be particularly ineffective with only 43% of patients were disease-free at the first year (1). By the increased secondary fluconazole resistance due to its overuse, the need of new approaches was proclaimed considering management of fluconazole refractory vulvovaginitis was reported to be difficult (9,14). In addition, new Candida agents were shown to be emerging that is also resistant to azoles (15).

The optimal duration of suppressive therapy with azoles is currently unknown (4). Long-term treatment with systematic agents was suggested to be given following in vitro susceptibility tests and therefore, boric acid may have a role in long-term maintenance treatment where the azole susceptibility is unknown (9).

Despite the lack of published data in the literature, it is a known fact that clinicians worldwide use long-term maintenance with intravaginal boric acid in the treatment of RVVC with high patient satisfaction and few adverse events (16). In a systematic review; boric acid step forward as an economic and safe alternative to azoles for the ordinary treatment of VVC with the advantage of not inducing drug resistance and of not discriminating between the Candida species (12). Boric acid has also shown to have dose-dependent antimicrobial activity against Trichomonas vaginalis (17).

RVVC is caused by host factors rather than infecting with a more virulent strain or reintroduction of the pathogen to the vulvovaginal tract (4). Host factors include persistence of Candida and the disturbance of vaginal microflora. Currently, boric acid is not used as a first-line treatment, mainly because less known about its mechanism of action. At this point, it is recently showed by Schmidt et. al. that boric acid may restore the persistence by inhibiting the several metabolic pathways including glycolysis, fermentation, and the mitochondrial activity (18). Boric acid was also shown to induce cell autofluorescence, decrease catalase activity and the cell size, and subsequently promote the programmed cell death (19).

The regimen used in the current study includes an intermittent maintenance therapy with monthly boric acid for

five nights starting with every fifth day of the menstrual cycles. This step aimed to keep the vaginal fungal load at reduced levels. As discussed by Sobel, there is currently no other method to control the host mucosal reaction but facilitating yeast antigen tolerance with decreased viral load (5). Guaschino et al. examined the efficacy of long-term use of vaginal boric acid in the cure and prevention of recurrent vulvovaginal candidiasis (20). They used a similar concept of maintenance regimen to the current study but not the same in dosage and the timing. Monthly maintenance therapy has given daily for five days from the first day of the menstrual cycle in contrary to our postmenstrual approach. We postulate that menstrual approach can seriously impair the patient compliance and variances in bleeding patterns can substantially decrease the effect of vaginal boric acid. In a similar manner, Reichman et. al. delayed the vaginal use of boric acid during the menses in their study assessing a similar regimen in treating bacterial vaginosis (21). Nevertheless, it should be noted that this is an empiric regimen that needs to be validated by further prospective trials.

Secondly, a vaginal estriol-lactobacilli combination was added following the treatment with boric acid. Lactobacilli are essential in maintaining the vaginal flora by preventing the colonization of pathogens (22). A recent systematic review that investigating the impact of vaginal probiotics on VVC cure has reached two important conclusions (23). Firstly lactobacilli-containing vaginal probiotics were found to be encouraging in cure and prevention of bacterial vaginosis, however, the benefits for VVC were not promotive. Secondly, the probiotic strains did not persist after cessation of the treatment that suggests probiotics did not colonize the vagina. At this stage, the lifestyle and the behavioral changes become prominent that provide the persistence of reduced viral load and decrease the triggering stimuli, as in the current study.

The safety of the vaginal use of boric acid was well documented (12). The adverse outcomes following the vaginal boric acid were observed to be low in this study with only mild and reversible side effects. To be noted, watery discharge was not considered as a side effect and has told to the patients during the counselling that this can often be seen. Expectedly, there was no complain or cessation of the treatment due to watery discharge that is common in the morning.

The high success of this study can be explained by several aspects. The rigorous lifestyle recommendations and the vaginal flora support should have enhanced the results. To the best of our knowledge, this is the first study that has performed a detailed urogynecological examination and excludes patients accordingly among the studies investigating the treatment of RVVC. Contrary to common popular belief, it is now known that a urinary microbiome exists (24). The urine may act as a reservoir for pathogens and cause recurrences for infections such as bacterial vaginosis (25). We also speculate that urogynecological problems including urinary incontinence and pelvic organ prolapse may disturb the vulvovaginal flora due to chronic irritation of urine or persistent exposure to the external environment.

The main limitation of this study was that the diagnosis of RVVC was not supported by culture in this study due to technical paucity. The absence of the control group to compare with the success of boric acid was another limitation. A further prospective case-control study is needed. There is also a causative bias that the success may not be solely linked to the use of boric acid. On the other hand, this study encompasses a realistic population and a vast number of patients with RVVC. The data derived from the current study can fill a gap in the lacking evidence for the efficacy, safety, and the need for long-term maintenance regimen with boric acid.

The overall success rate of boric acid-based approach reached to 95% at a one-year follow-up. Boric acid, along with a vaginal estriol-lactobacilli combination and lifestyle changes can be a safe and effective alternative in lieu of potent systemic antifungal drugs as a first-line treatment for the patients referred with RVVC.

Conflict of interest

The authors report no conflict of interest**.**

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The impact of the Covid-19 pandemic on the short and mid-term urological emergencies and the emergency department

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Abstract

Dynamic changes are observed in the delivery of health care services due to the COVID-19 Pandemic. Its effect in the short term is a dramatic decrease in service, however, its effect in the medium and long term is unknown. In this study, we aimed to investigate the effects of the COVID-19 pandemic on emergency department and emergency urological surgery in the short and medium term during the 8-month period, and the reasons for possible changes. Emergency department operations, urology operations and emergency surgical procedures between April and November were compared as the 2020 pandemic period and the 2019 non-pandemic period. The relevant information was obtained from the hospital management information system. Descriptive analysis and statistical methods comparing the two periods were used. In the early stages of the pandemic, significant decreases were observed in both urology procedures and emergency department operations. In the medium period, while emergency department operations and urology consultations returned to the non-pandemic periods, there was no such a change in in emergency urological surgeries. In the medium-term effects of the pandemic, emergency department operations returned to the non-pandemic periods due to reasons such as patients' abuse of emergency departments in line with their requests for rapid diagnosis and treatment. In accordance with the changing nature of the pandemic, it is necessary to make different scheduling for emergency department operations and emergency surgeries.

Keywords: COVID-19, emergency department, pandemic, urological emergencies

1. Introduction

At the end of 2019, the coronavirus disease (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which started in Wuhan, China and affected the whole world, was defined (1-3). With the virus affecting the whole world, the World Health Organization (WHO) named this disease COVID-19 and declared it as a pandemic (4). In this process, the first case in our country was seen on March 11, 2020 (4). Following the declaration of pandemic, increases were observed in the density of hospitals. For the patient density in hospitals observed all over the world, healthcare systems implemented significant changes to maintain the delivery of primary health care services and reduce the risk of collapse (5, 6). The Republic of Turkey Ministry of Health, which is the national health authority in Turkey, determined an action plan by declaring all national and private hospitals that fulfilled pre-defined requirements as 'Pandemic Hospitals' at the end of March. While Pandemic Hospitals mainly served COVID-19 patients, they also continued routine emergency service delivery and emergency surgeries.

Our aim in this study was to examine the short and medium term effects of the pandemic by comparing the emergency department operations and the change in urology health service delivery with the same period of 2019, especially the number of patients admitted to the emergency department of our hospital, which is the only tertiary care hospital in the province where it is located and continued to provide both pandemic patients and other health services during the pandemic, during the pandemic period.

2. Materials and methods

2.1. Study type

This study was a retrospective observational study and was approved by the local ethics committee (approval number; 369).

2.2. Data source and population

Our hospital is the only tertiary care hospital serving approximately 530.000 people in the province where we live. Its emergency department is a clinic where healthcare services are provided uninterruptedly, and adult and pediatric emergency services are separate. All procedures in our hospital (such as patient application, examination note, requested examinations, interventions, and consultations) are recorded in the hospital information management system. In our retrospective observational study in which the data were obtained from the hospital information management system, the data showing the operations of the emergency and urology

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departments in an 8-month period between April 1 and November 31, 2020, were retrospectively analyzed. Then, the data were compared with the non-pandemic period of 2019, which included the same 8-month period. The emergency department of our hospital provides emergency services for adults and children. Since pediatric emergency urological patients are examined by pediatric urology, emergency admissions under the age of 18 were excluded from the study.

2.3. Definitions

- Application to Emergency Department Outpatient; refers to the number of patients aged 18 and over, admitted to the emergency department,
- Application to Urology Outpatient; refers to the number of patients admitted to the urology outpatient clinics,
- Number of urology consultations; refers to the number of emergency consultations requested from the emergency department to the urology department,
- Percentage of urology consultation; refers to the ratio of the number of emergency consultations requested from the emergency department to the urology department to the number of patients admitted to the emergency department,
- Total number of urological surgeries; refers to total number of surgeries performed by urology,
- The Number of emergency urological surgeries; refers to the number of emergency surgeries performed by urology,
- Percentage of emergency use of the Urology Operating Room; refers to the ratio of the number of emergency urological surgeries to the total number of urological surgeries,
- Percentage of emergency urological surgery; refers to the percentage of the ratio of the number of emergency surgeries performed by urology to the number of patients admitted to the emergency department.

2.4. Statistical analysis

The data were analyzed using the SPSS 22 package program. The normal distribution of data was tested by the Kolmogorov-Smirnov/Shapiro-Wilks test. Number and percentage expressions were used for descriptive statistics. Mann Whitney-U tests were used to compare 2019 and 2020. $p<0.05$ was considered statistically significant.

3. Results

There was a decrease of 31.05% in the admissions to the emergency department of our hospital, which is the only tertiary care hospital in the province where it is located, in the 8-month period from the month following the declaration of the pandemic compared to the previous year. A similar situation was also observed with a decrease of 67.07% in the admissions to the urology outpatient clinic during the same period. There was a statistically significant change in the number of applications to emergency department outpatient and application to urology outpatient (p=0.006, p=0.001, respectively). An increase of 8.37%, which was not statistically significant, was observed in the number of urology consultations (p=0.674). A significant increase of 57.14% was found in the percentage of urology consultation (p=0.036). When the surgical procedures performed during the pandemic period were examined, the total number of urological surgeries decreased by 42.14%, however, the number of emergency urological surgeries increased by 33.3% (p=0.001, p=0.130, respectively). While the percentage of emergency urological surgery was 0.087% in 2019, it increased to 0.185% with an increase of 112.64% in 2020 (p=0.016), and it was observed that the percentage of emergency use of the urology operating room increased from 2.19% to 5.06% with an increase of 131.05% (p=0.016) (Table 1).

Table 1. Change of emergency and urology department parameters during pandemic and non-pandemic periods

	Non-pandemic Period (2019 Year)	Pandemic Period (2020 Year)	Variation (%)	p
Application to emergency department outpatient (n)	345393	238156	- 31.05	0.006
Application to urology outpatient (n)	26515	10321	- 67.07	0.001
Number of urology consultations (n)	490	531	+ 8.37	0.674
Total number of urological surgeries (n)	1775	1027	- 42.14	0.001
The number of emergency urological surgeries (n)	39	52	+ 33.3	0.130
Percentage of urology consultation (%)	0.14	0.22	+ 57.14	0.036
Percentage of emergency urological surgery (%)	0.087	0.185	+ 112.64	0.016
Percentage of emergency use of the urology operating room (%)	2.19	5.06	+ 131.05	0.016

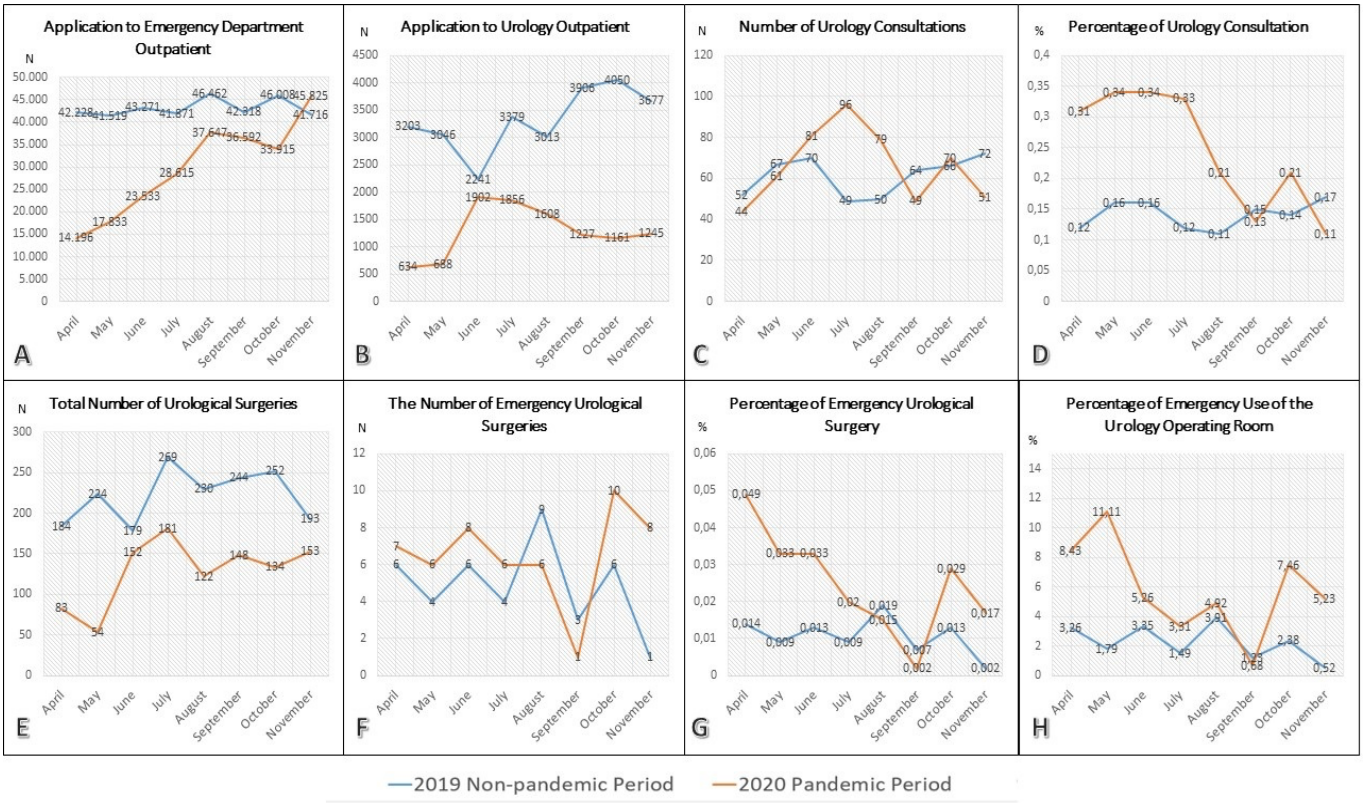

Fig. 1. A. Month-by-month change of application to emergency department outpatient during pandemic and non-pandemic periods. **B.** Month-by-month change of application to urology outpatient during pandemic and non-pandemic periods. **C.** Month-by-month change of number of urology consultations during pandemic and non-pandemic periods. **D.** Month-by-month change of percentage of urology consultations during pandemic and non-pandemic periods. **E.** Month-by-month change of total number of urological surgeries during pandemic and non-pandemic periods. **F.** Month-by-month change of the number of emergency urological surgeries during pandemic and non-pandemic periods. **G.** Month-by-month change of the percentage of emergency urological Surgery surgeries during pandemic and non-pandemic periods. **H.** Month-by-month change of the percentage of emergency use of the urology operating room during pandemic and non-pandemic periods

When the parameters mentioned were evaluated month by month, while the number of applications to emergency department outpatient decreased by 66.38% in April during the pandemic period compared to the non-pandemic period of 2019, in the following months, this ratio decreased by 57.05% in May, 45.61% in June 31.66% in July, 18.97% in August, 13.53% in September, and 26.28% in October, it increased by 9.85% in November (Fig. 1A). No such situation was encountered in the application to urology outpatient (Fig. 1B). The increase in the number of urological consultations, which occurred in the first periods after the declaration of the pandemic, started to decrease after June and decreased to levels like the previous year (Fig. 1C). The percentage of urology consultation reached similar rates to the previous year in the later stages of the pandemic, like the number of applications to emergency department outpatient and urological consultations (Fig. 1D). The total number of urological surgeries was lower during the pandemic compared to the non-pandemic period (Fig. 1E). The number of emergency urological surgeries followed a variable course (Fig. 1F). While the percentage of emergency urological surgeries was higher in the early pandemic period compared to the non-pandemic period of 2019 (April; 0.049% - 0.014%, May; 0.033% - 0.009%, June; 0.033% - 0.013%, July; 0.02% - 0.009%), it decreased in August and September (0.015% - 0.019%, 0.002% - 0.007%, respectively) and fell below the rates in the 2019 non-pandemic period, and then, it increased again in October and November (0.029% - 0.013%, 0.017% - 0.002%, respectively) and followed a fluctuating course (Fig. 1G). The percentage of emergency use of the urology operating room also followed a similar graphic course to the percentage of emergency urological surgery (Fig. 1H).

4. Discussion

The COVID-19 pandemic has profoundly affected the delivery of health care services around the world. While special and reserved areas were opened for COVID-19 patients, it was aimed to continue routine health care service delivery, especially for emergency and oncological patients. In this process, the European Association of Urology (EAU) defined guiding procedures for urological surgical practices during the pandemic period (7). In accordance with these recommendations, urological surgeries were reorganized, elective-postponable surgeries were postponed, and emergency and oncological surgeries continued, which ensured the continuation of emergency and non-deferrable surgeries along with the decrease in elective surgeries (7-10). Although these recommendations reduced the hospital workload in the short term during the pandemic period, the long-term effects have led to concerns about the complication of postponed surgeries or the need for urgent surgical

intervention (10-13). During the pandemic period, hospital's outpatient service delivery decreased since national and local administrations made assignments to reduce the hospital workload and to fight against the pandemic.

Our study showed that there was a decrease of 31.05% in admissions to the emergency department during the 8-month pandemic period compared to the previous year in a similar period. In the studies covering the early stages of the pandemic, it was reported that there was a decrease in admissions to the emergency department (14, 15). In a study covering the first 4-week early pandemic period in America, this decrease was found to be 42%, and another study found a decrease of 31%-45% although there was a difference by regions in America (16, 17). In our study, while the decrease in admissions to the emergency department was consistent with the literature, when the 8-month period was analyzed month by month, it was observed that the admissions to the emergency department reached similar numbers to the previous periods in the 8th month of the pandemic (medium term), which may be caused by the decrease in admissions to hospitals due to the fear of transmission and anxiety in the society at the beginning of the pandemic, as well as the abuse of emergency departments by those who could not benefit from outpatient health services sufficiently in the early period.

During the pandemic period, a decrease of 67% was observed in the delivery of urology outpatient services. In their multi-center study, Bozkurt et al. reported this rate as 73%, which may be due to the restrictions imposed by the country's government to reduce social movements, as well as the decrease in hospital admissions due to the fear of Covid transmission in the hospital (6). It can be expected that the decrease in outpatient service delivery will direct people to use emergency departments. Emergency urological surgeries can be expected to increase with the number of urology consultations requested from the emergency department and the percentage of consultations. In our study, an increase of 8.37% was found in the number of consultations requested. The number of consultations, which was smaller during the first months compared to the non-pandemic period, decreased to numbers like the non-pandemic period after reaching the highest number in the 4th month of the pandemic. In the studies in this regard in the literature, in their multi-center study covering a period of three months, Grasso et al. reported a decrease of 39% in the number of urology consultations (1). Similarly, in their study covering the 36-day pandemic period, Motterle et al. reported that urology consultations decreased three times during the pandemic period (18). As it is stated in the results of our study and in the literature, there was a decrease in the number of consultations at the beginning of the pandemic. Regarding the contribution of our study to the literature, it showed that the number of consultations would increase during the months when the pandemic continued and would return to non-pandemic periods. Since the number of urology consultations will be affected by the number of patients admitted to the emergency department, the percentage of urology consultation was also examined in our study. It was observed that the percentage of urology consultation was high in the first 4 months of the pandemic, and then, it decreased to the rates like the non-pandemic period after the 6th month.

Regarding urological surgery practices, total urological surgeries decreased by 42.14% during the pandemic period, like the literature. In the literature, this rate is seen between 18%-79.5% (5, 6, 18). Although elective surgeries were postponed, these surgeries may be complicated and require immediate intervention during the postponed period. Therefore, an increase in the number of emergency urological surgeries can be expected. Indeed, in our study, this increase was observed in the 7th and 8th months of the pandemic. In our study, although the number of emergency urological surgeries was not statistically significant, an increase of 33.3% was observed. In the percentage of emergency urological surgeries, an increase from 0.087% in the non-pandemic period to 0.185% in the pandemic period and up to 112% was determined. It was observed that the percentage of emergency use of the urology operating room increased from 2.19% to 5.06%. In their study, Grasso et al. reported that this ratio increased from 7% to 11.8% during the pandemic period (1).

Our study is the first study examining the mid-term experience and reporting the change in urological emergencies, however, it has some limitations, it is a single-centered study, and our hospital admitted all referrals as a central hospital in the city where it is located, which may also lead to high number and rates of emergency urological surgeries and consultations.

In conclusion, Studies have demonstrated that the pandemic period has a unique variable structure. While the number of outpatient clinics has decreased due to both restrictions and fear of social transmission, emergency department operations and emergency surgical interventions are going on. In this case, different approaches are required in the early stages of the pandemic and in the medium and even long periods. Since emergency departments can be abused for rapid examination in the medium and long periods, it is needed to make different scheduling for emergency department operations and emergency surgeries.

Conflict of interest

There is no conflict of interest.

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Investigation of the effect of *N*-Acetylcysteine on colistin mic values in *Acinetobacter Baumannii* strains isolated from clinical samples

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Abstract

Acinetobacter baumannii is an opportunistic pathogen which colonize inpatients and cause severe infections, septic shock and death. With emergence of multi-drug resistant gramnegative species and being effective in *A. baumannii* infections, colistin becomes a treatment option again. N-acetylcysteine (NAC), is a mucolitic agent which used commonly in lower respiratory tract infections especially patients who have cronic respiratory disorders like Chronic obstructive pulmonary disease, cystic fibrosis and bronchiectasis. In this study we aim to investigate the effect of NAC, which commonly added in lower respiratory tract infections tratment regime, on MIC values colistin used in *A. baumanni* tratment. Fifty A.*baumannii* isolates were included in the study. The isolates were identified by automated identification system. With broth microdilution method, we investigated and compared the MIC (minimum inhibitory concentration) values of colistin and NAC+Colistin combination. Colistin MIC50 value is 0.25 µg/mL and MİK90 value is 1 µg/mL, NAC+Colistin combination MIC50 value is 0.25 µg/mL and MİK90 value is 1 µg/mL. The screening for the effectiveness of clinical drugs may provide clinical strategy to improve treatment outcomes of *A. baumannii* and reduce hospitalization days.

Keywords: *A.baumannii,* colistin, lower respiratory tract infection, microdilution method, N-acetylcysteine

1. Introduction

The Acinetobacter genus is classified in the *Moraxellaceae* family and consists of bacteria in the morphology of immobile, oxidase-negative, Gram-negative coccobacillus (1). *A. baumannii* is an opportunistic microorganism that can colonize in hospitalized patients and cause serious infections, bacteremia, septic shock, and death (2). These pathogens most frequently cause urinary tract infections and lower respiratory tract infections, especially in patients hospitalized in intensive care units (3). Although the frequency of hospital-acquired lower respiratory tract infections caused by *A. baumannii* varies from country to country, region to region (27-50%), the mortality rate in these infections is between 30-70% (4). Colistin is a lipopeptide antibiotic that belongs to the class of polymyxins, and its use was reduced or completely discontinued due to its nephrotoxic and neurotoxic properties (5). Later, the emergence of multidrug-resistant gram-negative pathogens and its effectiveness in the treatment of *A. baumannii* infections made colistin a treatment option again (5). N-acetylcysteine (NAC) is a mucolytic drug commonly used in lower respiratory tract infections especially in patients with chronic respiratory disorders such as COPD, cystic fibrosis, and bronchiectasis (6). In this study, it was aimed to investigate the effect of NAC, which is frequently added to the treatment of lower respiratory tract infections, on the minimum inhibitory concentration (MIC) values of colistin used in the treatment of *A. baumannii*.

2. Materials and methods

The colistin susceptibility status of 50 *A. baumannii* strains that isolated from clinical samples, including 19 (38%) tracheal aspirate, 15 (30%) blood culture, 7 (14%) wound, 6 (12%) sputum, 2 (4%) CSF, 1 (2%) catheter samples were investigated with Vitek 2 (BioMérieux, Lyon, France) automated system. Of the 50 *A. baumannii* strains investigated, 47 were found to be sensitive to colistin, and 3 to colistin-resistant. Colistin MIC values of these strains with and without N-acetylcysteine using N-Acetyl-L-Cysteine (A7250 Sigma Aldrich, USA) were investigated by the broth microdilution method. Using colistin sulphate (C4461, Sigma Aldrich, USA), between 128 and 0.125 µg / ml concentrations, prepared in double-fold dilutions in microplates according to ISO-standard broth microdilution method (20776-1) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (7) studied according to their recommendations.

As the medium cation-adjusted Mueller Hinton Broth (MHB, Merck KGaA, Darmstadt, Germany) was used. The stock solution of colistin antibiotic (512 µg/ml) was dissolved in distilled water, portioned, and stored at -20°C. For management of serious lower respiratory disorders, generally

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used therapeutic concentration of NAC ranges between 4 and 10 mM per day (8), therefore a NAC concentration of 10 mM was used in this study. N-Acetyl-L-Cysteine was dissolved in distilled water and the stock solution (10mM, 1.6 mg/ml) was prepared daily when the test was performed (9). Inoculum suspension was added to each well at a final bacterial concentration of $5x10^5$ cfu/ml. In addition, bacterial growth control (MHB+microorganism) for bacteria and sterility control (MHB) for each microdilution plate was studied. Microdilution plates were incubated at 35°C for 24 hours in an oven under aerobic conditions. The results were evaluated according to the breakpoint values determined in EUCAST standards (≤2 µg / ml sensitive and> 2 µg / mL resistant) and the MIC values of N-acetylcysteine-Colistin combination and colistin were compared.

3. Results

The MIC (µg/mL) values of *50 A. baumannii* strains isolated from clinical samples, determined by the broth microdilution method, are shown in Table 1.

Table 1. MIC (µg/mL) values of 50 *A. baumannii* strains isolated from clinical samples determined by broth microdilution method

	n	Min	Max	Average	*Sd(±)
Colistin MIC	50	0.125	4	0.55	0.91
NAC+Colistin MIC	50	0.125	8	0.73	1.39

*Sd: Standard deviation

The mean colistin MIC value of the strains was 0.55 ± 0.91 µg/mL, and the mean NAC+Colistin combination MIC value was 0.73±1.39 µg/mL. Colistin MIC50 value of 50 *A. baumannii* strain isolated was determined as 0.25 µg/mL and MIC90 value as 1 µg/mL, NAC+Colistin combination MIC50 value was determined as 0.25 µg/mL and MIC90 value was determined as 1 µg/mL.The MIC (µg/mL) values of 47 *A. baumannii* strains, which are known to be sensitive to colistin, determined by the broth microdilution method are shown in Table 2.

Table 2. MIC (µg / mL) values of 47 *A. baumannii* strains known to be sensitive to colistin by broth microdilution method

	n	Min	Max	Average	*Sd(±)
Colistin MIC	47	0.125	1	0.33	0.27
NAC+Colistin MIC	47	0.125	4	0.43	0.60

*Sd: Standard deviation

The mean colistin MIC value was 0.33 ± 0.27 µg/mLand average NAC+Colistin combination MIC value was 0.43± 0.60 µg/mL. Colistin MIC50 value of 47 *A. baumannii* strain known to be sensitive to colistin was determined as 0.25 µg/mL and MIC90 value as 1 µg/mL, MIC value for NAC+Colistin combination MIC value as 0.25 µg/mL and MIC90 value as 1 µg/mL.

Table 3. MIC (µg/mL) values of 3 *A.baumannii* strains known to be colistin-resistant by broth microdilution method

	n	Min	Max	Average	*Sd(±)
Colistin MIC	3	4	4	4	0
NAC+Colistin MIC	3	4	8	5.5	2.3

*Sd: Standard deviation

The MIC (µg/mL) values of 3 *A. baumannii* strains, known to be colistin-resistant, determined by broth microdilution method are shown in Table 3. The mean colistin MIC value of colistin-resistant strains was 4 µg / mL and the mean NAC + Colistin combination MIC value was 5.3 ± 2.3 µg/mL. Colistin MIC50 value of 3 *A. baumannii* strains known to be colistin-resistant was 4 µg/mL and MIC90 value was 4 µg/mL, NAC+Colistin combination MIC50 value was 4 µg/mL and MIC90 value was determined as 8 µg/mL. Colistin and NAC+Colistin combination MIC values of all strains are shown in Table 4.

Table 4. Colistin and NAC + Colistin combination MIC values of 50 *A. baumannii* strains isolated from clinical samples

Colistin MIC Values	Strain No.	NAC+Colistin MIC Values	Strain No.
0.125 µg/mL	5,9,10,17, 20,21,22, 23,24,27, 32,36,37, 38,39,40, 41,46	0.125 µg/mL	5,7,8,9,10, 12,14,17,19,2 0,21,22,23, 25,2,29,31, 36,37,38,39,4 0,45
0.25 µg/mL	1,3,4,8, 13,14,15, 16,19,25, 28,33,34,45	0.25 µg/mL	1,3,6, 24,34,45
0.50 µg/mL	6,712, 26,29,31, 33,43,48,50	0.50 µg/mL	2,4,13, 15,16,27, 28,32,35, 42,47,50
1 µg/mL	2,42,44,47, 49	1 µg/mL	41,43,44, 46,48,49
2 µg/mL	18	2 µg/mL	18
4 µg/mL	11, 30	4 µg/mL	30, 33
8 µg/mL	-	8 µg/mL	11

4. Discussion

In some cases, there is a discrepancy between MIC values detected and treatment results; while in-vitro test results indicate susceptibility to antibiotics, *Acinetobacter baumannii*, becomes intrinsically resistant to various antimicrobial agents and immune system products due to biofilm production and eradication of colonization cannot be achieved (10). The use of NAC is considered as an alternative approach in the control of diseases caused by biofilm-producing bacteria in humans. Previous studies have shown that NAC inhibits biofilm formation or disrupts the biofilm structure in various bacteria (11, 12). Pollini et al. (13) showed that the colistin / NAC combination showed synergy against the *A. baumannii* biofilm structure and NAC could reverse the colistin-resistant phenotype of this pathogen.

The chequerboard method, which is the reference method, was not preferred in our study because Rodríguez-Beltrán et al. (14) showed that the antimicrobial effect of NAC was due to the low pH caused by NAC and Landini et al. (15) stated that there was no antimicrobial effect at the concentration ranges used in humans. Instead of different concentrations of NAC, the highest concentration that can be used for humans

was tested in our study. Goswami and Jawali (9) did not observe growth inhibition in their experiments with various bacteria on agar containing the maximum dose of 10mM N-acetyl cysteine in their study based on the 4 and 10mM/day dose range, which is widely used in the treatment of severe respiratory diseases.

When the effect of *N*-acetyl cysteine on the MIC values of various antibiotics was investigated, it was observed that the MIC values of fluoroquinolones and aminoglycosides increased in *E. coli, Klebsiella aerogenes* and *P. aeruginosa strains*, chloramphenicol and tetracycline did not cause a change in MIC values and caused a decrease in the MIC values of penicillin and ampicillin (9). The effect of NAC on the MIC values of antibiotics used in the treatment of different bacteria can be very variable. Therefore, the possible effects on colistin, which is often used as a last resort in treatment, should be well understood.

Zuin et al. (8) reported that the use of high-dose NAC in patients with chronic obstructive pulmonary disease led to an improvement in the clinical condition of the patients and a decrease in inflammation markers. Since it has been shown that NAC can reverse the *A. baumannii* colistin resistance phenotype, the risk of colistin monotherapy resulting in the selection of colistin-resistant strains can be avoided by using the colistin / NAC combination (13). In addition, in our study, by testing the in vitro effect of colistin / NAC combination on *A. baumannii*, it can give an idea about the potential in vivo effects of the inhaled colistin / NAC combination in the next step.

Antibiotics and mucolytic agents are used together in the treatment of lower respiratory tract infections in patients with chronic respiratory tract problems and nosocomial pneumonia in patients with a prolonged hospital stay. Landini et al. (15) showed in their study that the MIC values of colistin at two different NAC concentrations (10mM and 50mM) did not change, our results are consistent because it covers the concentration (10mM) we used in our study. In different studies, N-Acetylcysteine, a mucolytic agent, has been shown to have synergistic or antagonistic effects for different antibiotics. In our study, it has been shown that the MIC values of colistin, which is an important treatment option in gram-negative bacterial infections with multiple drug resistance, increase the mean MIC values in combination with N-acetylcysteine. It can be useful in developing clinical strategies.

Conflict of interest

The authors have no conflicts of interest to declare.

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Research Article

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Comparing rLH with hMG in embryo transfers at the stage of blastocyst and pregnancy outcomes in poor responders

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Abstract

Despite showing the beneficial effects of adding LH activity to FSH, based on the pregnancy rate (PR) in patients in the previous studies, no studies have been done to compare two different gonadotrophin preparations with activity of LH in the same group of poor responders. The present study was a single-center retrospective one conducted in January 2015 - December 2019 among 30 women under 39 years old who had reduced ovarian reserve and underwent ICSI cycles. The same patient group received rFSH combined with hMG or rLH. The ovarian stimulation cycle began on the second day of the menstrual cycle, and the initial doses of gonadotrophin were 225 IU/day rFSH in addition to 75 IU/day hMG or 75 IU/day rLH. In all cycles, a flexible antagonist protocol was used. Adding rLH significantly increased the mean number of MII oocytes and cumulus oophorus complex (COC) ($p < 0.001$). There was no significant difference between poor responders treated with rLH or with hMG in terms of blastocyst transfer, implantation rates and clinical pregnancy rates ($p>0.05$). There should be further studies to confirm the better effect of rLH addition to rFSH than hMG in young poor responders. Interventions for poor responders obviously need large, randomized studies which were designed properly, due to the lack of evidence-based treatment to date for this particular patient group.

Keywords: hMG, poor responder, rLH, rFSH

1. Introduction

Poor ovarian response (POR) occurs in 9-24% of IVF cycles. Roughly 80% of the IVF cycle cancelations in the U.S. occur due to the inability to obtain sufficient oocytes. Pregnancy rates (PRs) in poor responders vary from 14-34.5% (1). As is generally known, controlled ovarian stimulation (COS) is used to get enough quality oocytes to reach pregnancy (2). Although a poor response to ovarian stimulation creates difficulties in IVF practices, many new treatment strategies are being developed (3), one of which is the use of luteinizing hormone (LH) in assisted reproductive technology (ART) cycles (4). LH is effective in gonadal functions and is also involved in follicle growth and ovulation, exerting a synergistic effect with follicle stimulating hormone (FSH). LH has been shown to reduce cumulus cell apoptosis and cause an increase in oocyte maturity and quality (5). The developing follicles theca cells are also induced by LH to produce growth factors of androgens and polypeptide, enhancing the follicular response to FSH during follicular selection and recruitment (6). ART is therefore used during the LH hormone cycle for difficult patients with diminished ovarian reserve, advanced maternal age, and a lower chance of success. However, there are uncertainties in the literature on the use of LH, and there is no consensus regarding the patients for whom it should be used, during which cycle it should be started, or whether urinary or recombinant preparations should be used (7). Two sources of exogenous LH activity are used in the IVF cycles, rLH and human menopausal gonadotropin (hMG), the latter of which has both LH and FSH activity (7, 8). There are many studies in the literature comparing the effects of these two different LH preparations (9, 10); however, the patient heterogeneity in these studies is extremely high. Therefore, in the present study, to reduce the patient heterogeneity, we aimed to compare rLH and hMG cycles performed in the same poor responder patients during the same year.

2. Materials and methods

2.1. Participants

A total of 30 patients were included, all which cycles were performed in the Department of Assisted Reproductive Technologies and Reproductive Genetics, BAU Medical Park Göztepe Hospital, Turkey, between 2015 and 2019. The procedures were done in accordance with the regulations established by the Clinical Research and Ethics Committee

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and the Helsinki Declaration of World Medical Association. The study was carried out with the permission of the Local Institutional Review Board and Istanbul Medeniyet University Göztepe Research and Training Hospital (Permission granted/CAAE number. 2020, Decision no: 0669). Signed informed consents were obtained from all patients. Women who had a BMI of 18-30 kg/m^2, were eligible for IVF, ≥ 18 and < 39 years old, and were diagnosed with POR based on the 2011 ESHRE Bologna criteria were included in the present study. As the Bologna criteria showed, there should be at least two of the following three features: (a) an abnormal ovarian reserve test (i.e., AMH, 0.5-1.1 ng/mL or AFC, 5-7 follicles), (b) a previous POR (≤3 oocytes with a conventional stimulation protocol), and (c) advanced maternal age (≥ 40 years) or any other POR risk factor, (11). Those diagnosed with tubal pathologies, uterine anomalies, grade 3-4 endometriosis, or those with any lesion in the uterus were excluded from the study. The collected data included BMI (kg/m^2), age, FSH levels on cycle day 2, total dosage of gonadotropins, anti-Mullerian hormone (AMH), number of previous ART attempts, total number of oocytes retrieved, duration of stimulation, number of embryo transfers performed, total number of mature oocytes, peak estradiol level and endometrial thickness values, blastocyst formation ratio, implantation rates and clinical pregnancy rates.

2.2. Assisted reproduction procedures

Controlled ovarian stimulation (COS) was started on the second day of cycle in all patients. The starting gonadotrophin doses were 225 IU/day rFSH in addition to75 IU/day u-hMG or 75 IU/day rLH. For all cycles, the flexible antagonist protocol was used. When a minimum of two follicles achieved a mean diameter of > 17 mm, a single dose of 250 µg rec hCG (Ovitrelle amp 250 µg/0.5 mL, Merck-Serono, Istanbul, Turkey) or 10,000 IU urinary hCG (Pregnyl amp 5000 IU, Organon, Istanbul, Turkey) was administered. Oocyte retrieval guided by the transvaginal US, was performed 35-36 hours after the administration of rhCG. Standard intracytoplasmic sperm injection techniques were utilized to fertilize the oocytes. All frozen-thawed embryo transfers were performed by same highly experienced clinician, using the Wallace catheter with the after-load transfer technique guided by the transabdominal US and without using any sedation or anesthesia. The embryos with the best quality based on their morphology were selected for transfer. Based on the quality and number of the existing embryos, indication for IVF, and maternal age, there was transfer of one or two embryos on the fifth day. Daily vaginal progesterone gel (Crinone 8%, 90 mg; Merck Serono, Central Pharma Ltd, Bedfordshire, UK) was implemented as luteal-phase support.12 days after ET, serum quantitative β-hCG levels were obtained. A clinical pregnancy means that there is a gestational sac visualized through transvaginal US examination.

2.3. Statistical analysis

For statistical analysis, SPSS 15.0 for Windows was used. Descriptive statistics including the minimum, and maximum for numerical variables, standard deviation, mean, and percentages and numbers of categorical variables was given. dependent groups were compared using a paired sample t-test when the normal distribution conditions differences were met by the numerical variables, and the Wilcoxon test was used when the normal distribution conditions were not met. The difference between the dependent groups in the rates was examined through McNemar analysis. The statistical significance level was accepted as p> 0.05.

3. Results

In total, 30 patients with a mean age of 31.5±4.8 years were included in the present study. The mean BMI value was 24.3±3.9, the mean AMH value was 0.81±0.26, and the mean D3 FSH was 8.46±3.9/(4-11). The demographic characteristics of the patients are given in Table 1.

Table 1. The patients' demographic characteristics

	Mean ± SD/(min−max) (n = 30)
Female age (years)	31.5 ± 4.8/ (21-38)
Male age (years)	36.2 ± 4.1/ (27-42)
BMI (kg/m²)	24.3 ± 3.9/ (18-30)
AMH (ng/mL)	0.81 ± 0.26/ (0.23-1)
D3 FSH (mIU/mL)	8.46 ± 3.9/ (4-11)

AMH, anti-Mullerian hormone; FSH; follicle-stimulating hormone; BMI; body mass index

The treatment results for rLH and hMG that patients received in the same year are summarized in Table 2. In the rLH treatment group, total number of retrieved oocytes, and mean number of MII oocytes were statistically significantly higher compared to those in the hMG treatment (p = 0.001, p=0.003). The rate of blastocyst formation was 70% in patients using rLH and 53.3% in patients using hMG. No statistically significant difference was found regarding blastocyst formation during the treatment period (p=0.289, p=0.302). Also, there was no statistical significance for implantation rates and clinical pregnancy rates between two groups. (p = 0.137, p= 0.269).

4. Discussion

To date, no existing study proves the superiority of any gonadotropin used during COS in poor responder patients. Although many studies have been conducted on this subject, the heterogeneous and variable patient groups have produced contradictory results. The present study aimed to eliminate the variables. Therefore, we compared different cycles used hMG or rLH in the same patient group. We also compared the blastocyst formation rate and clinical pregnancy rate of both groups using rLH or hMG supplementation from the beginning of early follicular phase. Our study shows no significant difference in blastocyst formation or clinical rates of pregnancy following the addition of either hMG or rLH to rFSH.

Table 2. Clinical characteristics of the patients

		rLH	hMG	
		Mean ± SD/median (n = 30)	Mean ± SD/median (n = 30)	*p*
Previous IVF attempts		1.70 ± 1.49/1	1.63 ± 0.93/2	0.736
Total GND dose (IU)		2545.8 ± 356.7/2550	2542.5 ± 579.4/2362.5	0.959
Total stimulation days		9.20 ± 0.41/9	9.13 ± 0.35/9	0.480
Total retrieved oocytes		5.17 ± 2.36/5	4.17 ± 1.82/4	**0.001**
MII oocytes		4.03 ± 1.75/3	3.37 ± 1.35/3	**0.003**
PN		3.50 ± 1.70/3	3.17 ± 1.26/3	0.115
Estradiol on hCG day		715.8 ± 347.2/592.5	697.3 ± 352.8/467	0.848
Endometrial thickness		10.04 ± 1.27/9.95	9.83 ± 1.28/9.45	0.539
		n (%)	n (%)	*p*
Number of blastocysts	**N**o	9 (30.0)	14 (46.7)	0.302
	Yes	21 (70.0)	16 (53.3)	
Number of embryos transferred	**1**	24 (80.0)	26 (86.7)	0.625
	2	6 (20.0)	4 (13.3)	
Implantation rate (%)		17.1	15.2	0.137
Clinical pregnancy rate (%)		22.3	19.7	0.269

FSH: follicle-stimulating hormone; GND: gonadotropin; PN: pronucleu

Despite certain studies indicating the positive effects of rLH supplementation on the pregnancy rates of specific populations with diminished ovarian reserve, low serum LH levels, or an advanced age (12-14), the 2017 meta-analysis performed by Mochtar et al. found no difference between the live birth rates of women using rFSH alone and in combination with rLH. In another meta-analysis, however, a small RCT conducted among poor responders demonstrated a positive effect of pretreatment with rLH on the live birth rate (15). Nevertheless, a more recent, larger RCT showed no benefit to rLH addition for the clinical pregnancy rate of Bologna-classified poor responders (16). Also, in the present study, a clinical pregnancy rates were similar between two groups.

Although studies on hMG use in poor responders are more limited, a pilot study conducted by Polyzos et al. showed that hMG use in patients under the age of 40 increases the ongoing pregnancy rate and that the use of hMG is promising in the case of poor responders (17, 18). In the newest RCT, Drakopoulos et al. reported that hMG use in 152 poor responders, classified based on the Bologna criteria, did not change their pregnancy rate as compared to the group using only rFSH. In this study, the clinical pregnancy rate was found to be 14.3% in the hMG group and 17.1% in the rFSH group. Similarly, in our study, the clinical pregnancy rate was lower in the hMG group (19.7%) but it was not significant. Although no studies in the existing literature compare the use of rLH and hMG in poor responders, as classified based on the Bologna criteria, there are existing studies that compare two different LH sources. In a study on 4719 patients cured with a GnRH agonist protocol, Buhler and Fischer found higher clinical pregnancy rate in the rLH group than that in the hMG group (25.5% vs. 21.7%, respectively) (3). A retrospective study conducted by Dahan et al. showed the addition of rLH for the patients treated with a serum FSH level of > 10 IU/L to be more effective toward raising clinical rates of pregnancy than the addition of hMG (5). It was found that the number of oocytes in this study was significantly higher in the rLH group than in the hMG group. In accordance, the number of MII oocytes and retrieved oocytes in the present study was significantly higher in the rLH group (p=0.003, p=0.001). This effect may have resulted from the cAMP- and protein kinase-mediated proapoptotic effects of hCG in granulosa cells (19). A possible cause of the higher pregnancy rates in patients using rLH may be the longer half-life of hCG. This prolonged effect had a negative impact on the endometrium in terms of luteinizing hormone-chorionic gonadotropin receptor (LHCGR) downregulation (20, 21). Since we performed frozen embryo transfers in the present study, we did not see this effect, and we determined that there was a similar pregnancy rate in both groups. We applied rLH- and hMG-induced LH supplementation to poor responders classified based on the Bologna criteria. The advantage of this study was that both treatments were applied to the same patient group, which eliminated patient heterogeneity. Despite the higher number of MII oocytes and retrieved oocytes in the rLH group, the clinical pregnancy or blastocyst formation rates had no statistically significant difference. Further studies will confirm whether the addition of rLH increases higher pregnancy rate as compared to the addition of hMG in young poor responders. To achieve this, large and professionally designed randomized studies are required, since there is no evidence-based treatment to date for poor responders.is no evidence-based treatment to date for poor responders.

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgments

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Analyzing the biochemical, clinical, and hormonal characteristics of patients with polycystic ovary syndrome

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Abstract

To analyze the biochemical, clinical, and hormonal characteristics of patients with four phenotypes of Polycystic ovary syndrome (PCOS). A total of 225 patients admitted to Medistate Kavacık Hospital Gynecology and Obstetrics outpatient clinic and Giresun University Faculty of Medicine Gynecology and Obstetrics clinic between January 2019 and January 2020 diagnosed as PCOS and healthy controls were included in the study. The revised Rotterdam criteria were applied to diagnose PCOS. The patients with PCOS were divided into Type I classic, Type II classic, Ovulatory and Normoandrogenic PCOS. Biochemical, clinical, and hormonal values were compared. The mean age of the participants is 28 (±5.7) and the mean body mass index (BMI) is 26.15 (±5.36). The mean Ferriman Gallwey Score (FGS) is 7.4(±5.4), which is normal. There is a statistically significant difference between the four PCOS groups and control group in terms of age (p-value=0.000), BMI (p-value=0.000), Luteinizing hormone / Follicle stimulating hormone (LH/FSH) (p-value=0.000), and fasting blood sugar (p-value=0.01). There is a statistically significant difference among the four phenotypes in terms of BMI (p-value =0.002), LH/FSH (p-value =0.000), LH (p-value =0.000), free T4 (p-value =0.01), fasting insulin (p-value =0.001), total testosterone (p-value =0.000), FGS (p-value =0.000), etc. Age, BMI, LH/FSH, FSH, LH, fasting blood sugar, and hirsutism are good predictors of PCOS.

Keywords: anovulation, hyperandrogenism, hirsutism, phenotype, polycystic ovary syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) affects 5-8 % of women at reproductive age as the most frequent endocrinopathy (1). PCOS is characterized by chronic anovulation, biochemical or clinical hyperandrogenism, and polycystic ovaries morphology (2). It is a multifactorial disorder due to environmental and genetic factors. PCOS includes different phenotypes due to its heterogeneous nature (3). It is considered a metabolic and systemic disorder like insulin resistance and hyperglycemia, increasing the risk of type II diabetes mellitus (DM) and cardiovascular diseases (4), hyperinsulinemia, insulin resistance (IR), and dyslipidemia (5).

Women with PCOS have hyperinsulinemia and IR affecting the hypothalamo pituitary ovarian axis, increasing the secretion of Luteinizing hormone (LH) over Follicle stimulating hormone (FSH), producing ovarian androgen, and reducing follicular maturation and Sex Hormone Binding Globuline (SHBG) (4).

Obesity plays an important role in the clinical features and pathophysiology of PCOS due to increased circulation of free androgen in blood, causing to change in the function of ovarian granulosa cells and the development of follicles (6). Based on the four phenotypes defined by the Rotterdam (7), the hormonal and anthropometrical differences show more similarity of the phenotype D to the control group than the other PCOS phenotypes in a study by Yilmaz et al. in Turkey (8). According to Dewailly et al. (9), those with polycystic ovaries and oligo-anovulatory morphology had mild endocrine features of PCOS. The clinical variants and classical form of PCOS in a Bulgarian population show significant differences in hormonal and anthropometric indices (10).

The present study aims to analyze the biochemical, clinical, and hormonal characteristics of patients with four phenotypes of PCOS, compare them in the four phenotype groups and the five study groups.

2. Materials and methods

This retrospective study was conducted on 225 patients who admitted to Medistate Kavacık Hospital Gynecology and Obstetrics outpatient clinic and Giresun University Faculty of Medicine Gynecology and Obstetrics clinic between January 2019 and January 2020. The Ethics Committee approved this study of Beykoz University, Turkey (Date:26.02.2020 Decision No:1). All procedures conducted in studies, including human participants, conformed to the national or institutional research committee's ethical standards and the 1964 Helsinki Declaration and its later amendments or other ethical standards.

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Table 1. The descriptive statistics of variable

Variable	Minimum	Maximum	Mean	Sd
Age	18	41	28	5.7
BMI	16.9	44.9	26.15	5.36
LH/FSH	0.01	6.9	1.34	0.9
HOMA-IR	0.59	14.77	3.03	2.07
FSH	1.51	10.9	5.79	1.76
LH	0.04	52.57	7.37	5.4
Estradiol	6.98	330.9	56.5	46.2
Free T4	0.52	16.2	1.3	1.3
TSH	0.46	7.98	2.35	1.3
Prolactin	0.49	143	21.6	16.05
Fasting Sugar	73	130	93.1	8.9
Fasting Insulin	3.1	54.04	12.9	8.02
Cholesterol	20	352	191.05	49.4
LDL	-48.8	243.8	107.5	36.5
HDL	24	154	57.6	16.5
Triglyceride	31	341	105.7	52.1
Testosterone	3	141	32.9	18.4
DHEA-SO4	33.8	677.3	289.5	95.03
Leukocytes	3.75	13.5	7.9	2.1
Neutrophil	1.66	9.83	4.8	1.6
Basophils	0.01	2.47	0.06	0.2
Lymphocytes	0.04	4.89	2.3	0.7
Monocytes	0.03	1.36	0.5	0.1
Hb	9.6	24.5	13.09	1.3
Htc	30.7	45.2	39	3.02
PLT	116000	419000	262050.2	53405.4
PCT	0	1	0.2	0.07
RDW	10.9	18.4	13.3	1.3
MPV	6.9	12.8	9.5	1.002
MCV	69.9	98.2	85.01	5.08
FGS	0	22	7.4	5.4

The revised Rotterdam criteria were applied to diagnose PCOS. Biochemical, clinical, and hormonal values were compared. The patients with PCOS were divided into four groups: Type I classic PCOS (hyperandrogenism+chronic anovulatuary+PCOS) (n=72), Type II classic PCOS (hyperandrogenism+chronic anovulatuary+normal ovaries) (n=10), ovulatory PCOS (n=19), and normoandrogenic PCOS (normoandrogenism+chronic anovulatuary+PCOS) (n=38). The criteria for inclusion in the study was age between 18 and 41 years. The criteria for exclusion from the study were: smoking; having DM, endocrinopathy, or hypertension; use of drugs that alter the metabolism of insulin, lipids, or hormones up to three months before the study; deficiency of vitamins B6 and B12 or taking vitamin supplements up to 6 months before the study.

2.1. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 19). The student's t-test was used to compare the means of the two groups. A p-value of < 0.05 was considered statistically significant. The Kolmogorov–Smirnov test shows that only variables of FSH, Hematocrit (Htc), and mean platelet volume (MPV) have a normal distribution and other variables have no normal distribution. To investigate the significant difference among the groups for the normal variables, One-way ANOVA is used, and in nonparametric variables, Kruskal-Wallis test is used. Post-hoc Dunn test is used to analyze the significant results more.

Table 2. The comparison of five groups in terms of the studied variable

Variable	Type I classic PCOS (n:72)	Type II classic PCOS (n:10)	Ovulatory PCOS (n:19)	Normoandrogenic PCOS (n:38)	Control Group (n:86)	Chi-Square	Sig.
	Mean (sd)	**Mean (sd)**	**Mean (sd)**	**Mean (sd)**	**Mean (sd)**		
Age	**25.1 (2.07)**	**25.7 |(4.7)**	**27.05 (5.7)**	**26.6 (4.9)**	**31.4 (5.01)**	**52.02**	**0.000**
BMI	**27.3 (5.8)**	**20.9 (1.7)**	**27.04 (4.5)**	**27.4 (4.3)**	**24.9 (5.2)**	**23.09**	**0.000**
LH/FSH	**1.8 (1.1)**	**0.8 (0.2)**	**0.9 (0.6)**	**1.4 (0.60**	**0.9 (0.6)**	**56.8**	**0.000**
HOMA-IR	**3.8 (2.4)**	**2.04 (0.8)**	**2.6 (1.4)**	**3.8 (2.3)**	**2.2 (1.2)**	**46.5**	**0.000**
FSH	5.5 (1.5)	6.9 (1.6)	5.9 (1.7)	5.4 (1.8)	6 (1.8)	F=2.18	0.07
LH	**9.7 (5.01)**	**5.6 (1.6)**	**6.03 (4.9)**	**7.8 (5.1)**	**5.7 (5.5)**	**46.1**	**0.000**
Estradiol	66.8 (61.7)	34.1 (9.8)	48.6 (20.5)	51.7 (32.9)	54.3 (41.2)	7.7	0.1
Free T4	**1.4 (1.7)**	**1.05 (0.06)**	**1.2 (0.4)**	**1.6 (1.6)**	**1.3 (1.07)**	**11.5**	**0.02**
TSH	2.4 (1.4)	2.1 (1.4)	2.06 (1.2)	2.6 (1.2)	2.2 (1.2)	5.6	0.2
Prolactin	24.4 (17.5)	16.6 (4.1)	18.01 (9.8)	23 (13.3)	20.1 (17.5)	8.3	0.08
Fasting Blood Sugar	**94.9 (10.2)**	**98 (7.1)**	**90.8 (6.8)**	**94.3 (9.4)**	**90.9 (7.4)**	**11.8**	**0.01**
Fasting Insulin	**16.04 (9.5)**	**8.6 (3.8)**	**11.06 (5.03)**	**16.2 (9.2)**	**12.9 (8.02)**	**45.7**	**0.000**
Total Cholesterol	184.4 (47.2)	195.4 (45.4)	187.1 (55.8)	199.02 (51.5)	193.4 (49.6)	2.2	0.6
LDL	109.1 (40.1)	96.8 (36.7)	106.4 (33.3)	105.3 (31.9)	108.7 (36.3)	1.7	0.7
HDL	57.4 (17.7)	59.2 (13.6)	53.6 (13.2)	55.02 (16.3)	59.6 (16.6)	3.6	0.4
Triglycerides	106.8 (48.6)	85 (26.4)	116.2 (74.9)	112.1 (44.4)	101.9 (54.4)	4.2	0.3
Total testosterone	**45.03 (21.6)**	**30.7 (10.1)**	**41.02 (15.5)**	**29.3 (10.9)**	**22.9 (12.1)**	**65.2**	**0.000**
DHEA-SO4	**321.7 (104.4)**	**300.4 (45.4)**	**321.2 (88.2)**	**248.8 (80.5)**	**272.2 (88.5)**	**19.06**	**0.001**
Leukocytes	8.1 (2.1)	9.9 (1.8)	7.8 (2.1)	8.08 (2.3)	7.6 (2.08)	9.3	0.05
Neutrophil	4.8 (1.7)	6.06 (1.1)	5.05 (1.7)	4.9 (1.7)	4.7 (1.6)	7.3	0.1
Basophils	**0.07 (0.2)**	**0.07 (0.03)**	**0.04 (0.02)**	**0.09 (0.4)**	**0.04 (0.02)**	**16.8**	**0.002**
Lymphocytes	2.4 (0.7)	2.8 (0.7)	2.1 (0.6)	2.3 (0.8)	2.2 (0.7)	8.5	0.07
Monocytes	0.5 (0.1)	0.6 (0.2)	0.5 (0.2)	0.4 (0.1)	0.5 (0.2)	7.6	0.1
Hb	**13.5 (1.03)**	**12.9 (1.4)**	**13.2 (1.3)**	**12.9 (1.06)**	**12.8 (1.6)**	**20.5**	**0.000**
Htc	**40.2 (2.7)**	**37.5 (3.3)**	**38.9 (3.6)**	**38.8 (2.7)**	**38.1 (2.9)**	**F=5.8**	**0.000**
PLT	265684.7 (44511.4)	287000 (38108)	234000 (58727.2)	266736 (54102.3)	260232.5 (59611.8)	9.4	0.05
PCT	0.2 (0.09)	0.15(0.1)	0.2(0.04)	0.2(0.05)	0.2(0.05)	3.9	0.4
RDW	**13 (1.2)**	**13.1 (0.5)**	**13.2 (1.4)**	**13.5 (1.2)**	**13.6 (1.3)**	**13.9**	**0.007**
MPV	**9.3 (0.9)**	**9.9 (0.4)**	**10.1 (1.2)**	**9.4 (0.7)**	**9.6 (1.04)**	**F=3.03**	**0.01**
MCV	**86.1 (4.4)**	**82.1 (4.6)**	**83.9 (5.6)**	**85.5 (5.1)**	**84.4 (5.3)**	**11.4**	**0.02**
FGS	**13.3 (3.2)**	**12.5 (1.4)**	**9.7 (2.7)**	**4.4 (3.06)**	**2.8 (1.9)**	**161.6**	**0.000**

3. Results

Table 1 shows that the mean age of the participants is 28(±5.7). The mean BMI of the participants is 26.15(±5.36). The mean LH/FSH, FSH, fasting blood sugar, fasting insulin, and total cholesterol are 1.34 (±0.9), 5.79 (±1.76), 93.1 (±8.9), 12.9 (±8.02), and 191.05 (±49.4), respectively. The mean FGS is 7.4 (±5.4), which is normal.

Table 3. The pairwise comparisons of 5 groups

		Test Statistics	Sig.
Age			
Control	**Type I classic PCOS**	-70.5	0.000
	Type II classic PCOS	-64.7	0.02
	Ovulatory PCOS	-53.8	0.000
	Normoandrogenic PCOS	-49.7	0.02
BMI			
Type II classic PCOS	**Ovulatory PCOS**	-80.8	0.01
	Normoandrogenic PCOS	81.2	0.002
Type II classic PCOS	**Normoandrogenic PCOS**	-87.6	0.002
LH/FSH			
Type II classic PCOS	**Type I classic PCOS**	78.2	0.004
Control	**Normoandrogenic PCOS**	52.3	0.000
Control	**Type I classic PCOS**	70.6	0.000
Ovulatory PCOS	**Type I classic PCOS**	65.5	0.001
HOMA-IR			
Type II classic PCOS	**Type I classic PCOS**	66.9	0.02
Control	**Normoandrogenic PCOS**	58.08	0.000
Control	**Type I classic PCOS**	62.6	0.000
LH			
Control	**Normoandrogenic PCOS**	37.03	0.03
Control	**Type I classic PCOS**	66.8	0.000
Ovulatory PCOS	**Type I classic PCOS**	66.06	0.001
Free T4			
Type II classic PCOS	**Normoandrogenic PCOS**	-71.2	0.02
Fasting Blood Sugar			
Control	**Type II classic PCOS**	55.5	0.01
Fasting Insulin			
Type II classic PCOS	**Normoandrogenic PCOS**	-72.5	0.01
Type II classic PCOS	**Type I classic PCOS**	73.6	0.008
Control	**Normoandrogenic PCOS**	58.3	0.000
Control	**Type I classic PCOS**	59.4	0.000
Total testosterone			
Control	**Ovulatory PCOS**	74.4	0.000
Control	**Type I classic PCOS**	79.7	0.000
Normoandrogenic PCOS	**Type I classic PCOS**	48.08	0.002
DHEA-SO4			
Normoandrogenic PCOS	**Type I classic PCOS**	47.4	0.003
	Ovulatory PCOS	53.5	0.03
Basophil			
Normoandrogenic PCOS	**Type II classic PCOS**	90.2	0.001
Control	**Type II classic PCOS**	76.5	0.004
Ovulatory PCOS	**Type I classic PCOS**	71.6	0.04
Ovulatory PCOS	**Type II classic PCOS**	-65.1	0.02
Hb			
Control	**Type I classic PCOS**	44.9	0.000
Htc			
Control	**Type I classic PCOS**	46.7	0.000
RDW			
Control	**Type I classic PCOS**	-32.9	0.01
MPV			
Type I classic PCOS	**Ovulatory PCOS**	-43.63	0.09
MCV			
Type II classic PCOS	**Type I classic PCOS**	60.8	0.05
FGS			
Control	**Ovulatory PCOS**	86.3	0.000
	Type II classic PCOS	114.4	0.000
	Type I classic PCOS	122.4	0.000
Normoandrogenic PCOS	**Ovulatory PCOS**	63.1	0.005
	Type II classic PCOS	91.2	0.001
	Type I classic PCOS	99.2	0.000

Table 2 shows that there is a statistically significant difference between the four PCOS groups and control group in terms of age (p-value=0.000), BMI (p-value=0.000), LH/FSH (p-value=0.000), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (p-value=0.000), LH (p-value=0.000), free T4 (p-value=0.02), fasting blood sugar (p-value=0.01), fasting insulin (p-value=0.000), total testosterone (p-value=0.000), Dehydroepiandrosterone Sulfate (DHEA-SO4) (p-value=0.001), Leukocytes (p-value=0.005), Basophils (p-value=0.002), Hemoglobin (Hb) (p-value=0.000), Htc (p-value=0.000) Platelet (p-value=0.05), Red cell distribution width (RDW) (p-value=0.007), MPV (p-value=0.01), mean cospusculer volume (MCV) (p-value=0.02) FGS (p-value=0.000).

Table 4. Comparison of 4 groups

Variable	Type I classic PCOS	Type II classic PCOS	OVULATORY PCOS	Normoandrogenic PCOS	Chi-Square	Sig.
	Mean (sd)	**Mean (sd)**	**Mean (sd)**	**Mean (sd)**		
Age	25.1 (2.07)	25.7|(4.7)	27.05 (5.7)	26.6 (4.9)	3.03	0.3
BMI	27.3 (5.8)	20.9 (1.7)	27.04 (4.5)	27.4 (4.3)	14.4	0.002
LH/FSH	1.8 (1.1)	.8 (0.2)	0.9 (0.6)	1.4 (0.60)	24.8	0.000
HOMA-IR	3.8 (2.4)	2.04 (0.8)	2.6 (1.4)	3.8 (2.3)	13.7	0.003
FSH	5.5 (1.5)	6.9 (1.6)	5.9 (1.7)	5.4 (1.8)	F=2.4	0.07
LH	9.7 (5.01)	5.6 (1.6)	6.03 (4.9)	7.8 (5.1)	19.8	0.000
Estradiol	66.8 (61.7)	34.1 (9.8)	48.6 (20.5)	51.7 (32.9)	7.3	0.06
Free T4	1.4 (1.7)	1.05 (0.06)	1.2 (0.4)	1.6 (1.6)	10.01	0.01
TSH	2.4 (1.4)	2.1 (1.4)	2.06 (1.2)	2.6 (1.2)	4.9	0.1
Prolactin	24.4 (17.5)	16.6 (4.1)	18.01 (9.8)	23 (13.3)	4.9	0.1
Fasting Blood Sugar	94.9 (10.2)	98 (7.1)	90.8 (6.8)	94.3 (9.4)	4.5	0.2
Fasting Insulin	16.04 (9.5)	8.6 (3.8)	11.06 (5.03)	16.2 (9.2)	16.1	0.001
Total Cholesterol	184.4 (47.2)	195.4 (45.4)	187.1 (55.8)	199.02 (51.5)	2.2	0.5
LDL	109.1 (40.1)	96.8 (36.7)	106.4 (33.3)	105.3 (31.9)	1.6	0.6
HDL	57.4 (17.7)	59.2 (13.6)	53.6 (13.2)	55.02 (16.3)	1.1	0.7
Triglycerides	106.8 (48.6)	85 (26.4)	116.2 (74.9)	112.1 (44.4)	3.06	0.3
Total testosterone	45.03(21.6)	30.7(10.1)	41.02 (15.5)	29.3 (10.9)	21.2	0.000
DHEA-SO4	321.7 (104.4)	300.4 (45.4)	321.2 (88.2)	248.8 (80.5)	15.2	0.002
Leukocytes	8.1 (2.1)	9.9 (1.8)	7.8 (2.1)	8.08 2.3)	6.8	0.07
Neutrophil	4.8 (1.7)	6.06 (1.1)	5.05 (1.7)	4.9 (1.7)	6.06	0.1
Basophils	0.07 (0.2)	0.07 (0.03)	0.04 (0.02)	0.09 (0.4)	14.9	0.002
Lymphocytes	2.4 (0.7)	2.8 (0.7)	2.1 (0.6)	2.3 (0.8)	5.5	0.1
Monocytes	0.5 (0.1)	0.6 (0.2)	0.5 (0.2)	0.4 (0.1)	8.1	0.04
Hb	13.5 (1.03)	12.9 (1.4)	13.2 (1.3)	12.9 (1.06)	8.3	0.03
Htc	40.2(2.7)	37.5 (3.3)	38.9 (3.6)	38.8 (2.7)	F=4.05	0.009
PLT	265684.7 (44511.4)	287000 (38108)	234000 (58727.2)	266736 (54102.3)	9.3	0.02
Pct	0.2 (0.09)	0.15 (0.1)	0.2 (0.04)	0.2 (0.05)	3.7	0.2
RDW	13 (1.2)	13.1 (0.5)	13.2 (1.4)	13.5 (1.2)	8.1	0.04
Mpv	9.3 (0.9)	9.9 (0.4)	10.1 (1.2)	9.4 (0.7)	F=3.9	0.01
MCV	86.1 (4.4)	82.1 (4.6)	83.9 (5.6)	85.5 (5.1)	10.09	0.01
Ferriman Galleway Score	13.3 (3.2)	12.5 (1.4)	9.7 (2.7)	4.4 (3.06)	82.4	0.000

The mean ages of Type I classic PCOS is 25.1(±2.07), the mean age of Type II classic PCOS is (25.7) (±4.7), the mean age of Ovulatory PCOS is 27.05(±5.7) and the mean age of Normoandrogenic PCOS is 26.6(±4.9), significantly lower than that of control Group (31.4(±5.01). The mean BMI of Type I classic PCOS (27.3(±5.8)), Ovulatory PCOS (27.04±4.5), and Normoandrogenic PCOS (27.4(±4.3) are significantly higher than that of the control Group (24.9(±5.2)). The mean LH/FSH of Type I classic PCOS (1.8(±1.1)) and normoandrogenic PCOS 1.4(±0.60) are significantly higher than that of the control group 24.9(±5.2).

The mean fasting blood sugar of Type I classic PCOS (94.9(±10.2)), Type II classic PCOS (98(±7.1)), and normoandrogenic PCOS (94.3± (9.4)) is significantly higher than that of the control group (90.9(7.4)). The mean FGS of Type I classic PCOS (13.3(±3.2), Type II classic PCOS (12.5(±1.4)), Ovulatory PCOS 9.7(±2.7), and Normoandrogenic PCOS (4.4(±3.06)) is significantly higher than that of the control group (2.8(±1.9)).

The pairwise comparison in Table 3 shows that there is a statistically significant difference between Type I classic PCOS (P-value=0.000), Type II classic PCOS (P-value=0.02), Ovulatory PCOS (P-value=0.000), and Normoandrogenic PCOS (P-value=0.02) and the control group in terms of age. There is a statistically significant difference between Type II classic PCOS and Ovulatory PCOS in terms of BMI (P-value=0.01). Type II classic PCOS and Normoandrogenic PCOS show statistically significant

differences in BMI (P-value=0.002). Type II classic PCOS and the control show statistically significant differences in fasting blood sugar (p-value=0.01). There is a statistically significant difference between ovulatory PCOS, Type II classic PCOS, Type I classic PCOS groups, and the control group in FGS. Table 4 shows that there is a statistically significant difference among the four groups in terms of BMI (p-value =0.002), LH/FSH (p-value=0.000), HOMA-IR (p-value =0.003), LH (p-value =0.000), free T4 (p-value =0.01), fasting insulin (p-value=0.001), total testosterone (p-value=0.000), DHEA-SO4 (p-value=0.002), Basophils (p-value=0.002), Monocytes (p-value=0.04), FGS (p-value=0.000),etc.

Table 5. Pairwise comparisons of 4 groups

		Test Statistics	Sig.
BMI			
Type II classic PCOS	**Ovulatory PCOS**	-47.8	0.01
	Type I classic PCOS	49.6	0.002
	Normoandrogenic PCOS	-51.7	0.002
LH/FSH			
Type II classic PCOS	**Type I classic PCOS**	49.7	0.001
Ovulatory PCOS	**Type I classic PCOS**	40.7	0.001
HOMA-IR			
Type II classic PCOS	**Type I classic PCOS**	-39.8	0.03
	Normoandrogenic PCOS	41.4	0.01
LH			
Ovulatory PCOS	**Type I classic PCOS**	39.7	0.001
Free T4			
Type II classic PCOS	**Normoandrogenic PCOS**	-44.03	0.01
Fasting Insulin			
Type II classic PCOS	**Normoandrogenic PCOS**	-44.5	0.01
Type II classic PCOS	**Type I classic PCOS**	43.9	0.007
Total testosterone			
Normoandrogenic PCOS	**Type I classic PCOS**	35.5	0.000
DHEASO4			
Normoandrogenic PCOS	**Type I classic PCOS**	29.2	0.002
	Ovulatory PCOS	32.9	0.02
Basophils			
Normoandrogenic PCOS	**Type II classic PCOS**	54.1	0.001
Ovulatory PCOS	**Type II classic PCOS**	42.8	0.03
Type I classic PCOS	**Type II classic PCOS**	-38.9	0.02
Monocytes			
Normoandrogenic PCOS	**Type II classic PCOS**	40.5	0.02
Hb			
Normoandrogenic PCOS	**Type I classic PCOS**	22.1	0.03
Platelet			
Ovulatory PCOS	**Type II classic PCOS**	45.6	0.02
MCV			
Type II classic PCOS	**Type I classic PCOS**	39.1	0.02
FGS			
Normoandrogenic PCOS	**Ovulatory PCOS**	34.9	0.01
	Type II classic PCOS	62.3	0.000
	Type I classic PCOS	71.6	0.000
Ovulatory PCOS	**Type I classic PCOS**	36.6	0.002

Pairwise comparison of four phenotype groups in Table 5 shows that there is a statistically significant difference between Type II classic PCOS and Ovulatory PCOS (0.01), Type II classic PCOS and Type I classic PCOS (0.002), and Type II classic PCOS and Normoandrogenic PCOS (0.002) in terms of BMI.

4. Discussion

The findings show that the mean age of the control group was the highest. Type II classic PCOS group had the lowest BMI, followed by the control group with the mean BMI (24.9±5.2). Type I classic PCOS group had the highest LH/FSH. Type II classic PCOS had the highest fasting blood sugar. Fasting insulin was the highest in the normoandrogenic PCOS group. The four phenotype groups and the control group showed a

statistically significant difference in age, BMI, LH/FSH, fasting blood sugar, fasting insulin, free T4, total testosterone, Hb, Htc, PLT, FGS, etc.

In the comparison of the four phenotype groups, BMI was the highest in the normoandrogenic PCOS. Type I classic PCOS group had the highest LH/FSH. Normoandrogenic PCOS had the highest fasting insulin, followed by Type I classic PCOS. Type I classic PCOS had the highest FGS as compared with the other three groups.

Carmina et al. (11) found the intermediate values between phenotype A PCOS and controls for total testosterone levels and BMI and that those with phenotype C PCOS had lower BMI than those with phenotype A PCOS while slightly higher BMI than the controls, which is in line with the study by Jamil et al. (12) Our study found that the third group had slightly higher BMI than the first group and the controls and the first group had the highest testosterone.

Jamil et al. (12) found a significant difference among the four groups in total testosterone, not in line with the study by Sahmay et al. (13) but consistent with the study by Guastella et al. (14), showing that phenotype C had higher testosterone than phenotype D and Dewailly et al. (9).

Jamil et al. (12) also found obesity in 50% of the women, which is consistent with a study by Pasquali et al. (6), while the mean BMI of the women in the present study was 26.15. Jamil et al. (12) found that the number of overweight people was the same in the control and PCOS groups, not consistent with our study results. Pehlivanov et al. (10) found that women with phenotypes A and B were more obese, while our study found that phenotype A group was more obese than the controls.

There were higher LH/FSH ratio and LH levels in all PCOS phenotypes than in the control group reported by Dewailly et al. (9), while LH/FSH in our study was higher in the first, third, and fourth groups than in the second and control groups. LH level was the highest in phenotype A compared with the other phenotype's groups and the control group.

Dewailly found no difference in serum FSH levels of all phenotypes (9), consistent with our study results and the study by Jamil et al (12). Yilmaz (8) and Jamil et al. (12) found that LH/FSH ratio in phenotype D was lower than that in phenotype A, consistent with our study.

In our study, the mean FGS was normal in the participants and was the highest in the type I classic PCOS group, and hirsutism was significantly higher in the PCOS phenotype groups than in the control group. The clini¬cal features of hyperandrogenism were significantly different among the PCOS groups and between the PCOS Group and the control group, not consistent with the study by Jamil et al (12), Thathapudi et al. (15) and Farhan et al. (16).

Kucur et al. (17) showed the difference between PCOS phenotypes in serum LH levels and FGS score. Phenotype B had higher IR but not statistically significant. Yilmaz et al. (8) also reported significantly higher serum LH/FSH and LH values in all phenotypes than the control group, in line with our study results.

Like our study, Yilmaz et al. (8) found the FGS score to be the highest in phenotype B and the lowest in phenotype 4, which is in line with our study results.

Despite the articles in the literature with the same results as our study, it was recently reported that High density lipoprotein (HDL) levels were significantly lower in hyperandrogenic PCOS phenotype than the non-hyper androgenic phenotypes (8, 18, 19).

It is concluded that the control group was older than the four phenotype groups. Women in the Type II classic PCOS group had the lowest BMI and the highest fasting blood sugar. Type I classic PCOS group had the highest LH/FSH. The normoandrogenic PCOS group had the highest fasting insulin. The four phenotype groups and the control group showed a statistically significant difference in age, BMI, LH/FSH, fasting blood sugar, fasting insulin, FGS, etc. Among the four phenotype groups, women in the normoandrogenic PCOS group had the highest BMI, and those in the type I classic PCOS group had the highest LH/FSH. In general age, BMI, LH/FSH, FSH, LH, fasting blood sugar, and hirsutism are good predictors of PCOS.

Conflict of interest

The authors declare that they have no competing interest.

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The relationship of bile acid with biochemical tests in the diagnosis of intrahepatic cholestasis of pregnancy

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Abstract

Intrahepatic pregnancy cholestasis (ICP) is associated with increased fetal complications. It is linked to an increased risk. Early diagnosis of this disease reduces these fetal complications. In this study, it was aimed to determine the sensitivity and specificity of biochemical tests according to cut off values. The values of 14 patients with bile acid ≥40 µmol / L diagnosed with intrahepatic cholestasis of pregnancy and 40 patients with bile acid <40 µmol / L were compared retrospectively with 60 control patients. In ICP patients, the ALT and AST values in patients with group 1 with bile acid ≥40 µmol / L and group 2 with bile acid <40 µmol / L were significantly higher than the control group (p = 0.0001 for both markers). Groups 1 and 2 with ICP patients have high sensitivity and specificity compared to the cut off value of ALT and AST. In our study, it was found that increased the risk of preterm delivery in ICP patients. Especially in cases where bile acid was ≥ 40 µmol / L, complications such as preterm delivery and low birth weight increased in proportion to the increase in bile acid. In the ICP patients, AST and especially ALT values increased in proportion to the increase in bile acid.

Keywords: ALT- serum alanine transaminase, AST-serum aspartate transaminase, bile acid, intrahepatic cholestasis of pregnancy

1. Introduction

Intrahepatic cholestasis of pregnancy (ICP) is one of the most common liver diseases seen in pregnancy and it occurs in 0.1% to 15.6% of pregnancies (1). ICP causes itching, especially in foot soles and palms, increase bile acids and increase in liver function test. The disease is mostly seen in the late second and third trimesters (2). The patient's clinic improves spontaneously 5-6 weeks after birth (3). Intrahepatic cholestasis of pregnancy causes bad perinatal outcomes due to gestational diabetes, preeclampsia, unreliable fetal condition, meconium amnion, and preterm labor (4-6). As in many organs during pregnancy, many physiological changes occur in the liver and biliary tract (7). Hormonal changes during pregnancy, genetic and environmental factors affecting biliary transport and secretion have also been accused of ICP etiopathogenesis (8).

The most sensitive test in the diagnosis of ICP patients is the measurement of serum bile acids (9). In patients with ICP, after 37 weeks of pregnancy, the rate of intrauterine death increases in the fetuses of mothers with bile acid ≥40 µmol / L (10). Measurement of liver function tests is important in diagnosis in these patients, but in one third of patients these values are within normal limits (11). In previous studies, it was found that bile acids are correlated with transaminases in the liver in ICP patients. This correlation relationship has also been linked to secretory phospholipase A2 damage, which is associated with complications in the patient (12-13). The reference intervals of liver function tests should be evaluated considering pregnancy.

The purpose of this study is to separate the patient groups according to serum bile acid 40 µmol / L above and below, determine the transaminase values in these groups, find the cut off values in these transaminases and determine the specificity and sensitivity of the transaminases in group diagnoses according to the cut off values found.

2. Materials and methods

The study files of 54 patients with intrahepatic cholestasis of pregnancy who were admitted to Mersin University Faculty of Medicine Obstetrics and Gynecology between 2015-2019 were reviewed retrospectively. For the control group, 60 patients' files were randomly selected during the same gestational week, with no additional disease in the same period. Among the patients in the ICP group, patients with bile acid ≥40µmol / L were called “Group 1” and patients with bile acid <40µmol / L were called “Group 2”, and the control group was called “Group 3”. Patients with twin pregnancies, cholecystectomy, diabetes mellitus during pregnancy, thyroid disease, hypertension associated with pregnancy, liver and bile disease were not included in the study groups. The ages, gestational weeks, baby weights at birth, liver function tests, fasting bile acid and drug dosage used were recorded.

SPSS 22.0 software (IBM Corporation, Amork, USA) was used for statistical analysis. Variance analysis and Post Hoc

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test were used in the One-Way ANOVA test to compare the average values of the data. The ROC test for Alanine transaminase (ALT) and Aspartate transaminase (AST), which differed from the control group, and the sensitivity-specificity, confidence interval AUC, cut off and significance for each marker were examined. At the end of the test, values with p <0.05 were considered significant.

3. Results

In ICP patients, the ALT and AST values in patients with group 1 with bile acid ≥40 µmol / L and group 2 with bile acid <40 µmol / L were significantly higher than the control group (p = 0.0001 for both markers). The birth weight of the babies of the patients in group 1 was significantly less than that of group 2 (Table 1).

Table 1. Demographic and average values of cases

	Group 1 ICP patient bile acid ≥40 µmol/L N=14	Group 2 ICP patient bile acid <40 µmol/L N=40	Group 3 Control Patient N=60	P
Age	27±2.3	27±6.3	29.4±6.3	0.37
GGT	12.6±4.0	14.4±1.3	14.5±3.6	0.42
ALP	193±54.6	**211±80[a]**	**137±85[a]**	**0.01***
ALT	**235±166[b]**	**160±134[b]**	**23±21[b]**	**0.0001***
AST	**142±103[c]**	**112±97[c]**	**25.5±4.5[c]**	**0.0001***
Bile Acid	85.3±5.5	27.4±2.44	N/A	N/A
Gestation Week	34.6±0.1	36.4±2.3	37±2	0.31
Newborn Weight	**2510±882[e]**	**3268±462[e]**	3060±577	**0.02***
Umblikal Artery Ph	7.31±0.06	7.32±0.05	7.28±0.09	0.31
Drug Dose	642±134	750±182	N/A	N/A

Groups 1 and 2 with ICP patients have high sensitivity and specificity compared to the cut off value of ALT and AST. Confidence intervals and significance levels were found in these values (Table 2). ALT and AST ROC Curves for CP ≥ 40 Cases are given in Fig. 1.

Table 2. ALT and AST ROC Curve Results in ICP Cases

		Cutt Off Value	Sensitivity	Specificity	AUC±SE	%95 confidence interval		p
						Lower Limit	Upper Limit	
Group 1 ICP bile acid ≥40µmol/L	**ALT**	72	86%	87%	0.76±0.140	0.484	1.032	0.048
	AST	41.5	86%	80%	0.76±0.137	0.486	1.025	0.049
Group2 ICP bile acid <40µmol/L	**ALT**	62	88%	87%	0.89±0.050	0.793	0.989	**0.000**
	AST	39.5	88%	80%	0.87±0.052	0.769	0.973	**0.000**

4. Discussion

Intrahepatic cholestasis (ICP) of pregnancy causes an increase in fetal complications during pregnancy. That is why it is important to diagnose the disease in a timely manner. The biochemical parameter commonly used in the diagnosis of the disease is serum bile acid measurement (14, 15). The upper limit of bile acids accepted during pregnancy is 10 µmol / L. Without other symptoms and biochemical findings of ICP, high bile acids can be seen in normal pregnancies. Approximately 2% - 3% of these patients develop ICP in the later weeks of pregnancy (16). Serum bile acid measurement is considered as the most appropriate biochemical marker in the diagnosis and follow-up of ICP patients (17). Large prospective studies have found that ICP is associated with perinatal outcomes. Spontaneous preterm labor, preterm delivery and intrauterine death rates increase especially in pregnancies where serum bile acid exceeds 40 µmol / L (18). Increased bile acid in mother blood causes increased bile acid in fetomaternal circulation and fetal blood (19-21). It has been found that fetuses of ICP patients have more bile acids in intrauterine bronchoalveolar fluid and more RDS develops in these fetuses (22, 23). Genes et al found that serum bile acid and low ALT levels correlated positively with preterm labor (5). A study in Sweden found that complication rates were higher in cases with maternal fasting serum bile acids> 40 µmol/L (18). Despite the low bile acid levels in the treatment

of ursodeoxycholic acid in ICP patients, there have been unexplained fetal deaths before 39th week of pregnancy (24). Although spontaneous preterm risk was reported to be 60% in ICP patients (25), most studies found that spontaneous preterm labor was 30-40% (26). In two studies, maternal serum bile acid level was associated with spontaneous preterm delivery. When the results in our study were evaluated, fetal death was not observed since the number of patients was low (18, 27). However, we observed that ICP patients gave birth earlier than control (preterm delivery) and patients in the group with bile acid ≥ 40 µmol / L increased spontaneous preterm labor rate and especially patients in group 1 with increased bile acid gave birth at a lower birth weight (table 1). It was found that the risk of preterm delivery increased in ICP patients. Especially in cases where bile acid was ≥ 40 µmol / L, complications such as preterm delivery and low birth weight increased in proportion with increased bile acid.

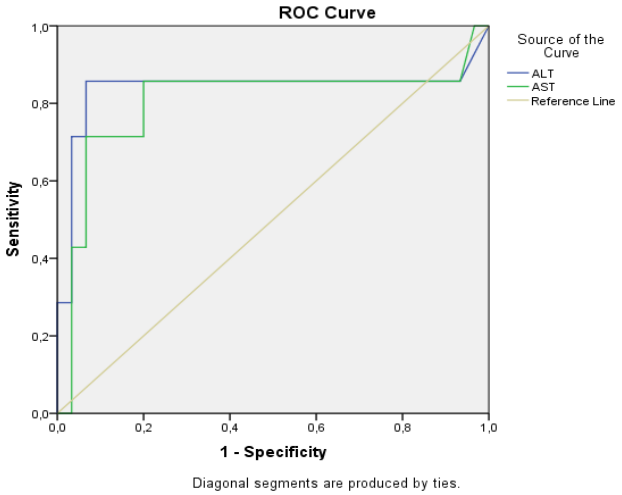

Fig. 1. ALT and AST ROC Curves in Cases with ICP ≥ 40 (ALT - serum alanine transaminase, AST– serum aspartat transaminase)

In normal pregnancy, reference intervals of ALT, AST (28) and GGT (29) should be reduced by 20% compared to non-pregnant women. The increase of transaminase enzymes indicates hepatocyte damage and hepatocellular damage. In ICP patients, ALT and AST values may also increase before or after bile acid increases (19, 30). In previous studies, there are results indicating that the height of transaminases in ICP is very variable and there is no cut off value (11). In recent studies, the cut off value for liver diseases was determined as 19 IU / L for non-pregnant women (31). In previous studies, it was found that ALT and AST increased 2-10 times in ICP patients and ALT was a better marker (30, 32, 33). In our study, it was observed that ALT and AST increased in two groups with the ICP patient with bile acid ≥ 40 µmol / L and <40 µmol / L than the control group. Especially, the increase rate in ALT was higher than AST, in line with previous studies. In our study, as seen in table 2, it was found that the cut off value of ALT was 72 IU / L and the cut off value of AST was 41.5 IU / L in patients with group 1 bile acid ≥ 40 µmol / L. It was found that ALT and AST have a high sensitivity and specificity in the diagnosis of ICP. Similarly, in group 2, it was found that the cut off value of ALT was 62 IU / L and AST was 39.5 IU / L in patients with bile acid <40 µmol / L and it was found that ALT and AST have a high sensitivity and specificity in the diagnosis of ICP. In our study, ALT was found to be a more specific marker in ICP diagnosis than AST (specificities were 87% for ALT and 80% for AST). It was found that AST and especially ALT values increased in proportion with the increase in bile acid in ICP patients. ALT and AST have a certain cut off value in the diagnosis of ICP. If this cut off value is used in the diagnosis of ICP, it has high sensitivity and specificity.

In normal pregnancy, since ALP value increases due to bone marrow and placental production, it has weak importance in the diagnosis of ICP. ALP increases in ICP patients, but since it is also synthesized from the placenta, it limits its effectiveness in diagnosis. In our study, it was found that ALP was significantly increased in group 2 (bile acid <40 µmol / L) (table 1). Although there are publications on the increased GGT in ICP patients (34-36), the common view is that it has not changed (33). As a matter of fact, it was seen that GGT did not change in our study.

In our study, it was found that the risk of preterm delivery increased in ICP patients. Especially in cases where bile acid was ≥ 40 µmol / L, complications such as preterm delivery and low birth weight increased in proportion with increased bile acid. It was found that AST and especially ALT values increased in proportion with the increase in bile acid in ICP patients. ALT and AST have a certain cut off value in the diagnosis of ICP. If this cut off value is used in the diagnosis of ICP, it has high sensitivity and specificity. The advantage of our study is that ALT and AST have a certain cut off value in the diagnosis of ICP and in determining the level of bile acid in this disease. The disadvantage is that the number of patients is low and it is a retrospective study.

Ethics Committee Approval

Ethical Approval was obtained from Mersin University's Ethical Committee. (6/1/2021, 01/20).

Conflict of interest

There is no conflict of interest to declare.

Acknowledgments

None to declare.

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Which COVID-19 patients should be recommended for home isolation and which should be hospitalized? Predictors of disease progression for mild COVID-19 patients

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Abstract

Each country has different treatment and home isolation recommendations regarding the management of mild COVID-19 patients, and there is not yet a standard approach. The aim of this study is to determine which patients are suitable for home isolation by identifying the variables that predict the progression of the disease in mild COVID-19 patients. This prospective observational study included laboratory confirmed mild COVID-19 patients older than 18 years. The primary outcome of the study was the disease progression in mild COVID-19 patients. A multivariate regression model was created according to the results of univariate analyses. A total of 254 patients included in the study. Median age of the patients was 34.5 years (27–42), and 132 (52%) of them were male. COVID-19 compatible thoracic computed tomography appearance (P<0.001, HR=6.58, 95% CI=2.60-16.65) and advanced age (P=0.008, HR=1.07, 95% CI=1.02-1.13) were significantly associated with the progression of the disease, and the use of hydroxychloroquine (P=0.002, HR=0.09, 95% CI=0.02-0.32) was significantly associated with a decrease in the disease progression. The advanced age and COVID-19 compatible thoracic computed tomography appearance were associated with progression of the disease, while hydroxychloroquine treatment was associated with decreased progression in mild COVID-19 patients.

Keywords: 2019 novel coronavirus disease, clinical deterioration, COVID-19 pandemic, disease progressions, SARS-CoV-2 infection

1. Introduction

On December 31, 2019, pneumonia cases with an unknown cause were reported from Wuhan, China. On March 11, 2020, the World Health Organization (WHO) declared a pandemic on the outbreak, which was officially understood to be caused by a new coronavirus strain (COVID-19) with the isolation of the novel coronavirus (n-CoV) on January 7, 2020 (1, 2). According to the latest WHO reports, on September 5, 2020, there were 26,415,380 cases associated with COVID-19 seen worldwide, and 870,286 deaths were reported (3).

COVID-19 may present as asymptomatic or mild; it may progress to adult respiratory distress syndrome (ARDS) or septicemia as well. While most of the patients experience the disease as so mild, they can be followed in home isolation, others require hospitalization (4). Since the beginning of the pandemic, many countries have been regularly updating their national guidelines according to the data in the available literature (5-9). However, since there is still not sufficient knowledge to create a clear understanding regarding the criterion for home isolation with this new disease, there are significant differences between the guidelines. Regarding prevention recommendations, for example, according to the Centers for Disease Control and Prevention (CDC), home isolation is recommended for the elderly and patients with comorbidities as long as they have a mild or moderate prognosis (those without hypoxemia and severe pneumonia). However, according to the Turkish Ministry of Health, regardless of disease severity, home isolation is not recommended for the elderly and patients with comorbidity. In contrast, the National Health Commission of the People's Republic of China recommends hospital admission for every patient diagnosed or suspected of COVID-19, regardless of age or disease severity (4, 8, 9).

To determine which patients are appropriate for home isolation and which are appropriate for hospital admission, in this study, we aimed to determine the variables predicting clinical deterioration in patients with laboratory confirmed mild COVID-19.

2. Materials and methods

2.1. Study design and setting

This prospective observational study was carried out between April 1, 2020, and May 9, 2020, in an emergency medicine department (ED) of a tertiary care training and research hospital. Ethical approval was obtained from Ümraniye Training and Research Hospital Clinical Research Ethics Committee.

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2.2. Selection of participants

Adult mild COVID-19 patients were consecutively included in the study. The definition of mild COVID-19 included patients with symptoms such as fever, myalgia, cough, shortness of breath, sore throat, nasal congestion, headache, diarrhea, or loss of smell/taste, and with none of the following severe disease criteria: age of >50 years, chest radiography or thoracic computed tomography revealing bilateral diffuse parenchymal infiltration, an O_2 saturation below 93% in room air, tachypnea (>22/min), the presence of comorbid diseases (chronic lung disease, immunity disorders, hypertension, malignancy, diabetes mellitus), or poor prognostic criteria in laboratory tests (D-Dimer >1000 ng/ml, blood lymphocyte count <800/µl, C-reactive protein (CRP) >40 mg/L or ferritin>500ng/ml) (9). Patients with those severe disease criteria were admitted to the hospital and excluded from the study. The medical treatment recommended in the guidelines published by the Ministry of Health (hydroxychloroquine 2x 200 mg for five days) was started and home isolation was recommended for patients who fit the mild COVID-19 definition. These patients were followed both by the Ministry of Health screening teams and by the hospital by phone. Patients who described any worsening in their clinical condition during their follow-up were called back to the hospital. After the re-evaluations, patients who met the above-mentioned severe disease criteria were accepted as progression of the disease and were hospitalized. Patients with at least one positive COVID-19 polymerase chain reaction (PCR) test result were included in the study. All patients with negative results, or who were younger than 18 years old, pregnant, had any abnormal vital signs (pulse, respiratory rate, arterial blood pressure, fever), who refused to participate in the study or had a lack of data were excluded from the study.

2.3. Measurements

The baseline characteristics of the included patients, complaints, close contact history, smoking status, home isolation status, another COVID-19 case in the household, thoracic computed tomography or chest radiography findings, and clinical deterioration during follow-up (requiring hospitalization) and treatments were recorded. Although hydroxychloroquine treatment is routinely initiated in all these patients as per the standard protocol in the country, some patients refused to use drugs due to their individual preferences, which was recorded during the follow-up. All patients were called daily and the presence of symptom worsening was questioned. Patients who described progression compared to their symptoms at the time of first admission were called back to the hospital and were re-evaluated. Patients who met the moderate-severe COVID-19 criteria were considered as patients with clinical progression. The clinical deterioration decision was made based on the moderate-severe COVID-19 criteria defined in the diagnosis and treatment of COVID-19 guideline published by Turkish Ministry of Health. Accordingly, if any of the following criteria was present, the patient was accepted as deteriorated and was hospitalized; not all COVID-19 related pneumonia but chest radiography or thoracic computed tomography revealing bilateral diffuse parenchymal infiltration, an O2 saturation below 93% in room air, tachypnea (> 22 / min) , or poor prognostic criteria in laboratory tests (D-Dimer> 1000 ng / ml, blood lymphocyte count <800 / µl, C-reactive protein (CRP)> 40 mg / L or ferritin> 500ng / ml) (9).

2.4. Outcome measures

The primary outcome was disease progression in mild COVID-19 patients. We aimed to determine which variables predicted the progression of the disease.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 26 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The distribution of normality for the continuous variables was tested with the Shapiro–Wilk test. The Student's T-test or Mann–Whitney U tests were used to compare groups. Chi-square and Fisher's exact tests were used for comparison of the categorical data. To determine the variables affecting disease progression, a multivariate regression model was created using variables having p values of <0.2 in univariate analyses. A two-tailed p value of <0.05 was considered statistically significant.

3. Results

After 569 patients were enrolled in our study, exclusion criteria were applied, and the remaining 254 patients were included in the study. The patient flow chart is given in (Fig. 1).

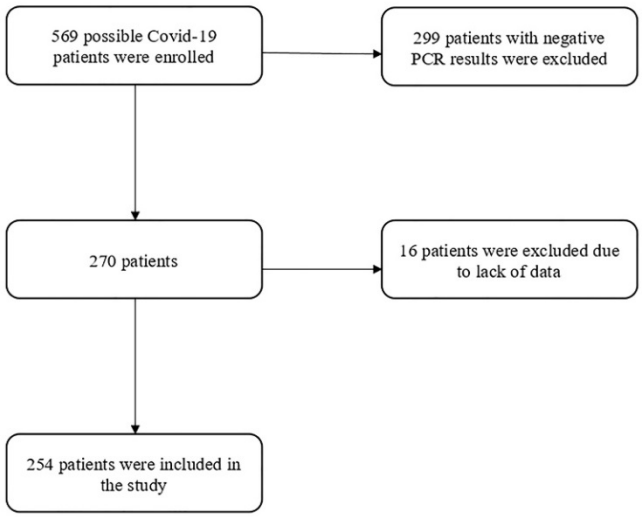

Fig. 1. Patient flow chart

The median age of the patients was 34.5 years (27–42), and 132 (52%) of them were male. The basic characteristics of the patients are shown in Table 1. The patients included in the study were divided into two groups: Group 1: patients whose disease did not progress (those who completed their treatment and follow-up in home isolation) and Group 2: patients whose disease progressed (those who required re-evaluation and then hospitalized).

Table 1. Basic characteristics of the patients

Variable	N (%)/Median (IQR)
Age, year	34.5 (27-42)
Gender, female	122 (48)
Patients with close contact history with Covid-19	115 (45.3)
Home isolation	
Strict isolation (patient lives alone)	54 (21.3)
Limited isolation (Isolation in a single room but in the same house with the family)	117 (46.1)
Poor isolation (Lives in the same house and room)	83 (32.7)
Another Covid-19 case in the household	
No other Covid-19 case	121 (47.6)
There is case of Covid-19 before the patient	69 (27.2)
There is case of Covid-19 after the patient	27 (10.6)
There is concurrent case of Covid-19	37 (14.6)
Smokers (current or former)	87 (34.3)
Healthcare professionals	25 (9.8)
Symptoms	
Fever	92 (36.2)
Cough	158 (62.2)
Shortness of breath	65 (25.6)
Myalgia	78 (30.7)
Fatigue	84 (33.1)
Loss of smell	32 (12.6)
Loss of taste	28 (11)
Diarrhea	15 (5.9)
Nausea	14 (5.5)
Vomiting	5 (2)
Hemoptysis	2 (0.8)
Headache	42 (16.5)
Sore throat	37 (14.6)
Nasal congestion	16 (6.3)
Conjunctivitis	13 (5.1)
Asymptomatic	24 (9.4)
Treatments	
Hydroxychloroquine	236 (92.9)
Oseltamivir	59 (23.2)
Azithromycin	12 (4.7)
Clarithromycin	3(1.2)
Other antibiotics	3 (1.2)
Patients undergoing thoracic CT	148 (58.3)
Findings compatible with Covid-19 in thoracic CT (for 148 patients)	55 (37.2)
Patients with clinical deterioration during follow-up (primary outcome)	29 (11.4)

CT; Computer tomography

In the univariate analysis, it was observed that COVID-19 compatible thoracic CT appearance and cough were statistically significantly associated with increased clinical progression, while the use of hydroxychloroquine was significantly associated with decreased clinical progression. In the multivariate logistic regression analysis, it was observed that COVID-19 compatible thoracic CT appearance and advanced age were significantly associated with the progression of the disease, while the use of hydroxychloroquine was significantly associated with decreased progression of the disease. The results of the univariate and the multivariate analyses are provided in Table 2. When the patients were divided into two groups according to the use of hydroxychloroquine, no statistically significant difference was observed between the groups in terms of basic characteristics such as age, gender, close contact history, smoking status, symptoms, or COVID-19 compatible thoracic CT appearance. Descriptive data of the hydroxychloroquine groups are provided in Table 3. No death was observed in any of the patients included in the study.

4. Discussion

In our study, none of the patients died who were initially recommended home isolation according to the guidelines of the Turkish Ministry of Health. From this perspective, it can be interpreted that the Ministry of Health's definition of mild COVID-19 is appropriate. However, considering that 11.4% of the patients had clinical deterioration, the current guideline might still require revision. According to the current data published by WHO, the number of deaths per million is as follows in some countries: Belgium 855, Spain 629, UK 612, Italy 588, US 567, Brazil 594, France 468, Turkey 78, South Korea 6, and China 3 (3). However, comparing the mortality rates of the countries based on the available data is a contentious issue because the PCR indications, treatment, and patient management policies are not standardized between the countries. Nevertheless, the low mortality rates in countries such as Turkey, China, and South Korea is striking. Regardless of the severity of the disease, early initiation of treatment with hydroxychloroquine of all patients with COVID-19 may be one of the causes of the low mortality rate in Turkey. The most striking finding of our study is the hazard ratio of not using hydroxychloroquine as 11.4. Although the patients in our study were not randomized in terms of hydroxychloroquine use, the fact that there was no difference between the hydroxychloroquine groups in terms of basic characteristics makes this finding remarkable.

The use of hydroxychloroquine in the treatment of COVID-19 is controversial (10). In vitro experiments have shown that hydroxychloroquine inhibits the SARSCoV2 virus (11). In a randomized controlled study, it was found that prophylactic hydroxychloroquine treatment initiated within the first four days after exposure did not prevent the disease in individuals with high and moderate COVID-19 exposure (12). When compared to standard therapy in a randomized controlled study, it was found that hydroxychloroquine use did not make a significant difference in the elimination (negativity) of the disease in hospitalized treatment-resistant mild-to-moderate COVID-19 patients (13).

Table 2. Univariate and multivariate analysis of variables associated with progression of disease in patients with mild Covid-19

Variable	Group 1 (Non-progression) N (%) / Median (IQR)	Group 2 (Disease progression) N (%) / Median (IQR)	Univariate Analysis		Multivariate Analysis	
			HR (95% CI)	P value	HR (95% CI)	P value
Age, years	34 (27-42)	39 (34-45)	0.366 (0.047-2.835)	0,484	1.070 (1.018-1.126)	**0.008**
Age <50	205 (91.1)	28 (96.6)	0.366 (0.047-2.835)	0.484	-	-
Gender, female	108 (48)	14 (48.3)	1.011 (0.466-2.192)	0.978	-	-
Patients with close contact history	103 (45.8)	12 (41.4)	0.836 (0.382-1.832)	0.654	-	-
Strict home isolation is provided	47 (20.9)	7 (24.1)	1.205 (0.485-2.991)	0.687	-	-
Smokers (current or former)	79 (34.7)	9 (31.0)	0.848 (0.369-1.951)	0.698	-	-
Healthcare professionals	22 (9.8)	3 (10.3)	1.065 (0.298-3.805)	1	-	-
Symptoms						
Fever	77 (34.2)	15 (51.7)	2.059 (0.945-4.487)	0.065	2.507 (0.971-6.470)	0,057
Cough	135 (60)	23 (79.3)	2.556 (1.001-6.524)	**0.044**	1.737 (0.612-4.927)	0.299
Shortness of breath	58 (25.8)	7 (24.1)	0.916 (0.372-2.257)	0.849	-	-
Myalgia	72 (32)	6 (20.7)	0.554 (0.216-1.421)	0.214	-	-
Fatigue	72(32)	12 (41.4)	1.500 (0.681-3.306)	0.312	-	-
Loss of smell	30 (13.3)	2 (6.9)	0.481 (0.109-2.130)	0.550	-	-
Loss of taste	26 (11.6)	2 (6.9)	0.567 (0.127-2.524)	0.752	-	-
Diarrhea	12 (5.3)	3 (10.3)	2.048 (0.542-7.737)	0.391	-	-
Nausea	11 (4.9)	3 (10.3)	2.245 (0.588-8.572)	0.205	-	-
Vomiting	3 (1.3)	2 (6.9)	5.481 (0.876-34.283)	0.101	11.962 (0.733-195.177)	0.082
Hemoptysis	2 (0.9)	0 (0)	1.515 (0.071-32.336)	1	-	-
Headache	39 (17.3)	3 (10.3)	0.550 (0.159-1.909)	0.434	-	-
Sore throat	30 (13.3)	7 (24.1)	2.068 (0.813-5.259)	0.157	1.812 (0.608-5.404)	0.286
Nasal congestion	15 (6.7)	1 (3.4)	0.500 (0.064-3.932)	1	-	-
Conjunctivitis	11 (4.9)	2 (6.9)	1.441 (0.303-6.851)	0.649	-	-
Asymptomatic	23 (10.2)	1 (3.4)	0.314 (0.041-2.414)	0.329	-	-
Patients given hydroxychloroquine treatment	214 (95.1)	22 (75.9)	0.162 (0.057-0.459)	**0.002**	0.087 (0.024-0.321)	**<0.001**
Findings compatible with Covid-19 in thoracic computed tomography	39 (17.3)	16 (55.2)	5.870 (2.613- 13.184)	**<0.001**	6.577 (2.599-16.646)	**<0.001**

HR; Hazard ratio, IQR; Interquartile range, CI; Confidence interval

Table 3. Basic descriptive data of hydroxychloroquine use groups

Variable	Use of hydroxychloroquine	Non-use of hydroxychloroquine	P value
Age, year	34 (27-43)	38 (29.5-39)	0.958
Gender, female	114 (48.3)	8 (44.4)	0.752
Patients with close contact history	108 (45.8)	7 (38.9)	0.572
Patients providing strict home isolation	50 (21.2)	4 (22.2)	1
Smokers (current or former)	79 (33.5)	8 (44.4)	0.344
Healthcare professionals	24 (10.2)	1 (5.6)	1
Symptoms			
Fever	89 (37.7)	3 (16.7)	0.073
Cough	147 (62.3)	11 (61.1)	0.921
Shortness of breath	60 (25.4)	5 (27.8)	0.784
Myalgia	75 (31.8)	3 (16.7)	0.180
Fatigue	80 (33.9)	4 (22.2)	0.310
Loss of smell	32 (13.6)	0 (0)	0.140
Loss of taste	28 (11.9)	0 (0)	0.234
Diarrhea	14 (5.9)	1 (5.6)	1
Nausea	13 (5.5)	1 (5.6)	1
Vomiting	4 (1.7)	1 (5.6)	0.310
Hemoptysis	2 (0.8)	0 (0)	1
Headache	40 (16.9)	2 (11.1)	0.746
Sore throat	33 (14)	4 (22.2)	0.310
Nasal congestion	14 (5.9)	2 (11.1)	0.316
Conjunctivitis	13 (5.5)	0 (0)	0.608
Asymptomatic	23 (9.7)	1 (5.6)	1
Treatments			
Oseltamivir	58 (24.6)	1 (5.6)	0.082
Azithromycin	10 (4,2)	2 (11.1)	0.205
Clarithromycin	3 (1.3)	0 (0)	1
Other antibiotics	3 (1.3)	0 (0)	1
Findings compatible with Covid-19 in thoracic computed tomography	51 (21.6)	4 (22.2)	1

Similarly, in a randomized controlled study conducted by Oriol et al. on patients with mild COVID-19, it was found that hydroxychloroquine use had no effect on the viral load for up to 7 days, and it did not have an effect on the improvement of symptoms or the incidence of hospitalization either (14). Although the increasing number of articles against the use of hydroxychloroquine is evident, there are also studies in the literature that assert that the medication may be beneficial. A study showed that hydroxychloroquine shortened the time of the clinical recovery and decreased the duration of coughing, and fever when compared to a control group (15). In a living systematic review where 32 randomized controlled studies were analyzed, it was reported that the use of hydroxychloroquine decreased the period of the disease up to 4.5 days, but that it increased the frequency of non-serious adverse effects (16). In consequence, the uncertainty in the literature regarding the use of hydroxychloroquine in COVID-19 patients still continues. According to the current study results, we consider that hydroxychloroquine treatment might reduce clinical progression, at least in mild COVID-19 patients.

The reason for the young population of this study is that the Ministry of Health of Turkey determined age of >50 years as a criteria for hospitalization at the time of the study, and our study examines patients referred to home isolation. For this reason, since they were already hospitalized, elderly patients were not included in our study. There are many publications in the literature indicating that advanced age is associated with poor prognosis in COVID-19 patients (17,18).

Interestingly, in our study, no significant difference was found in the hospitalization rate of mild COVID-19 patients who smoke compared to non-smokers. According to Francesca Polverino, ACE-2, synthesized by goblet cells due to metaplasia caused by cigarette smoke in epithelial cells, prevents invasion by creating a barrier for the pathogen (19). Similarly, according to the study of Eduardo Hernandez Garduna on 32,583 patients, it was determined that smoking is not a risk factor but is protective for COVID-19 (odds ratio 0.63 95% CI, 0.51–0.77) (20). However, some studies state that smoking will increase the inflammatory response and cytokine storm in patients with COVID-19 and thus have negative effects on the prognosis (21). Based on this inconsistent literature and the results we found in our study, we consider that further studies are needed to determine whether smoking is a risk factor or a protective factor for

COVID-19.

The limitation of this study is that it involves a limited patient population due to its single-center nature. The relatively small number of samples decreased its statistical power.

We recommend hospitalization of the patients with COVID-19 compatible thoracic CT appearance regardless of the severity of the infiltration and advanced age as these variables were found to be associated with progression of the disease. Considering that hydroxychloroquine was associated with reduced clinical progression in our study, we think that the use of the drug may be appropriate in mild COVID-19 patients who are recommended home isolation.

Conflict of interest

There are no conflicts of interest in connection with this paper.

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How have the obstetricians and gynecologists struggled in the clinics during the COVID-19 pandemic?

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Abstract

As all branches of the medicine, the obstetrical and gynecological clinical approach has been greatly affected by the pandemic. In our study, we aimed to reveal the effect of the pandemic on current obstetrics and gynecologic clinical approach and urgency/semi-urgency evaluations of physicians related with both obstetrical and gynecological cases. The obstetricians in Turkey from different hospitals and clinics have completed our online questionnaire-based survey using 'docs.google.com'. The survey questionnaire was created in three sections: Demographic, occupational analysis and thoughts about outbreak, clinical approach scale and clinical urgency scale. SPSS 22 program was used to analyze the validity and reliability of the scales. The outbreak has affected the clinical approach according to our study. The effect degree was 3.79, 3.15, 3.72 respectively at clinical effect, clinical functioning and struggle and prevention. The specialists regardless of the year in the occupation were more affected than research assistants in terms of clinical effect (p=0.017) and also postponed obstetrical (p=0.000) and gynecological (p=0.000) conditions more frequently. As the effect of the pandemic on clinical functioning increases, the delay of gynecological cases increases. With the postponement of obstetrics cases, the probability of delaying gynecological cases increases. In the current study, it was concluded that the pandemic has affected the clinical approach in obstetrics and gynecology clinics. We think that the study will help in determining our approach to obstetrical and gynecological cases in the future. Scenarios should be made patient-centered without neglecting the burden and possible damages on healthcare professionals.

Keywords: clinical approach, clinical effect, clinical urgency, gynecology, obstetrics, pandemic

1. Introduction

In late 2019, hospitals in Hubei province of China began reporting unusual cases of pneumonia among Huanan seafood market employees and visitors (1, 2). As the cases spread throughout the whole of Wuhan city in a short time, the World Health Organization (WHO) confirmed the public health problem on 31 December 2019 (3). In January 2020 as a result of examining the pneumonia cases, scientists achieved to isolate a novel beta coronavirus that 85% similarity to a bat SARS-CoV genome (4, 5). Because of the genomic similarity with SARS-CoV, the virus has been named SARS-CoV-2 and the disease has been called COVID-19 (3, 5, 6). By 11 March 2020 it was described as a pandemic because of the 118.223 confirmed cases and 4.291 deaths in 114 countries (3, 7, 8).

As it all branches of medicine, obstetricians and gynecologists delayed semi-urgent and non-urgent surgeries and decreased acceptance of patients and also extended the follow-up frequencies of some medical conditions through committee opinions of the concerned societies (9, 10). With the widespread of the outbreak, in the frame of precautions taken by Turkish Ministry of Health, all healthcare workers decreased elective cases and surgeries both in the state and private hospitals (8).

The obstetrical and gynecological clinical approach had been greatly affected by the pandemic. The obstetrical and gynecology practice shows both heavy patient and surgery burden. The surgical practice of obstetrics and gynecology includes deferrable, semi-urgent and urgent operations. The obstetrical clinical approach is more necessary that they require more stringent examination follow-up and the processes such as delivery cannot be postponed. Furthermore, disruptions that occur especially in the follow-up of high-risk pregnancies can lead to fetal and maternal complications (11). Many benign gynecologic cases are elective and can be delayed until the pandemic is brought under control, but the diagnosis and treatment processes of gynecological malignancies cannot be delayed for an extended period (9, 10).

In our study, we aimed to reveal the effect of pandemic on current obstetric and gynecological clinical approach and urgency evaluations of physicians on obstetrical and gynecological cases.

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2. Materials and methods

One hundred and twenty-two obstetricians in Turkey from different hospitals and clinics have completed our online questionnaire-based survey using 'docs.google.com'. Volunteer participants were invited through email and social media messages. Since the researchers do not have the opportunity to determine the participants in the digital environment, sampling management is chosen as simple random sampling method. Also, the reason for choosing the simple random sampling method is that it enables easier, cheaper and faster data collection than other sampling techniques.

The questionnaire was published online on April 18, 2020 and was available online for two weeks. Outbreak and dense business of obstetricians were the limitations of the data collection. The survey was anonymized by the name and place of work. Ethical approval was required from the ethics committee of Ordu University Medical Faculty, Ordu.

The survey questionnaire was created in three sections. In the first section, demographic, occupational analysis and clinicians' thoughts about outbreak was questioned. Eleven questions were asked to participants, six of which were open-ended. The details of the first survey section have been given on table 1.

Table 1. Demographic, occupational analysis and clinicians' thoughts about outbreak

Part I of the survey
Demographic questions
Age
Year in professional
Gender
Type of hospital
Professional title
Thoughts about outbreak
Do you work in pandemic hospital?
Have you encountered Covid-19 suspected patient?
Have you encountered a patient diagnosed with Covid-19?
What do you think is the most important point in the Covid-19 struggle?
Have you been tested related to Covid-19?
What is your opinion about applying Covid-19 test to healthcare professionals?

In the second section, clinical approach scale was interrogated. The clinical approach scale was conducted as three part including clinical effect (six multiple-choice and two open-ended questions), clinical functioning (ten multiple-choice and two open-ended questions), struggle and prevention (four multiple-choice questions). These questions of the scale querying how the clinical approach have been affected by the pandemic. The details of the second survey section have been given on table 2.

And the last section of the survey contains the clinical urgency scale that consist nine obstetrics and thirteen gynecologic conditions. The scale answers which obstetrical or gynecologic conditions are described as urgent or deferrable by the surgeon. Both the clinical approach and the clinical urgency scales were prepared by taking the suggestions of leading academicians, associations and societies. As a result, these two scales were used to investigate the effects of pandemic on clinical activities and surgeons' sense of urgency. The details of the third survey section have been given on table 3.

SPSS 22 program was used to analyze the validity and reliability of the scales. Factor analysis was conducted to understand the construct validity of the items of the scale. The clinical approach scale was firstly prepared as 24 questions. However, as a result of factor analysis, four expressions were excluded from the statistics because the factor loads were low or incompatible. As a result, the clinical activity scale was validated as 20 statements.

The Kaiser-Meyer-Olkin (KMO) test was performed for the sample number and it was found to be 0.664. In addition, Barlett's sphericity test, which was used to evaluate the scale's suitability for factor analysis, was found to be significant at the level of 0.001 (Approx. Chi-Square: 720.420/df:190/sig:0.000). In order to dimension the question items in the scale, 'Verimax' rotation was applied with the 'principal components' method. The clinical approach scale items were found to have factor loads between 0.404 and 0.788. The scale's results were conducted on a five-point likert scale and gathered around three factors. These factors were named as; clinical effect (6 expressions), clinical functioning (10 expressions), struggle and prevention (4 expressions) factors. The variance explanation level of the factors in the scale was calculated as 42.9%. Cronbach's Alpha coefficient was calculated as 0.670 for the reliability analysis of the clinical activity scale. This value was found to be confident at the acceptable level.

The clinical urgency scale was consisted of 22 questions that also conducted on a five-point likert scale and gathered around two factors. These factors were named as; obstetrics (9 expressions) and gynecologic (13 expressions). Factor loadings of the expressions in the scale vary between 374 and 885. Kaiser-Meyer-Olkin (KMO) test was performed for the sample number and it was found to be 0.789. Barlett's sphericity test was found to be significant at the level of 0.001 (Approx. Chi-Square: 1804.662/df:231/sig:0.000). In order to dimension the question items in the scale, 'Verimax' rotation was applied with the 'principal components' method. The scale was collected under two factors. The variance explanation level of the factors in the scale was calculated as 48%. Cronbach's Alpha coefficient was calculated as 0.90 for the reliability analysis of the clinical activity scale. And this value was found to be perfectly reliable.

In the research, whether demographic and occupational

characteristics of obstetricians and gynecologists have a differentiating effect on the factors of the scales in the survey was investigated by t and Anova tests.

3. Results

One hundred and twenty-two obstetricians and gynecologists participated in the study. The survey was consisted of three sections.

3.1. Demographic, occupational analysis and thoughts about outbreak

According to the results, 54.9% of the participants were women, 45.9% were in the age groups of 39 and under; 23.8% of the clinicians continued their education as research assistant. It was determined that 36.9% of the participants worked in state hospitals, 32.8% in university hospitals, and the others in private hospitals or private clinics. According to the results, 62.3% of the physicians stated that they have been working in a pandemic hospital and 6.6% stated that they have accepted suspicious patients even though they do not work in a pandemic hospital. 72.1% of the respondents confirmed that they had contact with patients with suspected COVID-19, 37.7% confirmed that they had contact with patients with the diagnosis of COVID-19. While 34.1% of the respondents stated that they had the COVID-19 test, the results of the five participants' COVID-19 test were positive. The details about the demographic, occupational analysis and clinicians' thoughts about outbreak have given in table 1.

3.2. The clinical approaches scale in Obstetrics and Gynaecology

The clinical approach scale was designed to show how the surgeons' clinical approaches were affected in the pandemic. It was concluded that the pandemic process has affected the clinical approach. The effect degree was 3.79, 3.15, 3.72 respectively at clinical effect, clinical functioning and struggle and prevention. The results of the clinical approach scale have been presented in table 2.

In open-ended questions about the clinical effect and functioning, 34.5% of the doctors who stated that they routinely received a separate consent form from the patients, but only 3.3% requested COVID-19 test in patients who needed hospitalization. In the future after the pandemic; 50% of physicians believe that online scientific congresses will be common and also 52.4% of them stated that online treatments will become more common.

3.3. The clinical urgency scale in Obstetrics and Gynaecology

The clinical urgency scale was designed to show how the surgeons' thoughts of urgency were affected in the pandemic. The scale was designed to answer which obstetrical and gynecological conditions was described as can be postponed regardless of time (<2.5) or can be postponed for a while (2.5-3.5) and cannot be deferrable so urgent (>3.5) according to the surgeon. In order to understand the details of the findings, the arithmetic means and frequency distributions of the clinical urgency scale have been shown in the table 3.

3.4. Statistical analysis

In the research, whether demographic and occupational characteristics of obstetricians and gynecologists have a differentiating effect on the factors of the scales in the survey was investigated by t and Anova tests. According to the results; it was observed that the year in the occupation had a statistically significant effect on both scales (clinical approach scale and the clinical urgency scale in obstetrics and gynecology). The clinicians who have been working as specialist for 6-10 years were more affected than research assistants in terms of clinical effect (p=0.008). Besides it was shown that physicians who have been working as specialist for 6-10 years have delayed both obstetrical (p=0.002) and gynecological (p=0.000) cases more than research assistants. Generally, the specialists regardless of the year in the occupation were more affected than research assistants in terms of clinical effect (p=0.017) and also postponed obstetrical (p=0.000) and gynecological (p=0.000) conditions more frequently. It was observed that the specialists were more sensitive about using protective equipment and besides, due to their professional experience, they postponed both obstetrical and gynecological cases more than research assistants. It has been observed that the type of hospital has created a significant difference on clinical functioning (p=0.021). Doctors who have been working in the state hospitals were more affected than the doctors working in private clinics in terms of clinical functioning (p=0.021). Also, it was observed that the clinicians that working in private clinics postpone gynecological situations more than the physicians working in the state and university hospitals (p=0.005). We think that private clinics have less affected in terms of clinical functioning than state hospitals due to their low out-patient load. Despite the effect in clinical functioning, it was observed that they postponed gynecological cases more due to the fact that they gave more importance to the follow-up of obstetric cases.

The clinical functioning of female physicians was more affected (p=0.017). The clinical functioning (p=0.026) of physicians working in the pandemic hospital were more affected, and it was also observed that they tend to delay gynecological (p=0.001) cases more. It was an expected result that the clinical procedures of the physicians working in the pandemic hospital would be affected more. We think that postponing the gynecological cases more is a measure to reduce the patient load in pandemics. The relationship between the factors of both scales was examined by Pearson correlation analysis and the results are given in table 4. A linear weak correlation was found between the clinical effect and clinical functioning at the level of p=0.05 error (0.210). It has been concluded that as the clinical effect degree of the pandemic increases, changes in clinical functioning would increase.

Table 2. The clinical approach scale in obstetrics and gynecology

Part II of the survey	Clinical approach scale in Obstetrics and Gynecology										x̄	SS
	Never agree		Not agree		Partially agree		Agree		Absolutely agree			
	n	%	n	%	n	%	n	%	n	%		
Clinical effect section											3.79	0.91
The number of patients admitted in the pandemic process changed	10	8.2	7	5.7	23	18.9	22	18.0	60	49.2	3.94	1.28
The number of patients admitted to the pandemic period has decreased significantly	12	9.8	13	10.7	17	13.9	29	23.8	51	41.8	3.77	1.35
I use surgical mask and/or face shield during the outpatient examination	26	21.3	13	10.7	10	8.2	10	8.2	63	51.6	3.58	1.67
I use respiratory mask and/or face shield in the operating room	16	13.1	14	11.5	9	7.4	13	10.7	70	57.4	3.87	1.51
I feel uncomfortable because of being away from my routine program during the pandemic process	10	8.2	11	9.0	31	25.4	39	32.0	31	25.4	3.57	1.19
I ask questions about the coronavirus related symptoms while accepting cases in the pandemic process	10	8.2	9	7.4	15	12.3	24	19.7	64	52.5	4.00	1.30
I receive a separate consent form related to Covid-19 from patients who are admitted to the hospital during the pandemic process; (yes 34.5%)												
I want Covid-19 test in patients who are admitted to the hospital during the pandemic process (yes 3.3%)												
Clinical functioning section											3.15	0,64
I renewed my routine diagnosis and treatment practice in the light of current guidelines during the pandemic process	8	6.6	7	5.7	20	16.4	56	45.9	31	25.4	3.77	1.09
I think that the Covid-19 condition mostly affects the diagnosis and treatment method in the pandemic process	5	4.1	10	8.2	35	28.7	42	34.4	30	24.6	3.67	1.06
I only care for emergency cases during the pandemic process	13	10.7	14	11.5	31	25.4	34	27.9	30	24.6	3.44	1.27
I reduced the follow-up frequency of pregnant women with low risk	7	5.7	11	9.0	24	19.7	45	36.9	35	28,.7	3.73	1.14
I reduced the follow-up frequency of pregnant women with high risk	45	36.9	27	22.1	23	18.9	17	13.9	10	8.2	2.34	1.32
I reduced the follow-up frequency of benign gynecological cases	6	4.9	4	3.3	19	15.6	47	38.5	46	37.7	4.00	1.05
I reduced the follow-up frequency of pre-malign/malign gynecological cases	39	32.0	32	26.2	28	23.0	11	9.0	12	9.8	1.36	0.85
Patients who have been given an outpatient appointment postpone their appointments	12	9.8	24	19.7	50	41.0	27	22.1	9	7.4	2.97	1.06
Patients who have been given a surgery date appointment delay their appointments	5	4.1	7	5.7	8	6.6	8	6.6	94	77.0	2.80	1.20
Patients requiring follow-up after treatment are not followed	19	15.6	31	25.4	41	33.6	17	13.9	14	11.5	2.42	1.14
I think that tele-congresses held during the pandemic process will be as common as classic-congresses												
I think that tele-patient/physician interviews made during the pandemic process will be as common as classic-examination.												
Struggle and prevention section											3.72	0.76
I do not have a problem in terms of supplying protective equipment	7	5.7	18	14.8	32	26.2	34	27.9	31	25.4	3.52	1.18
I use protective equipment properly	3	2.5	3	2.5	19	15.6	39	32.0	58	47.5	4.19	0.95
I think we are successful as a health system about struggling pandemic	2	1.6	9	7.4	38	31.1	41	33.6	32	26.2	3.75	0.98
I think we are successful as a country about struggling pandemic	7	5.7	17	13.9	42	34.4	30	24.6	26	21.3	3.41	1.14

In addition, a weak correlation was found between the clinical functioning and deferral status of the gynecological cases at p=0.01 error level (-0,278). In other words, as the effect of pandemic on clinical functioning increases, the delay of the gynecological cases increases. Finally, there was a linear moderate relationship between deferral status of the gynecological and obstetrical cases at p=0.01 error level (0.405). With the postponement of the obstetrical cases, the probability of delaying gynecological cases increases.

Table 3. The clinical urgency scale in obstetrics and gynecology

Part III of the survey	Clinical urgency scale in obstetrics and gynecology										$\bar{x}$	SS
	Absolutely deferable		Mostly deferable		Partially deferable		Mostly urgent		Absolutely urgent			
	N	%	n	%	n	%	n	%	n	%		
Obstetrics											3.51	0.75
Hyperemesis gravidarum	59	48.4	34	27.9	24	19.7	3	2.5	2	1.6	1.81	0.94
Abortusimminens	32	26.2	40	32.8	24	19.7	12	9.8	14	11.5	2.47	1.29
Preterm birth	4	3.3	12	9.8	28	23.0	27	22.1	51	41.8	3.89	1.15
Preterm rupture of membranes	3	2.5	5	4.1	18	14.8	22	18.0	74	60.7	4.30	1.02
Gestational hypertension	3	2.5	5	4.1	7	5.7	29	23.8	78	63.9	4.42	0.95
Gestational diabetes	2	1.6	9	7.4	38	31.1	32	26.2	41	33.6	3.82	1.03
Noninvasive prenatal tests	15	12.3	13	10.7	24	19.7	42	34.4	28	23.0	3.45	1.29
Second trimester sonography	13	10.7	8	6.6	20	16.4	46	37.7	35	28.7	3.67	1.25
Invasive prenatal tests	9	7.4	10	8.2	21	17.2	39	32.0	43	35.2	3.79	1.25
Gynecology											2.39	0.71
Vaginal discharge	80	65.6	24	19.7	17	13.9	1	0.8	0	0.0	1.50	0.76
Pelvic pain	35	28.7	49	40.2	31	25.4	5	4.1	2	1.6	2.09	0.92
Irregular menstrual bleeding	36	29.5	40	32.8	35	28.7	10	8.2	1	0.8	2.18	0.97
Heavy menstrual bleeding	13	10.7	24	19.7	44	36.1	27	22.1	14	11.5	3.04	1.14
Routine smear/HPV scanning	63	51.6	16	13.1	36	29.5	6	4.9	1	0.8	1.90	1.03
Colposcopy/cervical biopsy in CIN	24	19.7	19	15.6	32	26.2	26	21.3	21	17.2	3.00	1.36
Diagnostic hysterescopy	28	23.0	36	29.5	43	35.2	9	7.4	6	4.9	2.41	1.07
Operative hysterescopy	17	13.9	24	19.7	43	35.2	26	21.3	12	9.8	2.93	1.16
Diagnostic laparoscopy	47	38.5	36	29.5	29	23.8	6	4.9	4	3.3	2.04	1.05
Operative laparoscopy	30	24.6	19	15.6	40	32.8	23	18.9	10	8.2	2.70	1.25
Gynecological cancers	2	1.6	2	1.6	15	12.3	39	32.0	64	52.4	4.31	0.87
Infertility research	80	65.6	31	25.4	6	4.9	3	2.5	2	1.6	1.49	0.83
Infertility therapy	75	61.5	26	21.3	16	13.1	4	3.3	1	0.8	1.61	0.89

Table 4. Correlation analysis between clinical approach scale and clinic urgency scale in obstetrics and gynecology

	Clinic approach scale - Clinical effect	Clinic approach scale - Clinical functioning	Clinic approach scale - Struggle and prevention	Clinic urgency scale- Obstetrics	Clinic urgency scale- Gynecology
Clinic approach scale - Clinical effect	1				
Clinic approach scale - Clinical functioning	.210 (*)	1			
Clinic approach scale - Struggle and prevention	.077	.159	1		
Clinic urgency scale- Obstetrics	.139	-.018	.015	1	
Clinic urgency scale- Gynecology	.035	-.278 (**)	-.072	.405 (**)	1

*; Correlation is significant at the 0.05 level (2-tailed), **; Correlation is significant at the 0.01 level (2-tailed)

4. Discussion

There was considerable confusion regarding the clinical approaches in the early stage of the outbreak. However, at this stage, many associations have expressed their views on how clinical approaches can be applied in the pandemic process. In our study, we revealed that obstetrics and gynecology clinics are highly affected by the pandemic. Most of the authorities aim to reduce the clinical effect without disrupting clinical functioning. The current study has showed that the effect degree was 3.79, 3.15, 3.72 respectively at clinical effect, clinical functioning and struggle and prevention.

In the study, it was stated that the number of patients who applied to the obstetrics and gynecology clinic during the pandemic process changed significantly (3.94). This condition is directly correlated with suggestions of WHO and CDC (12, 13); so, the patients in Turkey rely on cautions about decreasing contact and staying at home (8). When the physicians have been asked about the symptom questioning of every patient about COVID-19, mostly answered as symptom

questioning of every patient have been done (4.0); but only 3.3% requested COVID-19 test in patients who needed hospitalization. That symptom questioning ratio is compatible with the suggestions of the societies. American Congress of Obstetricians and Gynaecologists (ACOG), The Royal College of Obstetricians and Gynaecologists (RCOG) and Society for Maternal Fetal Medicine (SMFM) suggest some patient acceptance charts, after the chart questioning, low risk population is accepted to make their examination in the routine clinical environment (14-16). Even it is mentioned, before application to hospitals, screening of the symptoms by telephone and then the scheduling of outpatient appointments can be done (17). In open-ended questions about the clinical effect, 34.5% of the doctors who stated that they routinely received a separate consent form from the patients for inpatient treatments for other health problems.

It was stated that physicians have greatly renewed their diagnosis and treatment practices in the light of current guidelines (3.77) and most physicians think that the COVID-19 situation has a major influence on diagnosis and treatment management (3.67). It was concluded in the present study that, the physicians postponed the follow-up and treatment of low risk pregnancies (3.73) and benign gynecological diseases (4.00). But the physicians preferred to preserve the routine diagnosis and treatment algorithms of high-risk pregnancies (2.34) and gynecologic oncologic diseases (2.34). The physicians stated that also the patients preferred to postpone their outpatient (2.97) and surgery date appointments (2.80).

In open-ended questions about the clinical functioning, 50% of the physicians stated that online scientific congresses and 52.4% of the physicians stated that online therapies would become more widespread after the pandemic. SMFM declared that community mitigation efforts are important but local practice and population factors and the resources like tele-health (including telephone with other remote services) is the critical point to practice (16). Also, it is important to categorize the cases as high-risk or low-risk and according to the risk assessment to decide urgent/semi-urgent/deferrable or non-deferrable cases. The tele-health and other remote services can come into question after the clinicians experienced and the societies definitely decided which cases are deferrable or non-deferrable.

The clinical urgency scale was designed to answer which obstetrical and gynecological conditions that described as deferrable, semi-urgent or urgent. Most of the obstetrical conditions had been found as non-deferrable and so urgent by the clinicians (3.51); but only hyperemesis gravidarum (1.81) and abortus imminens (2.47) was found to be deferrable regardless of time. Because hyperemesis gravidarum and abortus imminens require less hospitalization and these conditions do not cause severe obstetrical complications generally. Currently, there is no evidence to suggest that COVID-19 causes developmental problems or causes miscarriage. With regard to vertical transmission (transmission from woman to baby antenatal or intrapartum), emerging evidence now suggests that vertical transmission may be possible (18, 19). Preterm birth (3.89) and preterm rupture of membranes (4.30) were found to be urgent obstetrical complications so physicians considered both conditions to be non-deferrable. The clinicians have evaluated gestational diabetes (3.82) and gestational hypertension (4.42) as emergency obstetrical conditions which cannot be deferrable. The clinicians care about high risk pregnancies - such as preterm birth, preterm rupture of membranes, gestational diabetes and gestational hypertension- by considering both maternal and fetal morbidity/mortality, regardless of pandemic and maintain their routine practices. The risk of an asymptomatic pregnant progressing to severe COVID-19 disease is unknown, but is usually thought to be equivalent to that of health- and age-matched women. But if there is comorbidity accompanying pregnancy like gestational diabetes or hypertension disorders, that may contribute to increased risk related with COVID-19 (20, 21).

And clinicians stated that noninvasive prenatal tests (such as double, triple and quadruple tests) (3.45) can be postponed for a while. However, the clinicians stated that, second trimester ultrasound (3.67) and invasive prenatal tests (such as amniocentesis, chorionic villus sampling and cordocentesis) (3.79) are urgent and so non-deferrable situations for the prenatal diagnosis. According to the clinicians, while noninvasive prenatal tests have been found to be deferrable for a while due to their wide application time ranges and interchangeability, invasive prenatal tests and second trimester ultrasound cannot be postponed because of their limited application time intervals. The invasive fetal procedures in which the fetus/mother benefit ratio is high and the procedure's theoretical risk of vertical transmission is low or moderate, like chorionic villus sampling, amniocentesis, fetal blood transfusion, thoracoamniotic shunting, laser for twin-to-twin transfusion syndrome, spina bifida closure, are considered as safe procedures and can be done with the COVID-19 risk assessment of the pregnant (22).

The International Fetal Medicine and Surgery Society (IFMSS) and other societies have published guidelines for routine prenatal care and screening during COVID-19 pandemic (20). Routine antenatal visits can be spaced out, after first prenatal visit detecting the pregnancy and special risk factors, Down syndrome screening and other virtual ultrasound scan may be delayed until 12-13 week of pregnancy, blood test for routine controls can be taken together with noninvasive prenatal testing. Detailed anomaly ultrasound scanning should be delayed until 20-22 weeks (22).

The clinicians have evaluated the gynecological conditions to be deferrable either regardless of time or for a

while (2.39). As a result of the research, frequent gynecological symptoms such as vaginal discharge (1.50) and pelvic pain (2.09) were seen to be deferrable regardless of time. And irregular menstrual bleeding (2.18) was found to be deferrable regardless of time, while heavy menstrual bleeding (3.04) was found to be deferrable for a while but not urgent. The important point in irregular menstrual bleeding is to diagnose important diseases such as endometrial cancer and the clinicians think that the reasonable delay in the pandemic process is not important. However, increased morbidity in heavy menstrual bleeding is thought to be more important than the delay in cancer diagnosis. Therefore, the clinicians think that, while irregular menstrual bleeding can be postponed regardless of time, heavy menstrual bleeding can be delayed for a while. Irregular uterine bleeding is generally associated with decreased ovarian function. However, obese and late reproductive aged women have higher risk for endometrial cancer. RCOG, British Society for Gynaecological Endoscopy (BSGE) and British Gynaecological Cancer Society (BGCS) concluded that women at high risk for endometrial cancer must be screened by physicians as soon as possible (23). In our study, we stated that the number of patients admitted to the hospital during the pandemic period decreased significantly. Complaints like vaginal discharge, pelvic pain, irregular menstrual bleeding and demands of patients like routine smear screening, infertility treatments and infertility search constitute the main patient burden of gynecology practice in Turkey. That's why we think, the decrease in the number of patients depends on delaying the appointments of the patients who plan to apply to the hospital based on these several complaints.

According to the physicians, routine smear and HPV scanning (1.90) are mostly deferrable regardless of time. But colposcopy/cervical biopsy in cervical preinvasive lesions (3.00) was found to be deferrable for a while, not urgent. According to American Society for Colposcopy and Cervical Pathology (ASCCP), individuals with low-grade cervical cancer screening tests may have postponement for 6-12 months and individuals with high-grade cervical screening tests should have diagnostic evaluation within 3 months (24). This information is also correlated with our study results. While diagnostic hysteroscopy /laparoscopy procedures (2.41, 2.04) for gynecological diseases were founded to be deferrable regardless of time, operative hysteroscopy /laparoscopy procedures (2.93, 2.70) were found to be deferrable for a while. Generally according to our study, while the procedures used in the diagnosis of gynecological diseases can be postponed regardless of time, the procedures for treatment have only been postponed for a while.

In the current study, the only condition among gynecological causes, which was founded to be urgent and cannot be deferrable, was gynecological cancers (4.31). Even in the pandemic process, the delay in cancer treatment was found unacceptable by clinicians. But at that point, gynecologic and other cancer associations were more cautious. They evaluated the cancers as low-risk, high-risk and advanced disease and suggested degreed and softened therapy strategies. For example, in the low-risk endometrial cancer, they suggested non-surgical options like hormonal intrauterine devices. In ovarian cancer, in patients who have already taken neoadjuvant chemotherapy, extending the chemotherapy time to six cycles, rather than three, and then planning the interval cytoreductive surgery (24).

According to the clinicians both infertility research and infertility treatments were found to be deferrable regardless of time (1.49, 1.61). International Federation of Fertility Societies (IFFS) has conducted an online survey with respondents from 93 countries. The study showed that the fertility treatment was not considered as an essential health service during the pandemic. Most of the countries modified their policies about fertility treatment; and many artificial reproductive technologies (ART) center had been closed or presented limited opportunities for some special conditions (25). Unlike these considerations, according to the document prepared by the European Society of Human Reproduction and Embryology (ESHRE) COVID-19 working group, while our internet survey was continuing, as the COVID-19 pandemic is getting stabilized, the return to normal life will also need to restart of ART treatments. They say that infertility is an important disease and the spreading of the pandemic is decreasing, all ART treatments can be restarted for any clinical indication, in line with local regulations (26).

In our study, we investigated the effects of pandemic in obstetrics and gynecology clinics. We think that the study will help in determining our approach to obstetrical and gynecological cases in the future. The effects of a pandemic on health systems should be examined on the basis of each unit from smallest to biggest: clinic, hospital, city, country. Other studies together with this study, investigating delay times or urgency categorization in diagnosis and treatments of obstetrical and gynecological diseases will give directions to the clinics and health system managers. Scenarios should be made patient-centered without neglecting the burden and possible damages on healthcare professionals.

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Conflict of interest

The authors certify that they have no financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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The importance of aminoguanidine and methylprednisolone administration in lung contusion after chest trauma

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Abstract

This study aims to evaluate the effect of the antioxidant and anti-inflammatory properties of aminoguanidine and metylprednisolone (MP) on lung tissue in a pulmonary contusion model of rats and evaluate whether their combined use improves treatment efficacy. This study included 35 female Sprague Dawley rats weighing 250-300 grams. The rats were divided into five groups as following: Sham; Pulmonary Contusion (PC); PC+MP, PC group treated with i.p methylprednisolone; PC+AG, PC group treated with i.p Aminoguanidine; and PC+AG+MP, PC group treated with Aminoguanidine and methylprednisolone. Each group had seven animals. Blood and lung tissues were studied biochemically and histopathologically. When compared groups according to serum levels of biomarkers, serum YKL-40, nitrate-nitrite, catalase, and TBARS levels were significant different. Serum YKL-40 levels were decreased after treatments in three groups. The serum YKL-40 levels in PC+AG group were lower than the other treatment groups, especially compared to PC + MP ($p=0.028$). Serum nitrate-nitrite levels were decreased in all treatment groups (PC+MP, PC+AG and PC+MP+AG). The lowest levels were measured in PC+MP+AG; but there was no statistically significant difference compared to PC group ($p>0.05$). Serum catalase levels were increased in all treatment groups. The higher levels were measured in PC+MP+AG than the other single treatment groups; however, PC+MP+AG and PC+MP were statistically significant different compared to PC group ($p=0.001$ and $p=0.002$ respectively). Serum TBARS levels were decreased in all treatment groups compared to Sham group ($p<0.001$) and PC group ($p<0.001$). The lowest levels were measured in PC+MP+AG compared to PC group ($p<0.001$). Histopathologic and immunohistochemical staining scores were decreased at all the treatment groups, especially PC+MP+AG. We suggest the use of combined treatment of methylprednisolone and aminoguanidine for the treatment of pulmonary contusion.

Keywords: aminoguanidine, lung contusion, methylprednisolone, oxidative stress

1. Introduction

Trauma is one of the most common cause of mortality, morbidity, and hospitalization in the world. According to the Centers for Disease Control and Prevention in United States of America, 169.936 deaths occurred from unintentional injury in 2017 (1). Blunt chest injury can affect any part of thorax. Lung contusions are the most common injury occurred after blunt chest trauma in all aged trauma-patients. Patients' clinical status with lung contusions may vary from asymptomatic to severe acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) that requires intensive care and results in a high mortality rate.

At the molecular level, data obtained from animal experiments supports a mediator-driven inflammatory process that further leads to respiratory compromise after chest trauma. Endothelial cell activation occurs, and it may result in mediator generation. Similarly, pro-inflammatory signaling molecules (tumor necrosis factor (TNF), IL-1β, angiopoietin 2, vascular endothelial growth factor, platelet-activating factor and others) are released after blunt chest trauma (2). When these substances are released, systemic vascular permeability frequently increases, and it often contributes to hypovolemia and causes multiple organ failure (3).

To understand the pathophysiology at the molecular level may provoke to develop the new methods of treatment and improve the prognosis of trauma patients. Many studies have been demonstrated to clarify the pathological - physiological pathways, intra - extracellular changes, and inflammation in lung tissue (2, 4, 5). The objective of the present study was to determine the effect of aminoguanidine and metylprednisolone (MP) on lung tissue of rats and evaluate whether their combined use improves treatment efficacy in an animal model of pulmonary contusion.

2. Materials and methods

2.1. Ethical statement

This study was approved by The Ethics Committee of Ondokuz Mayıs University for Experimental Animal Studies. Efforts were made to minimize animal suffering and reduce the number of animals used in experimental groups. All animals were treated in accordance with The World Health Organization Ethical Code for Animal Experimentation.

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2.2. Experimental animals and procedures

A total of 35 healthy female Sprague Dawley rats with weights of 250-300 g were used as experimental animals. Feeding and tap water were provided ad libitum. All rats were kept in windowless animal quarters with temperature automatically maintained at 24ºC and controlled lighting (12-hour light/ 12-hour dark cycle) and humidity (55-60%).

The rats were divided into five groups as following: Sham (control); PC; PC+MP, PC group treated with i.p MP (20 mg Prednol-L; Mustafa Nevzat, Turkey); PC+AG, PC group treated with i.p AG (Aminoguanidine hemi sulfate salt; Sigma-Aldrich, St. Louis, MO, USA); and PC+AG+MP, PC group treated with AG + MP. Each group had seven animals.

The rats in the PC groups were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and subjected to chest trauma with 1.96 J of impact energy as described by Raghavendran K. et al. (2). The impact energy (E) of the falling weight was calculated from the following equation: E = m (0.4 kg) × g (9.8 m/s^2) × h (50 cm) (6). The rats in the PC + MP group were injected with MP i.p. once a day (30 mg/kg injected 5 min after the trauma on day 1 and 5 mg/kg from days 2 to 7) (7). The rats in the PC + AG group were administered AG i.p (150 mg/kg administered daily during one week after the trauma). The rats in the PC + MP + AG group were administered i.p. the 5 mg/kg/day doses of MP and the 150 mg/kg/day doses of AG for seven days. All rats were kept under observation until they recovered from the experimental procedure. After seven days of treatment, all rats were sacrificed with i.p. ketamine hydrochloride and xylazine injections. The lungs were removed from the thorax for histopathological and immunohistochemical analyses.

2.3. Histopathological studies

Lung tissue samples obtained from midsagittal slices of the lungs were fixed in 10% formalin and embedded in paraffin. The samples were stained with hematoxylin and eosin (H&E) for microscopic examination. A histopathological evaluation was performed in at least 8 randomly selected microscopic high-power fields from each tissue samples. Subsequently, all slides were examined by a pathologist blinded to the study groups, and they scored all microscopic slides according to degree of peribronchial inflammatory cell infiltration (PICI), alveolar septal infiltration (ASI), alveolar edema (AED), alveolar exudate (AEX), alveolar histiocytes (AHI), interstitial fibrosis (IF), granuloma (GRA), and necrosis (NEC) formation using the 4-point scale developed by Takil et al. (8) Table 1.

2.4. Immunohistochemistry procedure

Lung tissue samples were fixed in 10% neutral- buffered formalin and embedded in paraffin. All samples were sectioned at a thickness of 5 µm. Additional sections, placed on 3-aminopropyl triethoxysilane (Sigma, St. Louis, MO, USA) coated slides, were stained by the streptavidin-biotin-peroxidase complex (SBPC) technique (Histostain Plus Kit; Zymed, cat no: 85-8943, California, USA). Rabbit polyclonal anti inducible nitric oxide synthase (iNOS) antibody (1/250; Abcam, cat no: ab3523, UK) was used as primary antibody. Aminoethyl carbazole was used as the chromogen in H_2O_2 for 10 minutes, which was controlled by visual observation with a microscope. The sections were counterstained with Mayer's hematoxylin for 1 minute and rinsed with tap water. Subsequently, the sections were mounted with an aqueous mounting medium. Immunohistochemical iNOS staining of the lung tissue slides was evaluated semi quantitatively according to intensity for the differences between each experimental group (Fig. 1). Staining intensity of iNOS were recorded as faint (−/+), mild (+), moderate (++), and strong (+++). The evaluation of immunostaining was performed in at least 8 randomly selected areas per lung section, using 2 sections from each animal at 400×magnification. The final score calculated in each category for each individual rat was the mean of the scores from the sections of the lungs examined.

Table 1. Parameters for histopathologic evaluation (4-point scale)

	0	1	2	3
PICI	No	No Prominent germinal centers of lymphoid follicles	Infiltration between lymphoid follicles	Confluent bandlike form
ASI	No	Minimal	Moderate	Severe, impending of lumen
AED	No	Focal	In multiple alveoli	Widespread, involving lobules
AEX	No	Focal	In multiple alveoli	Prominent, widespread
AHI	No	Scattered in a few alveoli	Forming clusters in alveolar spaces	Filling the alveolar spaces
IF	No	Focal, minimal	No Focal, prominent fibrous thickening	Widespread, prominent fibrous thickening

2.5. Biochemical analysis

Sample preparation

After the rats were sacrificed, blood samples were collected in sterile test tubes without anticoagulants. Whole blood was allowed to clot at room temperature for 30 minutes. Then, the samples were centrifuged at 3000xg for ten minutes at 4°C. Following centrifugation, the serum was removed and transferred into a clean tube. All samples were stored at -80°C'de until analysis. A day before measuring, all samples were unfrozen at 2-80°C.

Measurement of serum TBARS levels

TBARS were measured using the TBARS assay kit (Catalog No. 10009055, Cayman Chemical Company, Ann Arbor, MI, USA). The principle of the test is the formation of the Malondialdehyde-Thiobarbituric acid (MDA-TBA) adduct by the reaction of MDA and TBA under high temperature (90-

100 °C) and acidic conditions. The concentration of the MDATBA adduct was measured colorimetrically at 530 and 540 nm. The results have been presented in micromoles per liter.

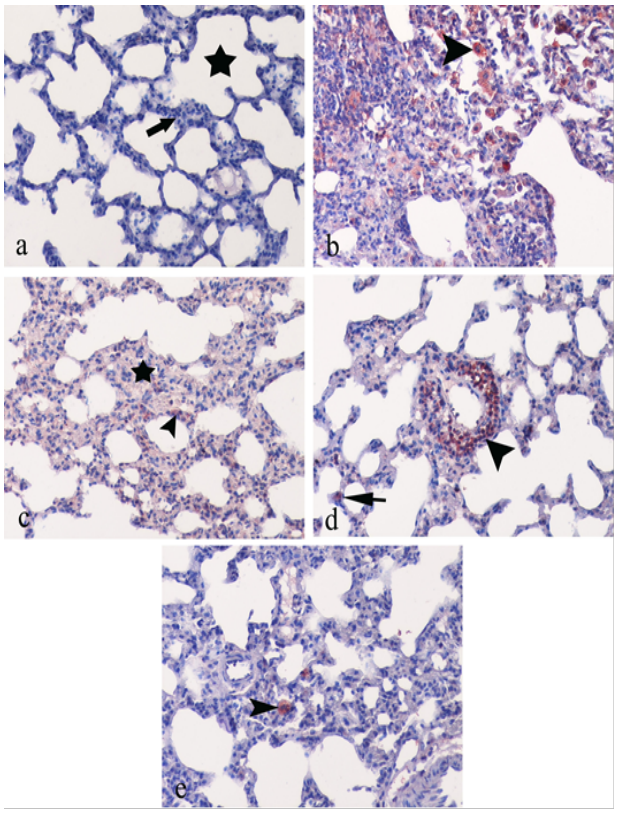

Fig. 1. Immunohistochemical evaluation of lung tissues. a) Sham group, normal alveolar structure (star) and there is no immunopositive cells in the alveolar septal tissue (arrow); b) PC group, severe immune-positive staining of alveolar macrophages (arrowhead) and interstitial tissue; c) PC + AG group, moderate alveolar septal infiltration (star) and minimal immunopositive reaction of interstitial cells (arrow head); d) PC+MP group, severe immunopositive reaction of the inflammatory cells in the perivascular area (arrow head) but minimal positive reaction of interstitial cells (arrow). e) PC+AG+MP group, there is minimal immunopositive reaction of interstitial cells (arrowhead). IHC staining, counter stain with Lille Mayer Alum Hematoxylin x 20. Sham, control; PC, pulmonary contusion; PC + MP, PC group treated with methylprednisolone; PC + AG, PC group treated with aminoguanidine; PC + MP + AG, PC group treated with MP and AG

Measurement of serum Cartilage Glycoprotein 39 levels

Serum Cartilage Glycoprotein 39 (YKL-40) levels were measured using the YKL-40 ELISA kit (CK-E90203, Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China) according to manufacturer's procedures. The results of test have been presented as ng/mL using a double antibody sandwich ELISA method.

Measurement of serum Nitrate/Nitrite levels

Serum Nitrate/Nitrite levels were measured in accordance with the procedures specified by the manufacturer using the Nitrate/Nitrite Colorimetric Assay kit (780001, Cayman Chemical Company, MI, U.S.A.). In this two-step colorimetric test, nitrate was first converted to nitrite, then nitrite to azo compound. The absorbance of this azo compound was measured photometrically at 540 nm. Total Nitrate / Nitrite concentration was given as µmol / L.

Measurement of serum Catalase levels

Serum catalase levels were measured in accordance with the procedures specified by the manufacturer using Catalase Assay kit (707002, Cayman Chemical Company, MI, U.S.A.), a colorimetric test based on formaldehyde formation. Test results were given in nmol/min/ml.

2.6. Statistical analysis

Biochemical results and histopathological scores were analyzed using IBM SPSS 21.0 for Windows. The results were presented as median (minimum/maximum) or mean ± standard deviation. Statistical differences between different groups were analyzed with one-way ANOVA and Tukey's and Tamhane tests were used for post hoc in parametric data. Kruskal Wallis were used for group analysis as a nonparametric test. Homogeneity of variance were made to all groups. Differences were considered significant at $p<0.05$.

3. Results

3.1. Biochemical examination

When compared groups according to serum levels of biomarkers, serum YKL-40, nitrate-nitrite, catalase, and TBARS levels were significant different ($p=0.002$, $p=0.006$, $p<0.001$ and $p<0.001$ respectively), (Table 2).

Table 2. The comparison of serum YKL-40, Nitrate-Nitrite, Catalase and TBARS levels in all experimental groups

	YKL-40 (ng/ml)	Nitrate-Nitrite (µM/l)	Catalase (nmol/min/mL)	TBARS (µmol/l)
Sham	0.62 ± 0.09	6.86 ± 1.37	120.85 ± 34.40	7.14 ± 1.01
PC	0.77 ± 0.04 [a]	21.14 ± 7.37 [a]	32.60 ± 20.23 [a]	17.50 ± 2.12 [a]
PC + MP	0.75 ± 0.03	18.66 ± 8.03	98.14 ± 32.30 [b]	12.42 ± 1.40 [b]
PC + AG	0.63 ± 0.11 [b]	18.27 ± 8.66	75.04 ± 31.27	10.84 ± 1.91 [b]
PC + MP + AG	0.68 ± 0.05	15.13 ± 6.59	101.75 ± 27.29 [b]	10.25 ± 0.92 [b]

The values were given as mean ± standard deviation. Sham, control; PC, pulmonary contusion; PC + MP, PC group treated with methylprednisolone; PC + AG, PC group treated with aminoguanidine; PC + MP + AG, PC group treated with MP and AG; YKL-40, human cartilage glycoprotein 39 (chitinase-3-like protein-1); TBARS, thiobarbituric acid reactive substances. The comparisons of groups were abbreviated as "a" and "b". "a" means significantly different (at $p<0.05$) from sham group. "b" means significantly different (at $p<0.05$) from PC group

According to serum YKL-40 levels, PC group was statistically significant different compared to Sham group (p=0.011). Serum YKL-40 levels were decreased after treatments in three groups. The serum YKL-40 levels in PC+AG group were lower than the other treatment groups, especially compared to PC + MP (p=0.028). There was a statistically significant difference only in PC+AG group compared to PC group (p=0.013). However, PC+MP group was significantly statistical different compared to Sham group (p=0.024) (Table 2).

According to serum nitrate-nitrite levels, PC group was significant different compared to Sham group (p=0.019). Serum nitrate-nitrite levels were decreased in all treatment groups (PC+MP, PC+AG and PC+MP+AG). The lowest levels were measured in PC+MP+AG; but there was no statistically significant difference compared to PC group (p>0.05), (Table 2). When PC+MP+AG compared to PC+AG and PC+MP, there was no statistically significant difference (p>0.05).

According to serum catalase levels, PC group with the highest levels was significant different compared to Sham group (p<0.001). Serum catalase levels were increased in all treatment groups. The higher levels were measured in PC+MP+AG than the other single treatment groups; however, PC+MP+AG and PC+MP were statistically significant different compared to PC group (p=0.001 and p=0.002 respectively). When PC+MP+AG compared to PC+MP, there was no statistically significant difference (p>0.05), (Table 2).

According to serum TBARS levels, PC group was significant different compared to Sham group (p<0.001). Serum TBARS levels were decreased in all treatment groups compared to Sham group (p<0.001) and PC group (p<0.001). The lowest levels were measured in PC+MP+AG compared to PC group (p<0.001), (Table 2). When compared to PC+MP, PC+MP+AG was statistically significant different (p=0.036); but there was no significant difference compared to PC+AG (p>0.05), (Table 2).

3.2. Histopathological and immunohistochemical findings

Images of histopathological sections belonged to all study groups are shown in Fig 2. All groups compared to PC and sham according to histopathologic scores including PICI, ASI, AED, AEX and AHI. Scores of histopathological and immunohistochemical staining belonged to all study groups are shown in Table 3. There were statistically significant differences in the distribution of PICI, ASI, AED, AEX and AHI without IF across groups (p<0.001, p<0.001, p<0.001, p<0.001, and p<0.001); IF scores were similar in all groups (measured IF score: 0), (p>0.05).

After the histopathological evaluations of groups, there was a significant statistical difference between Sham and PC group (p<0.001). Median of PICI scores in three treatment groups was 1. PC+MP group was significant different compared to Sham group according to PICI (p=0.043). There was no significant difference in PC+AG compared to PC according to PICI scores (p=0.077). PC+MP+AG was statistically significant different to compared to PC group according to PICI scores (p=0.008). In order to ASI scores, PC group was significant different compared to Sham (p<0.001). Although median of all treatment group in ASI scores were lower than PC group, there was no statistically significant difference compared to PC (p>0.05). But PC+AG was statistically significant different than Sham according to ASI scores (p=0.006). According to AED scores, PC was statistically significant different compared to Sham (p<0.001). PC+MP+AG had the lowest median according AED scores compared the other treatment groups. But there was statistically significant difference in PC+MP+AG compared to PC group (p=0.022). According to AEX scores, there was a statistically significant difference between Sham and PC groups (p=0.001).

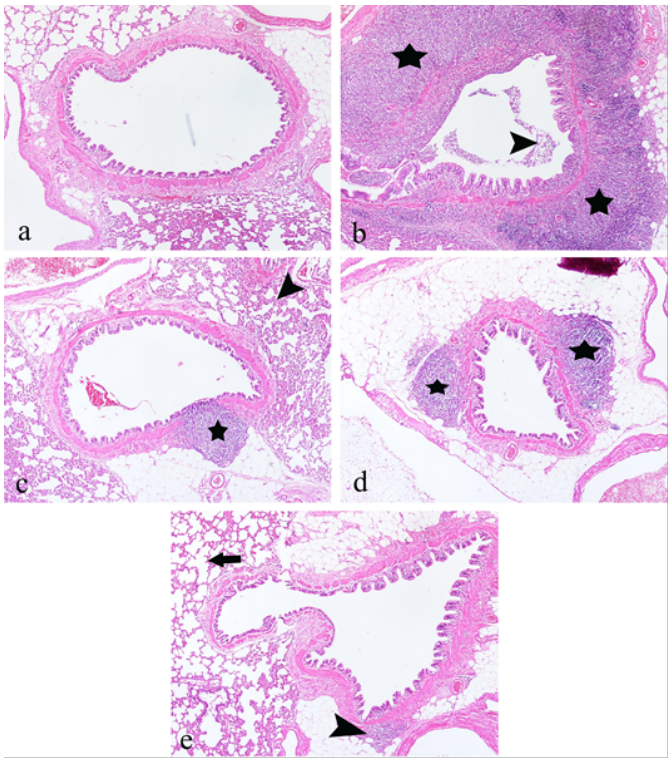

Fig. 2. Histopathological evaluation of lung tissues. a) Sham group, normal histopathologic appearance of lung tissues, there is no peribronchial infiltration and alveolar septal infiltration, HE x4; b) PC group, there is severe infiltration with lymphoid follicles in the bronchial wall (stars) and necrotic cells in the bronchial lumen (arrow heads) HE x 4; c) PC + AG group, there is only one lymphoid follicle with prominent germinal center in the bronchial wall (star) and minimal alveolar septal infiltration (arrow head) HE x 4; d) PC + MP group, there are two lymphoid follicles with prominent germinal center in the bronchial wall (stars) HE x 4; e) PC+AG + MP group, minimal mononuclear cell infiltration in the peribronchial area (arrowhead) and normal alveolar tissue (arrow) HE x4. Sham, control; PC, pulmonary contusion; PC + MP, PC group treated with methylprednisolone; PC + AG, PC group treated with aminoguanidine; PC + MP + AG, PC group treated with MP and AG

Table 3. The comparison of histopathologic and immunohistochemical scores between all groups

	Sham	PC	PC+MP	PC+AG	PC+MP+AG
PICI	0	3 (2-3) [a]	1 (0-2)	1	1 (0-1) [b]
ASI	0	2 (1-2) [a]	1 (0-2) [b]	1 (1-2)	1 (0-1) [b]
AED	0	2 (1-2) [a]	1 (0-1)	1 (0-1)	0 (0-1) [b]
AEX	0	2 (1-2) [a]	1 (0-1)	1 (0-1)	0 (0-1) [b]
AHI	0	2 (1-2) [a]	1 (0-1)	1 (0-1)	0 (0-1) [b]
IF	0	0	0	0	0
IHC	0	2 (1-3) [a]	2 (0-2)	1 (0-2)	0 (0-1) [b]

The values were given as median (min-max). PICI, peribronchial inflammatory cell infiltration; ASI, alveolar septal infiltration; AED, alveolar edema; AEX, alveolar exudate; AHI, alveolar histiocytes; IF, interstitial fibrosis; IHC, immunohistochemical; Sham, control; PC, pulmonary contusion; PC + MP, PC group treated with methylprednisolone; PC + AG, PC group treated with aminoguanidine; PC + MP + AG, PC group treated with MP and AG. The comparisons of groups were abbreviated as “a” and “b”. “a” means significantly different (at $p<0.05$) from sham group. “b” means significantly different (at $p<0.05$) from PC group

PC+MP+AG group has the lowest median according to AEX scores compared to the other treatment groups. PC+MP+AG was a statistically significant different compared to PC group ($p=0.004$). According to AHI scores, there was a statistically significant difference between Sham and PC groups ($p<0.001$). The lowest median of AHI score was in PC+MP+AG group between treatment groups. PC+MP +AG group was statistically significant different compared to PC according to AHI scores ($p=0.006$). IF score of all groups including Sham, PC and three treatment groups was 0/4 and all groups were similar.

Images of immunohistochemical staining sections belonged to all study groups are shown in Fig. 1. According to the immunohistochemical evaluation, there was a statistically significant difference between groups ($p<0.001$). There was no statistically significant difference between Sham and PC groups ($p=0.631$). The lowest median of IHC score was in PC+MP+AG group and this combined treatment group, PC+MP+AG was significant different compared to PC group ($p=0.008$). To IHC staining scores, PC+MP was statistically significant different compared to Sham ($p=0.033$). Histopathologic and immunohistochemical staining scores of all groups were shown in Table 3.

4. Discussion

The presence of pulmonary contusion is an important risk factor in the progression of ALI to ARDS with increased rate of mortality (2). Therefore, the effects of antioxidant and anti-inflammatory properties of aminoguanidine and methylprednisolone on damage lung tissue in a pulmonary contusion rat model were evaluated in this study.

According to results, the combined administration of aminoguanidine and methylprednisolone significantly reduced all the histopathologic scores of pulmonary contusions (PICI, AED, AEX, AHI and IHC). While the combined administration of AG and MP had lowest scores of PICI, AED, AEX, AHI and IHC; the combined treatment was not significant different compared to the single administration of AG and the single use of MP separately. At the same time, both single therapy of AG and MP reduced the histopathologic scores even though not as the combined administration of AG and MP. This suggests the availability of aminoguanidine as an alternative to methylprednisolone to avoid its side effects of steroidal drugs.

YKL-40 (chitinase-3-like protein-1 (Chi3-l1), breast regression protein 39 (BRP-39) and chondrex) is a proinflammatory glycopeptide that belongs to the family of chitinase like proteins. It is expressed in many cell types including ductal and airway epithelial cells (9) and YKL-40 increases in presence of inflammation, tissue remodeling and cancer. YKL-40 levels are a predictor of all-cause mortality in the elderly (10) and have a significant association with rates of overall and cardiovascular mortality (11). The exact biological activities are yet to be identified (9). In this study, the lowest level of YKL-40 was found in the group treated aminoguanidine which is significantly different compared to PC group. Interestingly, there was no significant difference between PC and combined administration of AG and MP which also reduced the YKL-40 levels compared to PC.

Nitric oxide (NO) plays many physiological and pathophysiological roles in widespread fields. NO, synthesized in various tissues and cells, is vasodilator substance, neurotransmitter, and a killer molecule in the immune system (12). The production of NO, synthesized by NO synthase (NOS) catalytic action. It is usually evaluated by measuring nitrates and nitrites as the final stable oxidized products of its metabolism.

The sum of nitrites and nitrates (named NOx) is a good indicator of NO formation, and it shows NOS activities in many pathophysiological conditions (13). However, it is difficult to measure NO directly because NO is quite short lived. Alternatively, NOx (nitrite and nitrate) is measured as an indirect marker of NO formation. However, serum or plasma NOx concentrations are influenced by diseases such as heart failure, sepsis, and liver cirrhosis (12). In this study, the PC group that had the highest level of serum nitrate-nitrite levels was significantly different compared to Sham group. However, the combined treatment group (PC+MP+ AG) had lower levels compared to PC, PC+AG and PC+MP, but there was no statistically difference between groups.

The term “oxidative stress” indicates that the antioxidants status of cells and tissues is altered exposure to oxidants that

cause the diseases. The reducing environment is related to the interaction of oxidative metabolism and antioxidant substances and enzymes which serve to remove reactive oxygen species such as thioredoxin, vitamin E and C, glutathione, superoxide dismutase (SOD), catalase, the selenium dependent glutathione and thioredoxin hydroperoxides (14). This situation plays an important role in lung damage.

Catalase, present in peroxisomes, catalyzes the decomposition of hydrogen peroxide (15, 16). In this study, PC group was significant different compared to Sham group. The combined treatment significantly increased the catalase levels as similar as in the group treated with MP.

To evaluate free radical generation and peroxidative damage in the airways, the measurement of hydrogen peroxide (H2O2) and thiobarbituric acid reactive substances (TBARS) is suggested. Hydrogen peroxide and/or TBARS increase in many inflammatory lung injuries. TBARS are occurred after polyunsaturated fatty acid peroxidation; however, they are also formed during oxidative injury of DNA, proteins, or carbohydrates. Therefore, TBARS is commonly used marker of oxidative stress (17). In this study, the increased levels of TBARS were found in PC group; controversially all the treatment groups had lower TBARS levels. The combined group was significantly different compared to PC as similar as the other treatment groups PC+AG and PC+MP.

Steroids become prominent with their strong anti-inflammatory functions. Although the administration of high doses of steroids are used in the practice, the use of steroids for the treatment of pulmonary contusion has rarely addressed in the literature and this does not provide enough evidence. Fransz et al. (18) administered methylprednisolone 30 minutes after experimental pulmonary contusion model in dogs. The weight ratio of contused to normal lung was significantly decreased in treated animals and the volume of injury was less on postmortem. Since the animals were killed, the effect of steroids on recovery and survival could not be assessed. In a small retrospective human study, Svennevig et al. (19) concluded that the mortality in severe chest injury was reduced using steroids (20). Although there are studies suggesting that steroids may be useful in lung contusion, the presence of defects on study randomization, administration of steroid and other deficiencies in these studies are noteworthy. In this study, PC+MP group was lower histopathologic scores compared to PC group; but PC+MP group was significant different in only ASI score compared to PC. In addition, PC+MP group was significant different according the TBARS levels compared to PC group; but there was no significant difference compared to combined therapy group (PC+AG+MP). However, in this study, single MP treatment increased the catalase levels and decreased the TBARS levels.

In a conclusion, we suggest the use of combined treatment of methylprednisolone and aminoguanidine for the treatment of pulmonary contusion. This treatment regimen seems to be more effective for treating lung injury after blunt chest trauma.

Conflict of interest

The authors declare that they have no conflict of interest.

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Comparison of subacromial corticosteroid injection and physical therapy in patients with subacromial impingement syndrome: A prospective, randomized trial

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Abstract

The aim of this randomized trial was to evaluate the efficacy of subacromial corticosteroid injection and physical therapy (PT) in patients with subacromial impingement syndrome (SIS). Forty patients who diagnosed as SIS were included in this study and were randomly assigned to the PT and injection groups. Pain during rest, sleep and motion were evaluated by visual analog scale (VAS). Disability was determined by Costant-Murley score. Physical and social functions were evaluated with Short Form-36 (SF-36). Active range of motion (ROM) was measured by goniometer. Patients were evaluated at baseline, 3rd and 8th weeks of the therapy. PT continued for 3 weeks with ultrasound and interferential current combined to local heat and exercise. For patients in the injection group, a single steroid injection of 40 mg triamcinolone acetonide into the subacromial space was combined with exercise. After eight weeks, significant improvements at pain, SF-36 and Costant-Murley scores were observed in both groups ($p<0.001$). Improvement rates of pain during sleep and motion were significantly higher in PT group than the injection group after 8 weeks ($p<0.01$). Significant improvements were determined at ROM in both groups ($p<0.001$). No statistically significant differences were found between two groups in terms of Costant-Murley and ROM scores. Results of the PT group were significantly better in terms of physical and social function, and pain subscores of SF-36 ($p<0.05$). Our results suggest that both PT and corticosteroid injection have beneficial effects on shoulder mobility and pain relief in SIS. PT should be an alternative and effective treatment method to corticosteroid injection in SIS.

Keywords: corticosteroid injection, interferential current, subacromial impingement syndrome, ultrasound

1. Introduction

Shoulder impingement syndrome is commonly referred to as painful arc syndrome, subacromial impingement syndrome (SIS), supraspinatus syndrome. SIS and rotator cuff tendinitis are considered to be the most common cause of shoulder pain and disability. SIS is a clinical syndrome that indicates pain and pathology related with the encroachment of the subacromial tissues as a result of the narrowing of the subacromial space (1).

The cause of SIS is considered to be multifactorial with both extrinsic and intrinsic factors involved in its pathogenesis. Extrinsic factors such as acromial shape and subacromial spurs, often associated with acromioclavicular joint arthritis, have been described. Intrinsic factors such as rotator cuff dysfunction (RCD) leading to superior head migration, and scapular dyskinesis leading to scapular malposition, also contribute and can be reversed with physical therapy. These injured tissues incite a local inflammatory response, which leads to tissue edema and pain (1, 2).

Many treatment modalities have been employed in attempts to relieve pain and restore function of the affected shoulder. Nonoperative treatment options for SIS include rest, ice, physical therapy (PT), electromagnetic radiation, corticosteroid injections, and systemic nonsteroidal anti-inflammatory drugs (NSAIDs) (3).

There is still little knowledge about the efficacy of most conservative treatment options for SIS. The injection of corticosteroids into the subacromial space is a procedure commonly performed for impingement. However, there is no consensus about whether subacromial injection is superior to other conservative treatment options. Corticosteroids have been reported to be associated with tendon rupture, subcutaneous atrophy, and articular cartilage changes. Due to the potentially serious side effects, frequent use of corticosteroid injections is avoided (4).

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There are limited number of studies evaluated the effect of PT intervention in patients with SIS. Although ultrasound (US) is the frequently used as a PT method for soft tissue disorders, there are limited and conflicting data regarding the effectiviness of US therapy in SIS (3,5). In the present study, we aimed to compare the efficacy of subacromial corticosteroid injection and ultrasound in the treatment of SIS.

2. Materials and methods

The study was planned as a prospective, randomized, clinical trial with an 8-week follow-up period. It consisted of 40 consecutive patients, who were suffering from shoulder pain and diagnosed as SIS, with physical examination and Magnetic Resonance Imaging (MRI) at Ondokuz Mayıs University, Medical Faculty, Department of Physical Medicine and Rehabilitation Clinic. Exclusion criteria were as follows; instability of shoulder, infection, decompensated heart failure, cardiac pace-maker, status angina, asthma, seizure, neurological deficiency, shoulder and neck surgery, history of PT and corticosteroid injection at last six months, pregnancy, positive drop arm test, rupture of RC, adhesive capsulitis (AC), rheumatologic disases, cervical radiculopathy, hemorrhagic diathesis and diabetes mellitus**.** The study was approved by the local ethics committee and all patients gave their written informed consent.

All participants were questioned about demographic and clinical characteristics. In physical examination Neer, Hawkins, Painful arc, Drop arm, Supraspinatus tests were evaluated. Active ROM degrees were measured by a goniometer, at supine position by the same physician blinded to the treatment of patients. Rotations were measured while shoulder was at 90° abduction. Degrees of abduction, flexion, internal rotation, and external rotation were recorded.

Pain at rest, motion and sleep periods were evaluated with 10cm (0= no pain, 10= severe pain) visual analog scale (VAS).

Shoulder functions were evaluated with Constant-Murley Shoulder Score. It is an easy, cheap, and reliable scale which has been used for the results of surgery, conservative treatment and traumatic shoulders since 1980s. There are 100 points including 15 points for pain, 20 points for daily activities, 40 points for active ROM and 25 points for strength (6).

Daily living activities were evaluated with Short Form-36 (SF-36), which was found reliable and valid in Turkish population (7). Forty patients were randomly assigned into two groups. In PT group (n=20), hot pack for 20 minutes, US for seven minutes (1.5 Watt/cm^2, constant mode, 1MHz) and interferential current (IC) for 20 minutes (100 Hz) were applied to the patients, five times per week for three weeks.

In injection group, subacromial injection was performed by the same physician with 40 mg/1ml triamcinolone acetonide. For every patient, a single injection was applied with lateral approach, by 5cc-21-gauge injector, in sterile conditions, to the subacromial space after marking the lateral side of acromion.

Passive ROM, stretching and pendulum exercises were started in both groups. After gaining full ROM, isometric-isotonic strenghtening exercises for RC and scapula stabilizer muscles were started with dumbbell and therabands. In PT group, exercises were applied in hospital twice per day with 10 repetitions while they were performed at home with the same conditions in injection group. All patients were allowed to use 275 mg Naproxen Sodium, twice per day at the first three weeks of the therapy. All subjects were evaluated by the same physician at the baseline, at the end of 3th week and 8th week.

2.1. Statistical analysis

Statistical analyses were performed using SPSS software, version 15.0. For continuous variables Mean ± Standard Deviation (Mean±SD), and for frequencies percents (%) were reported. All variables were analyzed for normally distribution by Shapiro Wilk test. In group comparisons Paired t-test and Wilcoxon test were used. Mann Whitney u test and t-test were used for intergroup comparisons. In group comparisons, Mc Nemar Chi square test was used for the countable variables, and in intergroup comparisons Chi square test was used. P<0.05 was accepted significant for all statistical measurements.

3. Results

Forty patients-23 female (57.5%) and 17 male (42.5%)-were included in the study. Ages ranged from 29 to 63. There were no differences between groups in terms of age, gender and duration of the pain (p>0.05)**.** The demographic characteristics of the patients are shown in Table 1**.**

Table 1. Demographic characteristics

		Injection Group Means±SD	Physical Therapy Group Means±SD	p
Age (years)		50.10 ±7.91	48.25 ± 9.80	0.745
Complaint Period (months)		10.95 ±6.64	9.55 ±10.10	0.287
		Number (%)	**Number (%)**	
Gender	**Female**	11(55)	12 (60)	0.749
	Male	9(45)	8 (40)	
Education	**Primary school**	14(70)	8 (40)	0.191
	Secondary school	1 (5)	2 (10)	
	High school	3(15)	3 (15)	
	College	2(10)	7 (35)	
Occupation	**Housewife**	9(45)	8 (40)	0.082
	Laborer	6(30)	2 (10)	
	Retired	4(20)	3 (15)	
	Officer	1 (5)	7 (35)	

p<0.05; significant, SD; standard deviation

Right side affected patients were 13 (65**%**) in injection group and 12 (60**%**) in PT group. Dominant hand of most patients was right; 17 (85**%**) in injection group and 18 (90**%**) in PT group, respectively. There were no differences between the groups for dominant hands and affected sides (p>0.05).

In injection group, Neer test which was positive in 90% of the patients at the baseline decreased to 35% and 15% at the end of 3rd week and 8th week. In PT group Neer test which was positive in all patients at the baseline decreased to 35% and 5% at the end of 3rd week and 8th week. Hawkins tests were positive in all patients in both groups at the baseline. In injection group they decreased to 65% and 40% at the end of 3rd week and 8th week. In PT group, these rates were 60% and 15% respectively**.**

The mean ROM measurements improved significantly in both groups at the 3rd and 8th weeks (p<0.001 for each). All measurements were better in PT group than injection group, but there were no significant differences between groups for active ROM at the end of 3rd week and 8th week (p>0.05) (Table 2).

Table 2. Comparison of range of motion

		Injection Group	Physical Therapy Group	p
		Means±SD	Means±SD	
Flexion	Baseline	126.5±39.1	126.5±35.13	0.978
	3th week	146.5±31.83	163.0±19.76	0.108
	8th week	159.0 ±28.26	171.5±20.84	0.091
Abduction	Baseline	122.75±42.41	120.0±37.55	0.838
	3th week	149.50±27.99	163.75±14.9	0.145
	8th week	160.50±24.59	170.5±20.89	0.123
Internal Rotation	Baseline	55.25±13.71	51.75±12.69	0.397
	3th week	64.00±12.31	67.75±8.02	0.333
	8th week	70.25±9.79	75.50±8.87	0.303
External Rotation	Baseline	64.50±15.03	61.00±17.13	0.527
	3th week	75.75±13.50	80.75±9.21	0.285
	8th week	80.25±13.42	86.00±0.94	0.060

p<0.05; significant, SD; standard deviation

At 3rd week, both treatment methods appeared to have similar efficacy on pain and disability**.** Both treatment options were similarly effective in improving the mean VAS activity pain and Constant-Murley scores. At the end of an 8-week follow-up period, the activity pain and Constant-Murley scores were significantly improved in both of the groups without significant difference between two treatment methods. There was a significant difference between the two groups with regard to the night pain and activity pain sores at 8th week. After 8 weeks, VAS pain scores at motion and at sleep were significantly better in PT group than the injection group (Table 3).

When we compared both groups for SF-36, physical function, social function, emotional role function and pain subscores in PT group were significantly better than the injection group at the end of 8th week (p<0.05 each other) (Table 4.) There were no differences for general health, change in health, mental health and fitness/fatigue scores in SF-36 (p>0.05). In both groups all SF-36 scores except for emotional role function in injection group improved significantly at the end of 3rd week and 8th week (p<0.05).

Table 3. Comparison of VAS pain and Constant-Murley scores

		Injection Group	Physical Therapy Group	p
		Means ± SD	Means ± SD	
VAS rest	Baseline	4.55 ± 1.50	5.10 ± 2.42	0.394
	3th week	1.35 ± 1.53	0.95 ± 1.05	0.341
	8th week	1.40 ± 1.53	0.85 ± 1.26	0.224
VAS motion	Baseline	8.20 ± 1.73	8.30 ± 1.75	0.814
	3th week	4.15 ± 1.84	3.50 ± 1.31	0.378
	8th week	**3.70 ± 1.89**	**2.00 ± 1.65**	**0.006***
VAS sleep	Baseline	5.90 ± 2.84	6.25 ± 3.16	0.663
	3th week	2.10 ± 1.94	1.45 ± 1.73	0.260
	8th week	**2.00 ± 2.10**	**0.55 ± 1.14**	**0.006***
Constant -Murley	Baseline	41.10±13.4	39.50±11.9	0.626
	3th week	57.70±10.3	62.20±7.47	0.616
	8th week	62.95±10.9	66.40±9.47	0.186

p<0.05; significant, SD; standard deviation, VAS; Visual anolog scale

Table 4: Comparison of Short Form-36 subscores

		Injection Group Means±SD	Physical Therapy Group Means±SD	P Value
Physical function	Baseline	48,80±16,64	59,00±22,74	0,106
	3th week	66,50±16,06	75,75±15,83	0,085
	8th week	**69,50 ±16,21**	**81,75 ± 17,18**	**0,011***
Social function	Baseline	41,00 ± 17,98	48,40 ± 21,23	0,394
	3th week	59,95 ± 16,53	69,85 ± 13,94	0,067
	8th week	**65,40 ± 21,87**	**78,70 ± 15,32**	**0,019***
Physical role function	Baseline	23,75 ± 18,97	28,75± 30,64	0,897
	3th week	**47,50 ± 31,30**	**69,15± 26,11**	**0,025***
	8th week	65,00 ±32,84	81,25 ± 29,10	0,066
Emotional role function	Baseline	54.65 ± 31,10	49,65± 33,23	0,681
	3th week	68,10± 33,40	74,75± 30,48	0,532
	8th week	**69,75 ± 32,38**	**88,20± 24,96**	**0,039***
Pain	Baseline	34,65 ± 16,46	35,75± 20,77	0,989
	3th week	63,25± 15,91	72,65± 14,10	0,117
	8th week	**68,80± 21,15**	**81,65± 15,61**	**0,040***

P<0,05 Significant SD: Standard Deviation

4. Discussion

The aims of the conservative treatment in SIS are to improve the shoulder functions, to provide full ROM and to obtain a painless shoulder by reducing the inflammatory response (8). There is not a standard conservative treatment for SIS. Corticosteroid injection is a very common treatment method for SIS. However, there is not a consensus on its effects and initial treatment is controversial for some authors (9).

Therefore, alternative methods are needed. Local and deep heat therapies and analgesic currents are the most commonly used physical therapy methods for SIS. In our study we compared the effectiveness of corticosteroid injection with the effectiveness of PT, including local heat, US and IC.

US is one of the most common investigated PT methods which may have the potential to induce biophysical effects within the soft tissue. It is assumed to have thermal effects on the target tissue resulting in an increase in blood flow, cell metabolism and tissue regeneration, and also reducing inflammation, edema and pain (10). There are several studies which have reported US as an effective or ineffective therapy at soft tissue pathologies of shoulder (11-12). Ainsworth et al have compared the effectiveness of US and sham US on 221 patients with shoulder pain. After 6 weeks, they have found no differences between the groups for VAS pain (12). But in their study, diagnoses of the patients have not been indicated and the US was applied to all patients with constant dose and same period. In another study, Yıldırım et al have compared four minutes or eight minutes US therapy for SIS treatment. They found that all four- or eight-minutes US therapy have benefical effects in treatment of SIS but eight minutes of ultrasound treatment was shown to be more effective than four minutes of ultrasound treatment (13).

Bicer et al. have compared the PT including US, Infrared and TENS with corticosteroid injection in shoulder pain**.** They have applied unique subacromial injection with methylprednisolone. At the end of the study, they have found no significant differences between the groups at VAS pain. But, in their study, there were patients diagnosed as tendinitis, bursitis and adhesive capsulitis as well as SIS (14). In our study we found that US threapy is effective as corticosteroid injection. We recruited only the patients who were diagnosed SIS. There was not any frozen shoulder as Ainsworth and colleagues's study that possibly US therapy is not effective on in. In addition, we applied US seven minutes longer like Yıldırım et al. but Ainsworth applied average four minutes per patient (13).

Our results indicate that US is effective on reduction of pain and improvement of function which are the main problem for a patient diagnosed SIS. Ultrasound therapy may have the potential to induce biophysical effects within the soft tissue. Ultrasound is assumed to have thermal effects on the target tissue resulting in an increase in blood flow, cell metabolism and tissue regeneration, and also reducing inflammation, edema and pain.

Corticosteroid injection is a very common and effective therapy on SIS. Tan et al. have compared conventional PT and corticosteroid injection as in our study. They have applied US, IC, hot-pack, exercise to PT group and corticosteroid injection with exercise to another group. As distinct from our study, they have found that VAS pain during motion and rest at first week and VAS pain during motion at 3rd week in injection group were significantly less than the PT group. However, they have not found differences between the groups for the pain scores at the 6th week (15). The early and significant improvement at pain can be explained with the anesthetic added to the corticosteroid injection treatment. Because, combination of corticosteroid and local anesthetics reduces the pain. Additionaly, in their study, Constant-Murley scores were significantly better in injection group at the end of the first week but, there were no differences between the groups at the end of 6th week.

Levendoglu et al have compared corticosteroid injection treatment at SIS, with 15 sessions-PT program including US, TENS and hot-pack. Similar to our study, they have found significant improvements at all parameters in both groups at the end of the study. Contrary to our study, they have observed better significant improvements in injection group at VAS pain during rest and active motion at 15th day, first month and 3rd month of the study (16). We can explain these differences with the local anesthetic added to the injection treatment and receiving physical therapy without hospitalization of the patients in the PT group. In our study VAS pain during active motion and sleep was better in PT group than the injection group at the end of 3rd week and 8th week, and we believe that it was due to hospitalization and being avoided from inappropriate activities and the emotional stress of the daily living as well as the exercise therapies with physiotherapists. In several studies it has been showed that corticosteroid injection is effective on reducing inflammatory response (16, 17) However, some side effects after injection including pain, tendon tears, blushing and infection have been reported (18). In our study there were no side effects and we found significant improvements at VAS pain, ROM and Constant-Murley scores at the end of 3rd and 8th weeks, when compared with the baseline.

There are limited studies in literature which compare the effectiveness of analgesic currents. In PT modalities, analgesic currents like IC and Transcutanose Electrical Nevre Stimulation (TENS) are important for pain relief. IC is often used for acute and chronic pain of superficial and deep tissues (19). As well as the analgesic effect, it is used for anti-inflammatory effect at joint sprains, for osteogenic effect at delayed fracture improvements and for sympathicolytic effect at Complex Regional Pain Syndrome (20, 21). Although the effect mechanism of IC is not clear, there are various suggested hypotheses, one of which is Melzack-Wall theory; increasing release of endogenic opiates, local vasodilatation and sympathetic blockade (22). There are studies which have showed that IC was as effective as TENS at the treatment of superficial and deep tissues pain. Ay et al. compared TENS with IC in patients with shoulder pain. In both groups US, hot-pack and exercise therapies were added to the treatment. At the end of the study, they observed significant improvement at pain scores and ROM in both groups, but they found no significant differences between two groups

(23). Facci LM et al also compared effects of TENS and IC in their study and found any statistical difference between two methods for reducing VAS score (24). Another supporting result was found in the study of Ucurum SG et al. They applied TENS or IC for their patients all of whom were diagnosed as SIS similar to our study. There was similar result in terms of pain and SF 36 physical component scores at fourth week and third month assesments (25). In our study, the main complaint was pain. Therefore, we combined US and local heat therapy with IC in PT group. We found that IC might have a beneficial effect on pain relief and improvement at daily living activities in PT group. Our results suggest that US combined with local heat, and IC, and exercise program has benefit effect similar local injection treatment on shoulder mobility, pain and disability in SIS.

Our study has some limitations, one of which is that subject number was fewer and follow up period was short. That's why, studies with large numbers of subjects and long follow up periods are needed to confirm these results.

Our results showed that both PT and subacromial corticosteroid injection treatments for SIS have similar effects on pain relief, providing ROM, shoulder functions and daily living activities**.** PT demonstrated comparable efficacy versus local injection treatment with similar clinical response. However, PT is more effective in long-term pain relief.

Conflict of interest

There is no conflict of interest to declare.

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None to declare.

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Characteristics of minor head trauma in toddlers

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Abstract

The objective of present study is to evaluate mechanisms and causes of head trauma, factors influencing management of pediatric minor head trauma, to highlight decision making processes in diagnostic imaging as well as searching for preventive measures for head trauma. Children younger than two years of age who were admitted to emergency department in one-year study period due to minor head trauma were included to the study. To be inside or outside of house did not significantly change the incidence of falls for children younger than two years of age (p=0.096). Incidence of falls was significantly increased at living rooms (p=0.01) and bathrooms (p=0.036). Incidence of scalp hematomas was significantly higher in symptomatic patients (p=0.006). Asymptomatic admission after a minor injury was not a significant factor on decision of diagnostic imaging. A patient's asymptomatic presentation should not be used as a criterion to rule out cerebral injuries. A lack of obvious signs and symptoms during evaluation does not exclude TBI. Existing serious symptoms should lead to a quick evaluation of patient to rule out a possible surgical emergency. Scalp hematomas are significantly associated with cerebral injury and are a predictor of brain injury. Clinicians should have a lower threshold for imaging in children<2 years of age.

Keywords: children, epidemiology, head trauma, imaging

1. Introduction

Trauma is the most common cause of death in children and the fifth most common cause of death in adults. Up to half of mortalities related to trauma are secondary to head injuries (1, 2). Children are more vulnerable to head trauma due to their larger heads relatively to their body size, incomplete brain myelination, and incomplete closure of the cranial sutures (1, 2).

Management of minor pediatric head trauma and the choice of diagnostic imaging modality are commonly based on the risk of traumatic brain injury (TBI) and age subcategory. Head trauma is a clinical challenge for healthcare providers in children <2 years of age (3). Clinical assessment is difficult in this age group, as these patients cannot describe the mechanism of the injury. In some cases, accidental or non-accidental causes of injury cannot be clearly identified (4, 5). In addition, anatomical differences in children <2 years of age pose a major problem and may lead to an asymptomatic presentation with subtle findings and/or late onset of symptoms (3).

In the present study, clinical and demographic characteristics of toddlers (<2 years of age) who were admitted to the emergency department (ED) for minor head trauma were evaluated. This study collected all injury data to highlight factors that may affect the outcomes of minor head trauma in toddlers. We investigated the mechanisms and causes of head trauma and the factors influencing the management of symptomatic and asymptomatic minor pediatric head trauma to highlight the choice of diagnostic imaging modality and search for preventive measures for head trauma.

2. Materials and methods

This prospective observational study was initiated following approval from the Institutional Review Board (HNEAH KAEK2013/275). Ethics committee approval was received for this study from the ethics committee of University of Health Sciences, Haydarpasa Training and Research Hospital (HNEAH KAEK2013/275). All consecutive children aged <2 years who were admitted to the ED during the one-year study period for minor head trauma were included in the study. Patients with no clear history of trauma, those >2 years of age, those who had returned for reassessment of the same head trauma, victims of child abuse, children with chronic

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diseases, and children whose parents refused to provide informed written consent were excluded from the study. All patients were followed for 12 hours in the ED observation room. Data about history of the trauma and symptoms were obtained from the parents and/or relatives of the children. Age and physical examination and radiological findings were recorded.

The choice of diagnostic imaging modality was based on the Pediatric Emergency Care Applied Research Network (PECARN) (6). Computed tomography (CT) scans of the head were examined by radiologists and were considered positive for TBI if pathological findings such as skull fracture or subdural, epidural, or subarachnoid bleeding were apparent. All head CT scans were re-evaluated by a senior radiologist, and no disagreement occurred.

2.1. Statistical analysis

The patients were divided into symptomatic and asymptomatic groups. Data were analyzed using SPSS ver. 17.0 software (SPSS, Inc., Chicago, Il, USA). Categorical variables were analyzed with Fisher's exact test, and continuous variables were analyzed with the independent sample *t*-test. The level of significance was set at 0.05.

3. Results

A total of 551 children aged <2 years were admitted to the ED during the study period. Among them, 48 were excluded from the study due to a lack of written informed consent. Thus, 503 patients were included in the study group. The mean age of all children was 12.4 ± 7.0 months. The mean age of the 234 (46.5%) girls was 12.1 ± 6.9 months, and the mean age of the 269 (53.5%) boys was 12.6 ± 7.1 months (p = 0.423). The age and sex distribution are shown in Table 1.

Table 1. Gender and age distribution

	Female	Male	Total
0-1 years	138(27.4%)	145 (28.8%)	283 (56.3%)
1-2 years	96 (19%)	124(24.6%)	220 (43.7%)

The most common mechanism of injury was falls, seen in 184 (36.5%) patients; falls from height were recorded in 26 (5.1%) patients, 34 (6.7%) children fell while a parent was holding them, objects fell on 20 (3.9%) children, 36 (7.1%) patients collided with an object, 2 (0.4%) were in vehicle accidents, 2 (0.4%) were in pedestrian accidents, 14 (2.7%) fell from a baby walker, 14 (2.7%) were injured by next of kin, 70 (13.9%) fell from a cradle, 41 (8.1%) fell from an adult bed, 40 (7.9%) fell from a couch/furniture, and 18 (3.5%) patients fell from a stroller. The distribution of the mechanisms of injury is shown at Table 2.

Ground-level falls inside the home were the most common type of injury (126 [68.4%] children), and 58 (31.5%) children fell outside the home. The location of the injury (inside vs. outside the home) did not significantly affect the incidence of falls (p = 0.096).

Table 2. Distribution of the mechanisms of injuries

Mechanism of injury	N (%)
Ground-level falls	184 (36.5%)
Falls from heights	26 (5.1%)
Parent fall while holding children	34 (6.7%)
Object falls on children	20 (3.9%)
Vehicle accident	2 (0.3%)
Pedestrian accident	2 (0.3%)
Fall from baby walker	14 (2.7%)
Sibling caused injuries	14 (2.7%)
Falls from cradles/baby beds	70 (13.9%)
Falls from adult beds	41 (8.1%)
Falls from couches	40 (7.9%)
Collision with an object	36 (7.1%)
Falls from strollers	18 (3.5%)
Unknown	2 (0.3%)
Total	503(100%)

The incidence of falls was significantly higher in the living room (p = 0.01) and bathroom (p = 0.036) compared to other parts of the home, such as the kitchen, hallway, or baby room. The most common injury locations due to falls and other injuries inside the home are shown in Table 3.

A total of 408 (81.1%) asymptomatic patients were included in the study group. No diagnostic images were taken in 16 (3.9%) of these patients. Patients who presented with symptoms underwent diagnostic imaging. Admission after a minor asymptomatic injury was not a significant factor when choosing the diagnostic imaging modality.

A repeat CT scan was obtained in 14 of 408 asymptomatic patients and in 14 of 95 symptomatic patients on admission. These repeat CT scans were performed in patients with new or persistent symptoms during the observation period. No repeat CT scan was taken after an asymptomatic observation period (p = 0.0001).

Twelve (12.6%) of 95 symptomatic patients and 18 (4.4%) asymptomatic patients had scalp hematomas. The incidence of scalp hematomas was significantly higher in symptomatic than in asymptomatic patients (p = 0.006).

Thirteen (13.6%) of the 95 symptomatic patients and 10 (2.4%) of 408 asymptomatic patients had fractures detected on a CT scan. Cranial fractures were significantly associated with a symptomatic admission (p=0.000), and 2.4% of asymptomatic patients also had cranial fractures.

Eight (8.4%) of the 95 symptomatic patients and 4 (0.98%) of the 408 asymptomatic patients had epidural hematomas on CT imaging. Epidural hematomas were significantly associated with the symptomatic admissions (p = 0.0003).

Table 3. Common injury locations of falls and other injuries inside the houses

Injury Location	Falls n (%)	Other n (%)	p
Living room	62 (33.6%)	64 (20.1)	0.01
Bathroom	10 (5.4%)	6 (1.8%)	0.036
Hallway	8 (4.3%)	18 (8.6%)	0.677
Baby room	30 (16.3%)	84 (26.3 %)	0.011
Kitchen	4 (2.1%)	18 (5.6%)	0.073
Balcony	6 (3.2%)	8 (2.5%)	0.53
Adult bedroom	6 (3.2%)	43 (13.4%)	0.008
Total in house	126 (68.4%)	241(75.54%)	
Total	184 (100%)	319 (100%)	

Six (6.3%) of 95 symptomatic patients and four (0.98%) of 408 asymptomatic patients had cerebral contusions on CT imaging. Cerebral contusions were significantly associated with symptomatic admissions (p=0.004). Four (4.2%) patients with intracerebral hemorrhages were in the symptomatic group, and none was in the asymptomatic group. Two patients in the study group had pneumocephalus, and all of these patients were in the symptomatic group. No cerebral edema was reported in the study group.

Eighty-nine (17.6%) of 503 patients consulted with neurosurgeons. The frequency of consultations was significantly higher in symptomatic than in asymptomatic patients (p=0.0001). Twenty-three (4.5%) patients were hospitalized, and symptomatic patients were more commonly hospitalized (p =0.000) than asymptomatic patients. Three patients in the symptomatic group underwent surgery. Six (1.2%) patients required a reassessment in the first 24 hours after discharge from the hospital.

4. Discussion

Falls were the most common cause of minor head trauma in this study group. Falls from a baby bed, an adult bed, or a couch, as well as falls while a parent was holding the child were common injury mechanisms. This high incidence of falls was related to toddlers' poor balance (1, 4). In the present study, the incidence of falls did not differ between those inside and outside the home in children <2 years of age. Toddlers generally live inside the home, and parents pay more attention when they are outside, which may have decreased the incidence of outdoor injuries (7, 8). Falls in the home were most common in the living room and bathroom. Protective measures against head injuries should be used in the living room and bathroom. These parts of the home have many possible risk factors that may lead to head injuries, and protective measures may decrease the incidence of head trauma (8, 9). Most head trauma cases are low-risk traumas. The clinical challenges when evaluating minor head trauma in children are to identify TBI and limit CT imaging and radiation exposure. A head CT scan is the gold standard for diagnosing patients with a head injury (10). Neuroimaging with CT is extremely sensitive for brain injury (10). However, clinical predictors of brain injury are commonly nonspecific in children aged <2 years (5, 6).

In the present study, symptomatic patients had a higher incidence of skull fractures, but these fractures were also seen in 2.45% of asymptomatic patients. Using initial symptoms as the sole predictor of TBI or as the basis of a choice of imaging modality has a high incidence of failure when trying to detect a fracture. In this study, the decision to obtain a head CT was not related to symptoms, physical examination findings, or clinical decision rules. Clinicians have a lower threshold for imaging in children <2 years of age (11).

Although PECARN is sensitive for this age group, treatment decisions depend on physical examination findings and the history taken from a parent (6). The CHALICE rules accept a wide range of patients, and the original article reported 98% sensitivity for minor head trauma (5). Decision rules fail to provide a management algorithm for head trauma. These rules simply assist the clinician with their choice of whether to obtain a head CT scan. For these reasons, clinicians prefer imaging to rule out TBI in this age group. The decision to obtain a second head CT was based on observation. In this study, clinicians obtained a second head CT in patients with persistent or new symptoms (p = 0.000).

Scalp hematomas are a common finding in pediatric cases of head trauma and are a sign for an increased risk of intracranial injury. Parietal, temporal, and occipital scalp hematomas should alert the physician to an increased risk of intracranial injury (7, 11, 12). Recent studies reveal that intracranial injuries are associated with scalp hematomas in 93% of cases. In this study, symptomatic patients had a significantly higher incidence of scalp hematomas than did asymptomatic patients.

The incidence of epidural hematoma was significantly higher in symptomatic than that in asymptomatic patients. However, an epidural hematoma cannot be ignored in an asymptomatic patient, and asymptomatic presentation does not rule out an epidural hematoma. No subdural hematomas were detected in the study group, so the present study could not reveal the features of subdural hematomas in this age group. Subdural hematomas should be further evaluated in a future study. Symptomatic patients had a significantly higher incidence of cerebral contusions than did the asymptomatic group in our study. All intracerebral hemorrhages were in the symptomatic group. Although all these diagnoses were more common in the symptomatic group, they were noted with a lower incidence in the asymptomatic group. Symptoms alone should not be used to predict a cerebral injury.

A patient's asymptomatic presentation should not be used

as a criterion to rule out a cerebral injury. Management of head injury in this age group should not be based solely on presenting symptoms. A lack of obvious signs and symptoms during the evaluation does not exclude TBI. Existing serious symptoms should lead to a quick evaluation of the patient to rule out a possible surgical emergency. Scalp hematomas are significantly associated with cerebral injury and are a predictor of brain injury. Physicians should be aware that decision rules do not provide an algorithm for managing head trauma (12). These rules are a guide for the clinician's decision about whether to obtain a head CT scan. Clinicians should have a lower threshold for imaging in children <2 years of age (3, 12-14). Head injuries in children aged <2 years are commonly due to preventable injuries (3). Family education may be effective and can contribute to lowering the incidence of these injuries (15). For example, family members should be aware that a baby can turn over after four months. Significant results can be realized by modifying the physical environment. Falls in the home were common in living rooms and bathrooms. Shock-absorbent floors or protective mats may reduce the risk of injury in homes. Similarly, protective measures should be taken against head injuries in living rooms and bathrooms (15). As the latency period for developing a malignancy may be decades, mortality due to malignancies caused by head CT radiation are often ignored. The incidence of head and neck malignancies caused by head CT radiation should be clarified (14). Further studies should focus on improving the sensitivity of clinical decision rules and designing a universal algorithm for managing minor head trauma in children aged <2 years.

Our study has several limitations. Because our data were collected in a tertiary hospital, they may not represent the entire population. We did not evaluate protective measures in the home. A standard decision rule could not be chosen due to the observational design of the study. Some injuries, such as subdural hematomas or brain edema, were not noted in the study group, and 48 patients were excluded from the study due to a lack of written informed consent. Excluding many patients from the study may have influenced the results.

A patient's asymptomatic presentation should not be used as a criterion to rule out a cerebral injury. Management of head injury in this age group should not be based solely on presenting symptoms. A lack of obvious signs and symptoms during the evaluation does not exclude TBI. Existing serious symptoms should lead to a quick evaluation of the patient to rule out a possible surgical emergency. Scalp hematomas are significantly associated with cerebral injury and are a predictor of brain injury. Clinicians should have a lower threshold for imaging in children <2 years of age.

Conflict of interest

The authors have no conflict of interests to declare.

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The effect of local endometrial injury on the success of intrauterine insemination

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Abstract

To determine the effect of local endometrial injury on implantation success in patients diagnosed with unexplained infertility and undergoing intrauterine insemination (IUI) after ovulation induction with gonadotropins. In this prospective randomized controlled trial, 82 infertile patients underwent IUI following ovulation induction with gonadotropin. In the study group (n:40), local endometrial injury (stratch) was performed to the posterior side of the endometrial cavity with a biopsy catheter between the 21-26th days of luteal phase of the cycle preceding ovarian stimulation. There was no statistically significant difference between the study and the control groups in terms of age of female, age of male, duration of infertility, BMI, serum FSH and LH levels, mean dose of gonadotropin and mean duration of ovulation induction ($p>0.05$). Clinical pregnancy was achieved in two patients (4.76%) in control group and four (10%) patients in the study group, with no significant difference between groups ($p=0.18$). All pregnancies achieved in the control and the study groups passed 12th gestational weeks and continued. Ectopic pregnancy, multiple pregnancy and abortion was not observed in any patient in both groups. In the study group, pain level immediately after endometrial biopsy procedure was evaluated with visual analog scale (VAS) and it was established that only one (2.5%) patient experienced severe pain after the procedure. Although local endometrial damage in the menstural period before ovulation induction and IUI cycle increases clinical pregnancy rates in the infertile patients, this increase is not statistically significant. Multi-center randomized controlled studies are needed for local endometrial damage to be recommended routinely in clinical practice.

Keywords: infertility, intrauterine insemination, local endometrial injury, ovulation induction

1. Introduction

If the cause of infertility can not be found in a couple who can not achieve pregnancy despite one year of unprotected sexual relation, this is termed as unexplained infertility and 10-20% of couples are diagnosed with unexplained infertility (1, 2). Of overall infertility cases, in 20-40% ovulatory dysfunction, in 30-40% tubal and peritoneal factors and in 30-40% male factor plays part, while unexplained infertility accounts for 10% (3).

Implantation is the process of attachment of blastocyst produced after fertilization to uterus wall. The period when endometrium is most receptive to implantation is midluteal period, i.e. the period between 19-24. days which is termed as implantation window (4-7). In this period, blastocyst should be implanted on endeometrium successfully by passing the stages of apposition, adhesion and invasion. For a successful implantation, in addition to a receptive endometrium, a functional embryo and synchronous communication between maternal and embryonic tissue are required (8, 9).

75% of pregnancy losses stem from implantation failure (6, 10, 11) and two third of implantation failures results from impairment in endometrial receptivity (12, 13). Although many problems associated with fertility have been overcome with assisted reproductive techniques (ART), mostly embryonal factors have been adressed. Therefore, implantation stage is a problem which still remains to solved (11).

There are many studies in the literature reporting that local endometrial injury caused by endometrial biopsy procedure leads to rise in implantation rate by increasing endometrial receptivity. In these studies, many hypotheses have been put forward regarding the probable impact of endometrial injury, which include proliferation of decidua like cells in endometrium (14, 15), alteration in endometrial gene expression (16, 17), release of various cytokines and growth factors (13) and development of a more synchronous environment between embryo and endometrium (17).

The aim of the present study is to determine the effect of endometrial injury on implantation success in patients diagnosed with unexplained infertility and undergoing intrauterine insemination (IUI) after ovulation induction with gonadotropins.

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2. Materials and methods

2.1. Patients

Overall 96 cases who presented to infertility outpatient clinic of Zeynep Kamil Gynecology and Obtretrics Training and Investigation hospital between 01.02.2013-01.07.2013 were included in the present study. This study was initiated after approval was obtained from Zeynep Kamil Gynecology and Obstretrics Training and Investigation Hospital ethics committee (approval dated 25.01.2013 and numbered 023). All cases were informed about the study and their informed consent was obtained.

Age, BMI, age of spouse, previous history of pregnancy, duration of infertility, menstruation pattern, smoking and drinking habits, history of chronic disease, drug use and history of previous operations were questioned. All patients underwent hysterosalphingography (HSG) examination. All patients were invited to outpatient control visit between 2nd-5th days of their menstrual cycle for antral follicle count and evaluation of pelvic pathology (uterine myoma, endometrial polyp, hydrosalpinx, endometrioma, ovarian mass etc) with transvaginal ultrasonography (TVUSG) and for assessment of FSH, LH, estradiol, TSH and prolactin values. Sperm counts of the spouses of patients were evaluated. Inclusion criteria were as follows:

- Age between 20-40
- BMI <30 kg/m^2
- Primary infertility and at least one year history of infertility
- Patent bilateral tuba in HSG
- FSH value of <10 mIU/ml and LH, estradiol, TSH and prolactin values within normal range
- No history of known systemic disease or of regular use of drugs
- No history of surgical intervention that can play part in the etiology of infertility (endometrial polypectomy, myomectomy, endometriosis surgery, congenital uterine anomaly surgery, ovary cyst surgery, hydrosalpinx surgery etc.)
- Normal pelvic USG
- No endometrial biopsy, endometrial curettage and hysterescopic procedure within the last three months
- Normal spermiogram results according to WHO criteria

All patients who matched these criteria were randomized and classified into two groups: the control group (n:42) and the study group (n:54). 8 patients in the study group were excluded from the study since endometrial biopsy could not be obtained from them because endometrial biopsy catheter could not pass from cervix. Further six patients were excluded from the study, although they underwent biopsy procedure, with the following causes: In two patients, ovulation induction with gonadotropin procedure was cancelled due to the risk of Ovarian Hyperstimulation Syndrome (OHSS). In addition, in four patients, ovarian cyst was detected with TVUSG examination carried out before treatment at the onset of menstrual cycle. Finally, study group included 40 patients and control group 42 patients.

2.2. Treatment protocol

In the study group, patients planned to undergo endometrial biopsy sampling, were invited to infertility outpatient clinic at a date between the 21-26th days of luteal phase of menstrual cycle. The scratch was performed by the same investigator, with a biopsy catheter (Endometrial Sampling cannula, Plastimed, Istanbul, Turkey), on the posterior side of the endometrial cavity under sterile conditions. The internal piston was withdrawn to create negative pressure. Biopsy catheter was moved back and forth four to five times. No medical treatment was administered to the patients after the procedure. The degree of pain experienced by patients after the procedure was evaluated using Visual Analog Scale (VAS). Patients in control group did not undergo endometrial scratching. Following these procedures, on the third day of menstrual cycle, ovulation inducton with gonadotropin was commenced in patients in control and study groups. For induction procedure, follitropin α (Gonal F, rec-FSH, Merck-Serono, Italy) 75 IU/day was administered subcutaneously. The size and number of follicles was measured at certain intervals using TVUSG. Serum estradiol levels were measured and gonadotropin doses adjusted. When at least one follicle reached the size of 18mm or more, 250 μcg recombinant hCG (Ovitrelle 250 μcg, Merck-Serono, Italy) was administered subcutaneously. During follicle monitorization performed with TVUSG, cases with 2 or more follicles larger than 14 mm or who have estradiol values over 1500 pg/ml had their cycle cancelled owing to risk of OHSS and they were excluded from the study. 32-36 hours after ovulation, intrauterine insemination (IUI) was performed by the same investigator under sterile conditions. 15 days after IUI procedure, β-hCG values were evaluated to determine pregnancy. Clinical Pregnancy Rate (CPR), was defined as the detection of intrauterine gestatinal sac and fetus with fetal cardiac activity with TVUSG between 5th-7th weeks of pregnancy and Ongoing Pregnancy Rate (OPR) was defined as pregnancy process which has passed 12th week of gestational pregnancy.

2.3. Statistical analysis

In the present study, for data analysis, IBM SPSS (Statistical Package for Social Sciences) for Windows 20.0 program was used. In data analysis for descriptive statistics, arithmetic mean, standard deviation, minimum, maximum, frequency and percentage were used. Whether the data were normallly distributed was evaluated with Kolmogorov Smirnov test. In the comparison of pregnancy rates in study and control groups, chi-square test was used. Data normally distributed were evaluated with t test in independent groups and with Mann-Whitney U test and Fisher's exact test in others. Results were evaluated with 95% confidence interval and p value of <0.05 was considered statistically significant

3. Results

Overall 82 patients (42 control, 40 study) were included in the present study. There was no statistically significant difference between study and control groups in terms of age of female, age of male, duration of infertility, BMI, serum FSH, LH, levels mean dose of gonadotropin, mean duration of ovulation induction (p>0.05) (Table 1). Clinical pregnancy was achieved in 2 patients (4.76%) in control group and four (10%) patients in study group, with no significant difference between groups (p=0.18). All pregnancies achieved in control and study groups passed 12th gestational weeks and continued (Table 2). Ectopic pregnancy, multiple pregnancy and abortion was not observed in any patient in both groups.

In the study group patients, pain level immediately after endometrial biopsy procedure was evaluated with VAS and it was established that 1 (2.5%) patient experienced severe pain after the procedure (Table 3). In addition, cases in the study group were followed for probable complications such as vaginal bleeding, pain and infection. On the day of procedure, in study group, spotting (mild vaginal bleeding) was detected in 6 (15%) patients and mild abdominal pain in 3 (7.5%) patients.

Table 1. Comparison of demographic and clinical characteristics of control and study groups

	Control group (n=42)	Study group (n=40)	P
Female age (year)	29.57±4.17	28.95±4.43	0.51
Male age (year)	31.81±5.1	32.78±3.79	0.34
Duration of infertility (year)	4.52±3.4	4.39±2.94	0.94
FSH (mIU/mL)	7.55±1.59	7.15±1.6	0.27
E2 (pg/mL)	51.26±25.76	43.29±13.18	0.08
LH (mIU/mL)	4.9±1.74	4.92±1.89	0.97
BMI (kg/m_2)	23.57±3.4	24.35±3.02	0.28
Antral follicle count	12.38±4.4	13.95±4.1	0.106
Total gonadotropin dose (IU)	744.05±259.6	737.19±364.32	0.24
Durataion of ovulation induction (day)	9.64±2.63	9.48±3.37	0.8

Table 2. Comparison of clinical pregnancy rates of control group and study group

	Clinical Pregnancy (-	Clinical Pregnancy (+)	p
Control	40 (95.24%)	2 (4.76%)	0.18
Study	36 (90%)	4 (10%)	

Table 3. VAS scores for study group

	Endometrial Biopsy group
VAS (for pain)	3.29±2.04 (0.1-7.7)
VAS (for pain) >7, n(%)	1 (2.5)

4. Discussion

In the present study, the effect of local endometrial injury exerted in previous cycle in infertile patients undergoing IUI following ovulation induction with gonadotropins on pregnancy outcome was investigated and no significant difference was found between study and control groups with respect to CPR and OPR (p=0.18).

Although endocrinological, immunological, genetic and reproductive physiology factors are considered among probable mechanisms of infertility (2), the most important cause is decrease in endometrial receptivity, associated with impairment in cellular or molecular mechanisms in endometrium, and consequent implantation failure (18). Development of a functional embryo, endometrial receptivity for implantation and complex molecular interactions between them are the required steps for implantation (4, 6, 9, 10, 12).

Some investigators have stressed that COH cycle exerts negative impact on implantation and reported that in COH cycle endometrial stromal development and pinopod development are at more advanced stage compared to natural cycle, which produces an environment without developmental synchrony between embryo and endometrium and influences implantation unfavourably (19, 20). In the study of Zou et al, it was stated that the most likely cause of the positive effect of local endometrial injury on implantation success may be that this advanced development process in COH cycle becomes slower during wound healing after endometrial injury and hence a more balanced environment is produced between embryonal development and endometrial development (17). Another mechanism known to be inducive to endometrial implantation is the release of cytokines and other growth factors during wound healing period following endometrial injury. With all these autocrine and paracrine effects, the release of sex stroids is regulated and uterus becomes receptive to embryo which will be implanted. (21). It is also known that cytokines such as IL-6, LIF, (TNF-α) and growth factors relaeased from immune system cells and endometrial cells after endometrial injury increases receptivity (13).

In literature review, it can be observed that the effect of local endometrial injury on pregnancy outcomes has mostly been investigated in ART (assisted reproductive technology) cycles. In the meta-analysis of Nahshon C. et al including 3016 cases from 17 randomized controlled studies (RCT), in women with at least one previous failed cycle, the effect of local endometrial injury on IVF outcomes was evaluated and CPR (RR 1.19 [95% CI 1.06–1.32]) and LBR (live birth rate) (RR 1.18 [95%CI 1.04–1.34]) were found to be significantly improved after local endometrial injury (22). However, in the meta-nalysis of Van Hoogenhuijze et al. including 14 RCT with 2537 participants, the effect of local endometrial injury in patient groups with a previous failed full IVF/ICSI cycle was evaluated and no difference was found between study and control groups with regard to LBR (RR 1.01 [95%CI 0.68–1.51]) and CPR (RR 1.04 [95%CI 0.74–1.45]) (23). Similarly, in the meta-analysis of Vitagliano et al, after local endometrial injury prior to first IVF cycle, nonsignificant difference was found between control and study groups with respect to LBR

(RR 0.99 [95% CI 0.57–1.73]) and CPR (RR 1.12 [95% CI 0.79–1.59]) (24). In the meta-analysis of Nastri et al, it was stated that local endometrial injury prior to IVF cycle had no significant effect on pregnancy in the groups which has not undergone IVF before or has undergone IVF once with failure while in the group with two or more failed IVF attempts CPR (RR 1.63 [95%CI 1.12–2.38]) and LBR (RR 1.96 [95%CI 1.21–3.16]) were significantly higher (25). The cause of discrepant results reported by meta-analyses is that different inclusion criteria are used in studies with different designs (randomized or nonrandomized studies, the number of previous IVF attempts, whether local endometrial injury is exerted before or during cycle). In conclusion, all of these meta-nalyses emphasized that well designed randomized controlled multicenter studies are warranted in order that the effect of local endometrial injury prior to IVF cycle on pregnancy outcomes can be determined more accurately.

In the meta-analysis performed by Vitagliano A. et al including 8 RCTs with 1523 participants, the effect of local endometrial injury on the outcome of intrauterine insemination (IUI) stimulated was evaluated (26). In included studies, local endometrial scratch injury was carried out either during the course of IUI stimulated cycle (C-ESI) or during the menstrual cycle preceding IUI treatment (P-ESI). In local endometrial injury group, higher rates of CPR (OR 2.27; P<.00001) and OPR (OR 2.04; P=.004) were found. Nevertheless, these findings were supported by moderate level evidence for CRP and low level of evidence for OPR. In subgroup analysis based upon the timing of endometrial injury, in the subgroup undergoing C-ESI, CPR (OR 2.57; P<.00001) and OPR rates (OR 2.27; P=.004) were higher. In addition, in patients with endometrial injury, even though the quality of evidence was low, the risk of multiple pregnancy (OR 1.09), induced abortion (OR 0.80) and ectopic pregnancy (OR 0.82) was not found to be increased. In the present study, although clinical pregnany rate was found to be higher in the study group, the difference between groups was not significant, which may be due to low number of patients. In the present study, multiple pregnancy, abortion and ectopic pregnancy was not observed. In the above mentioned meta-analysis, pain status after local endometrial injury was not evaluated in any study, except for the study of Wadha et al, who reported without using any pain scale that no patients had severe pain (27). In a study, patients were evaluated for pain with VAS and when number 4 karman cannula was used in the procedure, pain at the mean rate of 6/10 was experienced (28). In the present study, pain level after endometrial biopsy procedure was evaluated using VAS and it was established that only one (2.5%) patient experienced severe pain following procedure. In the studies evaluated in the aforementioned meta-analyses, there is no data on short and long term complications. In the present study, on the day of procedure, spotting (vaginal bleeding) was detected in in 6 (15%) patients and mild abdominal pain in 3 (7,5%) patients. Especially probable intrauterine adhesions that may develop after local endometrial injury are cause for concern. Therefore, long term studies which will clarify this tissue are required.

In an international survey performed in England, New Zealand and Australia, it was established that 92% of physicians recommend local endometrial injury (scratching) before IVF cycles used after repeated implantation failure while before IUI, it is recommended by only 3.2% of clinicians (29).

In conclusion, whether local endometrial injury exerts favorable effect on pregnancy outcome in patients administered ART is still debated at present. To reach definitive conclusions, well designed larger randomized controlled multicenter studies are required.

Conflict of interest

There is no conflict of interest to declare.

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Histopathological effect of platelet-rich plasma on cranial dura mater defects

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Abstract

There are many new studies in the selection of materials used in dura material repair. Platelet has important function in hemostasis and coagulation. Also, activated platelets initiate the wound healing process. They provide regeneration of tissue with the appropriate type of tissue. In this experimental study, we used platelet-rich plasma (PRP) on dura mater defect due to these properties. Materials and method: Our study started after we got the approval of Ondokuz Mayıs University Animal Experiments Local Ethics Committee. Thirty Wistar Albino female rats were used. The rats were divided into three groups, each one includes ten. Results: No significant difference was observed in the thickness and surface areas of dura mater in group 1 and group 2. Significant increase was observed in the thickness and surface areas of dura mater in the PRP applied group (Group 3) compared to other groups. New bone areas and new vasculature were observed more frequently in the PRP rooms group (Group 3). Statistical analysis of the data was done by using SPSS 21.0 for Mac (IBM Corporation) statistical package program and based on 0.05 significance level. A significant difference was observed between Group 1 and Group 3 ($p > 0.05$), a significant difference was observed between both Group 1 and Group 3 and between Group 2 and Group 3 ($p < 0.05$). Conclusion: Providing the regeneration of dura mater in defect situations will contribute to the protection of the barrier feature. In line with this idea, it is thought that PRP can be beneficial for us to reach this goal.

Keywords: cranial dura mater, histopathology, platelet-rich plasma

1. Introduction

The dura mater defect is a bothersome complication of neurosurgical practice. Dural tear can cause headache, cerebrospinal fluid (CSF) pseudocyst, meningitis, arachnoiditis, wound infection, and several other serious complications (1). These complications can be effectively decreased after the successful repair of dural tear (2). Dural repair is shown to decrease cystic cavity and connective tissue scar, increase tissue spare and reestablish function after dural injury in rats (3, 4). Thus, it is critical to repair it rapidly when dura mater tear arises. Suturing still remains the most common technique of dura mater repair. However, when dural tears are in moderately unreachable regions or enclosed by fragile dura mater, suture techniques are challenging to perform. This difficulty has led the neurosurgeons to support the use of several other methods. Cyanoacrylates, fibrin glue, and CO_2 laser have all been preferred to achieve dura mater support (5, 6). Platelet-rich plasma (PRP) is a by-product of blood (plasma) comprising numerous bioactive aspects which are included in tissue restoration and has more than a few benefits compared with other dural repair techniques. PRP can be prepared from autologous blood and its clinical use causes no postoperative complications or adverse effects (7). PRP is comprised of various growth factors such as platelet-derived, transforming-, insulin like-, fibroblast-, epithelial-, and vascular endothelial- growth factors (8). The presence of these growth factors is in charge for various factors involved in tissue regeneration such as angiogenesis, collagen production, increased cell proliferation, and induced cell differentiation (9). PRP-related products are used in several surgical interventions to stimulate the healing procedure after muscle, joint, and tendon injuries (10). There are numerous commercial products on the market for the repair of dura mater defects; however, the best technique of watertight dura mater closing has yet to be determined. In this animal study, we aimed to compare the histopathological effects of dural suture technique with combined suturing and topically applied PRP on dural healing in a rat model of dural injury and cerebrospinal fluid leakage.

2. Materials and methods

2.1. Experimental design

All animal studies were carried out with the approval of the Ondokuz Mayıs University Animal Care and Use Committee. Animals were housed at constant temperature (20-22ºC) and humidity 50-60 % with a 12-hour light and 12-hour dark cycle. They were allowed free access to water and standard rat chow. A total of 30 Wistar Albino female rats were allocated into three groups in random, which were control group (Group 1) (n=10), suturing group (Group 2) (n=10), and combined suturing and PRP group (Group 3) (n=10).

Group 1 (Control group): Craniectomy + dural incision

Group 2 (only suturing group): Craniectomy + dural incision

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+ suturing (The dura was sutured with 10/0 Vicryl)

Group 3 (PRP group): Craniectomy + dural incision + suturing + PRP (The dura was sutured with 10/0 Vicryl and PRP was applied over sutured dura.)

2.2. Surgical procedure

We induced anesthesia via intramuscular administration of 80 mg/kg ketamine hydrochloride (Ketalar, 5% solution; Levent, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, 2% solution; Bayer, Leverkusen, Germany). After we shaved the parietal region of each rat, the surgical site was sterilized by povidone. Craniectomy (2 cm in diameter) was performed with a high-speed drill on the right parietal bone of all rats. The dura mater was kept intact during this procedure. The dura was then opened transversely and CSF leak was observed with the operating microscope. For Group 3, 1 cc of fresh blood collected from the tail of the rats. The mixture was immediately centrifuged at 3000 rpm for 8 min. The top three-quarters of the supernatant, which consisted of the platelet-poor plasma, was aspirated and transferred to a new tube. The remaining fraction contained PRP. The surgical area was closed with 4/0 silk and the rats were kept under conventional laboratory conditions. 100 mg/kg cefazolin (Cefazolin, Mustafa Nevzat, Istanbul, Turkey) was administered intramuscularly. All animals were sacrificed 21 days after the operation by decapitation. Both cerebral hemispheres and dura mater were totally excised. The samples were stored in 10% formaldehyde for histopathologic examinations at room temperature. The samples were embedded in paraplast and sectioned exhaustively. Coronal sections, 5 µm thick, were cut at a 500 µm sampling interval and stained with haematoxylin and eosin. The total volume of each dura mater was estimated using the Cavalieri principle.

2.3. Cavalieri's principle

The volume (V) of dura maters were estimated from "V = ta(p)ΣP" where t is the mean slice thickness (0.5 mm), a(p) the area associated with each point of the point-grid (1mm2 in this study; corrected for magnification), and ΣP is the total number of points hitting each dura mater. In the Cavalieri principle, a researcher obtains a data called coefficient of error (CE) to evaluate the precision of estimates and to see the reliability of the point density of the grids and sectioning intervals. Since the cut surface areas of consecutive sections are not independent quantities, conventional statistical formulae of CE cannot be applied to determine the variance of their sum. Many of the researchers developed some formulas to obtain the CE for the Cavalieri estimation method. Those formulas not only provide the CE but also give information on the required number of slices and density of the point counting grid (Fig. 1).

2.4. Statistical analysis

Data were analyzed using the IBM Statistical Package for Social Sciences v13 (SPSS Inc., Chicago, IL, USA). The results for all items were expressed as mean±SD, assessed within a 95% reliance and at a level of $p<0.05$ significance. Parametric tests were applied to data of normal distribution and nonparametric tests were applied to data of questionably normal distribution.

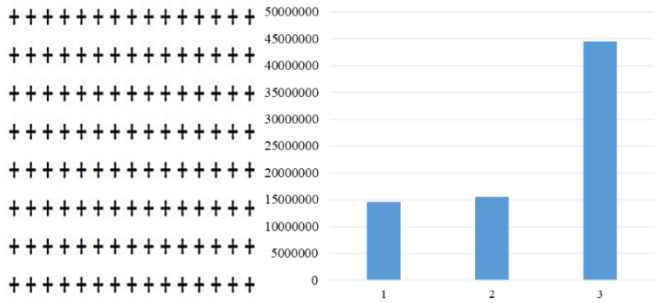

Fig. 1. Point counting grid used in the present study according to Cavalieri estimation method and graphical view

3. Results

No neurological deficits or wound infections were developed. In Group 1, regularly organized collagenous fibers were observed. In Groups 2 and 3, heterogeneous and regularly organized arrays of collagenous fibers were observed. In Group 3 (PRP Group), a significant increase in the volume of dura mater was determined when compared with other groups ($p< 0.05$). Areas of neogenesis and neovascularization were observed more frequently in the regions near the defect in group 3. On these regions, markedly higher osteoblastic activity was observed due to more intense peripheral infiltration tissue, while inflammation was less intense on the regions in the vicinity of neo-osteogenic areas. Newly formed bone tissue around the intact bone was extending into the defective area. In all experimental groups, new areas of bone, and vascularizations on the edges of the defective areas attracted our attention. In all groups patchy areas of narrowing were observed on arachnoid membranes. In all groups any macroscopic changes were not observed in pia mater (Fig. 2). Mean volumes of dura mater in Groups 1, 2 and 3 were calculated as 14616000 (455705) micrometer cubed (µm^3), 15558400 (264649) µm^3 and 44515200 (327769) µm^3, respectively. Any statistically significant difference was not found between Groups 1, and 2 ($p>0.05$), while a significant difference was observed between Groups 1, and 3, and also between Groups 2 and 3 ($p<0.05$) (Table 1).

Table 1. Statistical data table

Group	Estimated Area µm²	Estimated Volume µm³	Corrected Volume-µm³	Error Coefficient	Section Shear Thickness (µm)	Census
1.Group	487200	14616000	14048000	0.064	10	1218
2.Group	534000	16020000	15368000	0.064	10	1335
3.Group	1507200	45216000	43464000	0.048	10	3768

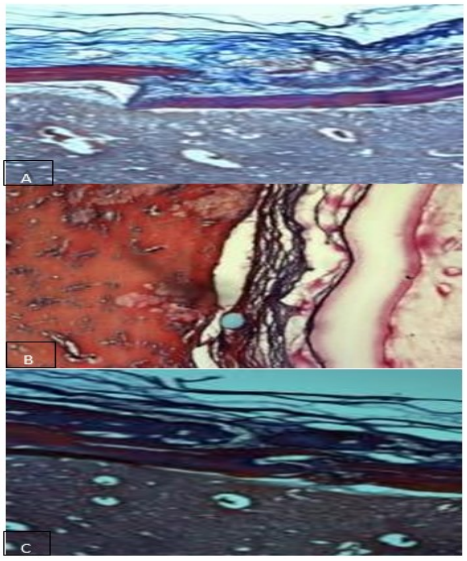

Fig. 2. The samples were embedded in paraplast and sectioned exhaustively. Coronal sections, 5 µm thick, were cut at a 500 µm sampling interval and stained with haematoxylin and eosin. **(A)** Group 1 (Control group): Craniectomy + dural incision **(B)** Group 2 (only suturing group): Craniectomy + dural incision + suturing **(C)** Group 3 (PRP group): Craniectomy + dural incision + suturing + PRP. A. In Group 1, regularly organized collagenous fibers were observed. B. In Groups 2, heterogeneous and regularly organized arrays of collagenous fibers were observed. C. In Groups 3, heterogeneous and regularly organized arrays of collagenous fibers were observed. Areas of neogenesis and neovascularization were observed more frequently in the regions near the defect in group 3. On these regions, markedly higher osteoblastic activity was observed due to more intense peripheral

4. Discussion

The dura mater is a collagenous tissue including various fibroblasts, collagen and elastic fibers. The dura mater may be damaged by trauma or surgery. Dural repair has recently garnered increased attention because CSF leakage leads to various secondary complications. Herein, we aimed to compare dural suture technique with combined suturing and PRP on the dural volume. Many neurosurgical interventions necessitate dural opening to maintain entree to central nervous system; besides, unplanned dura mater tears may arise frequently. Several harmful concerns of imperfectly treated dural CSF leaks are described (11). Collection of fluid avoids appropriate wound healing and can lead to incision infection and/or breakdown (12, 13). A constant leak of CSF may also lead to pseudo meningocele which may develop nerve roots trapping and neurological symptoms such as cranial nerve palsies, particularly of cranial nerve VI, which manifests as strabismus (14). The principles for the ultimate tissue adhesive comprise anticipation of CSF leak, no increase in infection rate, minimal foreign body or allergic reaction, cost effective, simplicity of application and availability. Consequently, the most essential characteristic of management of a CSF leak is avoidance. Dural suture is a simple and reliable method extensively preferred in cranial surgery (15, 16). Watertight suturing of a dural defect is not an easy surgical procedure in the deep, narrow surgical fields. After the hematological mediators were discovered, they were revealed to help wound healing. These mediators have proliferative cellular effects, which is also true for PRP. Farrag et al. applied PRP in cases with facial nerve injuries, and reported its favorable effects on the nerve regeneration (17). Sariguney et al. applied PRP in cases with sciatic nerve injuries, and reported its favorable contribution to axonal regeneration (18). In the present study, heterogeneous and regularly organized arrays of collagenous fibers were observed in suturing groups (Groups 2 and 3). Elgazzar et al. used cyanoacrylate sealants, and PRP in combination in cases with end-to-end sciatic nerve anastomosis, and reported successful anastomosis (19). Lichtenfels et al. used autologous nerve grafts in combination with PRP in anastomosis of sciatic nerves and published their successful results (20). Ding et al. and Wu et al., detected prominent contribution of PRP to axonal remyelination in cases with cavernous nerve injuries (21, 22). Cho et al. used PRP and mesenchymal stem cells in combination in cases with facial nerve injuries, and revealed its favorable effect on regeneration (23). Zheng et al. reported favorable effects of PRP on proliferation, and migration of Schwann cells, and also synthesis of neurotrophic factors (24). Zhao et al. used PRP, brain-derived neurotrophic factor, and stromal cells of bone marrow in combination, and reported functional improvement through achievement of axonal remyelination in cases with spinal cord injuries (25). In the present study, a significant increase in the volume of dura mater was determined in Group 3 (PRP Group) when compared with other groups. Furthermore, in Group 3 neogenesis, neovascularization, and osteoblastic activity were more frequently encountered, while inflammation was less intense in the regions near the defect when compared with other groups. Animal studies showed that experimental animals infrequently develop meningitis regardless of marked CSF leakage and histologically confirmed inflammation. CSF leak in humans is challenging to avoid and also treat, and leads to morbidity and mortality after some special neurosurgical approaches (26). That is why a persistent dural seal makes a clinically important difference in the prognosis. One of the limitations of this study is the short follow up of eight weeks. However, this time period is the equivalent of 27 human months (27). Although we still consider our results as preliminary, they warrant a larger comprehensive study, one which would include more rats in order to evaluate more precisely the role of platelet rich plasma in dural tear pathogenesis.

When all these findings are taken into consideration, according to our study, PRP conferred a marked benefit in the reconstruction of dura mater defects. We conclude that PRP can be safely used for the repair of dura mater defects. Its neo-osteogenic activity also signifies its significant effect on bone defects.

Conflict of interest

The authors have no conflict of interest declaration.

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Investigation of in vitro activity of colistin and tygecyclin against *Stenotrophomonas maltophilia* isolates

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Abstract

Stenotrophomonas maltophilia has emerged as an important opportunistic pathogen, causing infections whose management is often problematic due to its inherent resistance to many antibiotics. In this study, we aimed to investigate the antimicrobial susceptibility of colistin and tygecyclin as an alternative treatment options for *S. maltophilia* infections. A total of 122 *S. maltophilia* isolates were tested. Minimum inhibitory concentration (MIC) values of colistin and tygecycline were determined by broth microdilution method. Susceptibility of TMP/SMX and levofloxacin (LVX) were determined by disc diffusion method and MIC value of ceftazidime (CAZ) was determined by using E-test. Out of 122 S. maltophilia isolates, 5 (4%) of them were resistant to TMP-SXM. MIC range was 0.125- >512 µg/ml and MIC_{50} 64 µg/ml, MIC_{90} 512 µg/ml for colistin. MIC range for tygecyclin was detected as 0.5- >8, MIC_{50} 2 µg/ml and MIC_{90} 8 µg/ml. Tygecyclin resistance was detected as 66.4% according to the EUCAST guideline and 13.1% according to the USA-FDA breakpoints. And colistin resistance was determined as 86.9% according to both guidelines.

Keywords: colistin, MIC, *S. maltophilia*, tygecyclin

1. Introduction

Stenotrophomonas maltophilia is an important nosocomial pathogen in certain patient populations, particularly in individuals who are immunosuppressed (1). *S. maltophilia* usually appears in immunocompromised and intensive care unit (ICU) patients, also frequently recovered from the respiratory tract of cystic fibrosis patients, and generally associated with infections of the respiratory tract, the organism is also a cause of bacteremia, endocarditis and urinary tract infections (2, 3). *S. maltophilia* infections are associated with high morbidity and mortality with the risk of mortality highest amongst patients receiving inappropriate initial antimicrobial therapy (4).

S. maltophilia is commonly resistant to several antimicrobial agents, including beta-lactams, due to heterogeneous production of b-lactamases (1). Reduced permeability and expression of efflux pumps could enhance this resistance phenotype (5, 6). Trimethoprim/sulfamethoxazole (TMP/SMX) is the main antimicrobial of choice for the treatment of *S. maltophilia* infections with ticarcillin/clavulanate, ceftazidime, minocycline, fluoroquinolones, tigecycline, and the polymyxins are described as alternative therapies (7). TMP/SMX resistance has been described and as high as 10% of isolates in Europe (8, 9). Tigecycline is the first glycylcycline antimicrobial and licensed for clinical use. Tigecycline binds to the 30S ribosomal subunit and inhibits synthesis of protein. It has a wide range of activity against both Gram-positive and Gram-negative organisms (9).

Colistin, also known as polymyxin E, is an old antibiotic and has in vitro activity against some multi-resistant Gram-negative bacteria, including *P. aeruginosa, A. baumannii* and *Klebsiella pneumoniae.* Beta-lactams, aminoglycosides, or quinolones are ineffective, colistin, remain drugs of last choice (10). Colistin has also been shown to possess in vitro activity against *S. maltophilia* strains (83%–88%) of the tested isolates were susceptible to colistin in two recent studies) (8, 11, 12). The aim of this study was to assess the antimicrobial resistance in *S. maltophilia* against colistin and tigecycline.

2. Materials and methods

A total of 122 *S. maltophilia* isolates recovered from hospitalized patients in medical, surgical wards and in intensive care units were tested. Bacterial identification was made by using standard algorithms (microscopy, culture characteristics, oxidase reaction) followed by an automated system (Vitek MS, bioMeriux USA). Minimum inhibitory concentration (MIC) values of colistin and tigecycline were determined for all isolates based on the Clinical Laboratory Standards Institute (CLSI) (13) broth microdilution method. For tigecycline susceptibility fresh cation-adjusted Mueller-Hinton agar was used. Susceptibility of TMP/SMX and levofloxacin (LVX) were determined by disc diffusion method and MIC value of ceftazidime (CAZ) was determined by using E-test.

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Tigecycline breakpoints established by the USA-FDA for Enterobacteriaceae (≤2 / ≥8 µg/ml for susceptibility/resistance) and EUCAST for Enterobacteriaceae (≤1 / >2 µg/ml for susceptibility/resistance) as well as colistin breakpoints established by the CLSI for P. aeruginosa (≤2 / ≥8 µg/ml for susceptibility/resistance), and the EUCAST for P. aeruginosa (≤4 / >4 µg/ml for susceptibility/resistance), were applied for comparison purposes (14-16). MIC50 and MIC90 values were determined for each antimicrobial. TMP/SMX, LVX and CAZ susceptibilities were interpreted according to the CLSI criteria established for *S. maltophilia* (14).

3. Results

Clinical sites of infection for *S. maltophilia* were primarily bloodstream (35.3%) and respiratory tract (33.6%) (Table 1).

Table 1. Distribution of clinical specimen

Specimen	n(%)
Respiratory tract	41 (33.6)
Bloodstream	43(35.3)
Urine	19 (15.6)
Wound	10 (8.2)
Catheter tip	5 (4.1)
Sterile body fluid	2 (1.6)
Conjonktiva	2 (1.6)

Out of 122 S. maltophilia isolates, 5 (4%) of them were resistant to TMP-SXM. LVX and CAZ resistance were determined as 6.5% and 56.5%, respectively.

MIC range was 0.125- >512 µg/ml and MIC_{50} 64 µg/ml, MIC_{90} 512 µg/ml for colistin. For tigecycline, MIC range was detected as 0.5->8, MIC_{50} 2 µg/ml and MIC_{90} 8 µg/ml. Tigecycline resistance was detected as 66.4% according to the EUCAST guideline and 13.1% according to the USA-FDA breakpoints. And colistin resistance was determined as 86.9% according to both guidelines (Table 2).

Susceptibility rates according to the clinical sites were specified in Table 3. Tigecycline susceptibility was determined highest in conjunctiva and sterile body fluids as 50%, however the specimen number is very low (n=2) for these clinical sites. For bloodstream and respiratory tract specimens tigecycline susceptibility was determined as 39.5%-88.4% and 12.2%-78.0%, according to the EUCAST and USA-FDA breakpoints, respectively. The highest colistin susceptibility were determined for bloodstream isolates as 21%. And for catheter tips, conjunctiva and sterile body fluids tigecycline seems more effective than colistin *in vitro.*

Table 2. Resistance rates for tigecycline and colistin according to the EUCAST and CLSI/USA-FDA criteria

	EUCAST	CLSI/USA FDA	MIC_{50} MIC_{90}
	R	R	
Tygecyclin	81 (66.4%)	16 (13.1%)	2 8
Colistin	106 (86.9%)	106 (86.9%)	64 512

Table 3. Distribution of tigecycline and colistin susceptibilities according to the clinical specimens

	Tygecyclin EUCAST(S) CLSI(S)	Colistin (S) EUCAST CLSI
Blood stream (n:43)	39.5% 88.4%	21% 21%
Respiratory tract (n:41)	29.3% 78.0%	12.2% 12.2%
Urine (n:19)	36.8% 79.0%	5.3% 5.3%
Wound (n:10)	30.0% 60.0%	10.0% 10.0%
Catheter tip (n:5)	0% 60.0%	0% 0%
Sterile body fluid (n:2)	50% 100%	0% 0%
Conjunctiva (n:2)	50% 100%	0% 0%

4. Discussion

In this study, in vitro effectiveness of tigecycline and colistin was investigated as an alternative for treatment of *S. maltophilia* isolates that were isolated from clinical samples in our hospital.

S. maltophilia is accepted as a pathogen with gradually increasing importance recently. The reason for this may be increasing number of immune-compromised patients, prolonged hospital stays and increasing use of wide spectrum antibiotics like carbapenems (17). *S. maltophilia* was detected to be the most common non-fermentative bacillus following *P. aeruginosa* and *Acinetobacter spp.* between 1997-2001 and isolation ratio was found as 8% in clinical samples (18).

S. maltophilia may lead to respiratory tract, bloodstream, urinary tract and wound infections. In the studies, *S. maltophilia* was shown to be isolated from different sample types. While vast majority of the isolates were isolated from blood samples in this study, respiratory tract samples, urinary tract samples were the most common in some other studies (19-22).

TMP-SXM has been considered as the first therapeutic option against *S. maltophilia* infections, but this is primarily based on in vitro susceptibility data (7). However, increasing resistance to trimethoprim/sulfamethoxazole has been reported (23, 24), mostly related to the horizontal spread of mobile genetic elements which are carrying resistance genes (25, 26).

The fluoroquinolones are one of the other main alternative treatment options for the *S. maltophilia* infections (7). According to reports, primarily ciprofloxacin, levofloxacin and, particularly, moxifloxacin can have more potent in vitro activity (l5, 27,29). Also, it is reported that resistance to the fluoroquinolones can arise during therapy. In our study, the in vitro resistance to levofloxacin was 6.5% (30-32). Among the beta lactams, ceftazidime is the agent that can be considered as potential therapeutic options against *S. maltophilia*

infections (23). In our study, the susceptibility to this agent was rather low as 43.5%, in agreement with other relevant studies (33).

New treatment options are required due to limited number of antimicrobial agents and resistance development against the agents used for treatment of *S. maltophilia*. Colistin and tigecycline are among these new options. In a study investigating colistin susceptibility in *S. maltophilia* isolates, colistin resistance was found to elevate to 60% in 2010 while it was 8% in 1996. Authors reported that this was associated with increasing use of colistin (34). Colistin resistance was found between 24-100% in a few studies which was conducted with small number of *S. maltophilia* isolates (8, 35-37). Samonis et al. (19) found colistin susceptibility as 91.2%. The clinical breakpoints determined for *P. aeruginosa* by CLSI was used in these studies. Susceptibility method variabilities were reported as the reason for differences in the resistance rates (36). Geographic region and patient population were also reported to be able to be effective on colistin resistance profile (33, 37, 38).

Insa et al. (39) investigated the effectiveness of tigecycline in 120 *S. maltophilia* isolates and found susceptibility of isolates as 98% when they accepted breakpoint value as ≥2µg/ml. Farrel et al. (33) found tigecycline susceptibility as 95% (USA-FDA criteria were used as limit value for tigecycline) in their study investigating tigecycline susceptibility in *S. maltophilia* isolates isolated from different regions of the world. Tigecyclin was found as the most effective agent following TMP-SXM also in this study. Colistin susceptibility was found as 64.6% in the same study (33). Absence of a clinical breakpoint value for *S. maltophilia* in categorical assessment of tigecycline susceptibility and use of different clinical breakpoint influence resistance rates (14-16). When tigecycline susceptibility was evaluated in this study, while resistance rate is 13.1% according to USA-FDA criteria, this value elevated to 66.4% according to EUCAST criteria.

Absence of a specified clinical breakpoints for assessment of colistin and tigecycline susceptibility for *S. maltophilia* isolates seems to be one of the reasons for detecting different susceptibility rates. Determination of clinical breakpoints against these agents with future studies conducted with larger series is suggested to be useful for treatment of *S. maltophilia* infections which is gradually increasing.

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Research Article

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Affecting factors on the publication rate of surgical theses from different departments in Turkey

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Abstract

The aim of the study is to investigate and compare the factors affecting publication rates of otorhinolaryngology (ORL) theses and plastic and reconstructive surgery (PRS) theses. In order to examine ORL and PRS specialization theses published between 2013 and 2017, the author scanned the Council of Higher Education Thesis Center's browsing system that contains a list of all published theses for the terms "ear, nose, and throat" and "plastic and reconstructive surgery". All accessible theses (in total, 689), including 454 ORL and 235 PRS theses were included in the study. Most ORL and PRS theses authors were male (72.5% and 84.3%, respectively). Most of the ORL theses were conducted in state universities (76.9%), whereas most of the PRS theses were conducted in public universities (87.2%). More than half (50.9%) of the ORL advisors were professor doctors, compared to 46.3% of the PRS advisors. Most of the ORL studies were clinical studies (81.7%), while 74.9% of the PRS studies were experimental animal studies. In total, 24.5% of the ORL theses were published, and 29.7% of them were accepted in SCI-indexed journals. In comparison, a total of 19.6% of the PRS theses were published. The publication rates of experimental PRS and ORL studies were significantly higher than clinical studies. In order to increase the quality of medical theses and to publish them in scientific journals, the frequency of thesis production should be increased and that the factors affecting publication rates should be carefully examined, monitored, and improved.

1. Introduction

The Regulation on Specialist Education in Medicine and Dentistry in Turkey states that "It is obligatory (for specialization students) to prepare a master's thesis, in order to take the final examination of specialization in a main branch." (1). In writing a thesis, specialization students must develop their ability to establish a scientific hypothesis, evaluate the hypothesis using appropriate methodology with the guidance of data, and compare the results with the literature. It can be said that each thesis is a contribution to the knowledge of its field. Publication of a thesis in a peer-reviewed journal is one of the most effective methods of evaluating its quality. In particular, publication in journals that belong to certain indexes is an essential indicator of the importance and accuracy of a study. In addition, publications in these indexes are often accessed and evaluated by more people.

Previous studies have investigated the publication rates of theses from different countries and different fields. The publication rates of theses vary by country, ranging from 17% to 23% (2-4) The publication rate of theses related to family practice in Turkey has been estimated as 11% and 12% in two different studies (5, 6). In addition, as of 2014, 15% of theses published were in the field of orthopedics; as of 2013, 27% of theses published were in the field of emergency medicine (7, 8). Various studies have identified different publication rates for theses in different fields: infectious diseases (10%), public health (34%), and ophthalmology (50%) (9-11). It has been observed that 35% of speciality theses related to the field of otorhinolaryngology (12), 34% of master's and doctorate theses related to the field of audiology between 2007 and 2012 (13) and 22% of the papers submitted to otorhinolaryngology congresses (14) between 2008 and 2010 have been published.

The factors affecting the publication rates of theses have not been adequately examined and evaluated using appropriate statistical methods in the literature. These factors include the gender of the researcher, the academic institution of the researcher, the academic title of the thesis supervisor, the type of study, the number of discussion pages written in the thesis, and the total number of references used in the thesis. In order for a greater number of qualified scientific publications to be derived from specialization theses, the relationship between the criteria for publication and the publication rates of theses should be investigated.

In the present study, I aimed to investigate the factors affecting publication rates of otorhinolaryngology (ORL) theses in Turkey and compare with the factors affecting publication rates of plastic and reconstructive surgery (PRS) theses between 2013 and 2017.

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2. Materials and methods

2.1. Study design

In order to examine ORL and PRS specialization theses published between 2013 and 2017, I scanned the Council of Higher Education Thesis Center's browsing system that contains a list of all published theses for the terms "ear, nose, and throat" and "plastic and reconstructive surgery" (15). All accessible theses performed by relevant departments and clinics were included in the study.

A total of 689 abstracts of theses were accessible, including 454 ORL and 235 PRS theses. I accessed the full text of the 537 theses and recorded the number of discussion pages and the number of references used. I also recorded the gender of the author, the year of the thesis, the type of institution where the thesis was produced, the academic title of the thesis supervisor, and the type of study (clinical study/experimental animal study). I determined the publication status of theses by searching the authors' surnames in Google Scholar and PubMed Central indexes. I recorded the country of publication (Turkey, International), the journal index (Science Citation Index/ Science Citation Index Expanded/Other), and the year and language of publication.

2.2. Statistical analysis

I analyzed and uploaded the research data via SPSS for Windows 15.0 (SPSS Inc., Chicago, IL). I presented descriptive statistics as median (min-max), frequency distribution, and percentage. I used Pearson's chi-square test to evaluate categorical variables and examined the convenience of the variables to normal distribution using visual (histogram and probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk test). For variables that were not found to fit the normal distribution, I used the Mann–Whitney U test to determine the presence of a statistical significance between two independent groups, and the Kruskal–Wallis test for three independent groups. When I detected a significant difference between the three independent groups, applied a post-hoc Bonferroni correction test to determine the source of the difference. I accepted the level of statistical significance as $P < 0.05$.

3. Results

Within the scope of the research, a total of 689 specialty theses were accessible: 454 ORL theses and 235 PRS theses. Most of the ORL and PRS theses authors were male (72.5% and 84.3%, respectively). Most of the ORL theses were conducted in state universities (76.9%), whereas most of the PRS theses were conducted in public universities (87.2%). More than half (50.9%) of the ORL advisors were professor doctors, compared to 46.3% of the PRL advisors. Most of the ORL studies were clinical studies (81.7%). In comparison, 74.9% of the PRL studies were experimental animal trials (Table 1).

In total, 24.5% of ORL theses were published, and 29.7% of these were accepted in SCI-indexed journals. In comparison, 19.6% of the PRS theses were published, and 19.6% of these were published in SCI-indexed journals. Most ORL and PRS theses were published in international journals (70.3% and 80.4%, respectively). The majority of ORL and PRS theses were published in English (94.6% and 93.5%, respectively) (Table 2).

Table 1. Descriptive characteristics of the theses analyzed

The year of the publication	ORL (n=454) n (%)	PRS (n=235) n (%)
2013	73 (16.1)	47 (20.0)
2014	106 (23.3)	61 (26.0)
2015	93 (20.5)	44 (18.7)
2016	88 (19.4)	33 (14.0)
2017	94 (20.7)	50 (21.3)
The gender of the first author of the thesis		
Male	329 (72.5)	198 (84.3)
Female	125 (27.5)	37 (15.7)
The institution where the thesis was performed		
State University	349 (76.9)	205 (87.2)
Training and Research Hospital	87 (19.1)	22 (9.4)
Foundation University	18 (4.0)	8 (3.4)
Academic title of the advisory of the thesis		
Professor	231 (50.9)	109 (46.3)
Associate Professor	167 (36.8)	65 (27.7)
Assistant Professor or Lecturer	56 (12.3)	61 (26.0)
Type of the study		
Clinical Study	371 (81.7)	59 (25.1)
Experimental animal study	83 (18.3)	176 (74.9)

ORL, Otolaryngology diseases; PRS, Plastic and reconstructive surgery

The publication rate (27.0%) of all theses derived from experimental animal studies was significantly higher than that of those derived from clinical studies (20.2%) (P = .039) (Table 3). However, the gender of the author, type of institution, academic title of the thesis supervisor, specialty branch, number of discussion pages, and number of references were not statistically significant (P>0.05) (Table 3). When the theses were evaluated separately according to the two different branches, ORL and PRS, the effect of the independent variables did not change (P>0.05).

The median length of the discussion sections of theses published in SCI journals was seven pages (min: 2, max: 14). The median number of references was 91 (min: 51, max: 236). The median length of discussion sections of theses published in journals non-SCI journals was seven pages (min: 3, max: 20). The median number of references was 91 (min: 29, max: 233). There was no statistically significant difference between the theses published in SCI or non-SCI journals in terms of the length of discussion sections and the total number of references (z = -0.86, P = .392; z = -0.481, P = .631, respectively).

A statistically significant difference was found between the publication status of the theses and the year in which they were published (P <0.05). The lowest publication rates occurred in 2017. A statistically significant difference was also found between the academic titles of thesis advisors and the amount of time elapsed between the completion of a thesis and its publication (P <0.05). Post-hoc paired comparisons revealed that the time interval between completion and publication was significantly shorter for theses advised by assistant professors or lecturers compared to those advised by professors (Table 4).

Table 2. Publication status of the theses analyzed, their citation indices, origin and language of the journals that published these theses

Publication status of the theses	ORL (n=454) n (%)	PRS (n=235) n (%)
Published	111 (24.5)	46 (19.6)
Unpublished	343 (75.5)	189 (80.4)
Citation indices (n=157)		
Science Citation Index (SCI)	33 (29.7)	9 (19.6)
SCI-Expanded	52 (46.8)	23 (50.0)
Others	26 (23.4)	14 (30.6)
Origins of the Journals (n=157)		
Turkey	33 (29.7)	9 (19.6)
Abroad	78 (70.3)	37 (80.4)
Language of the Publication (n=157)		
Turkish	6 (5.4)	3 (6.5)
English	105 (94.6)	43 (93.5)

ORL, Otolaryngology diseases; PRS, Plastic and reconstructive surgery

Table 3. Distribution of some characteristics of the thesis published

Variables	Publication status		χ^2/Z	P
	Not published (n=532)	Published (n=157)		
Responsible author of the thesis, n (%)				
Male	405 (76.9)	122 (23.1)	0.17	682
Female	127 (78.4)	35 (21.6)		
The institution where the thesis was performed, n (%)				
State University	427 (77.1)	127 (22.9)	1.37	505
Training and Research Hospital	87 (79.8)	22 (20.2)		
Foundation University	18 (69.2)	8 (30.8)		
Academic title of the thesis advisor, n (%)				
Professor	260 (76.5)	80 (23.5)	2.18	337
Associate Professor	186 (80.2)	46 (19.8)		
Assistant Professor / Lecturer	86 (73.5)	31 (26.5)		
Type of the study, n (%)				
Clinical study	343 (79.8)	87 (20.2)	4.24	039*
Experimental animal study	189 (73.0)	70 (27.0)		
Specialty, n (%)				
ORL	343 (75.6)	111 (24.4)	2.09	148
PRS	189 (80.4)	46 (16.6)		
Number of pages reserved for the "Discussion" section, median (min-max)	7 (2-38)	7 (2-20)	0.05	0.960
Total number of references, median (min-max)	96 (25-347)	91 (29-236)	0.28	0.778

*P<0.05

Table 4. Investigation of the academic title of the thesis advisor, and the time to publication of the thesis

Academic title of the thesis advisor, n (%)	n	Time to publication (years) median (min-max)	χ^2	P
Professor	80	2 (1-5)	27.7	<0.001*#
Associate Professor	46	2 (1-6)		
Assistant Professor / Lecturer	31	1 (1-5)		

*P<0.05; # the significant difference in post-hoc pairwise comparisons was ensourced from the "assistant professor or lecturer" group

4. Discussion

The publication of medical specialization theses on critical scientific topics in peer-reviewed journals is an essential criterion in the evaluation of the quality of the study. In the present study, I aimed to analyze publication rates of theses written in the fields of ORL and PRS between 2013 and 2017 and the factors affecting them.

I found that 72.5% of the authors of ORL theses were male. A similar study found that 79.3% of ORL theses written between 2007 and 2012 were written by males (12). This is unsurprising, as fewer women than men in Turkey choose to pursue medical careers in surgical branches. This trend has not changed much within the last five years.

In the present study, I found that 76.9% of the ORL theses were performed in state universities, while 19.1% were performed in education and research hospitals. Aslı Çakır Çetin et al. (12) examined theses published between 2007 and 2012 found that 87% of the theses were conducted in state universities and 11.7% in education and research hospitals. As explained by authors of mentioned study, theses and dissertations can only be identified using the Council of Higher Education's browsing system, so the inability to identify theses realized in some hospitals may be the cause of this discrepancy (12). I identified two factors affecting the publication rates of theses: the type of study and the time elapsed between completion of the thesis and publication. My study and mentioned study found that approximately one-fifth of the ORL theses were experimental animal studies. Experimental animal studies were published significantly more frequently. This may indicate that there are some problems in the design of clinical trials that affect the publication rates of theses. Although three-quarters of the PRS theses examined were experimental animal studies, the fact that PRS theses were published less frequently than the ORL theses shows that this is not a factor in itself. Reasons for this preference for experimental animal studies over clinical studies in PRS theses may be the subject of new research.

In the present study, I found that 50.9% of the ORL thesis

advisors were professors, and 36.8% were associate professors. In comparison, 46.3% of PRS thesis advisors were professors, and 27.7% were associate professors. Also, when I examine possible reasons for the length of time between thesis completion and publication, the only significant variable is the academic title of the thesis advisor. The theses that were supervised by assistant professors and lecturers were published in a shorter time. This may be due to the fact that assistant professors and lecturers more frequently feel the need to produce publications than do full-time professors. The only comparable study is that of Mengüllüoğlu et al. (6), who evaluated family practice theses between 2005 and 2015 and found that 34% of thesis advisors were associate professors and 19% were professors. This result may be related to the fact that the Departments of Family Practice is a new medical discipline in Turkey; thus, the field lacks a sufficient number of academics. It may also suggest that the academic staff of ORL departments is more qualified than that of the PRS departments in Turkey.

This study aimed to compare the publication of ORL and PRS theses. In this study, no statistically significant difference was found in terms of publication rates of theses of the two branches. The publication rates of ORL and PRS theses were 24% and 16%, respectively. My findings revealed that 24.5% of ORL specialization theses written between 2013 and 2017 were published. In a similar study, Aslı Çakır Çetin et al. (12) reported that 35.6% of ORL theses were published. This difference is probably due to the fact that the theses analyzed by these authors were completed no later than 2012 (four years before the time study was conducted). Mentioned research chose to conduct a retrospective examination covering 2007 and 2012 years. The lowest publication rate of these theses reviewed in the present study was detected in 2017. If I was to repeat our study four years from now, I could expect that additional theses will have been published. Similar publication rates were detected for master's and doctorate theses in the field of audiology: a study found that 34% of master's theses and 39% of doctorate theses were published (13). One study examined the publication rate of theses in the field of orthopedics over a very long interval but found they remained at 15% (7). In comparison, 11% of family practice theses were published, (5, 6) 34% of public health theses were published (9). Another study reported that approximately 50% of ophthalmology theses were published (11). However, since the methodology of this study was based on self-reports of accessible specialists, it is unlikely that the result is accurate.

In a large-scale study evaluating theses in all branches of medicine, 6.5% were published in SCIE journals (16). The same study found that 18% of all ORL theses examined were published in SCI and SCIE journals, which corresponds to approximately three-quarters of the theses published. Approximately 13% of all PRS theses were published in SCI and SCIE journals. According to the literature, publication rates in SCI journals vary between 3.5% and 32.7% (17). The findings in my study are consistent with the literature.

The time elapsed between completion and publication of a thesis is a controversial issue. Of the theses examined, at least one year has passed between completion and publication. However, I found a significant difference in the publication rate of theses in 2013. When evaluating the publication rates of these theses, it should be noted that at least five years have passed since their completion. I also found that theses submitted in 2017 were published significantly less frequently than those submitted in other years. This condition also indicates that a minimum of two years should be passed over the completion of the thesis. In the present study, we found no significant difference between publication rates in 2014, 2015, and 2016.

One way to evaluate the quality of a thesis is to look at the number of pages in its discussion section. It is expected that approximately one-third of a thesis will consist of discussion. This criterion is rarely met by medical theses published in Turkey. The number of references used is also thought to affect the quality of the thesis. However, in the present study, the number of discussion pages and the number of references used in a thesis did not appear to affect publication rates. This shows that the quality of a thesis is based more on the product of quality content than the quantity of content.

In order to increase the quality of medical theses in Turkey so that more may be published in scientific journals, the frequency of medical thesis completion and publication must be increased. Therefore, the factors that influence publication rates in all disciplines should be studied further. The significantly lower publication rates of clinical trials may indicate problems with clinical trial designs. I believe that the evaluation of thesis subjects and study designs by a scientific committee authorized by academic departments within universities can solve this problem.

Conflict of interest

None to declare

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Is there any possibility of uterine sarcoma, STUMP and benign myoma variants in the patients operated for myoma uteri

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Abstract

Malignant pathologies may be observed in the histopathological examination of the patients who were operated with the diagnosis of myoma uteri. We aimed to investigate the rates of detection of uterine sarcoma, smooth muscle tumor of uncertain malignant potential and benign myoma variants who were operated due to myoma uteri. Patients who were operated with the diagnosis of myoma uteri between 2012-2018 were included. Patients with and without malignant pathology were compared in terms of their characteristics. The malignancy was encountered in 39 patients (1.5%) among 2583 patients. A significant difference was found between the patients with and without malignancy in terms of age, admission complaints, and cervical smear results. Patients in the malignant group were found to be older (52.5±11.0 vs 48.1±6.1, p=0.016). Postmenopausal bleeding was significantly a more common complaint in the malignant group (p=0.028). The rate of abnormal cytology in the cervical smear results in the malignant group was 5.1% (p=0.004). Pathology reports of the patients who were operated for myoma uteri may result with malignancy. In the preoperative evaluation, it is necessary to pay attention to the patients' characteristics, to evaluate the risk factors for the possibility of a malignancy.

Keywords: hysterectomy, myoma uteri, myomectomy, STUMP, uterine sarcoma

1. Introduction

Myoma uteri which is the most common benign, monoclonal tumor in the reproductive-aged women (1). It has been reported in the literature that women have myoma uteri with an incidence ranging from 5% to 40% (2). When the hysterectomy specimens are histopathologically examined, approximately 70% of the cases are found to have myoma uteri (3). The clinical symptoms and signs of myoma uteri depend on the location, size and number of myoma uteri. The most common symptoms are abnormal uterine bleeding, pelvic pain, dysmenorrhea, and infertility. The most common indication for hysterectomy is myoma uteri and 40-60% of hysterectomies are performed due to myoma uteri (4, 5).

Epidemiological factors including age, race, body mass index (BMI), heredity, reproductive factors, sex hormones, obesity, lifestyle factors (diet, caffeine and alcohol consumption, cigarette smoking, physical activity and stress), environmental factors, comorbid conditions, and genetic factors are responsible for the development of myoma uteri (6-10). Women with a history of familial myoma uteri are diagnosed at an earlier age, with more than one myoma uteri and undergo hysterectomy at an earlier age (11).

The main surgical methods in the management of myoma uteri are myomectomy and hysterectomy. Surgical treatment of myoma uteri is currently performed by laparoscopy rather than laparotomy due to the widespread use of non-invasive methods and increasing minimally invasive surgical skills of surgeons.

Pure mesenchymal tumors (sarcomas) such as leiomyosarcoma and endometrial stromal sarcoma and smooth muscle tumor of uncertain malignant potential (STUMP) are rarely diagnosed aggressive tumors and constitute approximately 3% of all malignant uterine tumors (12). Risk factors of uterine sarcomas include nulliparity, advanced age, history of pelvic radiation, number of spontaneous abortion and history of tamoxifen use. Uterine leiomyosarcoma with a similar ultrasonographic characteristics to myoma uteri is the most common uterine sarcoma (13).

There is no safe method to be used in the preoperative diagnosis of leiomyosarcoma. However, myoma uteri showing rapid change in appearance or in size in postmenopausal patients should be investigated. Furthermore, age, imaging characteristics including lacuna with necrotic spaces and

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increased central vascularization indicate increased risk of leiomyosarcoma at a relative annual incidence reported to be between 0.014-0.28% (14, 15).

The incidence of undiagnosed leiomyosarcoma and endometrial stromal sarcoma in patients who underwent hysterectomy with a diagnosis of myoma uteri has been reported between 0.05% and 0.28% (16). In these patients, performing hysterectomy due to myoma uteri delays the diagnosis and treatment for uterine malignancies. In addition, if an endoscopic morcellation is used without an endobag, there is a risk that the underlying malignant tissues may scatter around and be implanted into the abdomen.

Our aim was to determine the incidence of malignancy depending on the histopathological results and to compare their clinical, demographic, and preoperative ultrasonographic characteristics of the patients who had been operated for myoma uteri.

2. Materials and methods

This retrospective cohort study was conducted at Health Sciences University Istanbul Kanuni Sultan Suleyman Training and Research Hospital. Approval for the study was obtained from the local ethical committee (KAEK/2018.06.14). The principles stated in the Helsinki Declaration were followed and informed consent was obtained from the participants. All patients who underwent hysterectomy and myomectomy performed via abdominal, vaginal, or laparoscopic methods due to the preliminary diagnosis of myoma uteri between January 2012 and December 2018 were included in the study. Myoma uteri was diagnosed preoperatively in all patients using transvaginal and/or transabdominal ultrasonography according to koital status. Computed tomography and magnetic resonance imaging were performed in the case of cancer susceptibility. Patients who were not operated and those who underwent myomectomy with a hysteroscopic method were excluded from the study. Patients who had missing data were also excluded. A total of 2583 patients who met the eligibility criteria for the study were included.

Demographic characteristics of the patients including age, gravity, parity, history of abortion, mode of delivery, menstruation or menopausal status, comorbid conditions, history of previous operations, complaints during the consultation, cervical cytology results, ultrasonographic findings, endometrial sampling results, route of operation, and the presence of malignancy based on the histopathological results were examined. All these characteristics were compared among the patients with and without malignancy depending on the histopathological results.

2.1. Statistical analysis

Data analyzes were done with the Statistical Package for Social Sciences 22.0 (SPSS Inc, Chicago, Illinois, USA) package program. Descriptive statistical methods (Percentage, Average, Standard Deviation) were used while evaluating the study data. Independent samples two test was used because the data was normally distributed. Chi-square test was used to compare categorical data. The significance level of $p < 0.05$ was considered statistically significant in the 95% confidence interval. Thresholds for the association of age of the patients with malign tumors were determined by initially using receiver operating characteristics (ROC) curves to ascertain the optimal cut-off value. The sensitivity, specificity, positive likelihood ratio values were presented. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) to associate exposure variables with risk of developing malign tumors in both univariable and multivariable-adjusted models.

Table 1. Demographic and clinical characteristics of patients

Characteristics	mean±SD (min-max) or number (%)
Age (years)	48.1±6.2
Gravide	
-Nulligravid	94 (3.6)
-Multigravid	2489 (96.4)
Parity	
-Nullipar	104 (4)
-Multipar	2479 (96)
Abortus	
-Absent	1337 (51.8)
-Present	1246 (48.2)
Labor route	
-No birth	83 (3.2)
-Vaginal birth	1991 (77.1)
-Cesarean section	252 (9.8)
-Vaginal birth+cesarean section	257 (9.9)
Personal history	
-Absent	1989 (77)
-Present	594 (23)
Gynecological operation history	
-Absent	2374 (91.9)
-Present	209 (8.1)
Operation history	
-Absent	1870 (72.4)
-Present	713 (27.6)
Menstrual regularity	
-Irregular	1478 (57.2)
-Regular	711 (27.5)
-Menopause	394 (15.3)
Complaint	
-Control	103 (4)
-Menstrual irregularity	1618 (62.6)
-Pelvic pain	676 (26.2)
-Incontinence, prolapsus	76 (2.9)
-Postmenopausal bleeding	110 (4.3)

3. Results

The demographic characteristics of the patients are shown in Table 1. The mean age of the patients was 48.1±6.2 years. While 104 patients (4%) were nulliparous, 2479 patients (96%) were multiparous. Most patients gave birth vaginally (1991 patients, 77.1%). Among them, 23% of the patients had

some chronic diseases such as hypertension, diabetes mellitus, and thyroid dysfunction. In 209 patients (8.1%), there was a history of gynecological operations. Of those, 394 patients (15.3%) were postmenopausal, 1478 patients (57.2%) had irregular menstruation cycles, and 711 patients (27.5%) had regular menstruation cycles. The most common complaint of the patients (62.6%) was menstrual irregularity.

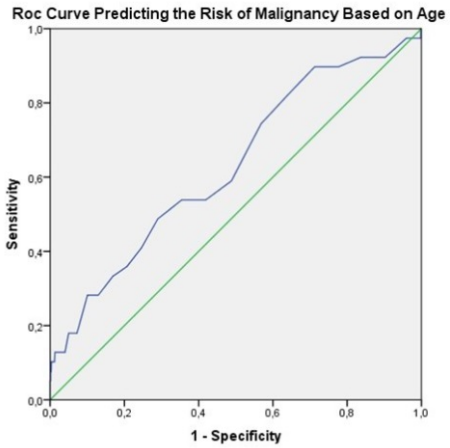
Table 2. Preoperative and postoperative clinical characteristics of patients

Characteristics	Mean±SD (min-max) or number (%)
Cervical smear results	
-Absent	318 (12.3)
-Normal	2228 (86.3)
-Preinvasive lesion	37 (1.4)
Adnexial pathology	
-Absent	2307 (89.3)
-Present	276 (10.7)
Myoma size (cm)	5.3±3.3
Endometrial thickness (mm)	11.4±5.2
Endometrial sampling result	
-Absent	1143 (44.3)
-Normal	1006 (38.9)
-Polyp	345 (13.4)
-Hyperplasia	89 (3.4)
Operation route	
-Abdominal	2013 (77.9)
-Laparoscopic	493 (19.1)
-Vaginal	77 (3.0)
Pathology	
-Myoma uteri	1902 (73.6)
-Adnexial mass	31 (1.3)
-Endometriosis	91 (3.5)
-Myoma uteri+endometriosis	520 (20.1)
-Cancer	39 (1.5)
Malignity	
-Absent	2544 (98.5)
-Present	39 (1.5)

The findings of detailed physical and gynecological examination preoperatively are presented in Table 2. While cervical smear was not performed in 318 patients (12.3%), 2228 patients (86.3%) had normal cervical smear results, and 37 patients (1.4%) had preinvasive lesion which were managed using additional diagnostic tests such as colposcopy and no cervical and/or vaginal malignant lesion was detected. The mean size of myoma uteri and endometrial thickness were 5.3±3.3 cm and 11.4±5.2 mm, respectively. Preoperative endometrial sampling was not performed in 1143 patients (44.3%). In most of the patients, the operation was performed abdominally (77.9%). When the postoperative histopathological results were evaluated, the diagnosis of myoma uteri was confirmed in 1902 patients (73.6%), while malignancy including uterine sarcoma, and STUMP was detected in 39 patients (1.5%).

The comparison of patients with and without malignancy depending on their demographic and clinical characteristics is shown in Table 3. There was significant difference between the groups regarding age (52.5±11.0 vs 48.1±6.1 years, p=0.016). When the gestational history of the patients was compared, no significant difference was found. It was determined that 5.1% of the patients with malignancy were nulligravid while this rate was 3.6% in benign cases (p=0.617). It was also determined that 5.1% of the cases having malignancy were nulliparous whereas 4% of benign cases were nulliparous (p = 0.724). It was observed that complaint of postmenopausal bleeding was more common in the malignant group compared to the benign group (12.8% vs 4.1%, p=0.028).

The only significant difference between the groups in terms of preoperative clinical characteristics of the patients was cervical smear results (5.1% vs 1.4%, p=0.004) (Table 4). While the mean size of myoma uteri were similar between the groups, the endometrial thickness was found to be thicker in the malignant group (5.3±3.4 vs 5.3±3.3 cm, p=0.979; 12.9±7.9 vs 11.3±5.2 mm, p=0.070; respectively). The most common route of operation was found to be the abdominal route in both groups (84.6% vs 77.8%, p=0.429). Although no statistical significance was detected in the logistic regression analysis, presence of comorbid conditions and previous history of gynecological operations increased the risk of malignancy (RR=1.77 (0.91-3.44), p=0.095; RR=1.32 (0.45-3.90), p=0.611) (Table 5).



Cut-off value for age	Area under curve	Sensitivity (%)	Specifity (%)	Positive likelihood ratio	p
49.5	0.63	53.8	64.6	1.52	0.005

Fig.1. The receiver operating characteristics (roc) curve predictand the risk of malignancy based on age

When the age-based roc curve was drawn to predict the risk of malignancy, statistical significance was found (p=0.005) (Fig. 1). The risk of malignancy increased with cut-off values for age more than 49.5 years. The sensitivity and specificity of that age to detect malignancy were 53.8% and 64.6%, respectively.

Table 3. Comparison of the patients regarding their demographic and clinical characteristics

Characteristics	Malignity		p
	Absent (n=2544)	Present (n=39)	
Age (years)	48.1±6.1	52.5±11.0	**0.016**
Gravide			
-Nulligravid	92 (3.6)	2 (5.1)	0.617
-Multigravid	2452 (96.4)	37 (94.9)	
Parity			
-Nullipar	102 (4)	2 (5.1)	0.724
-Multipar	2442 (96)	37 (94.9)	
Abortus			
-Absent	1318 (51.8)	19 (48.7)	0.702
-Present	1226 (48.2)	20 (51.3)	
Labor route			
-No birth	82 (3.2)	1 (2.6)	0.109
-Vaginal birth	1955 (76.8)	36 (92.3)	
-Cesarean section	250 (9.8)	2 (5.1)	
-Vaginal birth+cesarean section	257 (10.1)	0	
Personal history			
-Absent	1964 (77.2)	25 (64.1)	0.054
-Present	580 (22.8)	14 (35.9)	
Gynecological operation history			
-Absent	2339 (91.9)	35 (89.7)	0.617
-Present	205 (8.1)	4 (10.3)	
Operation history			
-Absent	1842 (72.4)	28 (71.8)	0.933
-Present	702 (27.6)	11 (28.2)	
Menstrual regularity			
-Irregular	1458 (57.3)	20 (51.3)	0.189
-Regular	702 (27.6)	9 (23.1)	
-Menopause	384 (15.1)	10 (25.6)	
Complaint			
-Control	103 (4)	0	**0.028**
-Menstrual irregularity	1591 (62.5)	27 (69.2)	
-Pelvic pain	669 (26.3)	7 (17.9)	
-Incontinence, prolapsus	76 (3)	0	
-Postmenopausal bleeding	105 (4.1)	5 (12.8)	

Data are presented as mean±SD or number (%), $p<0.05$ accepted as statistically significant

4. Discussion

Our aim was to investigate the detection rates of uterine sarcoma, STUMP and benign myoma variants in the patients who had been operated with the indication of myoma uteri. In our population, 39 patients (1.5%) were found to have malignancy according to their histopathological evaluation. We detected significant differences between the benign and malign cases in terms of age, complaint during the consultation, and cervical smear results. Myoma uteri, which affects 70-80% of reproductive-aged women, is the most common indication for hysterectomy, constituting 40-60% of all hysterectomy indications (4, 5, 12). The most common symptoms are abnormal uterine bleeding, pelvic pain, dysmenorrhea, and infertility. Myomectomy and hysterectomy are the main surgical approaches in the management of myoma uteri. With the increase of minimally invasive surgical skills of surgeons and the development of instruments used in laparoscopic surgeries, electromechanical morcellators have been developed, thus enabling hysterectomy with endoscopic methods even in the patients with large myoma uteri. The incidence of undiagnosed uterine sarcoma in the patients who underwent hysterectomy with a diagnosis of myoma uteri has been reported between 0.05% and 0.28% (16). The incidence of leiomyosarcoma was reported as 1/8300 in prospective studies, and 1/1700 in retrospective studies (17). The prevalence of malignant neoplasms was 0.34% according to a comprehensive study who underwent laparoscopic hysterectomy. However, histology of uterine malignant neoplasms was not reported in this study (18). In our study, 39 patients (1.5%) had malignancy in their histopathological results. The reason for higher incidence compared to other studies is that we deal with complicated cases at a tertiary referral center. Advanced age was determined as a predictor for uterine sarcoma according to our results. This supports the fact that advanced age is among the risk factors for uterine malignancy. In the most comprehensive study about this topic, it was concluded that the risk of malignancy was associated with increasing age (18). In a population-based study including uterine sarcomas, the rate of undetected uterine sarcoma was reported as 0.36% (19). In this study, the risk was found to be associated with older age. Adjusted relative risk was 2.5 times more between 50 and 59 years of age, compared to women aged younger than 50, and more than 12.8 times for older than 60 years of age. The risk of malignancy increased also significantly in the patients older than 49.5 in our results.

Table 4. Comparison of the patients regarding their preoperative and postoperative clinical characteristics

Characteristics	Malignity		p
	Absent (n=2544)	Present (n=39)	
Cervical smear result			
Absent	308 (12.1)	10 (25.6)	**0.004**
Normal	2201 (86.5)	27 (69.2)	
Preinvasive lesion	35 (1.4)	2 (5.1)	
Adnexial pathology			
Absent	2273 (89.3)	34 (87.2)	0.664
Present	271 (10.7)	5 (12.8)	
Myoma size (cm)	5.3±3.3	5.3±3.4	0.979
Endometrial thickness (mm)	11.3±5.2	12.9±7.9	0.070
Endometrial sampling result			
Absent	1129 (44.4)	14 (35.9)	0.111
Normal	990 (38.9)	16 (41)	
Polyp	340 (13.4)	5 (12.8)	
Hyperplasia	85 (3.3)	4 (10.3)	
Operation route			
Abdominal	1980 (77.8)	33 (84.6)	0.429
Laparoscopic	487 (19.1)	6 (15.4)	
Vaginal	77 (3)	0	

Data are presented as mean±SD or number (%), Used independent samples t-test or chi-square test; p<0.05 accepted as statistically significant

Black race has been reported to be another risk factor for both myoma uteri and uterine sarcoma with a 2-3 times higher risk than in white women (20). Other risk factors of uterine sarcomas include long-term tamoxifen use (5 years or more), pelvic irritation, history of childhood retinoblastoma, hereditary leiomyomatosis, and renal cell carcinoma syndrome (16). It was not possible to perform such an analysis in our study because we included only the patients from white race population and there were no patients having other risk factors listed above. Early menarche and nulliparity are accepted as risk factors for myoma uteri (16). It was shown in the literature that the risk of myoma uteri decreases with each pregnancy (21-23). However, the relationship of the parity with uterine sarcoma has not yet been proven. Although there was no significant difference in our population, the risk of malignancy increased in nulligravid and nulliparous patients when this possibility was compared between the groups.

Table 5. Results of logistic regression analysis

Risk factors	RR (95% CI)	p
Personal history	1.77 (0.91-3.44)	0.095
Gynecological operation history	1.32 (0.45-3.90)	0.611
Operation history	0.99 (0.48-2.06)	0.985
Adnexial pathology	1.28 (0.49-3.32)	0.609
Cervical smear result	0.53 (0.25-1.15)	0.107

Used binary logistic regression analysis, p<0.05 accepted as statistically significant RR, risk ratio; CI, confidence interval

Since abnormal uterine bleeding, pelvic pain, and pelvic mass can be a complaint of admission for both uterine myoma and sarcoma, it is not possible to use them in differential diagnosis (24, 25). In our study, although the main complaint was abnormal uterine bleeding in both groups, postmenopausal bleeding was significantly more common in the malignant group.

Cervical cytology is not a diagnostic tool for uterine sarcoma. Instead, this test is used to screen for cellular anomalies that may be associated with an increased risk of developing cervical cancer. In our clinic, cervical cytology is a screening test during preoperative examinations. The rate of abnormal cytology in our patients in the malignant group was found to be higher significantly compared to the patients in the non-malignant group. Thus, the patients having preoperative abnormal cervical cytology results should be further evaluated in terms of uterine sarcoma.

Endometrial sampling provides approximately 33% to 68% preoperative diagnosis for uterine sarcoma. There is no difference about the sensitivity between endometrial biopsy methods (26, 27). In our study, there was no significant difference in terms of endometrial sampling. This may be due to that endometrial sampling was not performed in 44.3% of the patients because of no suspicion of malignancy. However, endometrial sampling should be recommended in the patients with suspected sarcoma or whom intraperitoneal morcellation is planned (16).

There is a consensus that the presence of myoma uteri is not a risk factor for uterine sarcomas, except in rare cases. The most convincing data was obtained from the results of clinical and molecular genetic studies of a rare subset of myoma uteri with cellular or atypical histology (28-32). Sarcomas typically have complex karyotype and aneuploidy, while myoma uteri has characteristic rearrangements (33, 34).

Since rapid growth can be observed in both benign myoma uteri and sarcomas, it could not be a predictor for uterine sarcoma. In a study investigating 1332 patients who underwent myomectomy and hysterectomy due to myoma uteri, uterine sarcoma was diagnosed in 0.27% of the patients with rapid growth of uterus and 0.15% of the patients without rapid growth. Sarcoma was not detected in many premenopausal women with a rapidly growing uterus or uterine mass (32). On the other hand, the postmenopausal women having uterine mass with rapid growth or recently developed uterine mass should be evaluated in terms of malignancy. In our population, no anamnesis or physical examination about rapid growth was encountered.

In the literature, it is recommended that laboratory tests such as lactate dehydrogenase and cancer antigen 125 (CA-125) may be used for the differentiation of myoma uteri and uterine sarcoma although there was no sufficient evidence to support the clinical use of these tests. In our study, tumor markers were not examined since most patients had no suspicions of malignancy preoperatively.

The clinical prediction of uterine sarcoma is only possible by evaluating the factors together such as signs and symptoms, risk factors, non-responsiveness to treatment, magnetic resonance imaging (MRI) findings and endometrial sampling due to the difficulty of differential diagnosis preoperatively.

The investigation of morbidity rates among hysterectomy routes in the management of myoma uteri is still ongoing (35). In our study, no statistically significant difference was found in terms of hysterectomy routes between the patients with and without malignancy. We did not experience any intraoperative and short-term postoperative complications.

Retrospective design could be accepted as the limitation of our study. Despite this limitation, we contributed much to the literature. To the best of our knowledge, our study is the largest study about this topic. The analysis of all potential confounding factors, and examination of histopathology slides by the experienced pathologists' other strengths of our study.

As a conclusion, myoma uteri, the most common cause of gynecological operations, is too difficult to distinguish from uterine sarcomas. In the patients who are scheduled for operation with a diagnosis of myoma uteri, malignancy may be detected in the histopathologic examination. It is necessary to be careful about the demographic and clinical characteristics of the patients during the preoperative evaluation to determine the possibility of uterine malignancy.

Conflict of interest

The authors declare that they do not have any conflict of interest regarding this article.

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Determination of normal values of optic nerve sheath diameter in Turkish adult population using magnetic resonance imaging

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Abstract

The measurement of optic nerve sheath diameter (ONSD) is a reliable tool for the estimation of increased intracranial pressure (ICP). However, it is known that racial changes can occur in human anatomy. In the present study we aimed to determine the normal values of ONSD in Turkish adult population. This retrospective study involved the collection of data between June 1, 2015, and May 31, 2019, and was conducted on 291 adults known to have no increased cranial/intraocular pressure. The ONSD was measured at 3 mm posterior to the globe on MR images. 291 subjects were enrolled, 63.6% of whom were female. The total median ONSD was 4.40mm (4.20- 4.70). The median ONSD of the male participants was 4.50mm (4.25-4.75), of the female participants was 4.35mm (4.15-4.50) and the difference was statistically significant ($p = 0.001$). According to statistical analysis, it was found that only the presence of chronic renal failure altered the median of ONSD among the comorbid diseases ($p = 0.042$). Monitoring and treating increased ICP is crucial. Thanks to an ONSD with a known normal value, recognition and emergency management of this pathology would be easier. We think our study is noteworthy because it is the first study investigating the normal ONSD value in Turkish adult population.

Keywords: MRI, normal value, optic nerve sheath diameter, Turkish adult population

1. Introduction

The optic nerve, also called the second cranial nerve, transfers visual information from the retina to the vision centers of the brain via sensory nerve impulses. It is surrounded by cerebrospinal fluid, and its sheath is anatomically continuous with the duramater (1). This anatomical feature is so important. Such that, when the presence of increased intracerebral pressure (ICP), increased pressure will be transmitted from the subarachnoid space to the optic nerve and the surrounding nerve sheath. So, the detection of an increase in optic nerve sheath diameter (ONSD) due to this pressure may indicate an increase in the ICP (2, 3).

Nowadays, the mean upper limit for ONSD is accepted as 5 mm at most in adults (4). However, it may be wrong to assume that there is a universal ONSD value for all people around the world because it is known that there are variations in orbital anatomy with age, sex, and race.

A study conducted by Varma et al. was demonstrated that the mean optic disc area was 12% larger in Blacks compared to Whites (5). Wang et al. found significant differences in the lens position and relative lens position among various ethnic groups such as White, Asian, Hispanic, and Black populations (6). Samarawickrama et al. reported that after adjusting for age, gender, axial length, birth weight and optic-disc area, East Asian children had 30–43% larger mean vertical cup diameters compared to European Caucasians (7). The average cup/disc ratio in blacks (0.35) was significantly greater ($p < 0.0001$) than that in whites (0.24) for both right and left eyes (8). Moreover, there are also many other studies which have demonstrated that the ethnic background of the patients affects the optic nerve and eye anatomy (9-13).

We thought that it would not be surprising that a similar situation is also valid for ONSD. Based on this assumption, the aim of this study was to establish a reference scale of optic nerve sheath diameter in Turkish population using Magnetic Resonance Imaging (MRI).

2. Materials and methods

This retrospective study was approved by Ümraniye Training and Research Hospital local ethics committee and was retrospectively conducted on 291 patients who visited our hospital between June 1, 2015, and May 31, 2019. The data of the patients' descriptive characteristics and MR images included in our study were taken out from our hospital's

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medical records. Since the study was retrospective, the informed consent was not requested from the patients. This study conformed to the principles in the declaration of Helsinki.

The patients over the age of 18 who were requested orbital MRI for various reasons and then reported as normal MRI results were included to our study. The patients with any ophthalmologic or neurological disorders, patients using medications and/or eye examination findings (trauma, tumor, infection, papillary edema, glaucoma etc.) that affect cerebrospinal fluid pressure, and those with poor imaging quality due to movement or metallic artifacts were excluded from the study. The included eye examination findings were the findings admitted not needed rapid emergent intervention such as dry eye, retinopathy with no papilledema and hemorrhage, stye, hyphemia, scleritis etc.

Because of its greater accuracy, reliability, objectivity, and lower observer-dependent nature, we planned to use MRI for ONSDm in our study. MRI scans were performed on an Optima™ MR450W (1.5T, General Electric Company, United States) machine according to routine imaging protocols. The measurements were retrospectively analyzed with computer-assisted segmentation and performed manually by two neuroradiologists.

ONSD measurements were taken in the supine position. Axial T2-weighted MR images were used to measure the ONSD of each eye. ONSD is defined as the distance between the outer edges of the thick sheath layers covering the optic nerve. The measurement was performed from the image plane at the posterior aspect of the globe and at 3 mm behind the globe in an axis perpendicular to the optic nerve.

Statistical analysis was performed using the SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). The normality of the distribution of continuous and discrete variables was tested using histograms, Q-Q plots, and the Shapiro Wilk test. Categorical data were expressed as number and frequency. Continuous variables with normal distribution were described with mean (SD). The quantitative data with non-normal distribution were expressed by median and interquartile range (IQR). Mann Whitney-U and Kruskal-Wallis tests were used to compare quantitative data with non-normal distributions. P value of less than 0.05 was admitted statistically significant.

The primary outcome is a reference scale of optic nerve sheath in Turkish population. Secondary outcomes are changes in the measurement of ONSD (ONSDm) among with demographic and clinical characteristics of the patients.

3. Results

291 patients who met the inclusion criteria out of 920 patients performed orbital MRI during the study period was included, and their ONSD was measured. A patient flow chart is shown in Fig. 1.

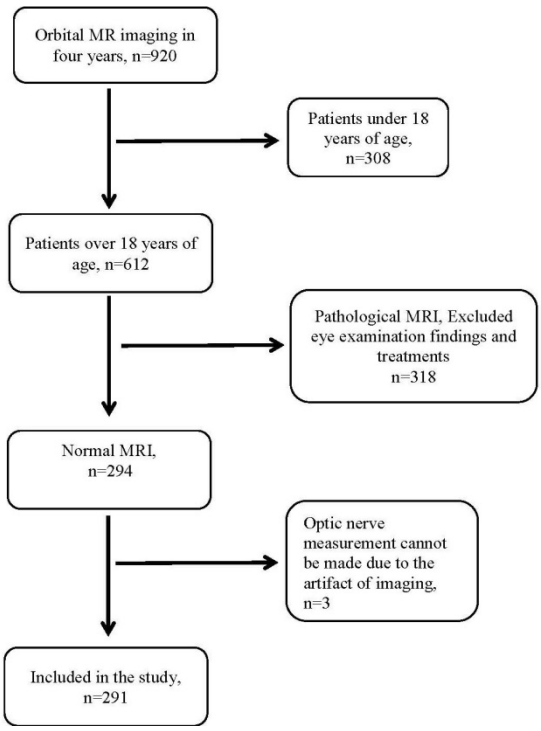

Fig.1. Patient flow chart

Of these 291 patients, 185 (63.6%) were women. The median age of the patients was 41 (30-52) and their descriptive characteristics are summarized in Table 1.

Table 1. Descriptive characteristics of the patients

Age *[Median (%25 - %75 IQR)]*	41 (30-52)
Gender (Female) *[N (%)]*	185 (63.6)
Comorbidities *[N (%)]*	
Diabetes Mellitus	34 (11.7)
Hypertension	48 (16.5)
Coronary Artery Disease	12 (4.1)
Hyperlipidemia	24 (8.2)
Chronic renal failure	3 (1)
Malignancy	3 (1)
Ischemic Stroke	1 (0.3)
Chronic obstructive pulmonary disease	21 (7.2)
Geographical region where the patient ID is registered *[N (%)]*	
Aegean	6 (2.1)
Marmara	55 (18.9)
Black Sea	115 (39.5)
Mediterranean	4 (1.4)
Central Anatolia	41 (14.1)
Southeastern Anatolia	7 (2.4)
Eastern Anatolia	63 (21.6)

The median of the right optic nerve sheath diameter (ONSD) of the patients was 4.40mm (4.20 - 4.70), the median of the left ONSD was 4.30mm (4.10 - 4.60), and the median of the total (right and left) ONSD was 4.40mm (4.20 - 4.70). When the difference between right and left ONSD was examined, no statistically significant difference was found (Mann Whitney-U test, $p = 0.319$). Primary outcome measures are summarized in Table 2.

Table 2. Primary outcome measures

	Median (%25 - %75 IQR)	p
Right ONSD (mm)	4.40 (4.20 - 4.70)	0.319
Left ONSD (mm)	4.30 (4.10 - 4.60)	
Total ONSD (mm)	4.40 (4.20 - 4.70)	

ONSD: Optical nerve sheath diameter; IQR: Interquartile range; mm: millimeter

When the age of the patients included in the study was divided into decades starting from the age range of 18-29, there was no statistically significant difference between decades and median ONSD. (Kruskal Wallis test, p = 0.489).

The median ONSD value of male patients was 4.50mm (4.25-4.75). It was also calculated as 4.35mm (4.15-4.50) for female patients, and this difference was statistically significant (Mann Whitney-U test, p = 0.001).

While the median ONSD value of patients without chronic renal failure (CRF) was 4.40mm (4.20-4.60), the median ONSD value of patients with CRF was 4.75mm (4.65-5.00). This result was found to be statistically significant (Mann Whitney-U test, p = 0.042). When the medians of ONSD of the patients were compared according to diabetes mellitus, hypertension, coronary artery disease, hyperlipidemia, malignancy and COPD groups, no statistically significant difference was found (Mann Whitney-U test, respectively p = 0.963, p = 0.739, p = 0.139, p = 0.881, p = 0.722, p = 0.658).

When the relationship between the ONSD and the geographical regions of the patients where they were registered was examined with the Kruskal Wallis test, it was found as p = 0.02. But no statistically significant difference was found in the comparisons between the groups (Bonferroni correction was applied, and significance threshold was accepted as p < 0.00238 after Bonferroni correction).

During our analysis, the eye examination findings of the patients included in the study were dichotomized as "normal examination finding" and "pathological examination finding", no statistically significant difference was found between the mean ONSD (Mann Whitney-U test, p = 0.058). Secondary outcome measures are summarized in Table 3.

Table 3. Secondary outcome measures

	ONSD (mm) [Median (%25 - %75 IQR)]	p	Statistical method used
Age (Categorical)	NA	0.489	Kruskal Wallis
Gender		**0.001**	**Mann Whitney-U**
Male	4.50 (4.25 - 4.75)		
Female	4.35 (4.15 - 4.50)		
Presence of comorbidity		0.844	Mann Whitney-U
Present	4.38 (4.20 - 4.60)		
Absent	4.40 (4.18 - 4.60)		
Geographical region where the patient ID is registered	NA	0.02*	Kruskal Wallis
Eye Examination Findings (Dichotomous)		0.058	Mann Whitney-U
Normal Examination	4.40 (4.20 - 4,60)		
Pathological Examination (mean +- SD)	4.30 (0.29)		

*Although p = 0.02, no statistically significant difference was found in the comparisons between groups (Bonferroni correction was applied). mm: millimeter, ONSD; optical nerve sheath diameter, SD; standard deviation

4. Discussion

Many invasive and noninvasive methods can be applied to monitor the ICP. While lumbar puncture one of the invasive methods is not reproducible, intraparenchymal probe and intraventricular catheterizations which are the gold standard in diagnosis cannot be applied under all conditions. Therefore, non-invasive procedures such as tympanic membrane replacement, CT, MRI, fundoscopy, transcranial doppler USG and USG-mediated optic nerve sheath diameter measurement (ONSDm) are used more (14, 15).

In today's practice, USG-mediated ONSDm has become more preferred compared to other non-invasive methods due to its bedside applicability, reproducibility, and easy accessibility to show pressure increase (16-18). And during the uses of this method, the accepted upper limit of normal ONSD values are < 5.0 mm for adults (>15 years of age), 4.5 mm for children and 4.0 mm for infants (19). Despite this accepted value, in studies conducted, this diameter was measured as 4.41 mm (4.25-4.75) in Bangladeshi and 5.1 mm (4.7 - 5.4) in Chinese (4, 20). In another study, where the average ONSD of both eyes was not given; The median right ONSD was 4.11 mm (3.36-4.86) and the median left ONSD was 4.35 mm (3.77-5.10) in Nigerian adult population.21 In our study, the median of the total (right and left) ONSD was found to be 4.40 mm (4.20 - 4.70). The different ONSD results obtained in all these studies strengthen the view that this diameter can vary between ethnic origins.

Our study is the first study that examined ONSD, and

demographic data associated with it in a population living in Turkey, according to our knowledge. In addition to this, the results of some studies performed in Turkey can allow the comparison of our ONSD findings, by using their control groups' ONSD values. In a study using CT that have 61 patients in its control group consisting of the patients with normal CT findings and discharged, the median ONSD mean of both eyes was reported to be 5.76 mm (IQR: 0.96).22 In another Turkish study using CT, this diameter was determined as 4.9. 23 Although they are made in Turkey, the racial characteristics of patients included to the study were not specified in both ones. Additionally, the results of our study are more reliable because of it is an MRI study and also it was performed with more patients.

In our study, the effects of comorbid diseases and the sex on ONSDm were also investigated. Differently from previous studies, a statistically significant difference in ONSD was found between genders in our study (21, 24). In addition, presence or absence of comorbid diseases included in the study did not change ONSDm statistically significantly except for CRF. However, only three of the patients included in the study were CRF patients. We think that studies with larger sample size are needed to make a more accurate interpretation. In addition to this, it should be kept in mind that the population we investigated did not include ICP and its complications. In the case of developing the ICP and its complication, we can see that the ONSD of patients with comorbid disease is affected more than those without comorbid disease.

There are some limitations to our study. In terms of representing the Turkish population, our study has been carried out with a relatively small sample size and a predominantly local population. However, one of the important factors limiting the large sample size is the preference of MR-mediated ONSDm in our study. Although the MR scan has low interobserver variability and greater accuracy, it is also difficult for ONSD associated studies to involve completely healthy volunteers and to use MRI to reach large sample sizes. Moreover, the funding of the study and allowing dedicated MR devices to be used for the study is not easy too. For these reasons, it was included the patients who applied to the hospital with any complaint required MRI examination and did not have ICP findings in this study. Despite the limitation about its sample size, this study gives the first insights into normal optic nerve sheath diameters in Turkish population. In addition to this, considering that our study covers a period of nearly 4 years, the sample size can be increased with multi-center studies using a similar method.

To identify increased ICP is very important for preventing possible herniation and death. ONSD has been proven as a parameter for measuring ICP indirectly. As a result, ONSD of 4.40 mm is the normal value in Turkish adult population according to our study. Despite its limitations, the results of our study are important for the management of these patients. Moreover, we think that the results we obtained will provide an idea for further studies in which pathological values will be investigated.

Conflict of interest

None to declare.

Acknowledgments

This retrospective study was approved by Umraniye Training and Research Hospital local ethics committee.

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Effects of Covid-19 pandemic in Turkey: Physical activity, smartphone usage, musculoskeletal system

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Abstract

This study aims to evaluate relationship between physical activity level, smartphone usage, back and neck health during Covid-19 pandemic. Participants between ages of 18-65 were included in study. Smartphone usage was evaluated with Smartphone Addiction Scale-Short Version, physical activity levels with short form of International Physical Activity Questionnaire. Oswestry Disability Index and Neck Bournemouth Questionnaire was used to evaluate back and neck problems. A total of 251 people (179 women, 72 men, age: 28.11±9.49 years, min-max: 18-62 years) participated in study. 134 participants (53.38%) had low physical activity levels; 35 individuals (13.94%) had sufficient physical activity levels while 82 participants (32.66%) were not physically active. A weak positive correlation was found between neck pain and total score of smartphone addiction scale, daily smartphone usage time, daily smartphone check frequency, and first check time after waking up (r=0.199, r=0.149, r=0.132, respectively). A weak negative correlation was found between neck pain and first check time after waking up (r=-0.145). As a result of study, it was observed that physical activity levels were insufficient in majority of individuals who participated in survey. The relationships we expected between physical activity level, smartphone usage characteristics, low back and neck health could not be demonstrated, only weak relationships were found between some features of smartphone use and neck health. We believe that finding solutions to increase physical activity levels of individuals during pandemic period will have both protective effects on health and will prevent problems by affecting musculoskeletal system positively.

Keywords: Covid-19, musculoskeletal system, smartphone

1. Introduction

The new coronavirus (Severe Acute Respiratory Syndrome-coronavirus-2 (SARS-CoV-2)), which was first reported in November 2019 and affected the whole world in a short time, caused a global health hazard and has been declared as a pandemic by the World Health Organization (WHO) (1). In countries with the Covid-19 pandemic, strict measures had to be taken to slow down the spread of transmission and thus prevent the health system from collapsing (2). Precautions such as preferring to work from home, closing schools, limiting shops, restaurants and business areas/services considered not in urgent need have been implemented and thus tried to ensure compliance with the rules of social distancing (2, 3). However, these measures have caused disruption of daily routines (3). There are significant concerns about how these changes in normal daily activities will affect public health and welfare (1).

As a result of home quarantine during the pandemic, physical activity and exercise levels of individuals have significantly decreased (2). Yet, physical activity is necessary for a healthy life. Being physically active is essential for the protection and improvement of musculoskeletal health, also.

The time spent on smartphones has also increased with the increase in free time at home during the pandemic period (8). In order to manage the anxiety caused by Covid-19, and the associated social isolation, many people may turn to excessive phone use, especially in the presence of home quarantine and in the absence of numerous other activities (8). Increased use of smartphones can also lead to prolonged stay in inappropriate postures and static positions, resulting in disrupting the biomechanics of the spine, causing pain, especially in the spine (9). It is stated in the literature that the first affected area is the neck due to the inappropriate posture taken while using smartphone (10).

Even before the Covid-19 pandemic, inadequate participation in physical activity has already been identified as a global public health problem, with more than a quarter of all adults have been reported that they do not reach the levels of physical activity required to maintain health (11). The sedentary lifestyle trend caused by the changes in lifestyle and technology taking an enormous place in our lives in recent years, has become more pronounced with the pandemic period. The sedentary lifestyle attitude a risk in terms of chronic health problems, mortality and morbidity as opposed

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to being physically active (12).

It is not known how long the Covid-19 pandemic will last and when societies can return to their normal lifestyle habits (13). Considering the harmful effects of physical inactivity as well as the treatment of people diagnosed with Covid-19, a second priority is the protection of health among people who have not been diagnosed with Covid-19 (3). Social distance and "staying at home" easily limit person-to-person transmission, but long-term physical inactivity as a result of quarantine procedures causes loss of condition that can easily impair overall health and well-being (3).

For this reason, it is necessary to determine how much the process caused by the pandemic affects the physical activity levels of individuals, how much it increases the dependence on technology, and how much the low back and neck health is affected in relation to these negative situations. For this reason, the aim of our study is to measure the relationship between physical activity level, smartphone addiction, and low back and neck health in individuals aged 18-65 during the Covid-19 pandemic.

2. Materials and methods

Participants between the ages of 18-65 who agreed to participate in the study were included in the study. Those with orthopaedic or neurological problems that prevent physical activity, congenital musculoskeletal deformities, uncontrollable chronic diseases, cognitive problems that prevent communication, and those who do not use mobile phones were excluded from the study. In addition, those who underwent Covid-19 were also excluded from the study so that the musculoskeletal system pain that can be seen due to Covid-19 disease does not affect the evaluation the parameters of our study.

The study initiated upon the permission of the Ethics Committee of the non-invasive Clinical Trials of Sivas Cumhuriyet University (Decision number: 2020-07/07, Date: 08.07.2020). The participants were informed about the purpose and content of the study and their informed consents were obtained online. In addition, necessary permits for the study were obtained from the Ministry of Health. The assessments were made through online forms since the face-to-face meeting may attitude a risk due to the pandemic. The first part of the form included information about age, gender, height, weight, marital status, body mass index, mobile phone/smartphone usage status, musculoskeletal system, and cardiovascular problems of the participants. Turkish translation of the 10-question Short Form of Smartphone Addiction Scale (SAS-SV) was used for the evaluation of smartphone addiction (14-16). The questionnaire questions were evaluated with six-point Likert scale. Scale scores vary between 10-60, while high scores indicate an increased risk of addiction. In the Korean sample, the cut-off score was determined as 31 for men and 33 for women. The Cronbach alpha coefficient of the original form's internal consistency and concurrent validity was 0.91 (15). The reliability of the Turkish form of SAS-SV was performed by Noyan et al (14).

The physical activity levels of participants were evaluated by the Turkish translation of the International Physical Activity Questionnaire-Short Form (IPAQ-SF) consisting of 7 questions (17, 18). In the short form, there are seven questions during the last 7 days about the time spent in walking, moderate and vigorous activities, and the frequency of activities. The time spent sitting was considered as a separate question. The times spent are multiplied by the metabolic equivalents (MET) present at the per activity scale and the average of the results for all materials gives the overall physical activity score. Physical activity levels are classified as physically inactive (<600 MET-min/week), low physical activity level (600-3000 MET-min/week), sufficient physical activity level (>3000 MET-min/week) (17).

The Turkish version of the Oswestry Disability Index (ODI) was used to determine back problems (19, 20). This scale includes 10 questions that examine the level of pain and the change in pain level and the differences in daily life activities due to pain. There are 6 options in each question with scores varying between 0 and 5, respectively. Participants were asked to mark the option they find closest to them as an answer to each question. The result value of the survey was obtained by summing the scores of the options they marked. The maximum score to be taken from this scale is 50 points. Option scores are 1 is 0 points, 2 is 1 point, 3 is 2 points, 4 is 3 points, 5 is 4 points, and 6 is 5 points. The evaluation is done as follows;

0 points: No functional impairment
1-10 points: Mild functional impairment
11-30 points: Moderate functional impairment
31-50 points: Severe functional impairment (20).

The Turkish version of the Neck Bournemouth Questionnaire was used to evaluate neck problems. It revised from Bournemouth Low Back Pain Questionnaire in 2002 by Bolton et al (21). The Neck Bournemouth Questionnaire is a survey in which pain intensity, daily social-functional level, anxiety, depression level, cognitive and behavioral aspects of fear-avoidance belief, and ability to cope with the pain are questioned. The Neck Bournemouth Questionnaire consists of seven questions and the answers are scored on a digital analog scale ranging from 0 to 10. The maximum score that can be obtained from the survey is 70, and a high score indicates a high disability (21).

2.1. Statistical analysis

The data obtained from our study were evaluated with the Windows-based SPSS analysis software (Version 22, Armonk, NY: IBM Corporation). The normality of the data was evaluated with Kolmogorov-Smirnov Test. Spearman Rank Correlation Coefficient was used to determine the relationship between physical activity levels, smartphone addiction, and low back and neck health, and Chi-square test

was used to evaluate the data obtained by counting.

3. Results

179 women (71.31%), 72 men (28.68%), totally 251 people (age: 28.11 ± 9.49 years, min-max: 18-62 years) participated in the study. The sociodemographic characteristics of the participants were shown in Table 1. Of the 251 individuals participating in the study, 178 (70.91%) stated that they or the people around them did not have Covid-19 infection. 69 people (27.49%) stated that they had family or friends' group who infected with Covid-19 and did not live in the same house, while 4 people (1.58%) stated that people living in the same house (0.39%) were infected. Physical activity levels of the individuals are given in Table 1. While 82 of the participants (32.66%) were not physically active, 134 individuals (53.38%) had low physical activity level, and only 35 individuals (13.94%) had sufficient physical activity level.

Table 1. Sociodemographic characteristics of the participants and study variables

		Frequency	Percentage
Gender	Female	179	71.31
	Male	72	28.68
Marital Status	Single	163	64.94
	Married	88	35.05
Education level	Primary/	4	1.59
	Secondary	3	1.19
	High School Graduate	101	40.23
	Some Collage Graduate	14	5.57
	Bachelor	98	39.04
	Postgraduate education (Master / Doctorate)	31	12.35
Working Status	Working	106	42.23
	Not Working	145	57.76
Smoking Habit	Yes	47	18.72
	No	204	81.27
Physical Activity Level	Not physically active	82	32.66
	Low physical activity	134	53.38
	Adequate physical activity	35	13.94

There was no correlation between the duration and frequency of smartphone use, and the time passed from waking up to using the smartphone, and the back, neck, and leg pain of the individuals participating in the study ($p>0.05$) (Table 2). When the relationship between neck pain scores and smartphone usage and physical activity levels was examined, a weak positive correlation was found between neck pain and smartphone addiction scale total score, daily smartphone usage time, and daily smartphone check frequency ($r = 0.199$, $r = 0.149$, $r = 0.132$, respectively). A weak negative correlation was found between neck pain and the first check time after waking up ($r = -0.145$). There was no relationship between back pain and smartphone usage and physical activity levels ($p>0.05$) (Table 3).

4. Discussion

As a result of the study, it was observed that the physical activity levels were insufficient for the majority of individuals (13.94%) who participated in the study. However, despite insufficient physical activity levels, no relationship was found between physical activity level and low back and neck problems. In other words, it has not been shown that people with insufficient or low physical activity level have more low back and/or neck problems. Similarly, no relationship was found between smartphone usage characteristics and waist, neck and leg pain. Only weak correlations were found between smartphone addiction and some features of smartphone use and neck survey results.

The disease caused by the Covid-19 virus, which has been in our lives since November 2019 (1) is expected to reduce the participation in physical activity and cause adverse effects on the general health of the population with the measures taken in countries where the pandemic is seen (2).

Physical activity is essential for a healthy life and being physically active is important for maintaining and improving musculoskeletal health (4-7). Physical inactivity is one of the most important risk factors for major disease morbidity (22). This applies not only to the general population but also to older adults and chronically ill populations, particularly those at high risk of death from Covid-19 (23). Our bodies require a relatively long period of time to take advantage of the healthy adaptations produced by physical activity (24-26). However, it takes only a few days to reverse these positive adaptations and the body returns to a physiological state similar to the initial condition or worse (27). This means that trying to lead an active lifestyle during home quarantine is necessary to avoid negative consequences (2). Despite all these beneficial effects, it was found that approximately 86.2% of the individuals participating in our study were not physically active or had a low level of physical activity. Even before the Covid-19 pandemic, inadequate physical activity was considered a public health threat (11). As a result of the measures taken with the pandemic period, physical activity and exercise levels have decreased significantly (2). For this reason, the results obtained from the study reflect the expected outcome. Regulations to protect against Covid-19 and keep people safe can cause a decrease in physical activity and an increase in sedentary behavior, which can lead to worsening of chronic health conditions and increased risks.

Today, the use of smartphones has increased with the area covered by technology in our lives (9). It is stated in the literature that long-term smartphone use is associated with mental health symptoms and many people seek to eliminate negative emotions, and emotional relief through easily accessible smartphones and devices that can be connected to the internet, but it is emphasized that such coping approaches may lead to negative consequences (8). Covid-19 pandemic (28) and the related home quarantine and social distance caused anxiety and negative emotions that increased greatly in society (29, 30). Given that social isolation causes

emotional distress and negative impact, anxiety specific to Covid-19 as well as general anxiety and depression can increase smartphone use (8). A study conducted in China revealed that there is a strong relationship between phone usage times and both general anxiety and anxiety specific to Covid-19. The strong association between excessive phone usage and Covid-19 anxiety has been interpreted as a response to environmental stressors to alleviate negative emotion (8).

Table 2. Comparison of smartphone usage characteristics of individuals with and without back, neck and leg pain

			No low back, neck, leg pain	With back and leg pain	With neck pain	Both with a back-leg and neck pain	p
Smartphone usage (hours / day)	**Less than 2 hours**	**Number**	35	18	9	21	0.465
		Percent %	42.2	21.7	10.8	25.3	
	3-4 hours	**Number**	29	21	16	19	
		Percent %	34.1	24.7	18.8	22.4	
	5-6 hours	**Number**	14	9	12	16	
		Percent %	27.5	17.6	23.5	31.4	
	More than 6 hours	**Number**	10	5	8	9	
		Percent %	31.3	15.6	25.0	28.1	
	Total	**Number**	88	53	45	65	
		Percent %	35.1	21.1	17.9	25.9	
Smartphone control frequency	**Less than 10 times a day**	**Number**	23	15	6	15	0.529
		Percent %	39.0	25.4	10.2	25.4	
	11-20 times a day	**Number**	22	14	7	16	
		Percent %	37.3	23.7	11.9	27.1	
	21-50 times a day	**Number**	24	14	18	20	
		Percent %	31.6	18.4	23.7	26.3	
	More than 51 times a day	**Number**	19	10	14	14	
		Percent %	33.3	17.5	24.6	24.6	
	Total	**Number**	88	53	45	65	
		Percent %	35.1	21.1	17.9	25.9	
Time after waking up to smartphone use	**In 5 minutes**	**Number**	33	18	22	34	0.171
		Percent %	30.8	16.8	20.6	31.8	
	In 6-30 minutes	**Number**	28	22	15	18	
		Percent %	33.7	26.5	18.1	21.7	
	In 31-60 minutes	**Number**	15	4	6	9	
		Percent %	44.1	11.8	17.6	26.5	
	After 60 minutes	**Number**	12	9	2	4	
		Percent %	44.4	33.3	7.4	14.8	
	Total	**Number**	88	53	45	65	
		Percent %	35.1	21.1	17.9	25.9	

Table 3. Relationships between neck pain and low back pain, smartphone use and physical activity level

		Neck Bournemouth Questionnaire Total Score	Oswestry Disability Index Total Score
Short Form of Smartphone Addiction Scale total score	r	**0.199****	-0.036
	p	0.002	0.565
Daily Phone Usage Time	r	**0.149***	-0.023
	p	0.018	0.712
Daily Phone Check Frequency	r	**0.132***	-0.084
	p	0.037	0.182
First check-up time after waking up	r	**-0.145***	-0.051
	p	0.022	0.424
Vigorous Physical Activity (minutes/week)	r	0.097	0.027
	p	0.124	0.671
Moderate Physical Activity (minutes/week)	r	0.021	0.015
	p	0.738	0.815
Walking (minutes/week)	r	0.024	0.091
	p	0.705	0.151
Sitting time (hours)	r	0.070	-0.014
	p	0.266	0.831

** Correlation is significant at the 0.01 level (2-tailed).

In parallel with the increase in smartphone usage, musculoskeletal problems associated with intense smartphone usage are also increasing (10). Studies indicate that with the increase in smartphone use, potential risks for musculoskeletal problems occur (31, 32). Neck flexion is the most commonly adopted posture by smartphone users while viewing the screen for a long time, and it can cause many neck disorders (10). In order to read the screen during smartphone use, it is necessary to look down sharply and keep the arms in front, which causes the head to move forward and an excessive anterior curve in the lower cervical spine and an excessive posterior curve in the upper thoracic region to maintain balance, which causes the stress on the cervical spine and neck muscles (31, 33). The forward head posture is one of the commonly recognized poor postures in the sagittal plane. It has been reported in the literature that this posture may contribute to the onset and continuation of neck and back pain syndromes and to further loss of cervical spine extension (34, 35). Incorrect head and neck posture has been associated with chronic musculoskeletal pain (36, 37). Similar to our study, a study in the literature comparing smartphone use and neck problems using a survey showed that smartphone use was associated with neck problems in healthy young adults (38). In another study, it was found that smartphone use in young people caused cervical disc degeneration and chronic neck pain, and excessive smartphone use was identified as a risk factor for cervical problems (39). As a result of our study, it was observed that there was a relationship between some parameters of smartphone usage characteristics and neck problems. As the time spent using the phone increases, the risk of experiencing neck problems increases.

It is reported in the literature that physical activity is a positive factor in the prevention of musculoskeletal diseases. Studies have similarly revealed a significant relationship between physical activity level and neck pain which means higher intensity of physical activity has been associated with a reduced rate of neck pain (38). Although the physical activity levels of the individuals participating in our study were mostly not sufficient, it was found that there was no relationship between neck pain and physical activity level. This may be considered a circumstance related to our study population. The fact that the individuals participating in the study consisted of mostly young people may be the reason for less musculoskeletal pain or it may not have enough time passed over the pandemic that the reduced physical activity level will adversely affect the neck region.

Every individual can experience problems related to the musculoskeletal system in a period of his/her life. It is stated in the literature that spine problems are most common in the low back region (40). It is stated in the literature that poor posture (41), lack of physical activity and depression play a role in back problems (42, 43). It is thought that with the restrictions during the Covid-19 pandemic period, the affected physical activity levels of individuals and the rose in stress and anxiety and the increased tendency to depression will cause the increase of back problems in this period (44). In a study examining the prevalence of low back pain in the Covid-19 pandemic period, it was found that back problems increased during quarantine compared to the pre-quarantine period, based on previous prevalence studies in the literature. The results were interpreted as an increase in prevalence due to the negative effects of the restriction measures taken for the Covid-19 pandemic, such as decreasing physical activity participation, increasing sitting time and increasing stress levels (44). Lumbar muscle activation is eliminated with prolonged sitting periods. This results in a decrease in the condition of the lumbar muscles and causes an overload of the passive structures of the body, such as the intervertebral disc and ligaments (45). Although we think that lumbar problems will be seen more commonly as the use of smartphones during the Covid-19 pandemic process reduces the level of physical activity and prolongs their sitting times, there was no relationship between physical activity level, smartphone usage characteristics, and low back pain of individuals who participated in our study. These results may be related to the fact that the assessment was made in the early period of the pandemic. The changes that the pandemic has brought to our lives may not yet have passed enough time to result in the formation of back problems by reflecting on the musculoskeletal system.

If we had the chance to compare the pre-pandemic and post-pandemic physical activity levels, smartphone usage characteristics, low back and neck problems of the individuals participating in the study, then we could more clearly reveal the effects of the pandemic on these parameters. This may be the limitation of our study. In addition, the physical activity levels and smartphone usage characteristics of the individuals participating in our study were determined through surveys. Using objective evaluation methods in further studies may help achieve more reliable results.

As a result of the study, it was observed that physical activity levels were insufficient in the majority of the individuals who participated in the survey. The relationships we expected between physical activity level, smartphone usage characteristics and low back and neck health could not be demonstrated, only weak relationships were found between some features of smartphone usage and neck health. Our study started to be implemented shortly after the Covid-19 cases began to appear in our country. For this reason, the results due to physical inactivity and smartphone usage habits may not yet be at a level that will affect the musculoskeletal system. We think that the evaluation results to be made will be different with the increase in the restrictions and the time elapsed over the duration of cases. We believe that finding solutions to increase the physical activity levels of individuals during the pandemic period will have both protective effects on health and will prevent problems by positively affecting the health of the musculoskeletal system. In addition, we

consider that creating leisure activities that will increase physical activity levels will change habits related to the use of smartphones and reduce the duration of usage.

Conflict of interest

The authors report no conflict of interest. There is no declarations of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Psychosocial assessment of patients with chronic pain

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Abstract

The main object of this study is to evaluate the psychological status of chronic pain patients (CPP) and describe the characteristics and frequency of psychological disorders of CPP. Two hundred sixty-three patients with complaints of chronic pain longer than 1 year and fifty healthy volunteers were included in the study. Patients with cancer were not included. Turkish version of Symptom Checklist-90 Revised (SCL-90-R) was used for the assessment of the psychological status of participants. CPP were divided into 5 subgroups regarding their painful regions: Headache, cervical or upper extremity pain, axial or radicular back pain, lower extremity pain, and diffuse pain. Global severity index (GSI) and subscales of SCL-90-R were analyzed. In CPP, GSI and almost all subscale scores of SCL-90-R were significantly higher than the control group. Headache patients had worse psychological symptoms than other subgroups of CPP. SCL-90-R scores of female patients were significantly higher than males. 24.7% of patients had moderate and 14.8% had severe psychological symptoms. While the intensity of the pain had a moderate correlation with increased psychological symptoms, the level of education and age had a weak negative correlation with SCL-90-R scores. Patients with chronic pain are convenient to have phycological symptoms. While almost half of the patients have increased psychological symptoms, the degree of the symptoms can be serious in some of them. Thus, treatment of chronic pain necessitates a multidisciplinary approach.

Keywords: anxiety, chronic pain, depression, headache, SCL-90-R

1. Introduction

It is well known that the psychological mood of individuals who suffer pain is worse than normal people. The two most prevalent psychological symptoms of chronic pain patients are depression and anxiety. Many patients with chronic pain and psychiatric illness have a physical basis for pain in the body since perception is worsened by overlying psychiatric illness (1-3). Decreased levels of serotonin and norepinephrine in depressive patients can be associated with the development or increased perception of chronic pain. Serotonin and norepinephrine are important neurotransmitters in the descending inhibitory system, and both inhibit nociceptive dorsal horn neurons when locally applied (4). This is the explanation of why selective serotonin-norepinephrine reuptake inhibitors (SNRIs) have effects both on depression and pain. Studies indicate that opioid analgesia is enhanced with antidepressant treatment and decreased after the depletion of serotonin and norepinephrine (5, 6) Patients with depression have been found to have higher levels of proinflammatory cytokines and acute-phase proteins even the patient does not have a medical illness or pain complaint. It is controversial if there is an association between the increase of pro-inflammatory cytokines and the development of depression (7-10).

When pain becomes chronic, sensory input plays a diminished role, while affective and cognitive pathways play a more prominent role in the creation of painful perceptions (11). Working for patients with an altered psychological state is a challenge for pain physicians. Taking history, getting enough information, performing a physical examination, ordering laboratory, or screening tests, giving medical advice, and providing patient compliance for the treatment may sometimes become difficult. Additionally, patients with psychological problems have a lower rate of treatment success. Research has indicated that psychiatric comorbidity harms treatments for chronic pain, such as rehabilitation, spinal cord stimulation, or opioid therapy (1, 12, 13).

Prolonged duration of pain increases the psychological symptoms of patients (14). While assessing patients with chronic pain, performing a psychological approach can be useful for preventing unnecessary, expensive, time and energy-consuming tests or procedures for diagnosis. Devoting a few minutes to the patient's psychological state may help the physician to decide the most proper diagnosis or treatment approaches. Medical physicians should not hesitate to consult patients with psychiatry physicians.

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Detection of psychological problems should not ignore medical complaints and signs. It should be kept in mind that a patient with medical issues may have psychological problems as well as a patient with psychological issues may have medical problems. The lifetime prevalence of suicide attempts in patients with chronic pain ranged from 5% to 14% and the rate of suicide attempts is double that was found in the general population (1). It is still unclear if their pain worsens the psychological state of chronic pain patients, or they feel more pain because of their worsened psychological state.

In this study, our purpose was to evaluate the psychological profile of patients with non-cancer chronic pain and detect the factors which show a correlation with psychological symptoms. We tried to determine the frequency, severity, and type of psychological symptoms accompanying chronic pain. We also evaluated the correlation between demographic features and SCL-90-R scores. We included 263 chronic pain patients with complaints for more than 1 year and 50 healthy volunteers. We created 5 subgroups from pain patients according to their painful body region: headache (Subgroup H), neck or upper extremity pain (Subgroup N-U), axial or radicular back pain (Subgroup B), pain originated from a lower extremity (Subgroup L), and diffuse pain (Subgroup D). We used the Turkish version of SCL-90-R as the psychological assessment tool of this study. SCL-90-R is a comprehensive questionnaire including 90 items and allows evaluating an individual for various psychiatric symptoms: general symptom severity, depression, anxiety, somatization, compulsivity, interpersonal sensitivity, anger-hostility, phobic anxiety, paranoid ideation, and psychoticism. The development of the SCL-90-R began as early as the 1950s under the direction of Parloff et al. and was revised by Degoratis et al. in the 1970s (15-17). A validity and reliability study of the Turkish version of SCL-90-R was performed by Dağ et al. in 1991 (18).

As the result of our literature research, we can say that this study is the first study assessing and comparing different psychological dimensions of chronic pain patients according to the classification of a painful body part.

2. Materials and methods

This study was conducted in the pain medicine clinic of University of Health Sciences Van Education and Research Hospital between the March and May of 2019. Approval of the institutional ethical committee was obtained before the study. All participants were informed about the study and a written informed consent form was taken from all participants. Two hundred sixty-three patients who suffer pain longer than 1 year were included in the study (Group P). Fifty healthy volunteers were included in the study as the control group (Group C). Patients younger than 18, suffering from pain due to cancer, inability to understand and answer the questions of the study, and individuals who do not want to participate in the study were excluded. Additionally, individuals who are relatives of the patients in Group P or who live with a chronic pain patient in the same house were also excluded from the study. For demographic data: age, gender, education level, marital status, and employment status were asked. The intensity of pain was assessed using a 4-point verbal rating scale (VRS) for Group P. 1: mild pain, 2: moderate pain, 3: severe pain 4: very intense pain. The total duration of the pain complaint was also asked for Group P. Group P was divided into five subgroups regarding the body part that patients complain of pain. Subgroup H (headache) was composed of patients who suffer headaches, Subgroup N-U (neck, upper extremity) was composed of patients who suffer axial or radicular cervical pain or other types of pain on the upper extremity (shoulder pain, carpal tunnel syndrome, elbow pain, etc.). Subgroup B (back) was composed of patients who suffer axial or radicular back pain. Subgroup L (lower extremity) was composed of patients who suffer pain on the lower extremity (gonarthrosis, coxarthrosis, ankle pain, etc.) and Subgroup D (diffuse) was composed of patients who suffer more than one of the above-mentioned regions of the body.

For the evaluation of the psychological states of participants, the SCL-90-R questionnaire was applied. SCL-90-R is a psychological assessment tool consisting of 90 items. These 90 items are rated according to 5-point Likert Scale by participants. 0: not at all, 1: a little bit, 2: moderately 3: quite a bit, 4: extremely. Results of the questionnaire were recorded as a global symptom index (GSI) and these 10 subscales: depression, anxiety, somatization, compulsivity, interpersonal sensitivity, anger-hostility, phobic anxiety, paranoid ideation, psychoticism, and additional scales.

Demographic features of Group P and Group C were compared. GSI and subscales of SCL-90-R results were compared between Group P and Group C and for subgroups of Group P. Association of SCL-90-R scores with demographic features was evaluated.

To determine the frequency of individuals with psychological problems, we used a method similar to Dağ and Radanov (18,19). We classified the individuals as ‘moderate problem’ if their GSI is more than 1 standard deviation (SD), but not more than 2 SDs, higher than the mean GSI score of Group C and ‘severe problem’ if their GSI is more than 2 SDs higher than mean GSI of Group C. Frequency of individuals with psychologic problems between groups were compared.

Statistical analyses were performed using Statistical Package for the Social Sciences 20.0 (SPSS 20.0) program. Parametric data were presented as mean ± standard deviation (SD) categoric data were presented as number and percentage. Demographic characteristics of the groups were assessed using the Chi-square test. The Shapiro–Wilk test was used to analyze normal distribution assumptions of quantitative outcomes. For parametric data, the Student’s t-test was used for the investigation of significance between

two groups and one-way ANOVA for more than two groups. For the non-parametric data Mann-Whitney-U test was used for the investigation of significance between two groups and the Kruskal-Wallis test was used for investigating the significance between more than two groups. Spearman correlation analysis was applied to investigate the correlation between SCL-90-R scores and the features of pain (intensity, duration), and the demographic features of participants. A p-value less than 0.05 was considered as 'statistically significant'. Correlation coefficients (CC) less than 0.25 were considered as 'weak correlation', CCs between 0.26 and 0.50 were considered as 'moderate correlation', CCs between 0.51 and 0.75 were considered as 'strong correlation', and CCs more than 0.75 were considered as 'very strong correlation'.

3. Results

Demographic features of all participants are presented in Table 1 and Fig. 1. There was no significant difference between groups in terms of age, marital status, and employment status. The level of education was significantly higher in Group C ($p < 0.001$). This difference was mostly because of the high rate of illiterate participants in Group P (47.1% versus 16.0%). The rate of females was higher than males in both groups, but there was no significant difference between groups ($p = 0.47$).

Table 1. Demographic data of participants

	Study Group (n=263)	Control Group (n=50)	P
Age	46.15 ± 13.2	44.5 ± 16.1	0.56
Number of females	206 (77.2%)	36 (72.0%)	0.27
Number of marrieds	231 (87.8%)	36 (72.0)	0.18
Number of non-workers	189 (71.8%)	33 (66.0%)	0.50

The total number of patients, according to painful regions, are presented in Fig. 2. Duration and intensity of pain regarding groups and subgroups are presented in Table 2. The mean duration of pain complaint was 7.61 ± 7.21 years. There was no significant difference in terms of duration of pain between subgroups of Group P ($p = 0.177$). Regarding the 4-point VRS assessment, the mean severity of pain was 2.78 ± 1.03. It is observed that the pain severity of Subgroup H was significantly more than subgroups B, D, and N-U. In the comparison of Subgroup H and N-U, the p-value was 0.002. While the p-value was 0.44 in comparison of the severity of pain between Subgroup H and B, it was less than 0.001 between H and D. We assumed that the difference between Subgroups H and L was not statistically significant ($p=0.09$) because of the relatively small number of patients in Subgroup L.

Results of SCL-90-R scores are presented in Table 3 and Fig. 3. We observed that scores of Group P were higher than scores of group C for GSI and all subscales of the SCL-90-R tool. This elevation was statistically significant, except for compulsivity, hostility, and paranoid ideation. SCL-90-R scores of subgroups of Group P are presented in Table 4. It is observed that scores of headache patients were higher than the other subgroups of Group P, but the difference was only significant between Subgroup H and B and between H and L ($p < 0.001$ and $p = 0.034$ respectively). The mean GSI score of Subgroup D was at the second rank, following Subgroup H.

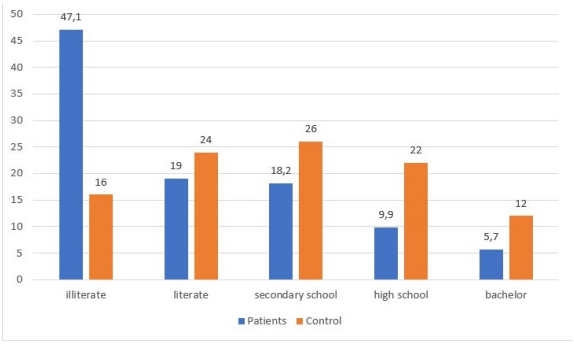

Fig. 1. Education levels of participants. (Results are given as percentages)

Table 2. Mean ± standard deviation of the duration of pain and verbal rating scales of patient group and subgroups

	Duration of Pain (years)	Mean VRS
Group P (n = 263)	7.61 ± 7.22	2.78 ± 1.03
Subgroup H (n = 78)	8.67 ± 9.53	3.30 ± 0.91
Subgroup D (n = 88)	7.78 ± 6.44	2.41 ± 1.10
Subgroup B (n = 57)	7.60 ± 6.13	2.82 ± 0.88
Subgroup L (n = 17)	5.18 ± 4.26	2.64 ± 0.78
Subgroup N-U (n = 23)	5.17 ± 3.78	2.43 ± 0.89

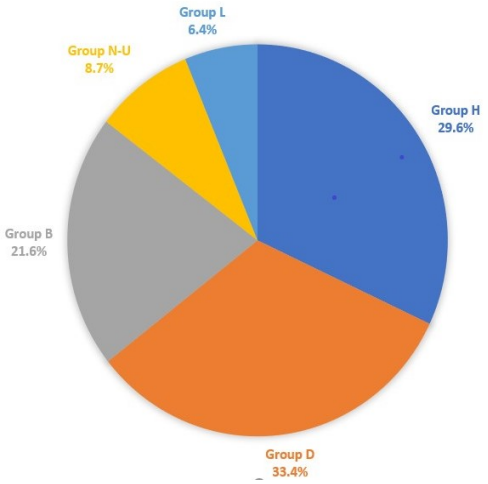

Fig. 2. Distribution of subgroups in chronic pain patients in this study

Correlation between demographic features and SCL-90-R scores was assessed. SCL-90-R scores of the participants regarding group and gender are presented in Table 5. Our results showed that SCL-90-R scores of females were higher in both groups. While this difference was not significant in Group C ($p = 0.07$), scores of females were significantly higher than the scores of males ($p < 0.001$) in Group P.

Table 3. SCL-90-R scores of patients with chronic pain and the control group

	Group P	Group C	p
Global severity index	1.04 ± 0 .50	0.70 ± 0.42	< 0.01
Somatization	1.46 ± 0.68	0.77 ± 0.60	< 0.01
Compulsivity	1.17 ± 0.63	0.99 ± 0.64	0.07
Interpersonal sensitivity	1.15 ± 0.66	0.83 ± 0.60	0.02
Depression	1.07 ± 0.67	0.85 ± 0.67	0.03
Anxiety	0.94 ± 0.66	0.57 ± 0.48	< 0.01
Anger-hostility	0.74 ± 0.72	0.66 ± 0 .65	0.88
Phobic anxiety	0.77 ± 0.68	0.19 ± 0.24	< 0.01
Paranoid ideation	1.00 ± 0.76	0.99 ± 0.71	0.95
Psychoticism	0.69 ± 0.56	0.37 ± 0.32	< 0.01
Additional scales	1.39 ± 0.73	0.88 ± 0.64	< 0.01

Results are presented as mean ± standard deviation

Regarding the ages of the participants, there was a weak negative correlation between the SCL-90-R scores and the age in Group P (p = 0.001, CC = - 0.20). No significant correlation was detected in group C (p = 0.325).

We observed a weak negative correlation between the level of education and GSI scores in Group P (p= 0.007, CC= - 0.166) while there was no correlation in control group (p = 0.841, CC = 0.03). According to the assessment of the correlation of education and GSI scores for all participants, there was a weak negative correlation (p = 0.001, CC = - 0.191).

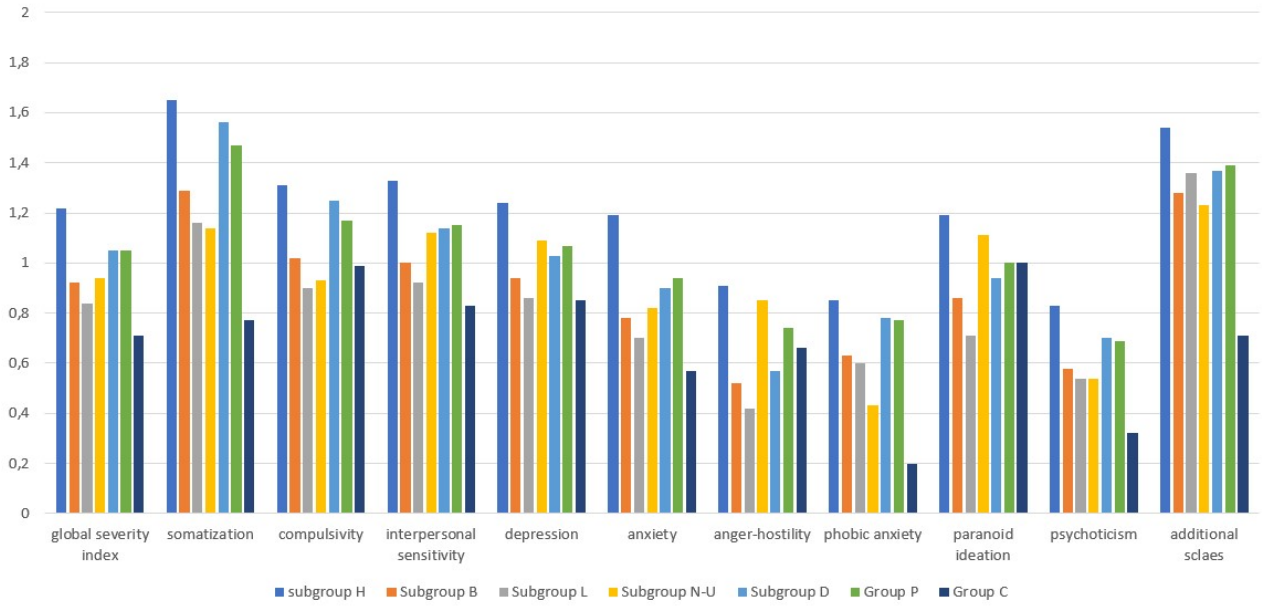

Fig. 3. SCL-90-R subscale scores of participants

Table 4. SCL-90-R scores of main groups and the subgroups of the study

	Subgroup H (n=78)	Subgroup B (n=57)	Subgroup L (n=17)	Subgroup N-U (n=23)	SubgroupD (n=88)	Group P (n=263)	Group C (n=50)
Global severity index	1.22±0.56 **(p<0.01)**	0.92±0.45 **(P=0.01)**	0.84±0.37 (p=0.20)	0.94±0.53 (p=0.05)	1.05±0.44 **(p<0.01)**	1.05±0.50 **(p<0.01)**	0.71±0.42
Somatization	1.65±0.74 **(p<0.01)**	1.30±0.60 **(p<0.01)**	1.16±0.66 **(p=0.04)**	1.14±0.61 **(p=0.02)**	1.56±0.63 **(p<0.01)**	1.47±0.68 **(p<0.01)**	0.77±0.61
Compulsivity	1.32±0.72 **(p=0.01)**	1.02±0.55 (p=0.73)	0.90±0.39 (p=0.70)	0.93±0.65 (p=0.91)	1.25±0.60 **(p=0.02)**	1.10±0.64 (p=0.07)	0.99±0.65
Interpersonal sensitivity	1.33±0.75 **(p<0.01)**	1.00±0.65 (p=0.17)	0.91±0.49 (p=0.47)	1.12±0.60 (p=0.10)	1.14±0.60 **(p<0.01)**	1.15±0.67 **(p=0.02)**	0.83±0.60
Depression	1.24±0.74 **(p<0.01)**	0.94±0.60 (p=0.37)	0.86±0.59 (p=0.66)	1.09±0.67 (p=0.23)	1.03±0.61 (p=0.07)	1.07±0.67 **(p=0.02)**	0.85±0.67
Anxiety	1.19±0.73 **(p<0.01)**	0.78±0.64 (p=0.12)	0.70±0.45 (p=0.26)	0.82±0.59 (p=0.11)	0.90±0.60 **(p<0.01)**	0.94±0.66 **(p<0.01)**	0.57±0.48
Anger-hostility	0.92±0.77 (p=0.04)	0.52±0.53 (p=0.24)	1.42±0.48 (p=0.57)	0.84±0.54 (p=0.45)	0.56±0.54 (p=0.43)	0.74±1.20 (p=0.88)	0.66±0.65
Phobic anxiety	0.86±0.75 **(p<0.01)**	0.63±0.60 **(p<0.01)**	0.60±0.46 **(p<0.01)**	0.42±0.63 **(p=0.02)**	0.91±0.64 **(p<0.01)**	0.77±1.04 **(p<0.01)**	0.20±0.24
Paranoid ideation	1.20±0.84 (p=0.17)	0.71±0.66 (p=0.34)	0.71±0.52 (p=0.15)	1.11±0.72 (p=0.64)	0.94±0.72 (p=0.65)	1.00±0.76 (p=0.95)	1.00±0.71
Psychoticism	0.83±0.68 **(p<0.01)**	0.54±0.49 **(p<0.01)**	0.54±0.42 (p=0.12)	0.54±0.49 (p=0.32)	0.70±0.48 **(p<0.01)**	0.69±0.56 **(p<0.01)**	0.37±0.32
Additional scales	1.54±0.93 **(p<0.01)**	1.28±0.61 **(p<0.01)**	1.36±0.68 **(p=0.01)**	1.23±0.64 **(p=0.02)**	1.37±0.64 **(p<0.01)**	1.39±0.73 **(p<0.01)**	0.88±0.64

Results are given as mean ± standard deviation. p-values present the differences comparing the control group (Group C)

When the results were assessed regarding the marital status of the participants, no significant correlation with SCL-90-R scores was observed. P values for GSI were 0.97 in Group P and 0.78 in Group C.

It is observed that there is no significant correlation in terms of an employment situation. There was no difference in any of the groups except somatization scores. Somatization was significantly higher in patients who do not work in a job. the p-value for Group P was 0.24.

As we did not find a significant correlation between SCL-90-R scores and the duration of pain complaint (p=0.419, CC = - 0.05), we observed moderate correlation between severity of pain and SCL-90-R scores (p<0.001, CC=0.324).

To determine the frequency of individuals who have psychological problems, we used the mean results and the standard deviations of the results of Group C. According to this method, we classified participants as 'no psychological disorder' if their GSI scores are in the range of standard deviations. We described individuals as 'moderate disorder' if their GSI scores are more than 1 SD but not more than 2 SDs higher than the mean GSI score of the control group. We described the participants who have GSI scores more than 2 SDs over the mean scores of the control group as 'severe disorder', and we suggest that these people should be referred to a psychiatrist. The mean GSI score of Group C was 0.71 ± 0.42. Thus, we described individuals as a moderate disorder if their GSI score is between 1.13 and 1.55 and as 'severe disorder' if their GSI score is more than 1.54. The classification of patients regarding their SCL-90-R scores is presented in Table 6. We observed that the frequency of individuals with moderate or severe psychological problems was significantly higher in Group P. Regarding the subgroups of chronic pain patients, the frequency of moderate or severe psychological symptoms was significantly higher in Subgroup H (p = 0.007).

Table 5. SCL-90-R scores regarding genders. p-values present the difference of scores between males and females in the groups

	Group P Female (n = 203)	Group P Male (n = 60)	Group C Female (n = 36)	Group C Male (n = 14)
Global severity index	1.12 **(p < 0 .001)**	0.81	0.77 (p = 0.07)	0.53
Somatization	1.59 **(p < 0.001)**	1.04	0.89 **(p = 0.02)**	0.44
Compulsivity	1.25 **(p < 0.001)**	0.89	1.07 (p = 0.27)	0.80
Interpersonal sensitivity	1.22 **(p < 0 .001)**	0.90	0.89 (p = 0.27)	0.68
Depression	1.17 **(p < 0.001)**	0.73	0.93 (p = 0.19)	0.65
Anxiety	1.02 **(p < 0.001)**	0.65	0.65 **(p = 0.04)**	0.37
Anger-hostility	0.78 (p = 0.09)	0.60	0.68 (p = 0.93)	0.60
Phobic anxiety	0.85 **(p <0 .001)**	0.50	0.22 (p = 0.39)	0.37
Paranoid ideation	1.03 (p = 0.12)	0.88	1.02 (p = 0.62)	0.92
Psychoticism	0.70 (p = 0.78)	0.63	0.41 (p = 0.74)	0.27
Additional scales	1.44 (p = 0.22)	0.82	1.00 (p = 0.64)	0.57

Table 6. Classification of the participants regarding psychological symptom severity

	Normal range (GSI< 1.13)	Moderate symptoms (GSI: .13-1.55)	Severe symptoms (GSI >1.55)
Group C (n = 50)	42 (84.0%)	5 (10.0%)	3 (6.0%)
Group P (n =263)	159 (60.5%)	65 (24.7%)	39 (14.8%)
Subgroup H (n = 78)	34 (43.6%)	23 (29.5%)	21 (26.9%)
Subgroup D (n = 88)	57 (64.8%)	18 (20.5%)	13 (14.8%)
Subgroup B (n = 57)	39 (68.4%)	14 (24.6%)	4 (7.0%)
Subgroup N-U(n=23)	17 (73.9%)	5 (21.7%)	1 (4.3%)
Subgroup L (n = 17)	12 (70.6%)	5 (29.4%)	0 (0%)

4. Discussion

In this study, our primary aim was to create a general idea on the psychological aspect of chronic pain patients for physicians who play role in the treatment of these patients. Our results showed a significant correlation between chronic pain and psychological problems. This correlation has been observed in several previous studies (20,21,22,23,24). We observed increased SCL-90-R results in patients with chronic pain. These results were statistically significant in almost all subscales of the questionnaire except compulsivity, hostility, and paranoid ideation. (Tables 3, 4) (Fig. 3).

Our secondary aim was to evaluate different types of chronic pain regarding psychological mood. For this purpose, we divided the patients into five subgroups regarding 'where the pain complaint is'. We did not create subgroups regarding the exact or possible diagnosis of pain for preventing the development of tens of subgroups. For instance, we did not create subgroups for migraine, tension-type headache, cluster headache, hemicrania continua, or occipital neuralgia. We counted all types of headaches in the 'headache subgroup'. We also did not create subgroups regarding the mechanism of pain for example neuropathic, somatic, or visceral. This method of pain classification may be a limitation of the study, but if we had created so many subgroups, statistical analysis would be complex and due to the smaller number of patients

in subgroups, results would not be meaningful.

Our subgroup analysis showed that GSIs of every subgroup were significantly higher than the control group. We also observed that patients with headaches have the worst symptoms amongst all subgroups. In previous studies, it is shown that patients with headaches have worsened psychological moods. Anxiety and mood disorders are the most relevant psychiatric comorbidities associated with migraine, influencing disease prevalence, prognosis, treatment, and clinical outcomes (25, 26). Peres et al. reported that in headache patients, the odds ratio for anxiety is 7.0 and for depression is 3.4 (25). In a comprehensive research study that includes 6624 patients with headaches, it is reported that the prevalence of depression, anxiety, and depression plus anxiety is 5.6%, 14.3%, and 3.8% respectively (27). In the same study regarding the types of headaches, patients with medication overuse headache have the worst psychological mood.

We observed that patients complaining of more than one region of their body had also high SCL-90-R scores in this study. This result was consistent with previous studies. In their study that consists of 1016 patients, Dworkin et al. reported that patients with two or more pain complaints are much likely to be depressed than patients with a single pain complaint (28).

Mean GSI scores of females were higher than males in Group P and C but as the difference was not statistically significant in Group C ($p = 0.07$), it was significant in Group P ($p < 0.001$) (Table 5). In Table 5, for some subscales of SCL-90-R, it is shown that there was statistical significance in Group P between the mean GSI scores of males and females as there was no significance in the control group. Regarding these results, we suggest that females are more vulnerable to the negative effects of chronic pain on psychological symptoms.

When we assess our study group, we can say that our patients are composed of individuals with low socio-cultural levels. Based on our results, almost half of our patients were illiterate, and only 15% of them were graduated from high school or university. Additionally, 71% of our study group was not working in a job. While we were selecting the participants for the control group, to prevent bias, we excluded the individuals who are relatives of people in Group P, and the ones who live in the same house with someone suffering chronic pain. It is known that relatives of chronic pain patients have also worse psychological moods. In a study including 270 participants, Hancı et al. showed that SCL-90-R scores of relatives of chronic pain patients were significantly higher than the control group (20).

When we assess the correlation between age and psychological symptom severity, we did not observe a significant relation in Group C and observed a weak negative correlation at GSI and some subscales in Group P.

We did not detect a relationship between the duration of pain and SCL-90-R results, but this should not cause a misunderstanding as ‘duration of pain is not associated with worsened psychological mood’. We did not compare the results of acute or subacute patients with chronic patients. Considering that our patient population consists of chronic pain patients who suffer pain for at least 1 year and psychological symptoms had already been established and became solid. The mean duration of pain of our study group (7.6 years) was longer than we expected.

For evaluation of pain severity, we preferred VRS rather than visual analog scale (VAS) or numerical rating scale (NRS) because as mentioned above, our patient group was composed of individuals with lower education level and regarding our clinical experience, trying to use VAS or NRS for the measurement of pain would be challenging, very time-consuming and may cause conflicting results. Jensen et al. suggested that a four-point VRS or the faces pain scale (FPS) would be the most appropriate in populations who might struggle with the 0-to-10 NRS (31). Herr et al. showed that VRS is the most sensitive and reliable tool among 5 single dimension pain assessment tools in both younger and elder patients (30)

Similar to several previous studies, we observed a moderate correlation between the intensity of pain and GSI (31, 32). We observed that patients with headaches tend to express their level of pain more intensely. Regarding the 4-point VRS, the mean result of headache patients was 3.30 and more than half of the individuals in Subgroup H declared their pain as ‘4 points’.

We did not find a correlation between the occupational status and the severity of symptoms neither in Group P nor in Group C. For the evaluation of occupational status, we asked only one question: Do you work in a daily job? We did not evaluate what job the participant has, or he/she is satisfied with the job. Poppel et al. reported that low job satisfaction is associated with the occurrence of low back pain episodes (33).

Patients with cancer pain are not included in the study for the protection of the study homogeneity. Since the clinical course and characteristics of cancer disease are different from non-cancer chronic pain symptoms, we did not include cancer patients in the study. The psychological mood of cancer patients was evaluated in many studies. More than half of patients with cancer suffer pain, and indeed, pain is an important issue in cancer management. In a study including 104 lung cancer patients, Lee et al. reported 50% of patients had a psychiatric diagnosis and the most prevalent psychiatric disorder was depressive disorder (25.0%) (34).

American Psychiatric Association (APA) published ‘Diagnostic and Statistical Manual of Mental Disorders’

(DSM-V) in 2013. (35). This manual is the most crucial tool of professionals in psychiatry for diagnosing psychiatric diseases. We emphasize that SCL-90-R is only a screening tool for the evaluation of psychological symptoms, and it solely has limited diagnostic value.

Patients with chronic pain tend to have psychiatric symptoms but one big mistake would be blaming patients because of their psychological mood or easily labeling their medical situation as 'psychogenic pain'. Pain physicians should not forget this fact while evaluating patients with chronic pain. Pain physicians should not hesitate to consult the patients who show psychological signs and symptoms to the psychiatrists. Regarding the results of this study, we suggest that cooperation between pain physicians and psychiatry physicians has the potential to improve the success of the pain treatment.

Conflict of interest

The authors declare no conflicts of interest regarding this study. Only the authors are responsible for the content and writing of this manuscript.

Acknowledgments

Approval of the institutional ethical committee was obtained before the study.

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Clinical use of the poisoning severity score in acute pediatric poisoning

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Abstract

To make an accurate diagnosis of poisoning and determine the severity of poisoning quickly without losing time are critical for managing the patient's medical treatment and predicting the prognosis. This study aimed to investigate PSS and Glasgow Coma Scale Score (GCS) effectiveness in predicting outcomes in acute pediatric poisoning. We retrospectively reviewed the data of intoxicated patients aged under 18 years who were admitted to the pediatric emergency service of Ondokuz Mayıs University Faculty of Medicine Clinical Research and Practice Hospital between January 1, 2018, and December 31, 2018. Two hundred twenty-two patients were admitted to our pediatric emergency department (ED) after drug ingestions. Of the patients enrolled in the study, 148 (66.7 %) were female, and 74 (33.3%) were male. The mean age was 105.8±75.3 months, and the median age was 65 (12-213) months. 96 (43.3%) poisoning cases were in the age range of 12-18 years. According to Poisoning Severity Score, it was found that 84 cases (37.4%) were asymptomatic (PSS score=0), 86 cases (38.7%) were minor (PSS score=1), 48 cases (21.6%) were moderate (score=2) and four cases (1.8%) were severe (PSS score=3). Combined using the Poisoning Severity Score with the patient's biochemical and physiological values may help improve an accurate diagnosis of poisoning and determine the severity of poisoning more accurately.

Keywords: intoxication, pediatric, poisoning severity score, prognosis

1. Introduction

Poisoning, especially in the pediatric population, remains to be an essential public health issue. While it is commonly seen in the first year of life due to the parents' dose errors, it is later caused by accidental ingestion of household cleaning materials and easily accessible chemical agents. At an older age, poisoning cases can be observed in these groups with excessive intake of medications kept in medicine cabinets or left open.

Although the management of poisoned patients is challenging for every emergency physician, this can be more difficult, especially in the pediatric population. Difficulties in taking an anamnesis and defining the hiding symptoms due to physiological differences in pediatric age subgroups or determining the association between symptoms and relevant toxidromes suggest that the approach to the pediatric poisoned patient will be more difficult than adults.

Therefore, to make an accurate diagnosis of poisoning and determine the severity of poisoning quickly without losing time are critical for managing the patient's medical treatment and predicting the prognosis. For this purpose, Poisoning Severity Score (PSS) has been used successfully in many poisoning types such as hydrocarbons, organophosphates, antipsychotics, and envenomations in adults (1-3).

The Poisoning Severity Score, a simple grading scale proposed by the European Association of Poison Centres and Clinical Toxicologists, uses a collection of clinical signs and symptoms to give a 0 to 4 (4). The score is applied according to the patient's most severe clinical effects, regardless of the timing of those effects. It is not meant to provide prognostic information. A score of zero (0) equates to being asymptomatic, one (1) is minor, two (2) is moderate, three (3) is severe, and four (4) is given if the patient dies. As such, all patients that die should only receive a score of 4 (5).

This study aimed to investigate the effectiveness of PSS and Glasgow Coma Scale Score (GCS) in predicting outcomes in acute pediatric poisoning. We also aimed to describe the detailed characteristics of acute pediatric poisoning cases admitted to our pediatric emergency department.

2. Materials and methods

2.1. Ethical statement

The Clinical Research Ethics Committee approved this study of Ondokuz Mayıs University (Ondokuz Mayıs University CREC protocol no: 2019/506).

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2.2. Study design and population

We retrospectively reviewed the data of patients aged under 18 years who were admitted to the pediatric emergency service of Ondokuz Mayıs University Faculty of Medicine Clinical Research and Practice Hospital between January 1, 2018, and December 31, 2018, after drug ingestion. The patients who had a history of ingestion related to recreational drugs (heroin, synthetic cannabinoids), alcohol, rodenticides, insecticide (organophosphate), and perfume were excluded from the study. After exclusion, 222 patients who met the inclusion criteria were identified.

The relevant information for each of the patients was recorded into the study form about demographic information (age, gender), the admission time (hour and month) and type (direct, referral), school degree, type of ingestion (intentional or nonintentional) the presence of multidrug ingestion, main complaints and symptoms at initial admission, the ingested drugs and their amounts (in grams), vital signs, treatment (hydration, activated charcoal, gastric irrigation), the name of a specific antidote, Glasgow Coma Scale score, Poisoning Severity Scores, the properties of electrocardiography, QTC time, hospitalization status, duration of hospitalization (hour) and type of discharge and prognosis. Clinical staging was performed using Poisoning Severity Score (PSS). While making a statistical analysis using PSS, those with a <2 PSS were described as a minor group, and the remainder with a PSS of 2-3 were described as a moderate group. No patient had a PSS of four points (severe). Understanding the statistical analysis was simplified by dividing the patient group into two subgroups according to PSS. We evaluated the hospitalization and discharge status using PSS and GCS scores.

2.3. Statistical analysis

Data were analyzed using IBM SPSS 21.0 for Windows. The descriptive statistics were presented as mean (±) standard deviation (S.D); median (minimum (min) – maximum (max)), and frequency distribution was presented as percentage (%). The variables' suitability to normal distribution was analyzed using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk Tests). Mann-Whitney U test was used to compare differences between two independent groups. Spearman's rho correlation test was used to investigate the relationship between different variables. Fisher's Exact test was used to examine the qualitative data. The significance level was accepted as $p < 0.05$.

3. Results

Two hundred twenty-two patients were admitted to our pediatric emergency department (ED) after drug ingestions. The poisoned patients represented 1.01% (222/21900) of overall pediatric emergency unit visits during 2018. Of the patients enrolled in the study, 148 (66.7%) were female, and 74 (33.3%) were male. The mean age was 105.8±75.3 months, and the median age was 65 (12-213) months. 96 (43.3%) poisoning cases were in the age range of 12-18 years (Table 1). There was a significant statistical difference between age groups and gender ($p \leq 0.001$), but there was no statistical difference between age groups and poisoning severity score ($p > 0.05$).

It was found that 56 admissions to our ED were in winter (25.2%), 34 in summer (15.3%), 74 in spring (33.3%), and 58 cases in autumn (26%), respectively. There was no statistical difference between admission month and gender as well poisoning severity score ($p > 0.05$). It was determined that the number of admissions between the hours of 00.00-08.00 were 55 cases (24.8%), 81 cases between 08.00-16.00 (36.5%), and 86 cases between 16.00-00.00 (38.7%) respectively. There was no statistical difference in admission hour according to gender and poisoning severity score ($p > 0.05$). When type of ingestion was compared according to gender, school status, admission month and hour, multiple drug ingestion and poisoning severity score; there was a significant statistical difference in gender ($p = 0.02$), school status ($p \leq 0.001$), admission hour ($p = 0.04$), and multiple drug ingestion ($p \leq 0.001$). Still, there was no statistical difference at admission month ($p > 0.05$) and poisoning severity score ($p = 0.217$), respectively.

Analgesic-antipyretics (49 patients (22.1%)) were the most ingested drugs among our pediatric poisoned group, which was consisted of 30 patients who ingested nonsteroidal anti-inflammatory drugs and 19 patients ingested paracetamol. There was no statistical difference between the PSS score and drug groups ($p > 0.05$). It was determined that four patients with high PSS score (PSS = 3) received antidepressants, antipsychotics, centrally acting muscle relaxants and antiepileptic drugs, respectively. A specific antidote was given to 11 (5%) of 222 patients. There was no statistical difference between the administration of specific antidote therapy and poisoning severity score ($p > 0.05$). According to data, 209 patients (94.1%) were hospitalized for treatment in the pediatric emergency observation unit, and 13 patients (5.9%) were hospitalized in pediatric intensive care unit. Of patients admitted to the pediatric intensive care unit, five patients had above 13 of Glasgow Coma Scale score (GCS score≥13), eight patients had 9-12 of GCS score. There was a statistical relation between PSS and GCS score ($p \leq 0.001$, $r = 0,35$). Of the 13 patients hospitalized in pediatric ICU, ten patients were poisoned by a single agent, and three of them were by multidrug intake. According to the data obtained from patients hospitalized in pediatric ICU, there was no statistical difference between multidrug intake and GCS and PSS ($p > 0.05$).

According to Poisoning Severity Score, it was found that 84 cases (37.4%) were asymptomatic (PSS score=0), 86 cases (38.7%) were minor (PSS score=1), 48 cases (21.6%) were moderate (score=2) and four cases (1.8%) were severe (PSS score=3). Of these patients with severe symptoms, three patients were female, and two patients were accidentally

intake and between the ages of 2-6 years. Contrarily, the remaining two patients were suicidal ingestion and between 12-18 years. Three patients with severe PSS have been poisoned by a single agent. Sixty-five cases (29.3%) refused the treatment left the hospital before the end of the observation period. The asymptomatic 156 patients (70.27%) were discharged after sufficient observation.

Table 1. Characteristics of acute pediatric poisoning

		Number of Patient n (%)
Gender	Female	148 (66,7)
	Male	74 (33,3)
Age (months)		105.8±75.3* 65 (12-213)**
Age Groups	0-2 years old	20 (9.0)
	2-6 years old	92 (41.4)
	6-12 years old	14 (6.3)
	12-18 years old	96 (43.2)
School Status	Out of School	121 (54.5)
	Nursery	23 (10.4)
	Preschool	3 (1.4)
	Primary school	11 (5.0)
	Middle school	21 (9.5)
	High School	43 (19.4)
Admission Time (Month)	Winter	56 (25.2)
	Spring	74 (33.3)
	Summer	34 (15.3)
	Autumn	58 (26.1)
Admission Time (Hour)	24.00 - 08.00	55 (24.8)
	08.00 - 16.00	81 (36.5)
	16.00 - 24.00	86 (38.7)
Type of Ingestion	Incidental	123 (55.4)
	Suicidal	99 (44.6)
Type of substance intake	Single drug	169 (76.1)
	Multiple drugs	53 (23.9)
Amount of substance intake	Antidepressants	36 (16.2)
	Nonsteroidal antiinflammatory drugs	30 (13.5)
	Antipsycotics	22 (9.9)
	Cardiovascular system drugs	21 (9.5)
	Paracetamol	19 (8.6)
	Antiepileptics	18 (8.1)
	GIS motility regulators	7 (3.2)
	Antibiotics	7 (3.2)
	Addictive substance	6 (2.7)
	Proton pump inhibitors	6 (2.7)
	Antihistaminics	4 (1.8)
	Antidiabetics	3 (1.4)
	Iron preperate	3 (1.4)
	Myorelaxants	3 (1.4)
	Acetylcysteine	3 (1.4)
	Alcohol	3 (1.4)
	Unknown	2 (1.4)
	Others	29 (13.1)
Complaints	Asymptomatic	91 (41.0
	Vomiting	55 (24.8)
	Altered mental status	35 (15.8)
	Syncope	13 (5.9)
	Seizure	5 (2.3)
	Dizziness	3 (1.4)
	Faint	2 (0.9)
	Abdominal pain	2 (0.9)
	Others	16 (7.0)
Decontamination	Activated charcoal – gastric lavage	184 (82.0)
	Activated charcoal	21 (9.5)
	Gastric lavage	5 (2.3)
	No application	12 (5.4)
ECG	Normal sinus rhythm	185 (83.3)
	Abnormal ECG finding	37 (16.7)
	Bradycardia	5 (2.3)

	Sinustachycardia	29 (13.1)
	PR elongation	1 (0.5)
	LongQTc	2 (0.9)
PSS	Asymptomatic -Minor (0-1)	170 (76.6)
	Moderate-Severe (2-3)	52 (23.4)
	Death (4)	0 (0)
GCS	Overall	15 (9-15)**
	Mild (≥13)	211 (95.0)
	Moderate (9-12)	11 (5.0)
Hospitalization	Pediatric Emergency / General service	211 (95.0)
	Pediatric intensive care unit	11 (5.0)
Duration of Hospital Stay (hours)		39.78±24.54* 35 (3-160)**
Antidote	Not used	211 (95.1)
	Specific antidote administered	11 (5.0)
	N-acetylcysteine	8 (3.6)
	Calcium and leucovorin	1 (0.45)
	Biperiden	1 (0.45)
	Deferoxamine	1 (0.45)
Final Status	Discharged with full recovery	155 (69.8)
	Treatment refusual	66 (29.7)
	Referred to Transplantation Center	1 (0.5)

*; The descriptive statistics were presented as mean (±) standard deviation (SD), **; The descriptive statistics were presented as median (minimum– maximum)

A 16-year-old girl who developed acute liver failure following massive paracetamol ingestion with the initial PSS score of two was referred to a transplantation center. The four patients who had severe symptoms at the admission (PSS score=3) were discharged from the hospital with complete recovery. The median (min-max) duration of hospitalization was 35 (3-160) hours. The median (min-max) duration of hospitalization in the single drug ingested patient and multiple drug intake were 35 (3-160) and 35 (6-120), respectively. The median (min-max) duration of hospitalization in the accidentally ingested group and suicidal group were 38 (6-96) and 32 (3-160), respectively. When patients were analyzed the type of ingestion (incidental, suicidal) according to admission time (month), there was no significant statistical difference at the seasonal variation (p=0.939). When we analyzed the patients' PSS and GCS scores according to hospitalization and discharge status, statistical differences were determined, respectively (Table 2 and 3).

Table 2. Evaluation of Poisoning Severity and Glasgow Coma Scale Scores according to hospitalization and discharge status

	Poisoning Severity Score (PSS)			
	Minor	Moderate	Total	p*
Hospitalization				
Pediatric Service	167 (98.2)	42 (80.8)	209 (94.1)	<0.001
IntensiveCareUnit	3 (1.8)	10 (19.2)	13 (5.9)	
Discharge				
Treatmentrefusual	57 (33.7)	8 (15.7)	65 (29.5)	<0.001
Dischargedwithfullrecovery	112 (66.3)	43 (84.3)	155 (70.5)	
	Glaskow Coma Scale Score (GCS)			
	Mild	Moderate	Total	
Hospitalization				
Pediatric Service	206 (97.6)	3 (27.3)	209 (94.1)	0.021
Intensive Care Unit	5 (2.4)	8 (72.7)	13 (5.9)	
Discharge				
Treatment refusual	65 (31.1)	0 (0)	65 (29.5)	0.036
Discharged with full recovery	144 (68.9)	11 (100)	155 (70.5)	

*Fisher's Exact test

Table 3. Evaluation of acute pediatric poisoning cases according to Poisoning Severity and Glasgow Coma Scale Scores

	N	Median	Minimum	Maximum	Mean	Std. Deviation	p*
PSS							
Minor	170	30.00	3.00	96.00	35.48	20.57	<0.001
Moderate	52	48.00	15.00	160.00	53.85	30.74	
GCS							
Mild	211	32.00	3.00	96.00	37.13	20.95	<0.001
Moderate	11	80.00	48.00	160.00	90.73	33.11	

*Mann Whitney U test

4. Discussion

In case of poisoning, determining the agent and administration of the specific antidote therapy without gratuitous delay is especially important, especially in the early ED admission period. Therefore, it is used in many clinical tools for early recognition of patients' prognosis. This retrospective study started with this idea. The poisoning severity score was used to determine the severity of acute pediatric poisoning cases admitted to a tertiary pediatric emergency department for one year.

The percentage of acute poisoning in our tertiary pediatric emergency department had a high level than the numbers of acute poisoning cases in western countries (0.28-0.66%) but also a similar ratio with the other pediatric emergency units in Turkey (0.21-2.31) (6). It supports that childhood poisoning is still an important issue; that is why it is a public health problem for Turkey and the other countries in the world. In our study, different from the others, acute poisoning was widespread in children between the ages of 12-18 years (7). In the various studies in the literature, the data that supports acute poisoning in children under five years of age are also available in our research. The female gender (66.7%) was dominant among all age groups in our study, like the other studies (8,9). Besides, there was no statistical difference between age groups and the poisoning severity score in our study. This result can be related to the small study size and duration because this study was planned as a pioneer study to determine the poisoning severity score as a valuable tool in pediatric aged, poisoned patients before a prospective study that will be conducted on the same field.

There was no statistical association between admission month and PSS. Still, in another study from Turkey, Sahin et al. (6) found that the highest numbers of pediatric poisoning cases have happened in the winter, contrary to our finding as spring. According to our data, antidepressant and anti-inflammatory drugs were the leading toxic agents, like the literature (7). While the younger patient group had more accidental single intakes, the older pediatric age group received higher rates of multiple suicide intake, like adults in our study.

Similarly, Mintegi et al. (7) emphasizes that the young pediatric patient group accidentally consumes a small number of drug-induced drugs at home, and the cases are benign. Although the symptoms or complaints are especially important for the clinician in the pediatric poisoning cases, it is noteworthy that most pediatric poisoning patients are asymptomatic in the studies conducted in the literature and our research. As the leading symptoms were vomiting (24.8%) and altered mental status (15.8%) in our research, neurologic symptoms, significantly altered mental status were often reported in the literature (7).

The fact that most patients are hospitalized due to the need for observation in pediatric poisoning cases appears with a high rate of hospitalization in our study. In contrast, the number of morbidities is deficient, and there was no mortality in the study group. We also think that it reduces the selectivity of scoring systems because most patients were mild or asymptomatic in our study. After all, a statistical difference was found between the admission to the intensive care unit and Glasgow Coma Scale score and Poisoning severity score. PSS and GCS's comparison is not exactly accurate, but it is noteworthy that both scores are in similar ratios in the pediatric poisoned group. When the PSS score was compared with gender, school status, admission hour, multidrug intake, hospitalization, and discharge status, significant statistical differences were detected. Then, it noticed that there were conflicting results about the poisoning severity score in specific poisonings in the literature. However, Akdur et al. (10) and Nauira et al. (11) did not find a relationship between pseudocholine esterase level and the PSS scores in two separate organophosphate poisoning studies like our study result. Tsao et al. (12) and Churi et al. (13) found contradictory results that there was a relationship between pseudocholine esterase level, clinical outcome, and PSS scores.

This retrospective study is a pilot study in a pediatric emergency department in a tertiary university hospital which contains only prescripted drug poisoning cases in a one-year limited time and reflects its data before the prospective research planned. Because of the low number of patients with severe symptoms and no mortality in our patient group, the PSS score was insufficient in the patient management in our study.

In conclusion, intoxication in childhood is a significant global public health problem causing mortality and morbidity that can be prevented. Even if the PSS score looks suitable for clinical and research uses, there is still no sufficient evidence in the literature. Also, the wrong usage of the PSS score was also emphasized in a few studies. Combined using the Poisoning Severity Score with patient's biochemical and physiological values may be helpful to improve an accurate diagnosis of poisoning and determine the severity of poisoning more accurately.

Conflict of interest

No conflict of interest was declared by the authors.

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This study was approved by The Clinical Research Ethics Committee of Ondokuz Mayıs University Medicine Faculty (OMU CREC protocol no: 2019/506).

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Laboratory risk indicator for necrotising fasciitis [LRINEC] score as a tool for differentiating necrotising fasciitis from other soft tissue infections

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Abstract

Necrotizing fasciitis (NF) is often fatal, characterized by extensive necrosis of the subcutaneous tissues and fascia. The present study was aimed to validate the Laboratory Risk Indicator for Necrotising Fasciitis (LRINEC) score as a tool to predict/diagnose NF and to differentiate it from other soft tissue infections depending on the score. A Prospective Observational study was conducted in ESICMC PGI MSR, Medical College Hospital, Rajajinagar, Bengaluru, from Jan 2019 to June 2020. Patients ≥18 years of age with severe soft tissue infections were included in the study. Based on the LRINEC score, the patients were categorised as low (≤5), moderate (6-7) and high risk (≥8) for the prediction of onset or diagnosis of NF. Data analysis was performed using SPSS version 21.0. A total of 55 patients were included in the study. A significant association was observed with age (p=0.042), LRINEC score (p=0.0001), C Reactive Protein (CRP; p=0.0001), haemoglobin (p=0.008), serum sodium levels (p=0.004), serum creatinine (0.001), and amputation (p=0.004). Amputation was done in 5 cases. Only 1 mortality was observed in LRINEC high risk group with NSSTI. To conclude, LRINEC scoring system showed a better positive predictive value in identifying the onset of NF and risk strategizing of the patients with severe soft tissue infections.

Keywords: debridement, LRINEC score, necrotizing fasciitis, soft tissue infections

1. Introduction

Skin and soft tissue infections (SSTIs) are caused by microbial invasion of the skin and underlying soft tissues (1). In few cases, severe infection causes skin necrosis leading to necrotizing skin and soft tissue infections (NSSTIs). NSSTI is also referred as Fournier gangrene, necrotizing fasciitis, necrotizing skin and skin structure infections, and meleneys gangrene (2). Among all variants, necrotizing fasciitis (NF) is a rare, rapidly progressive, potentially life-threatening, inflammatory infection of the soft tissue that cause necrosis of the muscle fascia and subcutaneous tissues (3, 4). Even with optimal treatment, the morbidity and mortality rate was reported to be 25% to 35% (4). The most common cause and route of infection is microorganism invasion of the subcutaneous tissue through a cut in the skin, open wound or surgical wound and insect bite. Once the pathogens enter the subcutaneous tissues, the pathogens releases exotoxins causing tissue necrosis. Patients with co-morbidities such as diabetes mellitus (DM), peripheral vascular diseases, malnutrition, liver disease, kidney disease, human immunodeficiency virus, and alcohol abuse were reported to be more susceptible to the disease (5).

The key to successful treatment and prognosis in patients with NF lies in early diagnosis, early operative debridement and appropriate antibiotic therapy. If not, it may lead to sepsis, systemic inflammatory response syndrome [SIRS] or Multi Organ Dysfunction Syndrome [MODS], and eventually causing mortality. The major challenge faced by clinicians is differentiation of NF from other soft tissue infections like cellulites or abscesses early in its evolution (6). Delayed diagnosis is one of the main reasons for the high mortality rate (7). However, diagnosis solely based on physical examination is highly impossible, though modalities such as magnetic resonance imaging (MRI), computed tomography (CT), and frozen section biopsy help in the early diagnosis of NF, cost and availability limits their use in routine application in the evaluation of soft tissue infections (8-10). To overcome these limitations, Wong et al., developed a scoring system from a clinical tool called as The Laboratory Risk Indicator for Necrotising Fasciitis (LRINEC). The tool is based on six routine laboratory variables: glucose, creatinine, serum sodium, haemoglobin, total white cell count, and C-reactive protein (CRP) (6).

The aim of the present study was to validate the Laboratory Risk Indicator for Necrotising Fasciitis (LRINEC) score as a tool to predict/diagnose NF and to differentiate it from other soft tissue infections depending on the score. This data can be vital in guiding clinicians to help and identify the disease, which can further help the patients in receiving definitive therapeutic interventions without much delay.

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2. Materials and methods

This prospective observational study was conducted at ESICMC PGI MSR Hospital, India, from January 2019 to June 2020. All ethical approvals were procured from Institute Ethics Committee (IEC) prior to study commencement. Written informed consent was obtained from all patients. Patients ≥18 years of age with severe soft tissue infections were included in the study. Patients ≤18 years of age, pregnant women, and patients refused to provide consent were excluded from the study. Ethics committee approval was received for this study from the Institutional Ethics Committee of ESIC Medical College & Post Graduate Institute of Medical Sciences & Research, India (Protocol No.– 532/L/11/12/Ethics/ESICMC & PGIMSR/Estt. Vol. IV).

Patients' age, sex, site and aetiology of infection, clinical manifestations, comorbidities, predisposing factors, vital signs, laboratory parameters at the time of admission were recorded in a prespecified proforma. In the present study, culture of pus was done to all patients admitted with soft tissue infection before giving antibiotics. Based on the LRINEC score, the patients were categorised as low (≤5), moderate (6-7) and high risk (≥8) for the prediction of onset or diagnosis of NF. LRINEC score risk categorization, the time interval between the admission and first surgery, the number of surgical procedures, the need for amputation and the mortality rate had been documented. All variables were statistically analyzed further to evaluate the significance of LRINEC score in predicting the clinical outcomes. On admission, general and medical treatment of necrotizing fasciitis, followed by wound debridement was done as the definitive procedure. Later, regular wound dressing, administration of antibiotics, and supportive therapy for maintenance of blood pressure and renal status and in few cases vacuum assisted dressings were done for faster healing. Once the wound was healthy, split skin grafting and secondary suturing was done in most cases. Some cases healed by secondary intention while some cases had to undergo major amputations for control of infection and its spread. Regular dressings and water beds were given to patients that had bed sores as a complication of necrotizing fasciitis. Patients that developed septicaemia were managed in intensive care units on ventilators under the guidance of anaesthetists and physicians. Post discharge patients were followed up to one month regularly for dressing, and also to review liver and renal parameters. Major amputation patients were advised for clutches.

2.1. Statistical analysis

Data analysis was performed using SPSS version 21.0. Data analysed by descriptive statistics was represented as mean ± standard deviation (SD) or percentages. Chi-square test and independent T-test was used to determine qualitative variables and significance difference between the two groups. P≤0.05 was considered as statistically significant.

3. Results

The study included a total of 55 patients, of which 12 (21.81%) were female and the rest 43 (78.18%) were male. Diabetes mellitus was observed to be most common comorbidity (89.09%) followed by systemic hypertension (50.91%), chronic renal failure (12.73%) and peripheral vascular disease (7.27%). The most common site of infection was extremity followed by scrotum and perineum. In 55 patients, 12 had soft tissue infections of unknown origin and the remaining 43 cases infection were attributed to injury as a cause; 12 were treated conservatively while 43 were debrided.

Based on LRINEC score, 17 patients were diagnosed with necrotizing fasciitis and 38 patients with other soft tissue infections (Table 1). Out of 17 patients with necrotizing fasciitis, one patient had low risk and the other 16 had high risk disease whereas among 38 patients with soft tissue infections, 19 were of low, 17 of moderate, and two were from high risk category. All the 16 NSSTIs cases underwent more than three debridement's (Figs. 1 and 2), 13 cases were treated with split skin graft (SSG), and five cases underwent major amputation to prevent septicaemia and its complications (Fig. 3). Out of five amputations, four patients had LRINEC high risk group with NSSTIs while only 1 had other soft tissue infection.

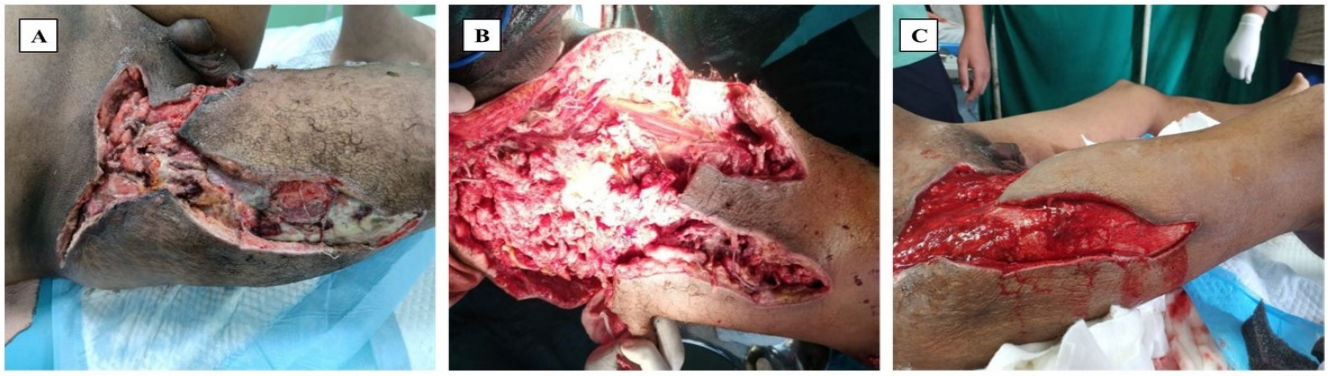

Fig. 1. Necrotising fasciitis of right lower limb [thigh] on arrival (A), after second debridement (B), and after negative pressure wound therapy or VAC dressing (C)

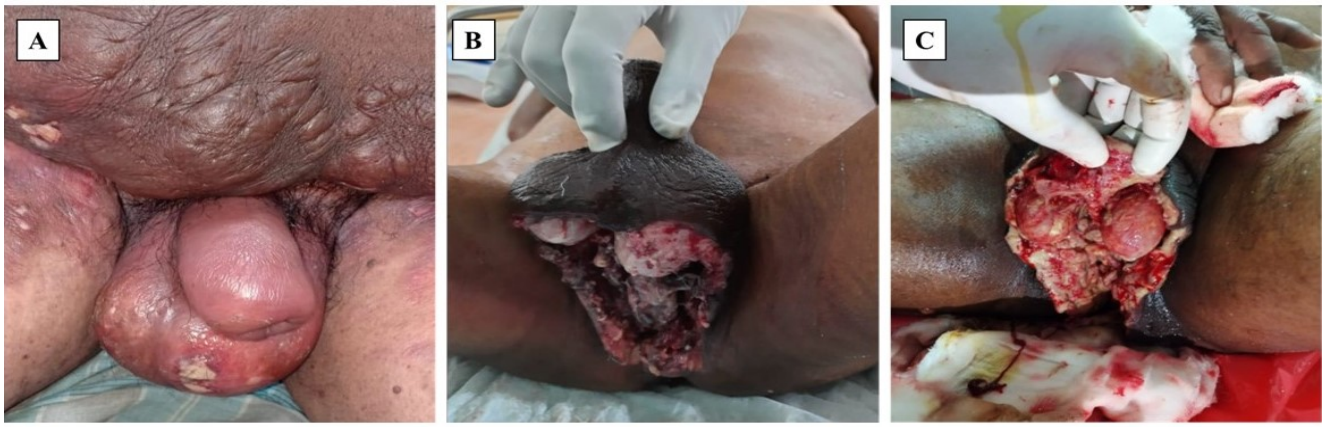

Fig. 2. A case of fournier's gangrene associated with meleneys gangrene (A), fournier's gangrene after second (B) and fourth debridment (C).

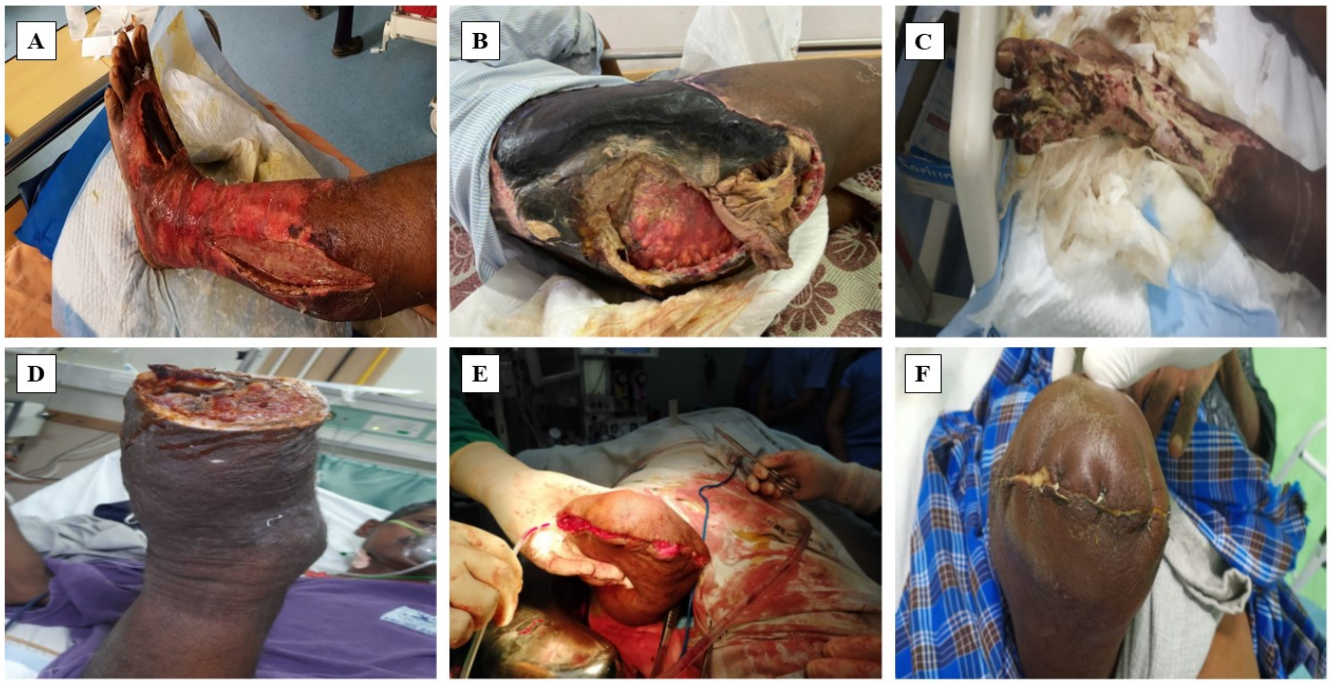

Fig. 3.

(A) A case of left lower limb cellulites with fasciotomy later developed necrotizing fasciitis
(B) A case of necrotizing fasciitis of right hip, gluteal region and upper thigh with maggots.
(C) A case of left lower limb necrotizing fasciitis did not heal with aggressive treatment and underwent above knee amputation.
(D) A case of right lower limb necrotizing fasciitis underwent emergency guillotine amputation
(E) Amputation stump on post op day 4 in a necrotizing fasciitis case
(F) Amputation stump immediate post-surgery on table in a necrotizing fasciitis case of right leg

Table 1. Association between LRINEC score and diagnosis

Risk category	Diagnosis		p
	Necrotising fasciitis	Other soft tissue	
Low	01(5.9)	19(50.0)	0.0001
Moderate	0(0.0)	17(44.7)	
High	16(94.1)	02(5.3)	
Total	17(100.0)	38(100.0)	

Table 2. Comparison of age by LRINEC score

Parameters	LRINEC Score			p
	Low	Moderate	High	
Age	51.00±14.89	62.94±13.27	55.94±13.44	0.042
LRINEC Score	4.75±2.07	6.76±0.56	9.61±1.38	0.0001

Table 3. Association between various parameters and their Laboratory Risk Indicator for Necrotising Fasciitis score

Parameters	LRINEC Score			p
	Low (n=20)	Moderate (n=17)	High (n=18)	
Male	17 (85.0)	12 (70.6)	14 (77.8)	0.571
Female	03 (15.0)	05 (29.4)	04 (22.2)	
	C Reactive Protein (CRP)			
Negative (≤150mg/dlL)	19 (95.0)	17 (100.0)	03 (16.7)	0.0001
Positive (≥150mg/dL)	01 (5.0)	0 (0.0)	15 (83.3)	
	White blood cells (cmm)			
15-25	04 (20.0)	03 (17.6)	08 (44.4)	0.135
>25	16 (80.0)	14 (82.4)	10 (55.6)	
	Haemoglobin (gm/dL)			
>13.5	01 (5.0)	0 (0.0)	0 (0.0)	0.008
11-13.5	12 (60.0)	2 (11.8)	4 (22.2)	
<11	07 (35.0)	15 (88.2)	14 (77.8)	
	Serum sodium (mmol/L)			
≥135	16 (80.0)	11 (64.7)	05 (27.8)	0.004
<135	04 (20.0)	06 (35.3)	13 (72.2)	
	Serum creatinine (mg/dL)			
≤1.6	18 (90.0)	05 (29.4)	12 (66.7)	0.001
>1.6	02 (10.0)	12 (70.6)	06 (33.3)	
	Blood glucose (mg/dL)			
≤180	04 (20.0)	01 (5.9)	2 (11.1)	0.425
>180	16 (80.0)	16 (94.1)	16 (88.9)	
	Diabetes mellitus			
Yes	17 (85.0)	16 (94.1)	16 (88.9)	0.675
No	03 (15.0)	01 (5.9)	02 (11.1)	
	Hypertension			
Yes	06(30.0)	10 (58.8)	12(66.7)	0.057
No	14(70.0)	07 (41.2)	06(33.3)	
	Amputation			
Yes	0 (0.0)	0 (0.0)	05 (27.8)	0.004
No	20 (100.0)	17 (100.0)	13 (72.2)	

Patients aged 55 years were found to be at higher risk with a LRINEC score of 9.61 (Table 2). Table 3 represents association between various laboratory parameters and their LRINEC scores. A significant association was observed with age (p=0.042), LRINEC score (p=0.0001), C Reactive Protein (CRP; p=0.0001), haemoglobin (p=0.008), serum sodium levels (p=0.004), serum creatinine (0.001), and amputation (p=0.004). No significant association was observed with gender, white blood cells (WBC), blood glucose, DM and hypertension. Only one mortality was observed in LRINEC high risk group with NSSTI.

4. Discussion

NF is one of the most difficult disease processes encountered by physicians and surgeons (11). It can often progress within a few hours, and is an invasive infection that complicates the skin, subcutaneous tissue, deep fascia (12). NF poses a challenge to clinicians by encompassing a wide range of infections that share the same diagnostic and treatment principles. Therefore, establishing the diagnosis itself is one of the biggest challenge to clinician. To distinguish the NF from other soft tissue infections, Wong et al. developed a novel diagnostic scoring system called LRINEC score (6). This LRINEC score was calculated from the routine laboratory investigations and such tests are easy to access, readily available, and cost-effective. This LRINEC score was calculated from the patient's glucose, creatinine, sodium level, haemoglobin, WBC count, and CRP values. The maximum score is 13. A score of ≥6 raise suspicion for NF, and a score of ≥8 is highly predictive of NF (6, 13, 14).

The present study was aimed to validate the Laboratory Risk Indicator for Necrotising Fasciitis (LRINEC) score as a tool to predict/diagnose NF and to differentiate it from other soft tissue infections depending on the score. In this study, ratio of male over female was predominant and the more common age group was found to be ~ 55 years. Similar male predominance was reported in a prospective study conducted by Espander et al. (12). In a recent retrospective study by Rampal et al, 60% of patients were male (15). Many reported studies showed the male predominance with highest number of cases among age groups between 50 and 60 years (5, 12, 15-17).

In the present study, the most common site of NF infection was extremities followed by scrotum and perineum which was in accordance with a study by Singh et al. The study reported most common anatomical sites with NF was lower extremities, followed by the upper extremity, and perineum (18). A prospective, cross sectional study also showed lower extremity, followed by

upper extremity, and other sites such as perineum, chest, buttocks as the most common anatomical sites infected (5).

The most pre-disposing factors observed from this study group were age, DM, and hypertension, which was in par with other reported studies (5, 18-20). In patients with DM, a delay in diagnosis of NF was apparent, especially in early stages. Due to these age related co-morbidities and progressive decrease in immunity, patients become more susceptible to infections. In some patients, diabetes related issues increases the risk of injuries which may act as triggering factors for infection (18). Kwan et al. and Shaikh et al. have also reported age, DM and hypertension as the most common co-morbid conditions making NF patients much more susceptible and immunocompromised (19, 20). Amputation rate was high in DM patients compared with other comorbidities, similar results were reported in a previous study by Espander et al (12). Such high rate of amputations in DM patients might be due to the development of severe atherosclerosis, especially in small-caliber arteries which results in ischemia and gangrene (16, 21).

In the present study, we have employed LRINEC score to differentiate NF from other SSTIs. The serum creatinine levels are often elevated in patients with septic shock and renal dysfunction, where, creatinine clearance is compromised with early signs of acute renal failure (16, 18). Overall, a strong positivity for NF from laboratory findings was observed with elevated CRP, elevated WBC, low haemoglobin, decreased sodium, and increased creatinine. Depending on the severity, treatment differed from patient to patient. Still the essential steps in the treatment is early diagnosis, surgical debridement, amputation of extremity in high risk patients, wound care, antimicrobial therapy, and intensive supportive care. According to our findings, the presence of multiple predisposing factors such as DM, low haemoglobin, high serum creatinine levels, gangrene, and skin necrosis were proven lethal.

To conclude, LRINEC score has shown a better positive predictive value in identifying the onset of NF and risk strategizing of the patients with severe soft tissue infections. The risk stratification was an important tool in differentiating NSSTI from other forms of SSTIs.

Conflicts of interests

None.

Acknowledgments

All patients provided written informed consent.

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Are neutrophil count and neutrophil/lymphocyte ratio useful as markers of polycystic ovary syndrome in early reproductive age?

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Abstract

Necrotizing fasciitis (NF) is often fatal, characterized by extensive necrosis of the subcutaneous tissues and fascia. The present study was aimed to validate the Laboratory Risk Indicator for Necrotising Fasciitis (LRINEC) score as a tool to predict/diagnose NF and to differentiate it from other soft tissue infections depending on the score. A Prospective Observational study was conducted in ESICMC PGI MSR, Medical College Hospital, Rajajinagar, Bengaluru, from Jan 2019 to June 2020. Patients ≥18 years of age with severe soft tissue infections were included in the study. Based on the LRINEC score, the patients were categorised as low (≤5), moderate (6-7) and high risk (≥8) for the prediction of onset or diagnosis of NF. Data analysis was performed using SPSS version 21.0. A total of 55 patients were included in the study. A significant association was observed with age (p=0.042), LRINEC score (p=0.0001), C Reactive Protein (CRP; p=0.0001), haemoglobin (p=0.008), serum sodium levels (p=0.004), serum creatinine (0.001), and amputation (p=0.004). Amputation was done in 5 cases. Only 1 mortality was observed in LRINEC high risk group with NSSTI. To conclude, LRINEC scoring system showed a better positive predictive value in identifying the onset of NF and risk strategizing of the patients with severe soft tissue infections. This study has aimed to contribute to the literature by investigating the value of inflammatory biomarkers of polycystic ovary syndrome (PCOS) that can be tested via a complete blood count. This retrospectively designed case-control study included 197 women in early reproductive age; who were in the age range of 18-24 years and who were admitted to the gynecology outpatient clinic. A total of 111 PCOS patients; in whom the diagnosis of PCOS was made based on Rotterdam criteria, were included in the study. A control group was formed by including 86 healthy women. All measurements of inflammatory biomarkers were obtained from the complete blood count test results. Of the inflammatory markers; the neutrophil count and the neutrophil/lymphocyte ratio were statistically significantly higher in the PCOS group compared to the control group (p=0.016 and p=0.002, respectively); however, the measured values of other parameters were similar between two groups. To evaluate whether or not the neutrophil count and neutrophil/lymphocyte ratio could be used as a screening tool to exclude PCOS, we constructed a receiver-operating characteristic curve (ROC). The ROC curve for the neutrophil count was 0.60 (p=0.016) and NLR was 0.627 (p=0.002). The neutrophil count and NLR were higher in the PCOS cases compared to the age-matched individuals in the control group. This finding confirms the presence of inflammation in PCOS cases of early reproductive age. However, it has been demonstrated that the diagnostic values of these markers are not strong in distinguishing PCOS patients from healthy individuals.

Keywords: Inflammatory markers, infertility, lymphocyte, neutrophil, polycystic ovary syndrome, ratio

1. Introduction

Polycystic ovary syndrome (PCOS) is a metabolic and ovulatory disorder; which becomes manifest in adolescence (1) and continues throughout the reproductive ages, causing infertility in most patients (2).

The major factor involved in the pathogenesis of both clinical and biochemical features of PCOS is the metabolic disorder caused by increased quantities of adipose tissue as observed in most cases. The emerging evidence points out the fact that metabolic changes such as hyperandrogenism, type 2 diabetes, insulin resistance, and cardiovascular disease occurring during the course of PCOS in the long term may be associated with low-grade chronic inflammatory environment (3-6). Studies have shown that a significant increase occurs in circulating inflammatory marker levels in PCOS and that proinflammation is an important component of PCOS (7, 8). It is known that inflammation affects many complex mechanisms and that detection of an increase in immune cells may indicate inflammation. In clinical terms, studies have shown that an increase in inflammatory biomarker levels in the complete blood count may be an indicator of inflammation (6, 9, 10). A literature review reveals that; of the hematological biomarkers, the neutrophil-to-lymphocyte ratio (NLR) may have prognostic significance in many cancers and conditions affecting the metabolic and cardiac systems (11-14). Studies examining the significance of hematological inflammatory biomarkers in different clinical phenotypes of PCOS found out that the levels of some biomarkers including the counts of white blood cells, neutrophils, leukocytes, and the mean platelet volume increased in the PCOS group (15-17). However, the results in

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the literature are controversial (18). Therefore; this study has aimed to contribute to the literature by investigating the value of inflammatory biomarkers that can be tested via a complete blood count but having no established diagnostic significance, yet.

2. Materials and methods

2.1. Study design and patient selection

This retrospectively designed case-control study included 197 women in early reproductive age; who were in the age range of 18-24 years and who were admitted to the gynecology outpatient clinic of Recep Tayyip Erdoğan University School of Medicine's Training and Research Hospital in the period between January 2016 and December 2019.

In accordance with the World Medical Association's 2008-revised version of the 1975-version of the Declaration of Helsinki, the study commenced after obtaining approval from the Ethics Committee of Recep Tayyip Erdoğan University (Approval No:2020/48).

Data; including the medical history, habits, and anthropometric parameters of participants in the PCOS and healthy control groups were obtained from hospital records. Our study excluded participants with the following conditions; including hyperandrogenism-related diseases such as androgen-secreting tumors, congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, and thyroid diseases; liver and kidney dysfunction, cardiovascular diseases, diabetes mellitus, chronic inflammatory disease, malignancy, pregnancy, acquired immune deficiency syndrome, and nicotine and alcohol consumption. Participants using drugs with potential effects on the inflammatory state such as oral contraceptives, steroid hormones, insulin-sensitizing drugs, antibiotics, or anti-inflammatory drugs were excluded, too.

A total of 111 PCOS patients; in whom the diagnosis of PCOS was made based on Rotterdam criteria, were included in the study. According to the Rotterdam criteria, making a diagnosis of PCOS requires the presence of at least two of the following three symptoms: [1] oligomenorrhea/polymenorrhea; [2] biochemical hyperandrogenis (Ferriman-Gallwey scores (FCS) of ≥8) (19) or the presence of clinical hyperandrogenism; [3] detection of polycystic ovaries in an ultrasonographic examination (20).

Blood pressure values, waist and hip circumferences, and the height and weight of participants were retrieved from hospital records. Body mass index (BMI) was calculated using the following formula: BMI=weight (kg)/height $(m)^2$. Complete blood counts and hormonal and biochemical tests were performed using the serum samples taken in the early follicular phase of the menstrual cycle. The results were retrieved from hospital records. All measurements including the counts of total white blood cells, neutrophils, monocytes, platelets, and lymphocytes, and all calculated ratios were obtained from the complete blood count test results.

The homeostatic model assessment-insulin resistance (HOMA-IR) values were calculated using the following formula: HOMA-IR = The fasting blood glucose level x the fasting insulin level / 22.5 (21).

2.2. Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences software version 23.0 (SPSS IBM, Armonk, NY, USA). Descriptive data were expressed as mean ± standard deviation. The chi-square test was used in the analysis of categorical variables. The normal distribution of the variables was tested by Kolmogorov-Smirnov test. The nonparametric distribution of the variables was tested by the Mann-Whitney U test. The optimal cut-off points for inflammatory parameters in distinguishing the PCOS patients were further evaluated by receiver operating characteristic curve (ROC) analyses. A probability level of $p<.005$ was considered statistically significant.

3. Results

Of the participants included in the study, 111 patients were included in the PCOS group and 86 participants were included in the control group. The anthropometric properties and hormonal and metabolic profiles of the PCOS and control groups are listed in Table 1. The age distribution was statistically found to be similar between the PCOS and control groups (p= 0.104). The waist circumference and BMI were statistically significantly higher in the PCOS group compared to the control group (p=0.001, p=0.001, respectively); however, the hip circumference was similar between the two groups (p=0.082). Compared to healthy women; FGS values, as an indicator of clinical hirsutism, were higher in PCOS patients (p<0.001).

Glucose and insulin values and HOMA-IR scores were not significantly different between the PCOS and control groups. As for the lipid profiles; triglyceride levels were statistically significantly higher in the PCOS group (p=0.007) but the levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) were not different between the groups. Women with PCOS had significantly higher levels of luteinizing hormone (LH), total testosterone (tT), and free testosterone (fT) compared to the control group (p=0.031, p=0.004, and p=0.001; respectively). The levels of *17-hydroxyprogesterone* (17-OH PG), dehydroepiandrosterone sulfate (DHEA-S), prolactin, and thyroid-stimulating hormone (TSH) were not significantly different between the groups.

Of the inflammatory markers; the neutrophil count and the NLR were statistically significantly higher in the PCOS group compared to the control group (p=0.016 and p=0.002, respectively); however, the measured values of other parameters were similar between the two groups. To evaluate

whether or not the neutrophil count and NLR could be used as a screening tool to exclude PCOS, we constructed a receiver-operating characteristic (ROC) curve (Fig. 1). Using a cut-off value of 4.955, the neutrophil count in the serum had a likelihood of excluding PCOS with a sensitivity of 0.39% and a specificity of 0.81%. Using a cut-off value of 1.755, NLR in the serum had a likelihood of excluding PCOS with a sensitivity of 0.64 % and a specificity of 0.62% (Fig.1). The area under the ROC curve for the neutrophil count was 0.60 (95% CI: 0.512-0.680, p=0.016). The area under the curve for NLR was 0.627 (95% CI: 0.549-0.706, p=0.002) (Fig.1).

Table 1. The anthropometric and biochemical findings in the polycystic ovary syndrome and control groups

Parameters	PCO (n=111)	Control (n=86)	p-value
Age (year)	21.13 ± 2.60	21.72 ± 2.43	0.104
WC (cm)	77.72 ± 10.22	73.36 ± 9.62	0.001*
HC (cm)	101.30 ± 9.27	98.85 ± 9.22	0.082
BMI (kg/m^2)	23.78 ± 4.88	21.69 ± 3.49	0.001*
FGS	7.77 ± 3.5	1.85 ± 2.69	<0.001*
Glucose (mg/dl)	90.14 ± 7.01	91.73 ± 10.53	0.454
Insulin (mIU/ml)	10.62 ± 7.46	9.39 ± 5.26	0.350
HOMA-IR	2.36 ± 1.84	2.04 ± 1.27	0.238
Triglyceride (mg/dl)	82.50 ± 36.41	68.44 ± 31.19	0.007*
TC (mg/dl)	181.04 ±28.85	199.15 ± 184.07	0.153
HDL-C (mg/dl)	60.47 ± 13.32	62.72 ± 13.67	0.339
LDL-C (mg/dl)	102.47 ±28.79	99.72 ± 31.72	0.149
LH (mIU/ml)	5.7 ± 5.22	5.7 ± 5.22	0.031*
tT (ng/ml)	44.67 ± 16.43	38.78 ± 11.49	0.004*
fT (ng/ml)	2.50 ± 1.09	2.06 ± 1.02	0.001*
17 OH P (ng/ml)	1.16 ± 0.46	2.27 ± 10.89	0.264
DHEA-S (mcg/dl)	275.01±110.02	257.06 ± 134.84	0.370
TSH (microIU/ml)	1.68 ± 0.81	1.73 ± 0.82	0.772
Prolactin (ng/ml)	20.40 ± 14.69	22.74 ± 35.81	0.375

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; FGS, Ferriman-Gallwey Score; fT, free testosterone; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; TC, total cholesterol; TSH, thyroid-stimulating hormone; tT, total testosterone; 17 OH P, *17-hydroxyprogesterone;* WC, waist circumference A p-value of <0.05 was accepted as statistically significant

4. Discussion

In our study, we found that; of the inflammatory biomarkers in the complete blood count, the neutrophil count and NLR were statistically higher in PCOS patients compared to the control group. In addition, we found that the neutrophil count and NLR values are poorly sensitive biomarkers to be used to distinguish PCOS patients from individuals in the control group. PCOS is a complex disease accompanied by metabolic and ovulatory dysfunction, obesity, and inflammation (7). Levels of circulating inflammatory markers have been investigated in PCOS. Studies in the literature have reported that levels of proinflammatory cytokines such as IL-1α, IL-1β, TNF-alpha, IL-6, IL-18, and CRP increase in the systemic circulation. This finding has been recognized as evidence of chronic inflammation in PCOS (22-24). However, the search for an inexpensive and easily accessible new marker to be used in making a diagnosis of PCOS is still a matter of interest for researchers.

Table 2. The levels of the measured blood parameters in the polycystic ovary syndrome and control groups

Parameters	PCO (n=111)	Control (n=86)	p
White blood cell count (10^3/uL)	7.88 ± 2.54	7.31 ± 2.19	0.094
Monocyte count (10^3/uL)	0.64 ± 0.79	0.66 ± 0.96	0.401
Lymphocyte count (10^3/uL)	2.24 ± 0.79	2.39 ± 0.79	0.362
Neutrophil count (10^3/uL)	4.89 ± 2.31	4.13 ± 1.67	0.016*
Platelet count (x10^3/mm^3)	258.41 ± 73.14	257.10 ± 81.24	0.722
Red cell distribution width (fl)	41.70 ± 3.68	42.70 ± 6.56	0.697
Mean platelet volume (fL)	9.64 ± 1.62	9.41 ± 1.54	0.210
Neutrophil/lymphocyte ratio	2.62 ± 2.53	1.84 ± 0.83	0.002*
Platelet/lymphocyte ratio	130.13 ± 63.34	112.94 ± 36.17	0.149
Monocyte /lymphocyte ratio	0.31 ± 0.31	0.28 ± 0.38	0.049
Mean platelet volume/ lymphocyte ratio (fl/10^3/uL)	5.03 ± 2.76	4.31 ± 1.51	0.103

p-value of <0.05 was accepted as statistically significant.

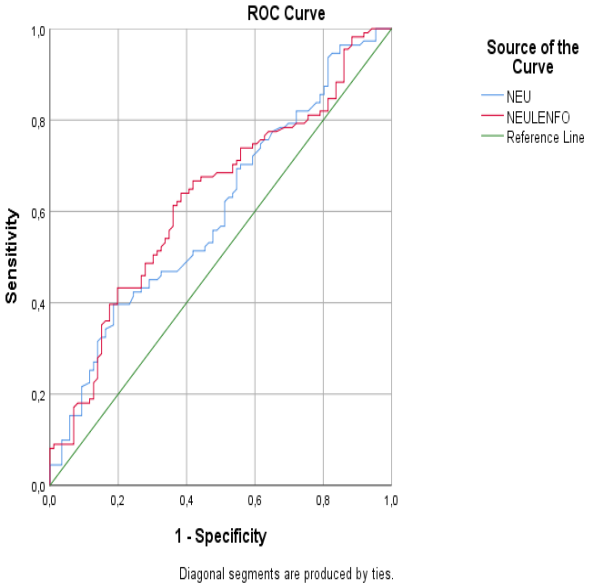

Fig. 1. The receiver-operating characteristic (ROC) curve to evaluate whether the neutrophil count and NLR can be used as diagnostic markers to exclude PCOS

Inflammatory marker levels derived from complete blood count tests and the ratios of such marker levels to each other are evaluated in most medical conditions as inexpensive and easily accessible methods to be used both in the diagnosis and the follow-up of the disease course. Being a parameter obtained from complete blood count tests as the ratio of the neutrophil count to the lymphocyte count; in the literature, NLR is correlated with the activation of diseases accompanied by chronic systemic inflammation including systemic hypertension, atherosclerosis, chronic obstructive pulmonary disease, and systemic lupus erythematosus (25-28). There are studies, too, emphasizing that the complete blood count parameters can be used in the diagnosis of PCOS, in the pathophysiology of which low-grade chronic inflammation is thought to have a role similar to the abovementioned diseases (29, 30). Studies have shown that; of the complete blood count parameters, especially both the WBC count and the neutrophil count occur at statistically higher levels in the PCOS group compared to the control group (10, 31, 32). In their study, Pergialiotis et al. found out that both the platelet-to-lymphocyte ratio (PLR) and NLR were correlated with some hormonal and metabolic indicators in PCOS cases (16). In another study, Yılmaz et al. found that neutrophil and basophil counts, NLR, and mean platelet volumes as the inflammatory biomolecules obtained from complete blood count tests were higher in the PCOS group compared to the control group (33). In another study by Rudnicka et al., PCOS cases were compared with healthy individuals in similar groups in terms of age and BMI. That study reported high WBC counts and CRP levels indicating the presence of low-grade inflammation (30). In our study; similar to the information in the literature, the neutrophil count and NLR were higher in PCOS cases of early reproductive age compared to the control group. However; levels of other parameters, including WBC counts, were not significantly different in the PCOS group. A review of the literature reveals studies suggesting that NLR can be an independent prognostic factor in many conditions under the influence of chronic inflammation and that NLR is an important marker to determine the severity of inflammation (25, 34). Different from the previous studies in the literature, our study may have provided evidence that only a high neutrophil count and a high NLR may play a more important role as biomarkers showing the impact of inflammation in PCOS compared to all other parameters of a complete blood count test. It can be thought that; compared to the other parameters in a complete blood count test, the neutrophil count and NLR may perform better as diagnostic criteria indicating the degree of inflammation severity and chronic effects of inflammation in patients with PCOS. In addition, it was determined in our study that both the neutrophil count and NLR have poor diagnostic values in differentiating PCOS cases from healthy individuals. However, this finding suggests that the diagnostic precision of these markers, which are accepted as inflammation indicators, is debatable. Inflammation in PCOS can be manifest at different levels of severity depending on the phenotype. We think that the diagnostic value of the neutrophil count and NLR may have been affected by the heterogeneity of the phenotypic distribution of the included cases in our study.

Our study had some limitations. The major limitation is the retrospective inclusion of PCOS patients in our study. The retrospective inclusion of the patients might cause failures in excluding some diseases with the potential to invoke inflammation. Another limitation is the small sample size resulting in the analysis of the results obtained from a small number of patients. In addition, inflammatory markers were examined without phenotypic grouping of PCOS cases. This prevented us to understand the effects of different phenotypes on the neutrophil count and NLR. Finally; because the PCOS cases included in our study were not grouped according to whether they had obesity or not, it was not possible to evaluate the effect of BMI on inflammatory parameters.

The neutrophil count and NLR were higher in the PCOS cases compared to the age-matched individuals in the control group. This finding confirms the presence of inflammation in PCOS cases of early reproductive age. However, it has been demonstrated that the diagnostic values of these markers are not strong in distinguishing PCOS patients from healthy individuals.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

None to declare.

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The role of triglyceride glucose index in predicting in-hospital adverse cardiovascular outcomes in patients with acute coronary syndrome

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Abstract

Previous studies have shown the association of triglyceride glucose (TyG) index with metabolic syndrome (MetS), cardiovascular disease (CVD) and long-term adverse cardiovascular outcomes. However, to best our knowledge, the relation between the TyG index and in-hospital adverse cardiovascular outcomes in acute coronary syndrome (ACS) has not yet been reported. Hence, in this study, we aimed to evaluate the role of the TyG index in predicting in-hospital adverse cardiovascular outcomes in ACS and to compare its performance with the Global Acute Coronary Events Register (GRACE) risk score. 170 patients diagnosed with ACS and underwent coronary angiography were analyzed retrospectively. The TyG index was calculated using the following formula: ln [fasting triglycerides (mg/dL)×fasting blood glucose (mg/dL)/2]. Receiver operating characteristics (ROC) curve analysis was used to evaluate the performance of the TyG index and GRACE risk score in predicting in-hospital adverse cardiovascular outcomes. A binary logistic regression model was applied to determine the independent predictors for in-hospital adverse cardiovascular outcomes. At the initial analysis, patients with adverse cardiovascular outcomes had higher TyG index and GRACE risk score ($p=0.011$, $p<0.001$). In ROC curve analysis, the GRACE score performed better in predicting in-hospital adverse cardiovascular outcomes compared to TyG index (AUC:0.716, $p<0.001$; AUC:0.588, $p=0.054$ respectively). In binary logistic regression analysis, left ventricular ejection fraction (LVEF), multi-vessel disease and GRACE risk score were independent predictors for in-hospital adverse cardiovascular outcomes (OR: 0.840, 95% CI: 0.791-0.891, $p<0.001$; OR: 3.581, 95% CI:1.382-9.282, $p=0.009$; OR= 1.017, 95% CI: 1.001-1.034, $p=0.04$ respectively). Our study findings revealed that the TyG index was scant in predicting in-hospital adverse cardiovascular outcomes compared to GRACE risk score. The independent predictors for in-hospital adverse cardiovascular outcomes were LVEF, multivessel disease and GRACE risk score.

Keywords: acute coronary syndrome, cardiovascular outcomes, GRACE, triglyceride glucose index

1. Introduction

Cardiovascular disease (CVD) is still one of the leading causes of mortality and morbidity today. Despite favorable advances in treatment, the increase in dysmetabolic diseases such as hypertension, diabetes and hyperlipidemia cause a slower decrease in CVD-related deaths (1, 2). Insulin resistance (IR) associated with glycolipid disorders has become an important risk factor for CVD (3). In addition, there is constant evidence that IR may lead to atherosclerosis process and adverse cardiovascular events through inducing of proinflammatory cytokines, impairment of endothelial dysfunction, triggering of pro-coagulant factor expression and increased oxidative stress (4).

Recently, the triglyceride glucose (TyG) index derived from triglyceride and glucose has been preferred for IR evaluation due to not requiring special techniques and low cost (5). The studies have shown that TyG index may be associated with coronary artery calcification, arterial stiffness, carotid atherosclerosis, and coronary artery disease (CAD) (6). In addition, some studies have revealed an association between the TyG index with adverse cardiovascular outcomes, both in the general population and in patient cohorts (7, 8). However, to the best of our knowledge, no study has been reported on the relation between the TyG index with in-hospital adverse cardiovascular outcomes and its comparison with The Global Registry of Acute Coronary Events (GRACE) risk score in acute coronary syndrome (ACS). Thus, in this study, we aimed to examine the relation between TyG index and in-hospital adverse cardiovascular outcomes in ACS and to compare its performance with the GRACE risk score.

2. Materials and methods

2.1. Study population

In this retrospective observational study, 198 consecutive patients who were hospitalized for ACS and underwent coronary angiography at our tertiary care center between January 2020 and September 2020 were included. Malignancy, acute infection, severe liver failure, kidney and thyroid dysfunction, pregnancy, being under fibrate

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treatment and missing data were defined as exclusion criteria, and as a result of the final analysis, the study was conducted with a total of 170 patients.

This study was in compliance with the Helsinki Declaration of Human Rights and was approved by the local institutional ethical committee (Pamukkale University Faculty of Medicine Hospital, Denizli, Turkey; 22.12.2020/24, protocol no: 60116787-020). Informed consent was obtained from each patient before participating in the study.

2.2. Data collection and definition

Demographic and clinical data including age, gender, smoking, medical history, standard laboratory parameters and, angiographic images were analyzed retrospectively. The TyG index was calculated using the following formula: ln [fasting TG (mg/dL)×fasting blood glucose (mg/dL)/2] (9). GRACE risk score consisting of age, systolic blood pressure, heart rate, presence of cardiac arrest, Killip class, ST segment deviation, serum creatinine, and positive cardiac markers was calculated for each patient using data from the registry system (10). ACS was defined as a collection of clinical syndromes, including unstable angina (UA), non-ST- elevation myocardial infarction (STEMI), and ST-elevation myocardial infarction (STEMI). Hypertension was defined as blood pressure ≥140/90 mmHg or currently taking antihypertensive treatment. Diabetes was defined as plasma glucose ≥200 mg/dL at any time or fasting blood glucose ≥126 mg/dL or under treatment. In-hospital adverse cardiovascular outcomes included cardiac death, cardiogenic shock, significant arrhythmia, recurrent revascularization and heart failure. The experience of any of these was defined as an in-hospital adverse cardiovascular outcome.

2.3. Statistical analysis

The data were analyzed using SPSS v.21.0 Windows (SPSS, Inc., Chicago, Ill., USA) programme package. Continuous variables were expressed as mean ± SD or median, and categorical variables were presented as frequency and percentage. Kolmogorov-Smirnov test was used to determine the normal distribution and the comparisons based on normality distribution were done with Student's t-test or Mann-Whitney U test. Categorical variables were compared using $\chi 2$ test. Pearson's or Spearman correlation analysis was used to evaluate the relationship between the continuous variables. A binary logistic regression analysis was used to determine whether the TyG index was an independent predictor for in-hospital adverse cardiovascular outcomes. The performance of the TyG index and GRACE scores were compared using the receiver operating characteristics (ROC) curve analysis in predicting in-hospital adverse cardiovascular outcomes, and $p<0.05$ was considered statistically significant.

3. Results

Patients without in-hospital adverse cardiovascular outcomes were assigned as group 1 (n=103) and those with in-hospital adverse cardiovascular outcomes were assigned as group 2 (n=67). The demographic and clinical characteristics of the groups are presented in Table 1. There were no significant differences in mean age, male gender, smoking, hypertension, previous MI or revascularization history between the groups. However, diabetes incidence was significantly higher in group 2 (p=0.044). 27%, 47%, 26% of the patients were diagnosed with UA, NSTEMI and STEMI, respectively in group 1. In group 2, 9%, 31% and 59% of patients had UA, NSTEMI and STEMI respectively. While the percentages of single and two-vessel disease were higher in group 1, multi-vessel disease was more common in group 2. There were significant differences in LVEF, glucose, HbA1c, creatinine, WBC between the groups. However, lipid parameters, hemogram, TG/HDL-C, LDL-C/HDL-C were similar. When the groups were compared in terms of TyG index and GRACE risk score, TyG index and GRACE risk score of group 2 increased significantly (9.00±0.70 vs 9.30±0.82, p=0.011; 114.42±25.68 vs 138.76±33.16, p<0.001 respectively).

A comparison of in-hospital adverse cardiovascular outcomes based on the median TyG index of the study population is shown in Table 2. There were no significant differences in incidence of cardiogenic shock, heart failure, cardiac death and significant arrhythmia in patients with low and high TyG index. However, the incidence of recurrent ischemia increased in patients with high TyG index compared to those with low TyG index (p=0.02). There was no difference between the groups in terms of single- or two-vessel disease, however multi-vessel disease was more common in patients with high TyG index (p=0.042) (Table 3).

In correlation analysis, the TyG index showed a significant association with hypertension, diabetes, NSTEMI, LVEF, HbA1c, lipid parameters, creatinine, WBC, and multi-vessel disease (Table 4). However, the performance of the TyG index in predicting in-hospital adverse cardiovascular outcomes was not at expected level in the ROC curve analysis (95% CI=0.501-0.674, AUC=0.588, 94% sensitivity, 25% specificity, p=0.054). The GRACE risk score predicted in-hospital adverse cardiovascular outcomes with 58% sensitivity and 81% specificity at a cut-off value of 135.50 (95% CI=0.633-0.799, AUC=0.716, p<0.001) and its performance was better compared to the TyG index (Fig. 1). In binary logistic regression analysis, the parameters associated with in-hospital adverse cardiovascular outcomes were LVEF, multi-vessel disease and GRACE risk score, regardless of all causes, as presented in Table 5.

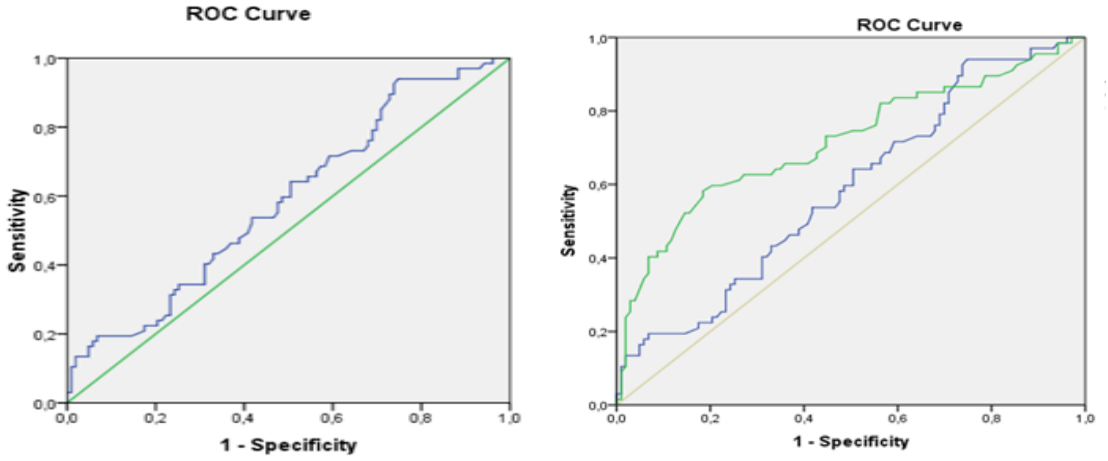

Fig. 1. Receiver operating characteristic (ROC) curves in predicting in-hospital adverse cardiovascular outcomes

Table 1. Baseline characteristics and clinical data of study population

Variables	Group I (n=103)	Group II (n=67)	p- value
Mean age (years)	65.01±11.28	67.78±15.29	0.177
Male gender, n (%)	65 (63)	40 (60)	0.412
Hypertension, n (%)	57 (55)	31 (46)	0.159
Diabetes, n (%)	33 (32)	31(46)	0.044
Current smoking, n (%)	34 (33)	25 (37)	0.339
Previous MI, n (%)	9 (9)	7 (10)	0.709
PCI history, n (%)	6 (6)	4 (6)	0.969
CABG history, n (%)	7 (7)	6 (9)	0.605
UA, n (%)	28 (27)	6 (9)	0.004
NSTEMI, n (%)	48 (47)	21(31)	0.034
STEMI, n (%)	27 (26)	40 (59)	<0.001
LVEF %, (median)	55.00	35.00	<0.001
Blood glucose, mg/dL (median)	120.00	174.00	<0.001
HbA1c %, (median)	7.60	8.80	0.047
Creatinine, mg/dL (median)	0.86	0.96	0.004
Tchol, mg/dL	173.99±39.19	171.37±41.45	0.678
LDL-C,mg/dL	104.14±34.86	106.12±34.70	0.717
HDL-C, mg/dL (median)	39.00	40.00	0.952
TG, mg/dL (median)	118.00	130.00	0.275
Hemoglobin, g/dL (median)	13.20	12.80	0.288
WBC, cells/μL (median)	9.08	10.70	<0.001
TyG index	9.00±0.70	9.30±0.82	0.011
LDL-C/HDL-C	2.7±1.16	2.8±1.19	0.548
TG/HDL-C	3.27	3.35	0.557
Single-vessel disease, n (%)	39 (38)	12 (18)	0.006
Two-vessel disease, n (%)	35 (34)	10 (15)	0.006
Multi-vessel disease, n (%)	29 (28)	45 (67)	<0.001
GRACE risk score	114.42±25.68	138.76±33.16	<0.001

MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; UA, unstable angina; NSTEMI, non- ST- elevation myocardial infarction; STEMI, ST- elevation myocardial infarction; LVEF, left ventricular ejection fraction; TChol; total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; WBC, white blood cells; TyG index, triglyceride glucose index

Table 2. The comparison of in-hospital adverse cardiovascular outcomes between the groups based on median TyG index

Variables	Low (<9.04)(n=84)	High (>9.04)(n=86)	p
Cardiogenic shock	-	2 (2)	0.160
Heart failure	29 (35)	32 (37)	0.715
Significant arrhythmia	7 (8)	14 (16)	0.115
Recurrent ischemia	-	9 (11)	0.002
Cardiac death	4 (5)	11 (13)	0.065

TyG index, triglyceride glucose index

Table 3. The number of diseased vessels according to median TyG index

Variables	Low (<9.04) n=84	High (>9.04) n=86	p
Single-vessel disease, n (%)	26 (31)	25 (29)	0.789
Two-vessel disease, n (%)	28 (33)	17 (20)	0.154
Multi-vessel disease, n (%)	30 (36)	44 (51)	0.042

TyG index, triglyceride glucose index

Table 4. The correlation analysis of TyG index

Variables	r	p
Age	-0.105	0.173
Hipertension	0.201	0.008
Diabetes	0.477	<0.001
NSTEMI	0.292	0.012
LVEF	-0.217	0.005
HbA1c	0.516	<0.001
Creatinine	0.228	0.003
Tchol	0.248	<0.001
LDL-C	0.189	0.013
HDL-C	-0.300	<0.001
WBC	0.282	0.018
Multi-vessel disease	0.346	0.027
GRACE risk score	0.046	0.549

NSTEMI, non- ST- elevation myocardial infarction; LVEF, left ventricular ejection fraction; TChol; total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; WBC, white blood cells; TyG index, triglyceride glucose index

Table 5. Binary logistic regression analysis for in-hospital adverse cardiovascular outcomes

Variables	OR	95% CI		p
		Lower bound	Upper bound	
LVEF	0.840	0.791	0.891	<0.001
WBC	1.098	0.968	1.246	0.146
Multi-vessel disease	3.581	1.382	9.282	0.009
GRACE risk score	1.017	1.001	1.034	0.040
TyG index	1.158	0.602	2.227	0.660

LVEF, left ventricular ejection fraction; WBC, white blood cells; TyG index, triglyceride glucose index

4. Discussion

In the current study, we investigated the impact of TyG index on in-hospital adverse cardiovascular outcomes in patients diagnosed with ACS at the first time and our main findings were as follows: (1) TyG index and GRACE risk score were higher in patients with in-hospital adverse cardiovascular outcomes; (2) There were no significant differences in in-hospital adverse outcomes including heart failure, cardiac death, cardiogenic shock, and heart failure between patients with high and low TyG index. However, recurrent ischemia was more common in patients with high TyG index. In addition, TyG index was significantly correlated with dysmetabolic conditions such as hypertension, diabetes, dyslipidemia, and multi-vessel disease; (3) GRACE risk score performed better in predicting in-hospital adverse outcomes compared to TyG index; (4) LVEF, multivessel disease and GRACE risk score were independent predictors for in-hospital adverse cardiovascular outcomes.

Many studies have shown that IR is associated with CVD and cardiovascular outcomes, in both short-term and long-term prognosis (11). However, the pathophysiological mechanisms by which IR plays a role in CVD have not been clearly determined. Inflammation, oxidative stress, lipid metabolism disorders, disruption of endothelial dysfunction through decreased NO release and inducing of the coagulation cascade are blamed mechanisms (4).

TyG index, a new method for evaluating IR, is associated with dysmetabolic conditions and CVD. Recently, the data reported that patients with increased TyG index have a higher risk of hypertension and diabetes. Moreover, the studies have found that the subclinical CAD may be more prevalent during screening with coronary CT angiography in patients with a high TyG index (12-14). Similar to these studies, there was a significant association between TyG index with hypertension, diabetes and impaired lipid parameters in our study. We also found a significant correlation between multi-vessel disease with TyG index and Mao et al.'s study supported our study by showing an increased incidence of multi-vessel disease in NSTEMI patients with a high TyG index (8). Recently, the relationship of the TyG index with cardiovascular outcomes has been investigated and in one study conducted with stable CAD patients; a high TyG index was associated with primary endpoints including all-cause death, non-fatal MI, recurrent revascularization and stroke (7). In another study, the TyG index showed successful performance in predicting cardiovascular events in patients with ACS, regardless of all causes (15). Additionally, the increased TyG index indicated the adverse cardiovascular outcomes in diabetic patients diagnosed with ACS undergoing PCI in one study (16). In this study, to the best our knowledge, we investigated the role of the TyG index on in- hospital adverse outcomes in ACS patients at the first time. Patients with in-hospital adverse cardiovascular outcomes at baseline showed a higher TyG index. However, the TyG index failed to predict adverse in-hospital cardiovascular outcomes compared to the GRACE risk score. Also, TyG index was not an independent predictor for in-hospital adverse cardiovascular outcomes after adjusting for confounding factors. In all above studies, it was aimed to determine the long-term prognostic significance of the TyG index in CAD patients, not in-hospital adverse cardiovascular outcomes. However, adverse cardiovascular events observed during hospitalization after ACS were reported in our study. in-hospital adverse

cardiovascular outcomes may be more affected by hemodynamic status at admission, late hospitalization due to atypical angina, presence of previous CAD, late referrals from rural areas, and inclusion of patients with unsuccessful revascularization from an external center, rather than TyG index. However, the TyG index was significantly correlated with in-hospital recurrent ischemia in our study. This may be due to the TyG index's association with dysmetabolic conditions such as hypertension, diabetes, hyperglycemia, and lipid metabolism disorders, which predispose to atherosclerosis.

Another finding of our study was that the GRACE risk score, a clinical scoring, performed better in predicting in-hospital adverse cardiovascular outcomes compared to the TyG index and was an independent predictor for in-hospital adverse cardiovascular outcomes. Indeed, clinical evaluation may be better than laboratory parameters in predicting in-hospital adverse cardiovascular outcomes that may occur immediately after ACS. As a matter of fact, the GRACE risk score, developed from multinational prospective patient registries, has been accepted as a strong predictor of short-term prognosis in patients with ACS, and its use has been recommended by ESC guidelines. (17). The other independent predictors for in-hospital adverse cardiovascular outcomes were LVEF and multi-vessel disease in our study. In a study with 8983 ACS patients, LVEF at admission was an independent predictor of death and adverse cardiovascular outcomes (18). Also, low LVEF may have led to clinical instability in patients with in-hospital adverse cardiovascular outcomes in our study. In another study, multivessel disease was a more important predictor of in-hospital adverse cardiovascular outcomes in patients with ACS compared to TIMI and age (19), and the findings of this study were consistent with our study.

Our study had some limitations. First, our study was retrospective and the study sample was relatively small. Second, the study was conducted in a Turkish population, and the study findings may vary by ethnicity. Third, patients using antidiabetic agents were not excluded. Therefore, we cannot ignore the effects of antidiabetic drugs. Fourth, due to retrospective design, we had missing data such as body mass index, exercise status, dietary habits, and energy intake, which could affect patients' TyG index.

As a result, the TyG index was higher in patients with in-hospital adverse cardiovascular outcomes. The performance of the GRACE risk score in predicting in-hospital adverse cardiovascular outcomes was better compared to the TyG index. Thus, the TyG index may not be a useful marker to predict in-hospital prognosis in patients diagnosed with ACS. According to the findings of our study, the independent predictors of in-hospital adverse cardiovascular outcomes were LVEF, multivessel disease, and GRACE risk score. However, a larger sample size, longer follow-up time, and multicenter studies are needed to confirm our findings.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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The effects of tubal sterilization on menopausal age in a cohort of postmenopausal women

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Abstract

To evaluate whether tubal sterilization (TS) has an adverse effect on menopausal age in a cohort of postmenopausal women. The medical records on TS were gathered from 1,228 postmenopausal women in menopause clinic at a tertiary hospital. The age at menarche, the parity, smoking and secondhand smoking status and mother's age at menopause did not show any significant difference between the groups. Likewise, marital status, educational and yearly income levels were comparable ($P > 0.01$). Although the mean BMI ($P = 0.06$) and the rate of oral contraceptive use ($P = 0.09$) were tended to be higher in non-TS women than in TS group, the difference did not reach statistical significance. The ever use of intrauterine device rate was significantly lower in TS group than in non-TS group ($P < 0.001$). The TS women, in comparison with the non-TS women had undergone earlier natural menopause ($P < 0.001$). The age at menopause had an inverse correlation with TS, and positive correlation with mother's age at menopause in univariate analyses. However, there was marginally significant correlation between secondhand smoking and age at menopause. Linear stepwise regression analyses revealed that TS and mother's age at menopause were independent predictors of age at menopause ($P < 0.05$). We found an earlier age at menopause in women with TS in this study. In this regard, this is the first report about the age at menopause in women with TS, as far as we know.

Keywords: menopausal age, tubal sterilization, menopause

1. Introduction

Tubal Sterilization (TS) is an irreversible way of the contraceptive method performed at the will of patients that have completed the fertility aspect (1). Although it is a highly effective and safe procedure, the existence of a series of symptoms referred to as post tubal sterilization syndrome has been debated (2, 3). It has been suggested that diminished blood flow due to the damaged vascular supply to the ovaries may cause ovarian dysfunction and decreased estrogen/progesterone production after TS (4, 3). This may even results premature loss of ovarian function, as reported in women who undergo hysterectomy with ovarian conservation (5). Since the first report of post tubal sterilization syndrome in 1951 by Williams et al. (6), any presence of ovarian reserve change has been extensively studied by many authors (7-9).

Several ovarian reserve tests, imaging methods and questionnaires were used to assess ovarian function after TS in many studies with conflicting results. Furthermore, these methods are amenable to subjective changes, making them unreliable for the determination of any deterioration in ovarian function. Anti-mullerian hormone (AMH) level is considered as a reliable and objective marker for prediction of ovarian reserve. However, even the studies that used AMH for the prediction of ovarian reserve after TS, yielded conflicting results (10-13).

There are also scanty reports investigating the effect of TS on women's health later in life. TS was found to be associated with decreased bone mineral density in elderly women (14, 15). Furthermore, some authors proposed increased menopausal complaints in women who had been operated on for TS (16, 17). The aim of the current study was to evaluate whether tubal ligation has an adverse effect on menopausal age in a cohort of postmenopausal women.

2. Materials and Methods

2.1. Subjects

The study included women who experienced natural menopause at an age > 40 years. Retrospective information on TS was gathered from 1,228 postmenopausal women who attended to our menopause clinics between April and October 2014. Natural menopause was defined according to the World Health Organization as at least 12 consecutive months of

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amenorrhea not due to surgery or other obvious cause, such as extreme weight loss (18). Women who reported that they had had a hysterectomy or oophorectomy (unilateral vs. bilateral was not specified, since in pretests women could not distinguish between the two) were excluded. Informed consents were obtained from all participants of this study. This project was approved by the local ethical committee of Mersin University (2014) and informed consent was obtained from all patients and controls according to the Declaration of Helsinki (revised in 2013).

2.2. Data collection

All independent variables of interest were obtained by self-report during the interview. Information regarding socio-demographic status (educational attainment, employment, marital status, ability to pay for basics), reproductive (age at menopause, age at menarche, parity, and oral contraceptive (OC) use), medical history, and lifestyle factors (smoking, physical activity).

The mother's age at menopause was retrieved mainly with interview of the subjects, and 1037 women of 1228 (84.4%) could provide information about this subject. Body mass index (BMI) was calculated as weight in kg divided by height in m^2. Due to similar ethnic background, ethnicity was not considered in the analyses.

The participants' educational level was divided into two levels: < high school and > high school. Marital status was categorized as: married, unmarried, widowed, or divorced or separated. The yearly income levels separated into two groups according to the national poverty line which was determined by the government: low and mid-to-high income levels.

The women were asked about current or past smoking, and women who smoked tobacco for more than one year were classified as "ever-smokers". Passive smoking status was collected by environmental tobacco smoke (ETS) questionnaire of International Agency for Research on Cancer. The questionnaire was validated by Nyberg et al. (19).

2.3. Statistical Analyses

Data were analyzed using SPSS for Windows 20.0 Demo. Data are shown as mean ± SD or as the number and percentage of subjects. Regarding baseline characteristics, if the distribution was normal, Student's t test was used for comparisons of variables, and the Mann-Whitney U test was used for variables that showed a skewed distribution. Dichotomous variables were analyzed by X^2 test. Pearson correlation was used to assess associations between the variables. Linear stepwise regression analysis was used to identify variables that best predicted the age at menopause. Statistical significance was defined as an alpha level at or below 0.05.

The analysis of first 200 patients showed that about 7% of patients had chosen TS as a contraceptive method. Regarding this ratio, assuming that women with TS experience two years earlier menopause (48 years vs 46 years with an SD of approximately 5 years), the power analysis dictated that 88 women in TS group and 1140 women in control group should be included at 5% significance level with 80% power (G*Power v3.1.7 Power Analysis Software).

3. Results

Demographic characteristics of women included in the study are shown in Table 1. The age at menarche, the parity, smoking and secondhand smoking status and mother's age at menopause did not show any significant difference between the groups. Likewise, marital status, educational and yearly income levels were comparable (Table 1, P > 0.01).

Although the mean BMI (27.9 ± 4.0 vs. 27.1 ± 3.8, P = 0.06) and the rate of oral contraceptive use (63.5% vs. 54.6%, P = 0.09) were tended to be higher in non-TS women than in TS group, the difference did not reach statistical significance. The ever use of intrauterine device rate was significantly lower in TS group than in non-TS group (21.6% vs 43.5%, respectively, P <0.001). The TS women, in comparison with the non-TS women had undergone earlier natural menopause (46.4 ± 2.8 vs. 48.4 ± 3.7, respectively, P <0.001).

Table 1. The characteristics of women with and without tubal sterilization (TS)

	TS group n = 88	Non-TS group n = 1140	P
Age at menopause (years)	46.4 ± 2.8	48.4 ± 3.7	<0.001
Age at menarche (years)	13.2 ± 1.3	13.3 ± 1.4	0.91
Body mass index (kg/m^2)	27.1 ± 3.8	27.9 ± 4.0	0.06
Parity	2.7 ± 1.8	2.7 ± 1.5	0.71
Ever oral contraceptive use	48 (54.6%)	724 (63.5%)	0.09
Ever intrauterine device use	19 (21.6%)	496 (43.5%)	<0.001
Ever-smoker	25 (28.4%)	327 (28.7%)	1.0
Secondhand smoking	53 (60.2%)	712 (62.5%)	0.68
Mother's age at menopause (years)	47.3 ± 3.6	48.1 ± 3.8	0.14
Marital status (married)	74 (84.1%)	1018 (89.3%)	0.13
Educational level (high school)	18 (20.5%)	223 (19.6%)	0.84
Yearly income level (medium-to-high)	74 (84.1%)	910 (79.8%)	0.93

Table 2 presents the results of Pearson correlation tests and linear stepwise regression analyses between the variables. The age at menopause had an inverse correlation with TS, and positive correlation mother's age at menopause in univariate analyses (Table 2, P < 0.05).

However, there was marginally significant correlation between secondhand smoking and age at menopause. Linear stepwise regression analysis revealed that TS and mother's age at menopause were independent predictors of age at menopause (P < 0.05, Table 2).

Table 2. Bivariate correlations and linear stepwise regression models of clinical variables on age at menopause

	Bivariate correlations			Linear stepwise regression models *		
	R	P		B-coefficient	Standard error of regression coefficient	P
Tubal sterilization	- 0.135	<0.001		- 2.108	0.464	0.001
Age at menarche (years)	- 0.005	0.86				
Body mass index (kg/m^2)	- 0.017	0.56				
Parity	- 0.021	0.46				
Oral contraceptive use	- 0.019	0.51				
Intrauterine device use	0.008	0.77				
Ever smoking	0.004	0.89				
Secondhand smoking	- 0.051	0.07				
Mother's age at menopause	0.297	<0.001		0.279	0.034	0.001
Marital status	0.010	0.74				
Educational level	0.011	0.70				
Yearly income level	0.010	0.72				

* Only the statistically significant variables were expressed in the regression model.

4. Discussion

The most consistent finding on age of menopause is that smokers have about 1.5 years earlier menopause before nonsmokers. Available studies are less clear regarding the relation of other factors to age at menopause. With varying frequency, studies have found less education, low relative weight, nulliparity or having few children, and not using oral contraceptives to be associated with an earlier menopause (20). In this study, we found TS as another possible cause of earlier menopause in both correlation and regression analyses. Women with TS in their history had experienced about 2 years earlier menopause than women without TS (46.4 $\pm$ 2.8 vs. 48.4 $\pm$ 3.7, respectively, $P < 0.001$). Regression analyses also showed TS as an independent risk factor for earlier menopause along with the mother's age at menopause.

Existing studies in the literature investigated short term effects of TS on menopausal age. Dede et al. (21) followed the patients for three months and found that there was no statistically significant difference in the serum FSH, LH and estradiol levels in preoperative and postoperative assessments, in fact, they proposed an improvement in ovulatory rate after the procedure. In another study from Turkey, on short-term follow-up (3 months), laparoscopic bipolar electrodesiccation and transection of tubes does not have a negative impact on ovarian reserve, revealed by insignificant changes in AMH, FSH, LH, and E2 levels, ovarian volume, and AFCs (10). Contrary, LH levels were found significantly higher, and progesterone levels significantly lower in another study with the observation period of three months. The authors tied this low level of progesterone to deficient production by the corpus luteum, probably caused by vascular changes after TS (22).

Changes in menstrual patterns and ovarian reserve after TS have been attributed to damage to the ovarian blood vessels leading to reduced ovarian blood supply. Earlier studies raised this question of ovarian vascular deterioration after the procedure. Sixteen laparoscopic tubal sterilizations using filshie clips were performed, and ovarian and uterine artery Doppler measurements were evaluated before and after surgery with 90 days follow-up. They found an increased vascular resistance both in ovarian and uterine arteries following the sterilization procedure (23). However, many aftercoming studies failed to reveal any vascular flow disorders by Doppler analyses (24, 25). But, Doppler studies may not be sensitive enough to reveal any disturbances to ovarian vascular blood supply due to tubal sterilization.

Many cohort and case control studies about menstrual function after tubal sterilization often failed to control for confounding factors such as previous contraceptive use, previous menstrual pattern, and advancing age, all of which can affect menstrual patterns. Furthermore, observation period changed generally from 3 to 12 months which may not capable enough to reveal any distortion to ovarian function. The Collaborative Review of Sterilization study found that, over a 5-year follow-up period, women who had undergone tubal ligation were more likely to experience a shortening of the duration of menses, a decrease in volume of menstrual flow, greater dysmenorrheal pain and an increase in cycle irregularity compared with those whose partners were sterilized (26). As a continuum of this study, the patients were followed-up to 14 years after sterilization, and it was found that women who underwent sterilization were likely to have decreases in the amount of bleeding, the number of days of bleeding, and the amount of menstrual pain and an increase in cycle irregularity (27).

Four years later, the subjects with tubal sterilization, who were now closer to the perimenopausal age range of 45–54 years were re-examined with respect to menopausal status, vasomotor and somatic symptoms and changes in menstrual patterns associated with the perimenopausal period (17). The author reported that women with TS had more flushing, they had higher psychological distress. Furthermore, the lifetime history of physician diagnosed depression was found marginally higher in those women. However, Nelson et al. (28) found that perimenopausal women with TS were not

significantly more likely to experience hormonal changes (FSH, LH, estradiol, testosterone, inhibin B) indicative of the transition to menopause or an increased severity of menopausal symptoms compared to the group of women without TS. Lastly, Wyshak (15) followed-up 3940 women for 15 years and observed that 3% of women with TS experienced vertebral fracture after age 20, whereas this rate was 1.6% in women without sterilization (P = 0.027). Among women aged 50 years and older, for the association between TS and vertebral fractures, the odds ratio was 2.7, for the association of chronic back pain was 3.3.

In conclusion; short-term follow-up studies could not reveal any conclusion in terms of ovarian function in women with tubal sterilization, because the end point in this situation is the menopausal age. We found an earlier age at menopause in women with TS in this study. In this regard, this is the first report about the age at menopause in women with TS, as far as we know. There are several reports indicating adverse outcomes of this procedure in perimenopausal women. However, prospective longitudinal studies have to be planned to reveal any association between TS and menopausal age.

Conflict of interest

None to declare.

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None to declare.

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Intramuscular meperidine analgesia at the beginning of active phase shortens labor duration without adverse effects on obstetric lacerations

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Abstract

The primary objectives of this study were to evaluate the impact of intramuscular meperidine on shortening of the active phase of labor, the neonatal outcome and the rate and severity of perineal lacerations in term pregnant women in the first stage of labor. A total of 571 primiparous term pregnant women delivered vaginally were included into this retrospective study. In 437 of them, meperidine (100 mg IM) at the beginning of the active phase was administered and 134 women did not receive any meperidine dose. The length of labor phases, obstetric lacerations, and neonatal outcomes were recorded. The results of this study showed that meperidine could be used safely as an obstetric analgesic with its additional benefit of shortening the active phase of the first stage and second stage of labor without increased risk of obstetric lacerations and perinatal adverse outcomes. In case of limited use of neuraxial analgesia in a busy state maternity hospital, intramuscular meperidine administration as obstetric analgesia seems beneficial in reducing the length of the active phase of the first stage of labor and the second stage of labor without adversely affecting obstetric lacerations and neonatal outcomes.

Keywords: meperidine, neonatal outcome, normal vaginal delivery, obstetric analgesia, obstetric lacerations, stages of labor

1. Introduction

Obstetric analgesia is used to relieve obstetric pain. Effective pain relief has become an essential part of obstetrics. The history of modern analgesia at birth dates back to 1847 with the use of ether and later chloroform (1). Parenteral opioids for labor pain relief are a common option for women worldwide and have been the subject of research for many years. The earliest documents of opioid use in the workplace appear in ancient Chinese scriptures describing the use of opium to relieve pain in the workplace (2). Examples include epidural analgesia and an opioid-based analgesic like meperidine (3). Pain during the first stage of labor is primarily visceral and results from cervical dilation and uterine contractions. Pain during the second stage occurs as the fetus descends through the birth canal, resulting in the stretching and tearing of fascia, skin, and subcutaneous tissue. This pain is somatic and is transmitted through the pudendal nerve; it can be treated with local anesthetics (4). Pain during labor is subjective, and other factors such as anxiety, depression, and neuroticism may contribute to it (4). The patient's age, parity, emotional status, and labor coaching are some other factors influencing labor pain. Pregnant women may benefit from antenatal childbirth training to reduce the intensity of pain. Physiological changes occurring later in pregnancy are a combination of hormonal factors and mechanical effects from the growing uterus (5).

Epidural analgesia is the most popular method for pain relief during labor. It is effective for both the first and the second stages. Although epidural analgesia is associated with slight prolongation of the second stage and an increased rate of operative vaginal delivery, it does not increase the risk of cesarean delivery or neonatal depression (6). Clinical conditions such as patients' refusal or inability to cooperate, increased intracranial pressure, coagulopathy, infection at the site of needle insertion, maternal fever or sepsis, thrombocytopenia, and hypovolemia preclude its use and leave systemic analgesia as the only option.

Meperidine (pethidine) was synthesized in 1939; it was first used at work in the early 1940s. Since then, it has been the most widely used opioid in the world for pain relief during childbirth (7, 8). Meperidine is generally preferred for labor analgesia; however, concerns about its analgesic efficacy and neonatal respiratory depression have limited its use. Four of five women who deliver by the normal vaginal

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route experience some degree of birth canal lacerations during labor beginning from the cervix to the rectum and anal sphincter (9). The impact of lacerations may range from very superficial to severe long-lasting ones that may deteriorate the quality of life of women of young age. Thus, it is recommended that the occurrence of obstetric lacerations should be prevented by proper management of all phases of labor (9, 10). Though widely used worldwide for many years (11), the role of meperidine use on obstetric lacerations has not been studied much. Based on long-term clinical experience regarding intrapartum analgesia including meperidine, we hypothesized that intramuscular meperidine administration, apart from pain relieving effect, would shorten the active phase of labor without increasing obstetrical lacerations and neonatal morbidity. This study aims to show the impact of meperidine use on the length of the active phase of labor, the rates and severity of obstetric lacerations and neonatal outcomes in term pregnant women delivered vaginally.

2. Materials and methods

In this retrospective study, 571 primiparous pregnant women with term, singleton, and vertex presented fetuses delivered by the vaginal route at the Samsun Training and Research Hospital, Samsun, Turkey between June 2018, and December 2018, where the annual birth rate was 6500/year and only 17% of deliveries were cesarean sections. This study was conducted after the approval of Human Research Ethics Committee of our Institution.

Multiparous women, women with multifetal gestation, high-risk pregnancy, macrosomia, breech presentation, preterm delivery, intrauterine fetal loss, obstetric analgesia other than meperidine, and operative deliveries were excluded to ensure homogeneity in the study. Patients were divided into two groups: 437 women who received meperidine 100 mg IM at 4-cm cervical dilatation and >70% cervical effacement with compressing head at level 0 and 134 women who did not receive meperidine or any other analgesia.

By convention, labor is divided into three stages (12, 13):

1. First stage: From onset of labor to full dilation of the cervix,

a) Latent phase: from the onset of regular uterine contractions to the beginning of the active phase,

b) Active phase: from the time, at which the rate of cervical dilation transforms at ~ 4 cm to full dilation,

2. Second stage: From full dilation of the cervix to delivery of the infant,

3. Third stage: From delivery of the infant to delivery of the placenta.

In our routine obstetric practice, appropriate time for meperidine use is defined as fetal head engagement at 0 level with 70% cervical effacement and 4 cm cervical dilatation.

All patients in the maternity hospital were followed by ac, and normal vaginal deliveries were performed by experienced midwifes. The duration of the active phase, time and dose of meperidine administration and time of delivery were all marked on the cartogram. All primiparous patients who need episiotomy repair, operative deliveries, laceration repairs, and cesarean section were managed by obstetricians. Therefore, all major complications and surgical procedures were recorded whereas minor lacerations were not sutured and recorded.

Obstetric analgesia is offered as opioid-based drug use rather than epidural analgesia in a busy maternity hospital if not otherwise contraindicated. Patients with severe pain who were about to pass the active labor phase at 4 cm with head compression and 70%-80% cervical effacement were given 100 mg meperidine IM for pain relief. Meperidine was not used routinely in patients with mild and moderate pain for obstetric pain relief.

All women who were interned for normal vaginal delivery had an IV line and an induction protocol with 10 IU of oxytocin in 1000 ml of lactated ringer solution beginning with 10 drops/min and an increase of 5 drops every 15 min until regular contractions were recorded in the nonstress test (NST). The maximum induction dose was 40 drops/min. The rectum was emptied by an intrarectal enema in the latent phase, and the bladder was emptied by a disposable catheter at the time of delivery. During delivery, the perineum was supported manually from posterior to anterior to minimize trauma to the perineal body and anal sphincter. Liquid vaseline was used to moisten the labial and vaginal tissues to ease the birth of the fetal head (crowning phase). At the same time, preserving the urethra and periurethral area with the aid of the midwife's other hand is very important to prevent lacerations and bleeding at that anatomical area. A mediolateral episiotomy is almost always added at the third stage of delivery in primiparous women. The second-most important issue to prevent severe lacerations is to warn the mother not to push anymore when the fetal head is out; the rest of the body should be taken out by the midwife. All deliveries of the participating women were conducted in the dorsolithotomic supine position without any extra interventions and abdominal compression to facilitate delivery. All deep and severely bleeding lacerations were recorded according to the ACOG classification (3). Cervical, vaginal, and perineal lacerations were carefully evaluated using bivalves and oval forceps and through rectal examination for anal sphincter damage.

2.1. Statistical analysis

Data were presented as mean, median, and percentage as appropriate. After normality test study variables were compared with Mann-Whitney test or chi-square test. P values below 0.05 were considered statistically significant.

3. Results

As presented in Table 1, the ages, gestational ages, and Apgar score at 1 min, and rates of meconium-stained amniotic fluid, NICU admission, neonatal intubation, cervical laceration, third- and fourth-degree perineal lacerations, vaginal laceration of pregnant women with or without meperidine administration were found comparable ($p>0.05$). The rate of gravidity 1 was significantly higher than the rates of gravidity 2 and 3 in both of the study groups ($p<0.05$); however, there was no significant difference between the rates of gravidity in the study groups ($p>0.05$).

The durations of active phase in the first stage of labor, and second stage of labor in the pregnant women with meperidine administration were significantly lower than those in the pregnant women without meperidine administration ($p<0.05$).

The birth weight and Apgar score at 5 min in the pregnant women with meperidine administration were significantly higher than those in the pregnant women without meperidine administration ($p<0.05$).

4. Discussion

The results of this study show that meperidine can be used safely as an obstetric analgesic with its additional benefit of shortening the active phase of the first stage and second stage of labor without increased risk of obstetric lacerations and perinatal adverse outcomes.

Quality in labor and delivery management should always be judged by the lowest maternal and perinatal morbidity and mortality and not by preset limits on specific interventions. Clinically, good-quality recommendations favor hospital births, delayed admission, support by midwife training birth assistants in developing countries, and upright position in the second stage. These labor and delivery techniques should be performed routinely (14, 15).

Pain is defined as an unpleasant sensory or emotional experience with actual or potential tissue injury. It is closely related to uterine contractions in labor, and patient satisfaction correlates closely to its management, which varies during the phases of labor. Opioids administered intramuscularly or subcutaneously or through repeated IV boluses are widely used in many centers, although they are not recommended in the era of epidural analgesia. In our clinic, meperidine is used as a 100 mg single intramuscular dose in patients with severe obstetric pain. A 2010 Cochrane review reported that parenteral opioids provide some pain relief in labor and were associated with maternal side-effects, including nausea, vomiting, and drowsiness (16). Meperidine may have negative neonatal effects owing to its long half-life (17). However, it has been the drug of choice in the pain relief of labor for years. The half-life of the active metabolite in the newborn may be 2–3 days; this may result in respiratory depression and may affect the consciousness and reflexes of the newborn (18).

Table 1. Baseline and selected obstetric parameters in labor in women received meperidine or not

	Meperidine (n=437)	No meperidine (n=134)	Significance
Age (y)	17-43 (23)	17-44 (24.5)	NS
Gravidity 1 2 3	 349 (79.9) 46 (10.5) 42 (9.6)	 89 (66.4) 22 (16.4) 23 (17.2)	 p<0.05
Gestational age (w)	39.78±1.69	39.50±1.49	NS
Duration Active phase of first stage of labor (h) Second stage of labor (min)	 1-5,5 (3) 5-60 (15)	 1-8 (4,7) 5-60 (25)	 p<0.05
Birth weight (g)	2550-3990 (3320)	2030-3990 (3255)	p<0.05
Apgar score 1 min 5 min	 8.68±0.98 9.74±1.03	 8.79±1.04 9.22±0.75	 NS p<0.05
MSAF (n, %)	41 (9.4)	9 (6.7)	NS
NICU admission (n, %)	41 (9.4)	13 (9.7)	NS
Neonatal intubation (n, %)	23 (5.3)	8 (6.0)	NS
Cervical laceration (n, %)	19 (4.3)	7 (5.2)	NS
Third- and fourth-degree perineal lacerations (n, %)	1 (0.2)	0 (0)	NS
Vaginal laceration (n, %)	45 (10.3)	13 (9.7)	NS

MSAF, meconium-stained amniotic fluid; NICU, neonatal intensive care unit

Most anesthetic drugs are safe for mothers who are breastfeeding and present a low risk to newborns who are breastfed when administered in a single dose. However, high doses and repeated drug administration significantly increase the risk of side effects in newborns. They should evaluate individual risk / benefit, paying special attention to premature

newborns or infants with concomitant disease, especially as they are more susceptible to side effects (19).

Meperidine, also called pethidine, is an opioid that is around one-tenth as potent as morphine; it can be prescribed and administered by midwives. The side-effects are similar to those of other opioids, namely, respiratory depression of the mother and neonate, delayed gastric emptying, nausea, vomiting, sedation, and hypotension (20). Meperidine crosses the placenta, and fetal exposure to this drug is maximal at 2-3 h after maternal intramuscular administration. Thus, the use of intramuscular meperidine at the right time will shorten the active phase and may prevent maternal and neonatal complications. This is the key issue of meperidine use in our setting for patients with severe pain who may react to the drug with pain relief and faster termination of the active phase of labor, which is beneficial to the mother and the newborn. The optimal time for the delivery of the baby following a dose of meperidine is within 1-4 h of dosing. Our time-range for the active phase of labor and delivery is consistent with this literature. Therefore, our neonatal results are good. Delayed delivery after meperidine use, which is not encountered commonly, necessitates consultation with a pediatrician to attend the delivery for possible neonatal respiratory distress. Meperidine use is not recommended in settings without pediatric support during labor (6, 20, 21, 22, 23). In a trial involving 407 women, 100 mg IV meperidine was used in women with term, singleton pregnancies who required oxytocin because of dystocia at 4-6 cm. The authors concluded that meperidine use does not worsen operative delivery rates and neonatal outcomes compared with placebo as in our study. Although many studies have investigated neonatal outcomes with meperidine use during labor, to the best of our knowledge, our study is the first to present the effect of meperidine use on the duration of labor.

In our maternity hospital with a high rate of daily deliveries, only operative procedures such as episiotomy and suturing for lacerations that were managed by obstetricians were recorded. That is why minor cervical, vaginal, and perineal lacerations were not found in the files for evaluation. This is the limitation of our study. Because minor lacerations have no severe effect on the quality of life of patients and our main outcome measure was shortening the duration of labor, this limitation was not a big bias. In a recent study, Mizrahci et al. argued with our results of obstetric lacerations. They studied all lacerations including minor ones, which were their primary objective. However, our primary objective was shortening the active phase of labor, and obstetric lacerations and neonatal outcomes were secondary outcomes. Another limitation is the retrospective nature of this study (9).

In conclusion, in case of limited use of neuraxial analgesia, meperidine use as an obstetric analgesic drug remains a safe, cheap, and noninvasive analgesic choice that reduces the duration of labor. Obstetric lacerations and neonatal outcomes are not different from those in patients not using meperidine. Thus, timely meperidine use affords benefits such as shortening the duration of the active phase of labor, pain relief, and good neonatal outcomes. Therefore, it can be an advantageous modality in obstetric analgesia where neuraxial analgesia is contraindicated or not available. Further prospective and large-scale studies are warranted.

Conflict of interest

None to declare.

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Research Article

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The evaluation of the triglyceride glucose index in symptomatic patients with high suspicion of coronary artery disease: A single center study

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Abstract

Triglyceride glucose index (TyG) plays an important role in metabolic syndrome (MetS), which predisposes to the development of cardiovascular diseases (CVD). Mostly, the association between the TyG index and coronary artery disease (CAD) has been investigated in healthy individuals on long-term follow-up or in asymptomatic patients with a mild and mild-moderate cardiovascular risk profile. However, there are few studies evaluating the association of TyG index with CAD in symptomatic patients. In hence, we aimed to determine the role of the TyG index in the presence and severity of CAD in symptomatic patients with high suspicion of CAD in this study. 100 patients who underwent coronary angiography were included and the patients were divided into two groups according to the presence of CAD. TyG index was calculated by the formula Ln [fasting triglycerides (mg/dl) fasting glucose (mg/dl)/2]. The severity of CAD was assessed by angiographic risk scores GENSINI, SYNTAX I, and the number of diseased vessels. TyG index did not differ significantly between patients with and without CAD. Also, there was no significant correlation between TyG index and GENSINI, SYNTAX I, and the number of diseased vessels. (for all $p>0.05$) In multiple logistic regression analysis, the only independent predictor of CAD was age. ($p=0.03$, OR=1.048, 95% CI=1.004-1.094) In conclusion, TyG index may not be a useful marker for predicting the presence or severity of CAD. According to the results of this study, age is the only predictor of CAD independent of all causes.

Keywords: coronary artery disease, gensini, syntax i, triglyceride glucose index

1. Introduction

Coronary vascular diseases (CVD) are still associated with high mortality and morbidity today, so early diagnosis and appoppriate treatment are important (1). Insulin resistance (IR) is the cornerstone for cardiometabolic diseases, and its increased levels may provide information in diagnosis and progression. Recently, triglyceride glucose index (TyG) derived from fasting blood glucose and triglyceride has been used as an alternative to the hyperinsulinemic-euglycemic clamp (HEC) and homestase model (HOMA-IR) due to its practical use, low cost, high sensitivity and high specificity. (2, 3). Although most of the research with the TyG index has focused on metabolic syndrome (MetS) and MetS related conditions, the cross-sectional studies have found that individuals in high TyG index quartiles are more likely to have arterial stiffness, coronary artery calcification (CAC), carotid atherosclerosis, and stroke than those in the lower quartiles (4-6). Recently, an increased risk of cardiovascular disease (CVD) has been reported in healthy individuals with a high TyG index (1). In addition, some studies have investigated the effect of TyG index on CVD prognosis (7). However, the importance of the TyG index in symptomatic patients with suspected CAD has not been clearly determined, and to the best of our knowledge, there is no study investigating the severity of CAD by using both SYNTAX I and GENSINI scores in symptomatic patients diagnosed with CAD. In hence in this study, we aimed to investigate the association between TyG index and CAD in symptomatic patients with high suspicion of CAD.

2. Materials and methods

2.1. Study population

In this retrospective observational case-control study, the medical records of 100 patients who met the inclusion criteria and underwent coronary angiography in our cardiology department between April 2020 and August 2020 were analyzed using hospital database. Inclusion criteria were being 18-90 age intervale, presence of angina or equivalent symptoms and a clinical diagnosis including diagnosis without advanced examination or history of a positive stress test and performing coronary angiography. Cardiomyopathy severe valvular heart disease, previous myocardial infarction or CAD, acute pericarditis or myocarditis, acute or chronic infection, chronic inflammation, malignancy, severe kidney dysfunction and severe liver disease were defined as exclusion criteria. Significant CAD was defined as 50% or more than 50% stenosis in at least one epicardial coronary vessel on coronary angiography. This study was consistent with the Helsinki declaration and was approved by our hospital's ethical review

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board (Pamukkale University Faculty of Medicine Hospital, Denizli, Turkey; 2020/20, protocol no:020/70506). All the participants gave an informed consent before enrolling in the study.

2.2. Data collection

The basic characteristics of the study population such as age, gender, hypertension, diabetes, current smoking was colllected from medical records. Fasting venous blood samples including blood glucose, lipid parameters, renal functional tests, complete blood counts and, left ventricular ejection fraction (LVEF) calculated by the modified Simpson method were reexamined using hospital database. The TyG index was calculated by the formula Ln [fasting triglycerides (mg / dl) x fasting glucose (mg / dl) /2] (8). Angiographic data were obtained from cardiac catheterization laboratory records were analyzed randomly by at least two experienced cardiologists who were blind to the study protocol.

2.3. Coronary lesion severity

Coronary lesion severity was evaluated by GENSINI score, SYNTAX I score and the number of diseased vessels. The Gensini score was calculated by assigning a severity score to each coronary lesion according to the stenotic degree of the coronary artery lumen and the importance of its localization. The total score was equal to the sum of the stenosis score and the location score for all diseased segments (9) (stenosis of 25%, 50%, 75%, 90%, 99%, and total occlusion were given Gensini scores of 1, 2, 4, 8, 16, and 32, respectively and location score was calculated by multiplying by the constant coefficient: left anterior descending coronary artery (LAD) × 2.5; the proximal segment of the circumflex artery (LCX)×2.5; the mid-segment of the LAD×1.5; the right coronary artery (RCA), the distal segment of the LAD, the posterolateral artery and the obtuse marginal artery×1; and others×0.5).

SYNTAX I score was calculated for stenosis diameter of 50% or greater in vessels of 1.5 mm or more in diameter and the final online updated version (2.11) was used (10).

2.4. Statistical analysis

All data were analyzed using SPSS v.17.0 for Windows (SPSS, Inc., Chicago, Ill., USA). Categorical variables were expressed as frequencies and percentages and continuous variables were presented as means ± SD. Kolmogorov-Smirnov test was used for determination of normal distribution. In independent groups comparisons, independent samples t test was used when parametric test assumptions were provided and Mann-Whitney U test was used when parametric test assumptions were not provided. χ 2 test was used for comparison of categorical values. Correlation between variables was done using Pearson's correlation analysis. Multiple logistic regression analysis was performed to determine the risk factors affecting CAD, which is the dependent variable. The threshold of statistical significance was considered as $p<0.05$.

3. Results

The baseline characteristics of the study population are summarized in Table 1. Age, the incidence of hipertension and diabetes, fasting blood glucose differed significantly in patients with and without CAD ($p<0.05$). However, no significant difference was found in gender, smoking, LVEF, HbA1c, creatinine, CRP, hemogram, WBC and lipid parameters between groups. The TyG index was calculated as 9.01 ± 0.49 and 8.82 ± 0.46, respectively, but no statistical difference was observed ($p = 0.064$). In addition, there was no significant difference in TG/HDL-C and LDL-C/HDL-C values.

The GENSINI and SYNTAX I scores of patients with symptomatic CAD were calculated as 51.74±31.38 and 24.42±10.14 respectively, and 42% had single-vessel disease, 28% had two-vessel disease, and 30% had multi-vessel disease. The TyG index was correlated with only TG/HDL-C which is another indicator used in IR evaluation. There was no significant correlation between TyG index with CAD severity scores identified by GENSINI, SYNTAX I and the number of diseased vessels (Table 2, Figs. 1-3) In the multiple logistic regression analysis, age was significantly associated with CAD regardless of all causes. ($p=0.03$, OR=1.048, 95% CI=1.004-1.094) (Table 3).

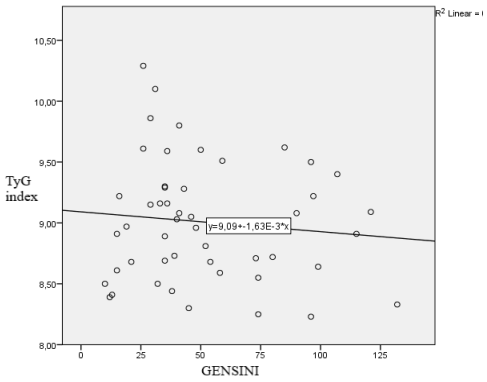

Fig. 1. The correlation between TyG index and GENSINI score. TyG index, triglyceride glucose index

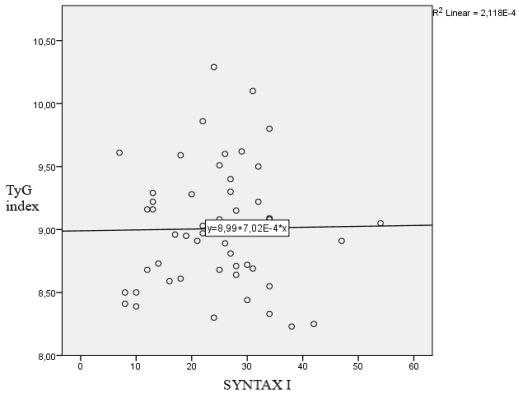

Fig. 2. The correlation between TyG index and SYNTAX I score. TyG index, triglyceride glucose index

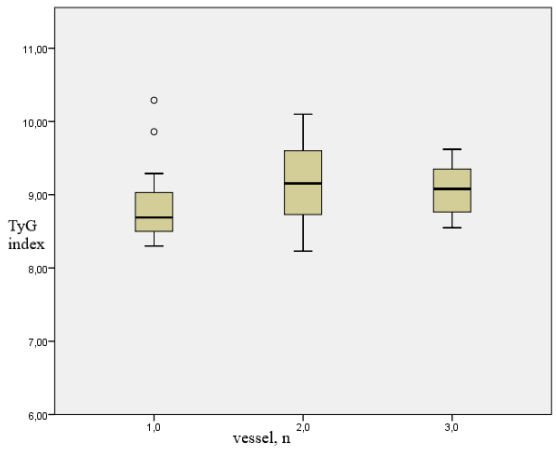

Fig. 3. The correlation between TyG index and the number of diseased vessels. TyG index, triglyceride glucose index

Table 1. Baseline characteristics of study population

Variables	Patients with CAD (n=50)	Patients without CAD (n=50)	p
Mean age (years)	63.80±10.63	57.56±13.37	0.016
Males, n (%)	37 (74)	31 (62)	0.209
Hypertension, n (%)	34 (68)	23 (46)	0.038
Diabetes mellitus, n (%)	22 (44)	11 (22)	0.020
Current smoking, n (%)	13 (26)	17 (34)	0.450
LVEF (%)	54.24±9.58	53.79±8.99	0.823
Fasting glucose (mg/dl)	130.60±51.04	111.72±29.24	0.042
HbA1c (%)	8.64±2.04	8.10±1.23	0.623
Creatinine (mg/dl)	1.01±0.30	1.00± 0.58	0.239
TChol (mg/dl)	178.44±41.21	176.56±40.87	0.831
LDL-C (mg/dl)	108.56±37.06	105.00±36.32	0.651
HDL-C (mg/dl)	40.82±10.80	43.90±11.78	0.203
TG (mg/dl)	145.22±64.04	133.59±46.51	0.342
Hemoglobin (g/dl)	13.28±1.97	15.63±2.95	0.209
WBC (cells/μL))	9.60±2.76	9.23±2.95	0.543
CRP (mg/dl)	1.35±3.14	3.20±5.55	0.060
TyG index	9.01±0.49	8.82±0.46	0.064
TG/HDL-C	3.82±2.07	3.35±1.76	0.260
LDL-C/HDL-C	2.82±1.18	2.47±0.82	0.126
GENSINI	51.74±31.38		
SYNTAX I	24.42±10.14		
Single- vessel disease	21 (42)		
Two-vessel disease	14 (28)		
Multivessel disease	15 (30)		

LVEF, left ventricular ejection fraction; TChol; total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; WBC, white blood cells; CRP, C-reactive protein; TyG index, triglyceride- glucose index; CAD, coronary artery disease

4. Discussion

In this study, we investigated the role of the TyG index in determining presence and severity of CAD in symptomatic patients with high suspicion of CAD. We found that TyG index did not predict neither CAD severity nor CAD presence in this population. The previously studies have shown the association of TyG index with CAD related conditions such as diabetes, hypertension and MetS. For these reasons, the TyG index has been claimed as an atherogenic marker (2, 11).

Table 2. Correlation analysis

TyG index	r	p
Mean age	0.031	0.773
LVEF	0.025	0.809
Fasting glucose	0.602	<0.001
HbA1c	0.183	0.465
Creatinine	-0.054	0.611
Tchol	0.075	0.481
LDL-C	-0.080	0.453
HDL-C	-0.158	0.137
TG	0.751	<0.001
Hemoglobin	-0.113	0.289
WBC	0.167	0.116
CRP	0.086	0.417
TG/HDL-C	0.687	<0.001
LDL-C/HDL-C	0.078	0.465
GENSINI	-0.104	0.470
SYNTAX I	0.014	0.920
Stenotic vesssel numbers	0.181	0.251

LVEF, left ventricular ejection fraction; TChol; total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; WBC, white blood cells; CRP, C-reactive protein; TyG index, triglyceride glucose index.

Table 3. Factors that independently correlated with the CAD in multiple logistic regression analysis

Variable	P	OR	95%CI
Mean age	0.031	1.048	1.004-1.094
Gender	0.121	2.426	0.792-7.436
Diabetes	0.486	1.005	0.991-1.019
Hipertension	0.605	1.303	0.477-3.560
TyG index	0.556	1.445	0.424-4.925

CAD, coronary artery disease; TyG index, triglyceride glucose index; OR, odd ratio; CI, confidence interval

In our study, the correlation between the TyG index and the TG / HDL-C, which is also one of the IR indicators, confirms that the TyG index may be used in evaluation of dysmetabolic conditions. Recently, the studies have been reported the effects of the TyG index on atherosclerosis. In one study, increased TyG index predicted the adverse cardiovascular event in healty population (12). Also, the TyG index was associated with arterial stiffness and the TyG index showed the CAC presence on computer tomography imagings in healty individuals with no cardiovascular risk factors in other studies (13, 14). In addition, a study has confirmed that the TyG index is associated with carotid atherosclerosis and arterial stiffness in lean postmenopausal women and another study showed that TyG index was increased in asymptomatic diabetic patients with coronary artery stenosis (15, 16). Moreover, one study has suggested that TyG index can be used in early detection of subclinical CAD in the absence of traditional CV risk factors (17). However, unlike these studies, we failed to show the relationship between the TyG index and the presence of CAD in our study. But these studies had some limitations such as performing with healthy population or using postmenopausal woman or including specific study population such as diabetic patients. Also, the individuals were asymtomatical in these

studies. However, we included symptomatic patients with high suspicion of CAD and with various cardiovascular risk factors in our study. As if to support our work, one study showed that high TyG index may indicate CAD risk profile only in healthy subjects, not patients with cardiovascular risk factors such as hipertension or diabetes and another study demonstrated that the TyG index may be an indicator for type 2 diabetes rather than CAD (18, 19). Inconsistencies in studies investigating the relationship between the TyG index and CAD may be linked to the the properties of study population, age distribution, ethnic diversity, the cardiovascular risk profile, and duration of exposure to dysmetobolic conditions. Also, providing glucose and lipid regulation by using antihyperlipidemic and antidiabetic drugs may weaken or eliminate this relationship. Indeed, Silvia et al reported that the TyG index was higher in patients with at least one cardiovascular risk factor and cardiac symptoms than in patients with known CAD and under treatment (20).

Recently, researchers have focused on the effect of the TyG index on atherosclerosis progression not only presence. A study involving prediabetic patients with acute coronary syndrome (ACS), there was a positive correlation between SYNTAX I and TyG index (21). In another study, Mao et al. showed that the patients with increased TyG index had high SYNTAX I scores in non-ST elevation miyocardial infarction (2). In addition, asymptomatic diabetic patients with high TyG index had severe CAD identified by stenotic degree and the number of diseased vessels during scanning with computer tomography in another study (22). But we could not find any relationship between the TyG index with GENSINI, SYNTAX I and number of diseased vessels unlike these studies. Also, these studies were performed in patients with ACS or asymptomatical patients and the patients using statins or fibrates were not included. Unlike these studies, Alizargar et al suggested that TyG index may be used in early stage of atherosclerosis and that TyG index is associated with presence of atherosclerosis not severity (23). In another study, Won et al. could not show the association of the TyG index with CAC progression in patients with heavy calcium burden (24). These studies findings support our results. Moreover, dynamic changes in the TyG index or prolonged exposure to cardiovascular risk factors may provide more accurately estimation of CAD severity than high TyG index.

The age was only independent predictor of CAD in our study and most studies have shown that age continues to be an independent predictor for CAD even if traditional risk factors are removed by using advanced analysis methods (25). This may be explained by the prolonged exposure to cardiovascular risks, higher cardiovascular burden and comorbidity increase with age.

There were some limitations in our study. The study was designed as retrospectively. Our study population was relatively small, and it was performed in a single center.

Therefore, the study results may not be generalized to the whole population. Also, triglyceride and fasting glucose were only mesured at baseline, body mass index or waist circumference and dietary habits were not recorded. In addition, we could not ignore drug use that could affect the results because of retrospective study design.

In conclusion, the use of TyG index in determining CAD severity or presence may not be aproppriate in symptomatic patients with high suspicion of CAD and the age is the only independent predictor in CAD according to our results. However multi-center prospective studies are needed to evaluate the role of the TyG index in CAD more accurately.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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Research Article

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The use of dinoprostone at third trimester in pregnant women with oligohydramnios

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Abstract

The aim of this study was comparing the efficacy of dinoprostone administration for labour induction in pregnant women with oligohydramnios and in pregnant women with normal amniotic fluid volume at third trimester. This retrospective study included 187 pregnant women between January 2015-December 2020. Four quadrant technique was used to determine amniotic fluid index. The cases in the study were divided into two groups as Group:1(the ones with oligohydramnios) and Group:2 (the ones with normal amniotic volume) Age ,gravida, parity ,gestational week according to the last date of menstruation, Bishop score ,dilatation ,hours elapsed after the first contraction, hours elapsed after active contraction, labour entry time (hour) , time elapsed until delivery (hour) , birth weight, Apgar score 0th minute and 5th minute and their distribution between the groups showed similarity ($p > 0.05$). The values of amniotic fluid index (AFI) ($p < 0.001$) and effacement ($p = 0.012$) were found to be significantly higher in Group 2 compared to the values of the cases in the Group 1 ($p < 0.05$). It was found in the analysis that the findings of premature rupture of membrane (PROM), oxytocin augmentation need, tachysystole, vaginal delivery rate, caesarean delivery rate, meconium stained amnion rate, neonatal intensive care unit (NICU) need, postpartum hemorrhage, and transfusion need ($p = 0.394$) were similar between two groups ($p > 0.05$). We can state that dinoprostone can be used safely and effectively to induce labor in third trimester pregnancies both with normal AFI and oligohydramnios.

Keywords: dinoprostone, induced labour, oligohydramnios

1. Introduction

Oligohydramnios is associated with increased morbidity and mortality. It is defined as five centimeters or less of amniotic fluid index that is measured by ultrasound (1). Almost 12% of pregnancies are complicated with oligohydramnios leading to fetal heart rate abnormalities and fetal hypoxemia due to cord compression during labor (2). The prevalence of oligohydramnios in early pregnancies is less than 1% (3). Maximal vertical pocket (MVP) and amniotic fluid index (AFI) are two important methods to diagnose oligohydramnios. The effects of oligohydramnios on neonatal outcomes are unclear; whereas some authors did not show any adverse effects, others have shown higher rates of neonatal intensive care unit (NICU) admissions, lower Apgar scores and higher rate of meconium aspiration syndrome (MAS). It is mostly an isolated finding, and it is recommended by the American College of Obstetricians and Gynecologists (ACOG) that delivery should be initiated between 36 0/7 and 37 6/7 weeks in pregnancies that is complicated by oligohydramnios in order to prevent antepartum stillbirth (4). Dinoprostone is the first option that is recommended for the induction of labour induction (IOL) in late-term pregnancy (LTPs) (5). It is a synthetic preparation which is chemically identical to prostaglandin E_2 (PGE_2) that is delivered with a cervical gel or vaginal insert, and which provides sufficient PGE_2 to local tissues to induce cervical ripening (6). Prostaglandin analogues are commonly administered to pregnant women in clinical practice to prepare the immature cervix for delivery (7).The sustained-release vaginal ovule form containing 10 mg of dinoprostone of these analogues is available in Turkey (PROPESS OVULES Ferring) and it has been approved by the Ministry of Health to be used in the induction of labour beginning from 37 weeks (8). In this retrospective study, we aimed to assess the possible effects of labor induction with Dinoprostone at third trimester pregnancies with or without oligohydramnios.

2. Materials and methods

This retrospective study included 187 pregnant women at third trimester, and it was conducted between January 2015-December 2020. Four quadrant technique was used to determine amniotic fluid index. Deepest, vertical length of each pocket of fluid is measured in each quadrant and summed

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up. Term pregnancy is diagnosed if at least 37 gestational weeks are completed according to date of last menstruation or first trimester ultrasound. Patients having single fetus in vertex presentation with estimated fetal birth weight <4000 g with normal non-stress test (NST) were included. Exclusion criteria were presence of twin pregnancy, spontaneous labor, any maternal condition complicating pregnancy (e.g., hypertension, Diabetes Mellitus, other systemic diseases), contraindications of labor induction e.g., cephalopelvic disproportion (CPD), history of uterine surgery, placental anomalies. After pelvic and ultrasonographic examination were completed, Dinoprostone (Propess 10 mg, Ferring AB, Limhamn, Sweden) was placed to posterior fornix. NST and pelvic examinations were repeated in 2-3 h intervals. Oxytocin augmentation was introduced in case of hypotonic uterine dysfunction, at least 6 h after removal of the ovule. Patients in active phase of labor were followed properly. In lack of active uterine contractions, ovule was removed 12 h after the application and the second ovule was introduced 24 h after the first administration. Lack of active uterine contractions and progressive cervical changes within 24 h following the second application was accepted as induction failure and Cesarean sections were performed. The statistical data analysis was conducted through Statistical Package for the Social Sciences (SPSS) 23.0 package program. The categorical measurements were summarized as numbers and percentages. Continuous measurements, on the other hand, were summarized as mean and standard deviation (median and minimum-maximum when needed). Chi-square test and Fischer's Precision Test were used in comparison of categorical variables. The variables' conformity to normal distribution was investigated by using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk Tests). In comparison of continuous measurements between the groups Mann Whitney U tests were used for parameters which were not showing normal distribution. Statistical significance level was taken as 0.05 in all tests.

3. Results

The cases in the study were divided into two groups as Group:1(the ones with oligohydramnios) and Group:2 (the ones with normal amniotic volume). Age (p = 0.696), gravida (p = 0.411), parity (p = 0.211), gestational week according to the last date of menstruation (p = 0.466),Bishop score (p = 0.326), dilatation (p = 0.687), hours elapsed after the first contraction (p = 0.508), hours elapsed after active contraction (p = 0.286), labour entry time (hour) (p = 0.133), time elapsed until delivery (hour) (p = 0.284), new-born weight (p = 0.080), new-born Apgar score 0^{th} minute (p = 0.586) and 5^{th} minute (p = 0.678) and their distribution between the groups showed similarity (p> 0.05). The values of AFI (p <0.001) and effacement (p = 0.012) were found to be significantly higher in Group 2 compared to the values of the cases in the Group 1 (p <0.05) (Table 1).

In Table 2, it was found in the analysis that the findings of premature rupture of membrane (PROM) (p = 0.119), oxytocin augmentation need (p = 0.564), tachysystole (p = 0.408), vaginal delivery rate (p = 0.434), caesarean delivery rate (p = 0.498), MAS rate (p = 0.530), NICU need (p = 0.753), postpartum hemorrhage (p = 0.533), and transfusion need (p = 0.394) (Table 2) were similar between two groups (p> 0.05).

Table 1. Demographic characteristics of both groups

	Oligohydramnios (n=59)	Normal (n=128)	All Patients (n=187)	p
	Mdn (Min-Max)	Mdn (Min-Max)	Mdn (Min-Max)	
Age(y)	25 (17-47)	25 (17-42)	25 (17-47)	0.696
Gravida	1 (1-6)	1 (1-11)	1 (1-11)	0.411
Parity	0 (0-3)	0 (0-4)	0 (0-4)	0.211
Gestational week according to the last date of menstruation(w)	39 (31-42)	39 (34-43)	39 (31-43)	0.466
Amniotic fluid index (AFI)(mm)	40 (10-50)	80 (60-180)	70 (10-180)	**<0.001**
Bishop	1 (0-3)	1 (0-3)	1 (0-3)	0.326
Dilatation(cm)	1 (0-2)	1 (0-2)	1 (0-2)	0.687
Effacement (%)	0 (0-60)	0 (0-70)	0 (0-70)	**0.012**
Hours elapsed after the first contraction(h)	4 (1-12)	4 (0-18)	4 (0-18)	0.508
Hours elapsed after the active contraction(h)	6 (2-18)	8 (0-24)	8 (0-24)	0.286
Labour entry time (hour)	10 (3-25)	12 (3-30)	11 (3-30)	0.133
Time elapsed until delivery (h)	14 (4-48)	18 (4-55)	17 (4-55)	0.284
New-born weight(g)	3080 (1685-3970)	3195 (1820-4680)	3170 (1685-4680)	0.080
Newborn Apgar scores 0^{th} minute	9 (4-9)	9 (2-10)	9 (2-10)	0.586
Newborn Apgar scores 5^{th} minute	10 (7-10)	10 (5-10)	10 (5-10)	0.678

Mdn:median, min:minimum, max:maximum, Amniotic fluid index (AFI)

Table 2. Clinical results of both groups

	Oligohydramnios (n=59)	Normal (n=128)	All patients (n=187)	*p*
	n(%)	n(%)	n(%)	
Premature rupture of membrane (PROM)				
No	56 (94.9)	112 (87.5)	168 (89.8)	0.119
Yes	3 (5.1)	16 (12.5)	19 (10.2)	
Oxytocin use				
No	39 (66.1)	79 (61.7)	118 (63.1)	0.564
Yes	20 (33.9)	49 (38.3)	69 (36.9)	
Tachysystole				
Yes	3 (42.8%)	4 (57.2)	7 (100)	0.408
Vaginal delivery rate (%)				
No	16 (27.1)	42 (32.8)	58 (31.0)	0.434
Yes	43 (72.9)	86 (67.2)	129 (69.0)	
Caesarean delivery rate (%)				
No	43 (72.9)	86 (67.2)	129 (69.0)	0.498
Yes	16 (27.1)	42 (32.8)	58 (31.0)	
Meconium Amnion				
No	57 (96.6)	120 (94.5)	177 (95.2)	0.530
Yes	2 (3.4)	7 (5.5)	9 (4.8)	
Neonatal intensive care unit (NICU)				
No	56 (94.9)	120 (93.8)	176 (94.1)	0.753
Yes	3 (5.1)	8 (6.3)	11 (5.9)	
Postpartum hemorrhage				
No	58 (98.3)	127 (99.2)	185 (98.9)	0.533
Yes	1 (1.7)	1 (0.8)	2 (1.1)	
Transfusion need				
No	55 (93.2)	123 (96.1)	178 (95.2)	0.394
Yes	4 (6.8)	5 (3.9)	9 (4.8)	

Mdn: median, min: minimum, max: maximum, premature rupture of membrane (PROM), Neonatal intensive care unit (NICU)

4. Discussion

In this study the clinical results of dinoprostone use for labour induction between two groups were similar. Oligohydramnios is a relatively common complication of pregnancy, and it is frequently experienced in clinical practice (9). It refers to the volume of amniotic fluid that is less than expected according to the age of pregnancy. It is typically diagnosed by ultrasound and it can be defined as qualitative (e.g. normal, decreased) or quantitative (e.g. [AFI] <5) (10). Like our results, in the study conducted by Çıkrık et al., it was demonstrated that labor induction with dinoprostone complicated with oligohydramnios was as safe and effective as it was in pregnancies with normal AFI (11). Approximately half of all PGE, ripening studies in term women's have indicated a significant decline in the cesarean delivery rate (12). In Carlan et al study 10 (45%) and 15 (68%) of the study and control group patients respectively had required a cesarean delivery (P=0.22). In our study, cesarian delivery rate was 27% in Group 1 and 32.8% in Group 2 respectively and it was lower than previous studies (13). Cervical ripening is an active biochemical process, similar to an apyretic inflammatory reaction controlled by multiple factors: progesterone, PGs, nitrous oxide (NO), and inflammatory cytokines, such as IL-1, IL-8, and TNF-α (14, 15). A study confirm that PGE analogs stimulate cervical NO release in pregnancy (16). Thus, dinoprostone could possibly activate a chain reaction in the cervix of pregnant women, as the initial NO stimulation caused by the PGE_2 analog is followed by a local secretion of PGs triggered by NO. Both responses account for cervical ripening in the pregnant patients (17). Prostaglandins have been reported to cause more tachysystole compared with mechanical methods (18). In another study, the rate of tachysystole was found to be significantly higher in cases which had caesarean delivery compared to cases which had vaginal delivery after dinoprostone administration (19). In our study, no significant difference was found between the groups in terms of the status of tachysystole. A study presented that there was no significant difference in terms of birth weight and APGAR scores between women who gave vaginal delivery after induction with dinoprostone and women who had caesarean section (19). Our study similarly revealed that there was no significant difference between the groups in terms of birth weight and APGAR scores. A study found lower birth weights in pregnancies complicated by oligohydramnios (20). But in our study the birth weights were similar between two groups. However, although there were no major maternal or fetal complications in our study, the retrospective study design precludes us from ruling out minor maternal or fetal complications or later neonatal complications.

We can state that dinoprostone can be used safely and effectively to induce labor in third trimester pregnancies both with normal AFI and oligohydramnios.

Conflict of interest

None to declare.

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None to declare.

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Investigation of MERS-CoV seropositivity among Umrah visitors from the Çorum Region of Turkey

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Abstract

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes Middle East Respiratory Syndrome (MERS). Since the vast majority of cases (more than 85%) are reported from Saudi Arabia, there is a pandemic potential for pilgrimage due to Hajj or Umrah. It is reported from Turkey that more than 400 thousand people went to Saudi Arabia for umrah and 61 thousand people for Hajj in 2014. In this study it is aimed to investigate the patients who had just returned from Makkah for Umrah and who also applied to the Infectious Disease Clinics at the Hitit University Erol Olcok Training and Research Hospital for having respiratory tract symptoms. Their serologic situations have been determined by ELISA whether there is any risk in terms of performing the Hajj and Umrah, and contracting MERS-CoV. Between January 1st to the 31st of October 2015, 40 people were included in this study, which were admitted to our hospital with upper respiratory tract complaints and had previously been in Saudi Arabia for Umrah within the last 15 days. As a control group, 40 healthy people without any complaints and travel histories to risky areas were selected. Their serum samples were taken and searched by MERS-CoV IgG ELISA (Euroimmun AG, Lübeck, Germany). The results ≤0.8 were considered as negative, ≥1.1 were as positive, 0.8-1.1 were suspected. All suspected and positive results have been revaluated and confirmed. Only two (5%) individuals from the patients' group were found as positive for the MERS-CoV IgG antibodies, but individuals from the remaining patients' group and also all control group members were determined as negative. Travels to Saudi Arabia could be a risk for exposure to MERS-CoV. Although there is no evidence, contamination could be realized by anthropologically due to crowds.

Keywords: corona virus, MERS-CoV, respiratory syndrome

1. Introduction

From all over the world 5.5 million Muslims perform Umrah, 1.8 million for the Hajj visit Saudi Arabia. In 2014, 61 thousand people performed the pilgrimage, and more than 400 thousand people from Turkey went to this holy area for Umrah (1). Because of the enormous crowds and close contact, from time-to-time outbreaks of communicable diseases can be seen during the pilgrimage or Umrah.

Middle East Respiratory Syndrome Coronavirus, (MERS-CoV) was identified in a patient from Saudi Arabia in 2012 for the first time and the disease defined as "Middle East Respiratory Syndrome (MERS)", which may progress to death with acute respiratory and multiple organ failure (2, 3). The virus causes high mortality in individuals who especially have underlying diseases. Camels are carriers of this virus and it's mainly transmitted to humans from them. However, the contamination risk from animals to humans is not high, and human-to-human transmission needs prolonged close contact (4, 5).

The World Health Organization (WHO) reported that at least 356 of 971 MERS-CoV cases confirmed by the reference laboratory have died by February 2015 (6). The vast majority of the MERS was seen in the Arabian Peninsula or the countries close to this area; almost 85% of the cases were from Saudi Arabia with a 35% mortality rate (6, 7).

Although detection of viral nucleic acid is the best method for diagnosis of MERS-CoV, where NAAT is not possible seroconversion in samples taken two weeks apart could be used for confirmation of the infection. Also, serological methods could be useful for the investigation of an ongoing outbreak and retrospectively assess the extent of an epidemics (8-11).

In this study, it is aimed to investigate the patients who had just returned from Makkah for Umrah and who also applied to the Infectious Disease Clinics at the Hitit University Erol Olcok Training and Research Hospita for having respiratory tract symptoms. Their serologic situations have been determined by ELISA whether there is any risk in terms of performing the Hajj and Umrah and contracting

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MERS-CoV.

2. Materials and methods

Forty patients who were admitted to our hospital's Infectious Diseases Clinics with upper respiratory tract complaints, and who had also previously been in Saudi Arabia within 15 days from January 1st to the 31st of October 2015 were included in this study. As a control group, 40 healthy people had been selected who had no history of visiting risky areas and respiratory tract complaints. Both suspicious and control groups have been examined by infectious diseases specialists and, their histories have been evaluated and recorded. From all people included in this study, 10 ml blood samples were collected, centrifuged, and stored at -20 °C.

MERS-CoV IgG antibodies in serum samples have been searched by a recombinant enzyme-linked immunosorbent assay (ELISA; Euroimmun, Lübeck, Germany) according to the manufacturer's recommendation as mentioned by Corman et al. (12). Briefly, serum samples were diluted as 1: 101 then incubated in the microplates coated with purified soluble MERS-CoV spike protein S1 domain expressed in HEK-293T cells, and the plates were read at 450 nm in a spectrophotometer. All serum samples were searched twice, and the arithmetic mean of the two measurements was considered. The results ≤0.8 were considered as negative, ≥1.1 were as positive, 0.8-1.1 were suspected. This study's ethical approval was taken from the Istanbul University School of Medicine Ethical Board (2015- 107113).

3. Results

The average age of the suspicious group with upper respiratory tract symptoms and who had a history of performing Umrah included in this study was 59.5 (29-89), the average age of the control group was 52.4 (36-93). In the suspicious group, 25 were female and 15 were male, while in the control group 26 were female and 14 were male. Only two (5%) individuals from the suspicious group were found as positive with the MERS-CoV IgG antibodies, but other individuals including the control group were determined as negative.

From the Hospital Database System, data of positive cases who had the IgG antibodies against the MERS-CoV were reviewed. There was no underlying disease history among the two seropositive patients. One of the cases was a 60-year-old female patient with positive antibody results determined as 1.15. Because she had a cough, her results were re-evaluated retrospectively, and it was found that the blood count results were normal, but her C-reactive protein (CRP) levels were high (24.5 mg/l, reference value: 0-5 mg/l). This patient and also her relatives were called to obtain control blood samples but could not be reached.

The other probable patient was a 40-year-old male who had a cough and a high fever history, whose antibody result was found to be 1.25. After evaluating retrospectively, his blood count results were found as normal while his CRP levels were high (22.6 mg/l). He and his relatives were invited to check for their blood samples. Samples were taken from this probable patient for a second time and the result was again positive (1.15) for MERS CoV IgG. Although his mother and his wife who had traveled with him at the same time had respiratory tract complaints, were found to be negative.

4. Discussion

MERS is a viral disease that can have pandemic potential. By the end of June 2018, 2,229 laboratory-confirmed MERS-CoV cases and 791 deaths (case-fatality rate 35.5%) were reported worldwide (13). Most of the cases were reported from Saudi Arabia (1,853 cases/717 deaths, case-fatality rate 38.7%). While most of the primary cases had a history of camel contact or consuming camel milk, most of the secondary cases were seen as nosocomial contamination in healthcare facilities (13).

Epidemiologic and genomic studies especially have taken place in home and hospital settings confirmed that the disease may be transmitted from human to human. (14, 15). In April and May 2013, hospital-borne outbreaks have occurred in Saudi Arabia and 23 patients from the intensive care unit and the hemodialysis unit were found to have the same genotype of the virus (14). It is thought that a large droplet and contact transmission were the main sources for human-to-human contamination, but also spread through the air or fomite should not be ignored (16). The average incubation period of the virus ranges 2-16 days, after contracting the infection, a healthy human can contaminate other people after 13-14 days (17, 18).

The disease is mainly caused by the contact of MERS-CoV-infected pilgrims who are hospitalized for any reason because camel contact is not expected during the Hajj/Umrah visit. But the risk of contamination should not be ignored, Since the Hajj period is almost a month (13). In our country, the first imported case of MERS-CoV was reported from Hatay in 2014 (19). This case was a 42-year-old male worker in Jeddah, who applied to the hospital with complaints of fever, weakness, respiratory failure, cough, and sweating but unfortunately, the patient lost his life in the intensive care unit. For this patient, the diagnosis was achieved by molecular methods based on the detection of the virus in the tracheal aspirate (19).

There is no vaccines or clinically applicable treatments are currently available for MERS-CoV, so rapid diagnosis is crucial for prompt treatment and protection against viral outbreaks. In the diagnosis of MERS-CoV, LLC-MK2 and Vero cell culture and in-house RT-rtPCR methods are considered as the gold standards (3, 20, 21). Cell culture techniques are slow and laborious, so PCR-based techniques are rather preferred (22, 23). But in any of the surveys conducted for infection risk of MERS-CoV and Hajj, the

virus has not been determined by molecular techniques. Memish et al. have taken nasopharyngeal samples from 3,210 people before and 2,025 people after Hajj; they searched these samples by RT-PCR but did not found the MERS-CoV genome (24). The same year, nasopharyngeal samples were taken from 839 African pilgrims just after returning to Ghana, the samples taken showed that there was no MERS-CoV genome by RT-PCR (25). The same results were also found in 129 French pilgrims just before returning to the country (26). Therefore, it is suggested that the PCR screening should be performed during the Hajj substantially (27). In this context, Barasheed et al. conducted a study during the Hajj in 2013 and nasal swab samples were taken from 1,038 pilgrims who had an influenza-like illness but none of them were found to have the MERS-CoV by molecular method (28). Besides the detection of the MERS-CoV RNA by real-time PCR, a humoral immune response against the infection is also the method used for diagnosis (29). Serological tests could be useful in the determination of asymptomatic cases because they are easy to use and inexpensive and can also be applied to investigate mass screening and epidemiologic researches (29). ELISA, antibody array, immunofluorescence, microneutralization plaque reduction neutralization, and MERS spike pseudoparticulate neutralization tests are used for detecting antibodies against MERS (30-32).

In our study, two (5%) of 40 people returning from Umrah were positive for the MERS-CoV IgG antibodies. In Saudi Arabia, serosurvey studies of the general population were conducted by using similar serological methods that we used, and the MERS-CoV antibodies were determined as 0.15% (15 of 10 009) of the population (33). Our higher rate could be explained that we searched symptomatic patients who visited the epidemic region. On the other hand, our study has some limitations since lower respiratory tract specimens had not been searched, either the budget restriction or the absence of molecular analyses infrastructure at that time. Even we realized probably we would not determine the virus molecularly since asymptomatic and mildly symptomatic patients' lower respiratory tract specimens could found PCR positive for only two weeks (23, 24). The Hajj period is almost a month, but the incubation period of the MERS-CoV infection is around 14 days (34). It is stated that even if individuals became infected, they probably would have recovered already by the time they returned to their homes. In several studies, none of the pilgrims were found as positive for the MERS-CoV when they returned to home and even during Hajj (23-28).

Fever, chills, sore throat, dry cough, shortness of breath, muscle pain, headaches, dizziness, and gastrointestinal symptoms are prodromal symptoms, but later serious systemic and respiratory symptoms could be developed (35, 36). MERS-CoV may progress to death with acute respiratory and multiple organ failure in individuals who especially have underlying diseases (2, 3). Our probable two patients who applied to our clinic with upper respiratory tract complaints healed completely that the reason might be explained due to the lack of underlying disease. These findings remain unclear because they were searched molecularly neither for MERS nor for other respiratory pathogens during their application.

In the Netherlands, two people having gastrointestinal complaints and returning from the Hajj's were determined as having MERS-CoV by RT-PCR method from throat swab samples but none of 78 people were positive although they had contact with these patients (35). In our study, only one of the patient's relatives could be achieved. This patient's wife and mother were found seronegative although they were together during Umrah.

For understanding of infection statistics at a population level, serology is the key element. The presence of MERS-CoV antibodies in a mildly symptomatic patients can last more than a year, after the first positive PCR results. In a study conducted in Abu Dhabi, it is found that 13 of 24 PCR-positive MERS patients' sera had detectable MERS-CoV antibodies for 45–348 days (34). Recent publications indicated that ELISA is 10-fold more sensitive than IFA, and appropriate as a screening tool for MERS (33). Both rS1- and rS-ELISAs maintained high sensitivity and specificity (≥90%) moreover showed better agreement and correlation with microneutralization assay which is the gold standard method for detecting antiviral antibodies, especially exhibiting virus-killing function (37, 38). On the other hand, cross-reaction with seasonal human coronavirus antibodies could be seen by rELISA, because of the higher sensitivity of the test (33, 39). In our study, none of the control group's members found as positive shows that there might be no cross-reactivity because we preferred to use ELISA based on purified soluble MERS-CoV spike protein S1 domain.

As a result, individuals traveling to Saudi Arabia for Hajj or Umrah have a risk of exposure to MERS-CoV. In our study, we defined two MERS CoV IgG positive cases in which the source was probably related to Umrah. Although there is no evidence for the source of the virus, if it is zoonotic or anthroponotic in origin, human-to-human contamination is more probable to the crowd and travel history to the risky area. Therefore, the people who are planning to visit this holly area should be informed about the risk of MERS-CoV and personal security measures should be taken.

Conflict of interest

None to declare.

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Information and behaviors of patients applying to chest diseases outpatient clinic regarding rational use of drugs

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Abstract

Rational Drug Use (RUD), which aims to use drugs, when necessary, in the appropriate amount and in an appropriate way, is required from the physician with the rational choice of drugs and prescribing; health policies cover a process that includes other individuals in the society, pharmacists, healthcare professionals and consists of many steps, from the conscious and correct consumption of drugs to the patient. In determining RUD, it is important to reveal the knowledge and behavioral characteristics of individuals at these stages. Irrational drug use is one of the major health problems in developing countries, including our country. In our study, it was aimed to evaluate the patient parameter in RUD. For this purpose, it was tried to determine the knowledge and behaviors of the patients who applied to Chest Diseases outpatient clinic for any reason. This descriptive study was carried out with 163 volunteers who applied to the 3rd Stage Health Institution Chest Diseases Policlinic due to any respiratory symptoms. The data were obtained by face-to-face descriptive questionnaire. The questionnaire consisted of 16 questions about the sociodemographic characteristics of the patients and their knowledge and behavior regarding drug use. The response rates and compliance with the RUD were evaluated. According to the analysis results, most of the patients were between the ages of 51-64 and primary school graduates (n = 71, 43.6%). The number of female patients was 80 (49.1%) and the number of male patients was 83 (50.9%), and the distribution of patients was found to be similar in terms of gender. 33.1% of the patients prescribed medication and kept them at home without being sick, thinking that it might be necessary, and most of these drugs were pain relievers (29.4%). Only 10.4% were taking medication or demanding a prescription from their doctor with the advice of their neighbors and / or relatives. 11.1% of them used antibiotics on their own, without being examined due to complaints such as flu, cold, and cold. In our study, the characteristics of knowledge and behavior in accordance with RUD were determined to the patient who applied to the chest diseases outpatient clinic. Rational drug use is very important in terms of preventing unnecessary national expense, side effects, adverse effects, and decreasing mortality and morbidity. In order to raise the awareness of individuals on drug use in the society; Health policy, healthcare professionals, media and educators have important responsibilities.

Keywords: rational drug use, medicine, chest diseases outpatient clinic, patients

1. Introduction

According to the definition of the World Health Organization (WHO), a medicine is "a substance or product that is used or intended to be used to change or examine physiological systems or pathological conditions for the benefit of the user" (1). Following the great progress that occurred with the discovery of Acetyl Salicylic Acid in the 20th century, many medicines were produced and medicines were discovered for the treatment of many diseases until the 1970s (2).

Prescribing more medicines than necessary, irrational medicine use, where there are problems such as incorrect or excessive use of medicines, is one of the important health problems in developing countries including our country (3). Irrational use of medicines increases the morbidity and mortality rates by causing the medicines not to reach the required places and not taking the required doses. Besides, the development of resistance mechanisms is an important result in inappropriate use (4). Also, irrational medicine use leads to failure to benefit from treatment, an increase in the side effects and incidence of complications (5, 6). The rapidly increasing number of medicines, the use of over-the-counter medicines on own in minor health problems and the rapid proliferation of over-the-counter medicines reveal the necessity of taking precautions for rational use of medicines (7).

WHO defined Rational Use of Medicine as "A set of rules that must be followed in order for patients to take medicines in accordance with their clinical needs, in doses to meet their personal needs, in sufficient time, at the lowest cost to themselves and the community" at the meeting in Nairobi in

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1985. And created the List of Essential Medicines in 1977 (8). The rational use of medicines, which aims to use medicines when necessary, in appropriate amounts and appropriately, includes a process consisting of many steps from the physician to health policies, other individuals in the society, pharmacists, healthcare professionals, and to the patient with the conscious and correct consumption of medicines to patient with rational medicine selection and prescribing (6).

In Turkey, the Rational Use of Medicine Branch Directorate was established in 2010 under the roof of the Ministry of Health, General Directorate of Pharmaceuticals and Pharmacy. Yet, for the regulations of Rational Use of Medicines, "Turkish Medicines and Medical Devices Agency" was established in November 2011. "A European Union Lifelong Learning Program Project for rational Use of Medicines education with distance education method has been realized and pilot studies have been completed by Refik Saydam Hygiene Center Presidency School of Public Health Directorate. Although Turkey does not have a list of essential medicines, recommendations have been made for the preparation of the "National List of Medicines" and "National Medicine Formulas" but have not yet been implemented (5, 9).

The "Turkish Medicine Guide" was first issued by Prof. Dr. S. Oğuz Kayaalp in 1999 after being adapted from the British National Formulas and was continuously updated and the 6th edition was published in 2011. Diagnosis and treatment guidelines were prepared by the Ministry of Health, but could not be updated continuously. In conclusion, we have serious deficiencies in terms of medicines and treatment with medicines (5).

In order to ensure rational use of medicines, it is important to determine the medicine use knowledge and behavior attitude of the society. In our study, it was aimed to evaluate the role of patients in RUM *(Rational Use of Medicines)*. For this purpose, it was tried to determine the knowledge and behaviors of the patients who applied to Chest Diseases outpatient clinic for RUM

2. Materials and methods

Ethics Committee Approval for the study was obtained from the Ahi Evran University Faculty of Medicine Clinical Research Ethics Committee with the decision No 2020-02/23, Date 11.02.2020. Written consent was obtained from each volunteer participant.

2.1. Study population

The descriptive study was conducted on 163 random volunteers who admitted to the Chest Diseases Polyclinic in February 2020 due to any respiratory complaint. After obtaining verbal and written consent from the volunteers participating in the study, a questionnaire was administered by the researchers, without asking their personal identity information, by face-to-face interviews (Table 1).

The questionnaire form consists of four questions that question age, gender, education level and descriptive features of chronic diseases and twelve closed-ended questions that question the attitude towards medicine use. The questions were prepared according to the "Rational Use of Medicines Guide" (6).

Questions questioning the knowledge and behavioral characteristics of medicine use are what they did after any treatment, where they kept the medicines without any warnings about the storage conditions, whether they prescribed medicines without being sick with the thought that it might be necessary, and which group of these medicines are, whether they use medicine with the recommendation of their neighbors and/or relatives or whether they want to prescribe them to their doctor, what they do when they are sick, whether they recommend medicine to the acquaintances with similar complaints, how they use the medicine that physician prescribed, whether they used antibiotics on own without being examined on complaints such as flu, cold, and influenza, where they learned about the use of the medicine and its possible side effects, how they behave if they encounter the side effects of the medicine, whether they bought the medicine from the pharmacy without a doctor's examination.

Answers were recorded for each patient, and response rates were associated with the descriptive characteristics of the patients and their knowledge and behavior about medicine use.

2.2. Statistical analysis

Categorical variables were shown as frequency (n) and percentage (%) in the analysis of the data. Chi-Square test was used for group comparisons of categorical variables. $p < 0.05$ was considered statistically significant. All analyzes performed with SPSS v.21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

3. Results

80 (49.1%) of 163 patients were female, 83 (50.9%) were male. Most of the female and male patients were between the ages of 51-64 (n=22, 44%; n=28, 56%, respectively). The education level of the majority of the patients was at the primary school level of 28 (56%) (Table 2).

In addition, 57 (35%) of the patients had Asthma/COPD (Chronic Obstructive Pulmonary Disease), 38 (23.3%) had hypertension, 24 (14.7%) had diabetes mellitus (DM), 32 (19.6%) were among the patients with other chronic diseases (such as Rheumatoid arthritis, Chronic Renal Failure, Chronic Liver Disease) and 54 (33.1%) of them did not have any chronic diseases (Table 3).

Table 1. Descriptive face-to-face questionnaire form

Date: …/…/….

City/District where you live:

1. Your age?
   - □ 18 -30
   - □ 31-40
   - □ 41-50
   - □ 51-64
   - □ 65 and older

2. Your gender?
   - □ Female □ Male

3. Your educational status?
   - □ Illiterate
   - □ Primary school
   - □ High school
   - □ Higher education
   - □ Master/PhD

4. Do you have any chronic diseases?
   - □ Asthma/COPD
   - □ Diabetes
   - □ Hypertension
   - □ Other

5. What do you do with the remaining medicine after any treatment?
   - □ I keep it for use when necessary.
   - □ I give it to a health institution.
   - □ I give it to a pharmacy.
   - □ I give it to the relatives who want it.
   - □ I throw it in the bin.
   - □ I flush it in the toilet.
   - □ Other

6. Where do you store medicines for which there are no warnings about storage conditions?
   - □ In refrigerator
   - □ In the freezer/deep freezer
   - □ At room temperature in a cool and dry place

7. Do you request to get a prescription or buy medicine and keep it at home without getting sick, thinking it might be necessary?
   □ Yes □ No (Please procedd to the question 9)

8. Which group of medicines are these?

Pain killers
   - □ Antibiotics
   - □ Cold medicines
   - □ Vitamins
   - □ Stomach medicines
   - □ Eye drops
   - □ Nasal sprays
   - □ Allergy medicines
   - □ Ointments
   - □ All
   - □ Other

9. Do you take medicine with the advice of your neighbors and/or relatives or would you like your doctor to prescribe it?
□ Yes □ No

10. What do you do when you get sick?
    - □ I consult the doctor.
    - □ I consult the pharmacist.
    - □ I consult with a nurse, health official or healthcare professional.
    - □ I consult with my acquaintance/neighbor/relative.
    - □ I try herbal remedies.
    - □ I try to be treated with medicines available at home.
    - □ I ask those who have had a similar condition to mine before.
    - □ Other (Please specify.).....

11. Do you recommend medicine to your acquaintances with similar complaints?
□ Yes □ No

12. How do you use the medicines prescribed by your physician?
- □ I use it until the medicine is over.
- □ I use it until my complaint passes.
- □ I use it as long as the doctor or pharmacist recommends.
- □ Other (Please specify.).....

13. Do you use antibiotics on your own without being examined for your complaints such as flu, cold, and influenza?
- □ Yes, I do.
- □ Yes, I start but stop using the medicine when I feel well.
- □ No; I do not use it without examination.

14. Where can you find information about the use of the medicine and its possible side effects?
- □ Pharmacist
- □ Assistant health personnel (nurse, midwife, health officer, etc.)
- □ Package leaflet of the medicine
- □ Internet
- □ Doctor

15. How would you behave if you experience side effects due to the medicine?
- □ I consult the doctor.
- □ I consult the pharmacist.
- □ I apply to assistant health personnel (nurse, midwife, health officer, etc.).
- □ I look for a solution myself.
- □ I do nothing.

16. Do you buy medicine from the pharmacy without being examined by a doctor?
- □ Yes; when I don't want to pay for money for the examination visit
- □ No; I don't take medicine without being examined, a serious disease may be missed.

There was no statistically significant difference between age groups and genders (p=0.512). However, there is a significant relationship between age groups and education level; while the illiteracy rate was higher in group of 65 years and above, the percentage of higher education graduates was found to be higher among those aged 18-30 (p <0.001) (Table 4).

Table 2. Age distribution rates of patients in their own gender groups

	Gender		**Chi-Square value**	**p-value**
Age Groups	**Female (n=80, 49.1%)**	**Male (n=83, 50.9%)**		
18-30	16 (53.3)	14 (46.7)		
31-40	16 (57.1)	12 (42.9)		
41-50	18 (54.5)	15 (45.5)	3.28	0.512
51-64	22 (44)	28 (56)		
65 and older	8 (36.4)	14 (63.6)		

Table 3. Chronic disease distribution characteristics of the patients

Asthma/COPD	**57**	**35**
Diabetes	24	14.7
Hypertension	38	23.3
Other	32	19.6
Have no chronic disease	54	33.1

The answers to the questions evaluating the medicine use knowledge and behavioral characteristics of the patients were as follows (Table 5): “What do you do with the remaining medicine after any treatment?”: 39% (n=64) said they would keep them for use when necessary, 26.2% (n=43) said they will give it to the health institution, 12.2% of them stated that they threw them away. "Do you request to get a prescription or buy medicine and keep it at home without getting sick, thinking it might be necessary": 33.1% of the patients (n=54) answered yes, and the medicines they mostly have at home were 29.4% (n=48) pain medicines, the least available were stomach medicines, and eye drops 1.2% (n=2). "Do you use antibiotics on your own without being examined for your complaints such as flu, cold, and influenza": 89.6% (n=146) of the patients answered "No". “What do you do when you get sick:” 92% (n=150) of the patients stated that they went to the doctor, 1.8% (n=3) consulted the pharmacist and healthcare personnel, and 4.9% (n=8) tried herbal treatment methods. ‘’How do you use the medicines prescribed by your physician": 54.9% (n=89) of the patients stated that they used the medicine for the time recommended by the doctor/pharmacist, 31.3% (n=51) stated that until the medicine is finished, and 14.1% (n=23) stated that they use it until the complaint recovers. ‘’Do you use antibiotics on your own without being examined for your complaints such as flu, cold, and influenza?’’: 89% of the patients (n=145) stated that they did not use medicine without being examined. "Do you buy medicine from the pharmacy without being examined by a doctor’’: 94.5% (n=154) of the

patients answered "No".

Table 4. Distribution characteristics of the patients by age group and education level

Age Groups	Literate	Primary school	High school	Higher education	Master/ PhD
18-30	0 (0)	4 (13.3)	9 (30)	14 (46.7)	3 (10)
31-40	1 (3.6)	8 (28.6)	12 (42.9)	5 (17.9)	2 (7.1)
41-50	0 (0)	18 (54.5)	10 (30.30)	4 (12.1)	1 (3)
51-64	2 (4)	28 (56)	13 (26)	6 (12)	1 (2)
65 and older	5 (22.7)	13 (59.1)	1 (4.5)	2 (9.10)	1 (4.5)

4. Discussion

Many factors are important in providing RUM. These factors can be classified as non-sick individuals, physicians, patients, nurses and other assistant health personnel, manufacturer company, pharmacy and health policy (10, 11, 12). In our study, the role of patients in RUM was tried to be evaluated.

People are expected to admit to the physician for the diagnosis and treatment of these diseases when they got sick. However, it can be seen that this behavior is not always exhibited (13). With a satisfactory result in our study, 92% of the participants stated that they went to the doctor when they got sick. In a study conducted on this subject, 75.7% of the participants answered the question "what do you do first when you get sick" that "I would go to the doctor", 15.0% answered that "I would use the medicines available at home", and 4.7% that "I would do nothing" (14, 15). The rate of answers given to this question was found to be high in our study; however, there are a few studies consistent with our result (16). The fact that this percentage was higher in our study may be due to the fact that patients were evaluated in a tertiary health institution, a specific outpatient clinic and patients with follow-up due to chronic diseases, and therefore, more conscious and continuous communication with the physician. It also suggests that the prohibition of over-the-counter medicine purchase by the Ministry of Health is effective in increasing this rate. Because, since the study mentioned above was conducted in 2011, that is, before the creation of the RUM National Action Plan, the rate may have been found lower than in our study. Studies on the RUM in the Ministry of Health is continuing for nearly 20 years, and RUM Unit was established in 2010, and RUM, Medicine Supply Management and Promotion Department was established within the Turkish Pharmaceuticals and Medical Devices Agency in 2012. In order to ensure coordination, RUM Provincial Representative was determined in Provincial Health Directorates in 81 provinces, and the RUM National Action Plan (2014-2017) was established. By all outward appearances, studies on RUM in specific branches and diseases are also rare (17).

In our study, most of the patients stated that they did not take medicine without medical examination, but it was observed that the rate of using the medicines prescribed by the physician during the period recommended by the physician decreased. In a study conducted on this issue, 77.3% of individuals used medicine without a physician's recommendation, 26.2% increased/decreased their medicine without consulting a physician, 84.4% followed the prescribed medicine hours, 77.3% of them stated that they stopped using medicine before the period recommended by the physician. In this study, the most important problem was that individuals had health behaviors that need to be developed in terms of RUM, and especially the use of medicines without consulting a doctor and discontinuing the medicine before the recommended time (16).

The rate of participants who got a prescription and purchased medicines without getting sick considering that it might be necessary was low in our study. The medicines that are most commonly available at home without getting sick are painkillers, cold medicines, antibiotics and vitamins, respectively. In a study conducted on this subject, it was stated that 31.3% of the participants bought medicines without a prescription from the pharmacy. Similar to our study results, the most common over-the-counter medicine was pain relievers (90.4%) (14). The higher percentage of admitting to doctors in our study may be due to the fact that the study was carried out in a tertiary health institution and the Ministry of Health prohibited taking medicines without a prescription.

The vast majority of the patient population in our study stated that they did not use any medicine with the advice of their neighbors and/or relatives and did not use antibiotics on their own for flu, cold, and influenza complaints, and they were examined by a physician. According to a study conducted, these rates on RUM were higher (15).

Table 5. Answer rates of the patients to the questions

What do they do with the remaining medicine after any treatment	n	%
I keep it for use when necessary.	64	39.0
I give it to a health institution.	43	26.2
I give it to a pharmacy.	17	10.4
I give it to the relatives who want it.	3	1.8

I throw it in the bin	20	12.2
I flush it in the toilet.	0	0
Other	17	10.4
Request to get a prescription or buying medicine and keeping at home without getting sick, thinking it might be necessary		
Yes	54	33.1
No	109	66.9
Which group of medicines are the medicines they keep at home		
Pain killers	48	29.4
Antibiotics	7	4.3
Cold medicines	12	7.4
Vitamins	7	4.3
Stomach medicines	2	1.2
Eye drops	2	1.2
Nasal sprays	4	2.5
Allergy medicines	6	3.7
Ointments	4	2.5
All	1	0.6
Whether they used medicine with the recommendation of their neighbors and/or relatives or whether they asked their doctor to prescribe		
Yes	17	10.4
No	146	89.6
What they do when they get sick		
I consult the doctor.	150	92
I consult the pharmacist.	3	1.8
I consult with a nurse, health official or healthcare professional.	3	1.8
I consult with acquaintances/neighbors/relatives	0	0
I try herbal remedies.	8	4.9
I try to be treated with medicines available at home.	4	2.5
I ask those who have had a similar condition to mine before.	0	0
Other	0	0
Whether or not recommending medicine to acquaintances with similar complaints		
Yes	12	7.4
No	151	92.6
How do they use the medicines prescribed by the physician		
I use it until the medicine is over.	51	31.3
I use it until my complaint passes.	23	14.1
I use it as long as the doctor and pharmacist recommends.	89	54.6
Cases of using antibiotics on their own without being examined due to complaints such as flu, cold, and influenza		

Yes, I do.	12	7.4
Yes, I start but stop using the medicine when I feel well.	6	3.7
No; I do not use it without examination.	145	89
Where can they learn information about the use of the medicine and its possible side effects		
Doctor	48	29.4
Pharmacist	26	16
Assistant health personnel	3	1.8
Package leaflet of the medicine	91	55.8
Internet	11	6.7
I do not learn	1	0.6
How they behave when faced with the side effects of the medicine		
I consult the doctor.	143	87.7
I consult the pharmacist.	9	5.5
Assistant health personnel	3	1.8
I look for a solution myself.	6	3.7
I don't do anything	3	1.8
Whether or not to take medicine from the pharmacy without medical examination		
Yes	9	5.5
No	154	94.5

When the patients were asked what they did the medicines after the treatment, the majority of the patients stated that they kept them for use when necessary or gave them to a healthcare institution. Unfortunately, a considerable number of patients also stated that they threw away the medicines. They stated that the majority of information about the use of medicines and possible side effects was obtained from the package insert and they applied to the physician when they experienced possible medicine side effects.

As can be seen from our results, the patient population we work with generally has information and behavioral characteristics suitable for RUM. We think that these results are effective because the patients were evaluated in a tertiary health institution and they had more frequent contact with physicians due to their chronic diseases. An important result is that pulmonologists are quite effective in patient knowledge and behavior in RUM in outpatient clinic practices.

One of the limitations of our study can be that we did not calculate the cost. Because, according to WHO data, the budget allocated for pharmaceutical expenditures in the whole world in 2006 is approximately 859 billion USD. The share allocated for pharmaceutical expenditures in the global gross product is 24.9% on average (3). According to 2009 data, medicine expenditures constitute 16% of per capita health expenditures in high income countries, 24% in middle income countries and 29% in low-income countries (17, 18). As the data supports, as the development level of countries increases, expenditure on pharmaceuticals decreases. According to the Turkish National Health Accounts Survey conducted in 1991-2000, the total expenditure of our country for medicines and non-durable medical consumables for 2000 was calculated as 2 Billion 763 Million TRY. Accordingly, the share of total medicine and medical consumable expenditure in total health expenditure is 33.5% (14). According to the data in the health expenditures research produced in accordance with the health accounts system of the Ministry of Health, General Directorate of Health Research (SAGEM), Organization for Economic Development and Cooperation (OECD), it was shown that the share of total health expenditure in the Gross Domestic Product (GDP), which was 4.9 billion in 1999, increased from 4.8% in 1999 to 5.9% in 2011 (7). In 2009, medicine expenditures constituted 45.7% of the total expenditures of the Social Security Institution and 24.7% of the total public health expenditures in Turkey (19, 20). Despite the measures of the SSI, the health and medicine expenditures in Turkey increased by 2.7% in 2011 and exceeded 15 billion TL (7). In Turkey where a total of 1 billion 700 million boxes of medicines are consumed,

antibiotics are in the first place in consumption, while the amount paid for medicines is 1 Billion 253 Million TRY for antibiotics and 939 Million TRY for painkillers (7).

Although compliance with RUM is observed in our study, the population we work with represents a small cross-sectional sample of the society. Therefore, the knowledge and behavioral characteristics of RUM will be revealed more clearly with studies that evaluate other factors effective in wider patient populations and RUM separately.

Another limitation of our study is the lack of questioning about the use of specific medicines such as inhaler medicines. The reason for this is the thought that objective results cannot be obtained with a specific outpatient clinic evaluation and a more conscious patient profile with follow-up due to chronic diseases. Besides, the fact that RUM was not evaluated in terms of both patients and physicians in specific patient groups such as Asthma and COPD can be considered among the limitations of the study and this issue can be evaluated in different studies. Because RUM is evaluated under sub-headings such as RUM in the elderly, rational use of antibiotics, inhaler medicine, rational use of painkillers, and rational use of acetyl salic acid (6). The main topic, RUM, was evaluated in our study. Because, according to WHO, more than 50% of medicines worldwide are prescribed improperly. Also, 50% of the patients do not take their medicines properly. Despite the current unnecessary and excessive use of medicines, nearly one third of the world population cannot even provide access to essential medicines (5, 9). In a study evaluating the RUM physician parameter on this subject and evaluating Inappropriate Antibiotic use in COPD exacerbations, it was observed that most pulmonologists tend to prescribe antibiotics for COPD exacerbations according to the defined GOLD (Chest Diseases COPD diagnosis and treatment guideline) criteria. However, it has been found that some physicians prefer to prescribe antibiotics on their own regardless of the GOLD criteria (19-21). In another study in which the physician parameter in the RUM was evaluated, researcher assistant physicians working in the Faculty of Medicine Hospital were evaluated. In this study, it was observed that the RUM knowledge and attitudes of physicians had deficiencies and revealed the necessity of updating this subject within the scope of continuous medical education (17, 22).

Providing and disseminating RUM is very important in terms of both our unnecessary national medicine expenses and the prevention of unwanted medicine effects and side effects, mortality and morbidity. In this regard, individuals, patients, physicians, health policies, pharmacists and pharmaceutical companies approach and practices are effective in the society. Limiting the use of non-prescription antibiotics, restricting the use of some medicines without a related branch report, and limiting the medicines intake by calculating the usage dose according to the duration were useful practices (21-24).

It is important that patients, even non-sick individuals, be informed about this issue. Hence, we think that it will be beneficial to inform and remind every patient who is examined in outpatient clinics by family physicians in public health centers at regular intervals, and at least RUM brochures are given together with a prescription. These approaches should be supported by health policies in line with the data obtained from broader studies, and Turkish National List of Essential Medicines, National Medicine Formulas and Medicine Guidelines, which have not yet been determined for our country, should be prepared and implemented.

In conclusion, it is important to raise the awareness of individuals about medicine use in the society, to provide RUM, in terms of our national medicine expenditures, treatment and side effects. Health policies, healthcare professionals, non-sick individuals, patients, media and educators have important responsibilities in ensuring and disseminating RUM.

Conflict of interest

None to declare.

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Poplar-type propolis provides protection of blood cells, testosterone levels and sperm motility in cisplatin-induced toxicity

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Abstract

Cisplatin is a frequently used chemotherapeutic in many types of cancer, especially urological cancers. Despite its efficacy in the treatment of cancer, it causes various toxic side effects such as nephrotoxicity, neurotoxicity and ototoxicity. The aim of this study was to determine the protective role of olive oil extract of propolis (OEP) with biochemical and histopathological approaches to cisplatin induced toxicity. Sixty-four adult male Wistar rats were divided into eight groups, control, propolis (100 and 200 mg/kg, orally daily for 10 days) and combined therapy (propolis 10 days before and after CP injection). Haematological (Wbc, Rbc, Mpv, Hgb, Htc, Mcv, Mch, Mchc, Lym, Neu), biochemical (triglyceride, total cholesteol, HDL and LDL-cholesterol, glucose, BUN, uric acid and testosterone level), epididymal sperm concentration, sperm motility, and histological parameters were analyzed. According to the results, cisplatin has negative effects on hematological, biochemical parameters, and sperm motility compared to control group. Administration of pre-cisplatin propolis ameliorated wbc, hct, mcv, mchc, neu levels. The administration of OEP prior to CP normalized the increased BUN and uric acid levels induced by CP. Interestingly it was also revealed that the testosterone levels increased in the OEP groups compared to the control group. Additionally, the combined administration of CP with OEP normalized the decreased testosterone levels induced by CP, particularly pre-treatment OEP. As a result, propolis, a natural product with numerous useful biological effects, was shown to a have protective as well as amelioration and normalizing effect on CP-induced damage.

Keywords: biochemical parameters, cisplatin, heamatological, propolis, sperm

1. Introduction

Cisplatin (CP; cis-diamminedichloroplatinum (II)) is an effective agent widely used in the treatment of numerous solid tumors (testis, bladder, breast, or ovarian cancer, etc.). After entering the cell, CP interacts with DNA, thereby leading to local denaturation of the DNA chain, inhibiting ATPase activity, modifying the cellular transport system, and ultimately causing apoptosis, inflammation, necrosis, and death in cells (1, 2). High CP concentrations lead to necrosis in the proximal tubular cells and low concentrations lead to apoptosis (3). Moreover, despite its clinical benefits, CP therapy has been shown to have several adverse effects including nephrotoxicity, neurotoxicity, and ototoxicity (4).

It is commonly known that many plants protect their leaves, flowers, fruits, and buds from frost and bacterial invasion by producing a potent antimicrobial, waterproof, and heat-resistant resinous substance. This substance, termed 'propolis', is collected by honeybees (*Apis mellifera* L.) with the aid of their mandibular glands and mixed with secretions from the mandibular and wax glands to form pellets and are then carried to the hive. Raw propolis cannot be used in food and pharmacology industries; therefore, it is subjected to extraction with ethyl alcohol, propylene glycol, glycerol, and water. However, because of the limited use of alcohol due to religious beliefs and in children and patients with alcohol intolerance and metabolic diseases, researchers have recently tested water and oil extracts of propolis (OEP) (5). Propolis typically has numerous biological and pharmacological properties such as immunomodulatory, antitumoral, anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, and antiparasitic activities (6-8). Additionally, OEP have been shown to have antimicrobial, antitumoral, antioxidant, and antidepressant properties (9, 10).

In the present study, we aimed to investigate the potential protective role of olive oil-propolis (poplar-type) extract (OEP) against CP-induced toxicity.

2. Materials and methods

2.1. High-performance liquid chromatography (HPLC) analysis of propolis

Detection of phenolic acids was performed using High-Performance Liquid Chromatography (HPLC). The analysis

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of samples was performed with an Agilent 1100 HPLC system equipped with a photodiode array detector and an ion-trap mass spectrometer detector (Agilent Technologies, Waldbronn, Germany). The mobile phase of the method consisted of Solvent A (methanol) and Solvent B (0.5% (v/v) acetic acid in water). The elution profile was 10% A in B, 0 min; 60% A in B, 28 min; and 10% A in B, 30 min. All the gradients were linear. A volume of 10 µL of sample was injected onto the column operating at room temperature at a flow rate of 1 mL/min. Ultraviolet (UV) detection was performed at 290 nm and UV chromatograms were recorded at 280 and 360 nm with a bandwidth of 8 nm. The eluted components were identified based on the retention time by comparison with the retention time of the reference standard.

2.2. Administrations of propolis and cisplatin

The olive oil-propolis (poplar type) extract used in the study (25% propolis) was manufactured by Nutral Therapy Ltd., Kayseri, Turkey. Propolis was administered orally at doses of 100 and 200 mg using an oral cannula. Cisplatin (Sigma-Aldrich, USA) injection was administered intraperitoneally according to the body weight of the rats in a single dose of 7 mg/kg.

2.3. Animals and experimental design

A total of 64 adult male Wistar albino rats weighing 250-260 g were used in the study. The animals were kept in a special room at a constant temperature of 22°C±1°C with 12-hour light/dark cycles and had free access to food and tap water. The study protocol was approved by the Animal Care and Use Committee at Erciyes University School of Medicine. All the experimental procedures were conducted in accordance with the Guide to the Care and Use of Laboratory Animals. The 64 rats were randomly divided into 8 groups with 8 rats each: (I) Control group; 0.9% saline (10 mg/kg) was injected i.p through the tail vein of the rats, (II) CP group (CP); a single dose of CP (7 mg/kg) was injected i.p., (III) OEP 1 group (OEP1); OEP was given orally once a day at a dose of 100 mg/kg for 10 days, (IV) OEP 2 group (OEP2); OEP was given orally once a day at a dose of 200 mg/kg for 10 days, (V) CP + OEP 1 group (CP+OEP1): a single dose of CP was injected i.p. and OEP was given orally once a day at a dose of 100 mg/kg for 10 days, (VI) CP + OEP 2 group (CP+OEP2): a single dose of CP was injected i.p and OEP was given orally once a day at a dose of 200 mg/kg for 10 days, (VII) OEP 1 + CP group (OEP1+CP); OEP was given orally once a day at a dose of 100 mg/kg for 10 days and then a single dose of CP (7 mg/kg) was injected, and (VIII) OEP 2 + CP group (OEP2+CP); OEP was given orally once a day at a dose of 200 mg/kg for 10 days and then a single dose of CP (7 mg/kg) was injected.

2.4. Sample collection

The animals were fasted for 6 h before the collection of testis tissue and blood samples. The animals were maintained under light ether anesthesia which was induced immediately before the collection of blood samples, and samples were collected from each animal by insertion of a cannula in the heart and then transferred into tubes both with and without anticoagulants. Blood samples were obtained from all 8 animals in each group and were centrifuged at 3.000 g for 10 min. Testis samples were cleared from adhering connective tissue and weighed. One testis was fixed in 10% formalin for histopathologic examination. Plasma and other testis samples were stored at -20°C until biochemical analysis. The blood parameters analyzed included white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), nucleated red blood cells (NRBC), mean platelet volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and lymphocyte (LYM) and neutrophil (NEU) counts. Serum was separated and used for the analysis of certain biochemical parameters. A Konelab 60i auto-analyzer (Thermo Clinical Labsystems, Finland) and a Konelab label kit (Thermo Clinical Labsystems, Finland) were used for the determination of serum glucose (GLU), creatinine (CRE), uric acid, triglyceride (TRIG), total cholesterol (CHOL), LDL-cholesterol (LDL-CHOL), and HDL-Cholesterol (HDL-CHOL) levels.

2.5. Epididymal sperm concentration and motility

The epididymis was finely minced with anatomic scissors in 5 mL of physiological saline, placed in a rocker for 10 min, and incubated at room temperature for 2 min. After incubation, the supernatant fluid was diluted at a ratio of 1:100 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%), and 25 mg eosin per 100 mL of distilled water. Total number of sperms was determined with a hemocytometer. Approximately 10 mL of the diluted sperm suspension was transferred to each counting chamber and could stand for five min for counting under a light microscope at 200x magnification. Sperm progressive motility was evaluated by a method described earlier (11). For this purpose, fluid was obtained from the caudal epididymis with a pipette and diluted to 2 mL with Tris's buffer solution. The system was prewarmed (35°C) and the percentage of motility was evaluated visually at 400x magnification. Motility estimations were performed from 3 different fields in each sample. The mean value was used as the final motility score.

2.6. Histopathologic examination

Testis samples were fixed in 10% formaldehyde solution and then embedded in paraffin blocks after being subjected to routine tissue processing sequences. Sections of 5-6 µm thickness were prepared from the paraffin blocks and mounted on glass slides. The slides were incubated for a certain period using histological techniques, deparaffinized through xylene, and hydrated with a graded series of alcohol. The sections were stained with hematoxylin-eosin (H&E) staining for general histologic features. Histopathological examination of the testis samples was achieved in 50 areas using Johnsen's mean testicular biopsy score (MTBS) criterion under an Olympus BX51 microscope (12).

Data were analyzed using SPSS for Windows version 22.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). Descriptive were expressed as mean ± standard deviation (SD). Groups were compared using One-Way ANOVA followed by post-hoc Tukey test. A p value of <0.05 was considered significant. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Approval number: 17/029)

3. Results

Fig. 1 presents the chemical composition of OEP. Caffeic acid phenethyl ester (CAPE; an active component of honeybee propolis) had the highest concentration, followed by dimethoxycinnamic acid, caffeic acid, ferulic acid, p-coumaric acid, and vanillin.

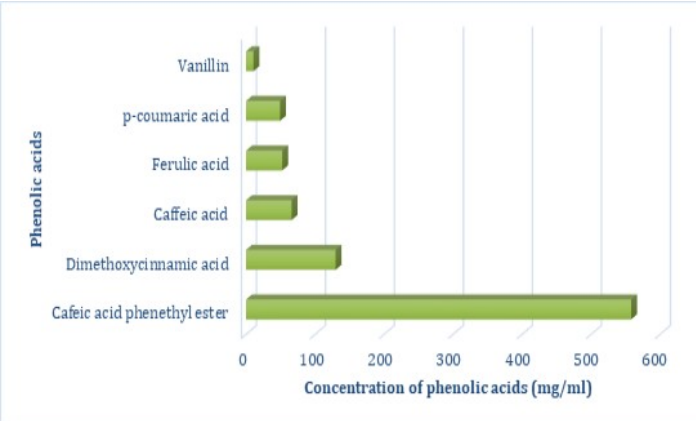

Fig.1. Chemical composition of OEP

3.1. Changes in Hematological Parameters

In terms of WBC, significant difference was observed in the group administered CP compared to the control group. While WBC levels were decreased in the groups that received CP, OEP1+ CP and CP+OEP1, an increase was found in the other groups near the control group (p<0.01). In terms of RBC, the highest RBC levels were observed in the CP group and the lowest in the control group and a significant difference was found among the experimental groups (p<0.01). In particular, the improvement in the groups that were administered OEP prior to CP was highly remarkable and a significant difference was found among the groups (p<0.05).

In terms of MPV, no significant difference was found among the groups and the CP group had the lowest MPV level. Additionally, a remarkable improvement was observed in all the OEP groups, although no significant difference was found among the OEP groups (p>0.05). On the other hand, the administration of CP decreased the HGB levels while the administration of OEP increased the HGB levels; however, no significant difference was found among the CP groups (p<0.01). The administration of OEP resulted in similar HCT levels to those of the control group and this improvement was statistically significant (p<0.05). No significant difference was found among the groups about MCH levels although a numerical improvement was observed in the OEP2 groups. A significant difference was found among the groups about MCV levels, with the lowest MCV levels found in the CP group and no significant improvement observed among the OEP2 groups. In the groups administered a combination of CP and OEP, the results were similar to those of the control group although this improvement was not statistically significant (p>0.05). MCHC levels were higher in the OEP2 groups compared to the control group and this improvement was particularly more noticeable in the OEP2+CP group. The LYM counts in the OEP groups were similar to those of the control group when compared to the CP group and the neutrophil counts were significantly improved in all groups except for the OEP1+CP group (p<0.05). Table 1 presents the changes in hematological parameters in the groups administered with a combination of CP and OEP.

3.2. Changes in biochemical parameters

Administration of CP led to increased triglyceride and LDL-cholesterol levels and decreased HDL-Cholesterol levels, although no significant difference was established among the groups (p>0.05). However, a significant difference was found among the groups about cholesterol levels, with the highest cholesterol levels found in the CP group. Additionally, the cholesterol levels in the groups administered a combination of OEP and CP were decreased to those of the control group. In terms of glucose levels, a remarkable decrease was found in the OEP groups compared to the control group and the improvement in the glucose levels in the groups administered a combination of OEP and CP was statistically significant (p<0.05). On the other hand, no significant difference was found among the OEP groups with regard to blood urea nitrogen (BUN) levels, whereas the BUN levels in the OEP2 group implicated that the administration of OEP reduced the CP-induced increase in BUN levels (p<0.05). The uric acid levels in the CP group were significantly higher than those of other groups and a significant decrease was observed in the uric acid levels in the OEP2+CP group. The testosterone levels in the OEP groups were higher than those of the control group, although no significant difference was established. However, it was revealed that the administration of OEP prior to CP had a normalizing effect on the decreased testosterone levels induced by CP. Table 2 presents the changes in biochemical parameters in the groups administered with a combination of CP and OEP.

3.3. Comparison of sperm parameters

Although no significant difference was observed among the groups about epididymal sperm concentration, a significant difference was found about sperm motility. A remarkable improvement was found in sperm motility in the groups administered OEP prior to CP (Fig. 2).

3.4. Histopathological evaluation results

Normal histological structure was observed in the control group. The OEP2 group and the groups administered a combination of CP and OEP showed nearly normal histological structure, whereas the CP group showed

significantly decreased MTBS compared to the control group.

Table 1. Hematological parameters measured before and after the administration of CP

Groups	WBC (10^3/mm^3)	RBC (10^6/mm^3)	MPV	HGB (g/dL)	HCT (%)
Control	11.33±1.3^{b}	9.31±0.4^{d}	7.44±0.3^{c}	16.23±04^{b}	53.15±1.1^{c}
OEP1	9.88±1.2^{b}	8.65±0.4^{bcd}	7.32±0.2^{bc}	15.50±0.7^{ab}	47.62±4.2^{ab}
OEP1+CP	6.34±1.3^{a}	8.56±0.6^{bcd}	7.10±0.4^{abc}	15.35±1.3^{ab}	46.46±2.1^{ab}
CP+OEP1	6.37±2.2^{a}	8.34±0.4^{ab}	6.95±0.3^{ab}	15.02±0.6^{ab}	45.58±2.3^{ab}
OEP2	10.62±1.2^{b}	9.18±0.3^{cd}	7.38±0.2^{bc}	16.23±0.4^{b}	53.33±2.13^{c}
OEP2+CP	9.66±1.4^{b}	8.42±0.2^{abc}	7.20±0.1^{abc}	15.27±0.7^{ab}	49.68±2.5^{bc}
CP+OEP2	9.79±1.4^{b}	8.09±0.6^{ab}	7.15±0.4^{abc}	14.23±0.8^{a}	46.07±1.3^{ab}
CP	4.88±1.3^{a}	7.64±0.9^{a}	6.78±0.2^{a}	14.18±1.4^{a}	44.34±4.8^{a}
Control	58.10±1.13^{c}	18.58±0.4^{b}	32.23±0.3^{c}	65.48±1.3^{a}	16.0±4.8^{a}
OEP1	57.40±1.02^{bc}	18.12±0.7^{ab}	32.32±0.1^{c}	79.48±5.3^{b}	15.95±3.9^{a}
OEP1+CP	55.72±1.09^{ab}	17.68±0.5^{a}	31.23±0.8^{ab}	82.97±3.7^{bc}	15.56±2.7^{a}
CP+OEP1	55.60±1.92^{ab}	17.58±0.4^{a}	30.53±0.6^{a}	83.43±2.7^{bc}	22.96±1.7^{ab}
OEP2	57.95±1.20^{c}	18.22±0.5^{ab}	33.15±0.9^{d}	74.44±9.3^{b}	15.13±4.8a
OEP2+CP	57.30±0.60^{bc}	17.92±0.5^{ab}	31.98±0.3^{bc}	81.60±3.9^{bc}	15.38±2.7^{a}
CP+OEP2	56.45±2.08^{abc}	17.93±0.3^{ab}	31.22±0.8^{ab}	83.44±2.3^{bc}	16.93±2.7^{a}
CP	54.70±0.84^{a}	17.45±0.3^{a}	30.43±0.6^{a}	84.73±4.4^{c}	30.23±1.9^{b}

Values are expressed as mean, SD; p<0.05; CP: Cisplatin, OEP: Olive oil extract of propolis. a, b, c, d. The groups in the same column with different letters are statistically different

On the other hand, seminiferous tubule diameter was significantly decreased in the CP, OEP1, OE2, and CS+OEP2 groups and was insignificantly decreased in the OEP1+CP, OEP2+CP, and CP+OEP1 groups compared to the control group (p<0.001). The histopathological results were shown in Table 3 and Fig. 3.

4. Discussion

The present study was designed to investigate the effect of CP supplementation with propolis on hematological parameters such as WBC and anemia indices in Wistar albino rats. The results revealed significant differences in various hematological parameters in the experimental groups compared to the control and the CP group. The results also implicated that hemolytic anemia could result from CP and this is likely to be associated with the reaction of an antibody directed against red cell membrane-bound CP. Anemia has been shown to be a common side effect of CP, with its primary mechanism to be a myelosuppression resulting from CP's interference with iron metabolism, thereby leading to a lower count of red cell precursors (13). The examination of hematological parameters in our study indicated a significant difference among the groups in terms of RBC and MCHC (p<0.05). Moreover, WBC counts were significantly increased in the CP group compared to the control group (p<0.05) and were like those of the control group in the OEP groups.

Table 2. Biochemical parameters measured before and after the administration of CP

Groups	Triglyceride (mg/dL)	Total Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)
Control	95.5±20.5	51.33±7.6^{a}	40.46±5.23	4.7±0.6
OEP1	95.0±34.2	52.75±4.2^{a}	53.39±5.4	4.4±1.3
OEP1+CP	108.75±24.3	55.33±9.6^{a}	34.97±3.1	7.5±1.2
CP+OEP1	123.20±37.2	58.8±8.9^{a}	36.25±4.3	7.4±1.3
OEP2	93.83±34.5	51.0±4.8^{a}	54.88±1.7	4.2±0.7
OEP2+CP	106.17±32.0	53.75±5.2^{a}	38.23±3.3	7.0±1.0
CP+OEP2	106.75±23.2	57.33±4.4^{a}	36.17±6.1	7.13±0.9
CP	132.33±31.1	69.75±6.6^{b}	32.19±4.81	8.0±0.8
Control	239.4±25.5^{ab}	15.50+0.20^{d}	19.0+2.82^{bc}	227.67±38.8^{b}
OEP1	214.83±30.0^{a}	17.38 +0.29^{cd}	21.63+5.32^{abc}	282.0±12.7^{b}
OEP1+CP	251.25±45.7^{ab}	24.33+1.08^{ab}	27.50+4.01^{abc}	198.83±14.2^{b}
CP+OEP1	282.25±32.6^{b}	26.20+1.83^{a}	29.50+2.90^{ab}	188.13±12.8^{a}
OEP2	213.5±26.9^{a}	13.14+0.42^{d}	16.25+1.32^{c}	288.17±14.1^{b}
OEP2+CP	242±28.40^{ab}	17.32+0.84^{cd}	20.0+2.12^{bc}	215.47±15.4^{ab}
CP+OEP2	252.00±46.8^{ab}	20.50+1.47^{bc}	23.83+4.82^{abc}	186.25±07.9^{a}
CP	280.25±30.65^{b}	26.40+1.72^{a}	32.14+1.52^{a}	69.88±06.4^{a}

Values are expressed as mean ± SD; p<0.05; CP: Cisplatin, OEP: Olive oil extract of propolis BUN: Blood urea nitrogen. ^{a,b,c,d} The groups in the

same column with different letters are statistically different

Table 3. Histopathological results of rat testis tissues

Groups	Control	OEP1	OEP2	CP	OEP1+CP	OEP2+CP	CP+OEP1	CP+OEP2	*p*
MTBS	9.60±0.49^{a}	8.86±1.02^{bd}	9.20±0.67^{ad}	6.73±0.82^{f}	9.33±0.66^{ad}	8.30±0.87^{bce}	8.93±0.94^{abde}	7.96±1.42^{c}	**0.001**
Seminiferous tubular diameter (µm)	310.22±35.58^{a}	272.60±39.76^{b}	272.72±52.71^{b}	258.84±23.33^{c}	284.62±33.8^{ab}	277.39±59.7^{ab}	278.2±41.1^{ab}	236.27±30.3^{c}	**0.001**

Values are expressed as mean ± SD; $p<0.05$; CP: Cisplatin, OEP: Olive oil extract of propolis; MTBS: Johnsen's mean testicular biopsy score; ^{a,b,c,d} The groups with the same letter are statistically different

Additionally, although the administration of OEP both before and after CP administration resulted in similar levels to those of the control group, no significant difference was established. Lymphocyte levels were significantly increased in the CP groups compared to the control group. On the other hand, the analysis of anemia indices revealed that the HGB levels in the OEP groups were similar to those of the control group ($p>0.05$). However, the CP group had decreased HGB levels compared to the control group. Hematocrit (HCT) indicates the volume of RBC compared to total blood volume. In other words, HCT is the percentage of cellular portion of blood to the liquid portion of blood. A low HCT level indicates anemia. MCV indicates the average size of RBC, whereas MCH indicates the weight of HGB in an average red cell. HGB indicates the amount of HGB present in blood while MCH indicates the amount of HGB in RBC alone. MCHC is the proportion of MCH to total amount of RBC independent of red cell count and size. In our study, CP had an adverse effect on blood parameters and the administration of OEP2 normalized the HCT, MCV, and MCHC levels. Meaningfully, since the administration of CP led to adverse effects on anemia indices, all the CP groups suffered anemia. This finding agrees with previous research results by Dufour et al (14). Similarly, CP causes oxidative stress in human platelets and lymphocytes, which might reflect on their life expectancy, induction of apoptosis, and ultimately reduce the number of these cells in the blood (15).

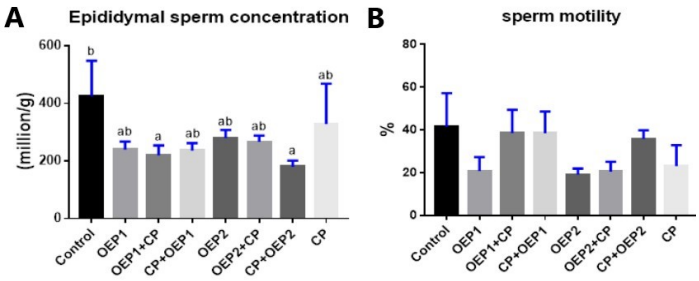

Fig. 2. (A) Epididymal sperm concentration of experimental groups, (B) Sperm motility of experimental groups

The biochemical analysis performed in our study indicated that both doses of OEP normalized the increased cholesterol levels induced by CP. Moreover, the administration of OEP prior to CP normalized the increased BUN and uric acid levels induced by CP. Interestingly; however, it was also revealed that the testosterone levels increased in the OEP groups compared to the control group. Additionally, the combined administration of CP with OEP normalized the decreased testosterone levels induced by CP, particularly in the groups administered OEP prior to CP. Propolis and its primary component, CAPE, have been shown to have antitumoral effects and to be effective on CP-induced toxicity. A previous study investigated the cytotoxic effects of ethanol extracts of Turkish propolis on human tumoral cell lines and reported that propolis showed high cytotoxic effect on five tumoral cell lines (16). Ibrahim administered CP both before and in combination with propolis in rats and reported that CP led to significantly decreased testicular weight, induced distorted seminiferous tubules, cellular disorganization, wide separation of intertubular space, cytoplasmic vacuolation, and pyknotic nuclei (17). Additionally, CP also increased the area of collagen fibers, increased optical density of nuclear factor-KB (NF-KB) immunoreactivity, and decreased the area of claudin 11 immuno-expression in the spermatogenic cells. The authors concluded that the blockade of NF-KB activation was achieved by propolis, and this activation could be an effective strategy for protection against CP-induced testicular damage if propolis is administered prior to the administration of CP.

CAPE is an active component of poplar-type propolis with antioxidant, anti-inflammatory, antiviral, immunomodulatory and wound-healing acceleration properties (18). CAPE has been shown to completely block the production of reactive oxygen species (ROS) in human neutrophils at a concentration of 10 µmol, to protect liver against diabetic oxidative damage, to provide protection against CP-induced hepatic and renal damage (19-22). Yılmaz et al. investigated the anticlastogenic effect of CAPE on CP-induced chromosome aberrations in rat bone marrow cells and reported that the administration of a single dose of CAPE led to a significant reduction in the total number of chromosomal aberrations and abnormal metaphases induced by CP (23). In addition, the total number of aberrations and abnormal metaphases was lower in the CAPE+CP group compared to the CP group, although no significant difference was established. In another study, Tohamy et al. examined the anti-histopathologic, antioxidant, and anti-mutagenic effects of water extracts of bee pollen (140 mg/kg bw per day) and water-soluble derivative of propolis (2.8 mg/kg bw per day) on CP-induced hepatic, renal, testicular toxicity, and

genotoxicity in mice and on the oxidant/antioxidant status in the tested organs (24).

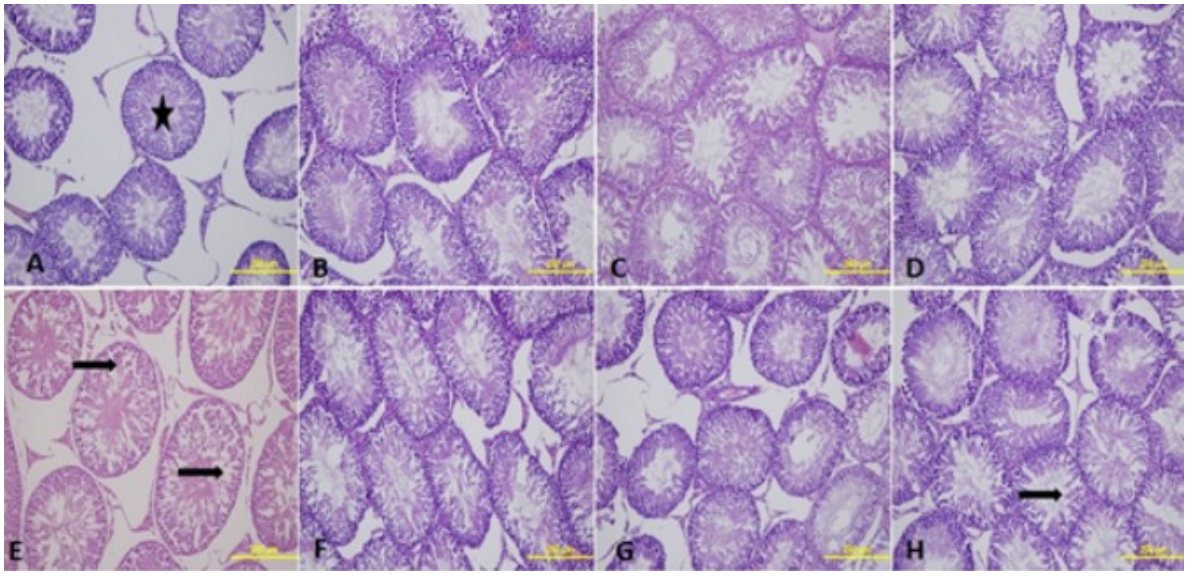

Fig. 3. (A) Normal seminiferous tubule in the control group (star). Seminiferous tubule degeneration and epididymis tissues in the (B) OEP1, (C) OEP2, (D) OEP1+CP, (E) CP, (F) OEP2+CP, (G) CP+OEP1, and (H) CP+OEP2 groups (arrows) (H&E X200)

The authors reported that both bee pollen and propolis provided significant protection against CP, leading to a significant decrease in the lipid peroxidation level and a significant increase in both glutathione content and catalase activity. Propolis and its compounds such as caffeic acid, galangin, quercetin, and chrysin have been extensively shown to have a protective role against ROS (25). For instance, Kart et al. reported that CAPE, an active component of propolis, exerted a protective effect against CP-induced hepatotoxicity and normalized the tissue glutathione (GSH) level and xanthine oxidase (XO) activity (26). Moreover, although histopathological alterations such as necrosis in hepatocytes, mononuclear cell infiltration, Kupffer cell proliferation, sinusoidal congestion, and hydropic degenerations were observed in the CP group, these alterations were less frequent in the CP+CAPE group. The increased XO activity induced by CP leads to increased production of superoxide and hydrogen peroxide, both of which are responsible for the toxic effects of CP. A previous study demonstrated that CAPE decreased XO activity and ameliorated CP-induced ototoxicity (27). In our study, combined administration of CP and OEP resulted in a histological structure like that of control group in the OEP1+CP, OEP2+CP, CP+OEP1, and CP +OEP2 groups. The adverse effects of CP on the histological structure of kidney, liver, and testis have been extensively documented. Shirwaikar et al. reported that the administration of CP (5 mg/kg bw) in rats resulted in interstitial edema, inflammatory cell infiltration, epithelial degeneration, blood vessel congestion, tubular casts, and glomerular congestion (28). Ozen et al. revealed that CP led to extensive epithelial vacuolization, proximal tubular necrosis, swelling, and tubular dilatation in rat kidney (21). Sawhney et al. showed that CP led to a significant decrease in seminiferous tubular diameter and a severe disruption of seminiferous epithelium and decreased the populations of specific cells and spermatids (29). Nephrotoxicity induced by CP morphologically involves necrosis and apoptosis in proximal tubules of distal nephron (30). Additionally, a previous study reported that CP led to multiple histopathological alterations including fibrosis necrosis, and hydropic degenerative changes in liver, tubular, and glomerular degeneration with albuminous cast deposition in the kidney as well as a disruption of seminiferous tubular cells with germ cell loss, particularly spermatids and sperms, and congestion of blood vessels in the interstitial tissue of testes (26). Finally, Ceylan et al. argued that CP has a negative effect on the testicles and that these negative effects can be prevented with CAPE (31).

As a conclusion, in addition to its therapeutic effects, CP was shown to induce toxicity in numerous organs and systems due to its side effects. The presence of these side effects can be confirmed by the aberrations in blood and biochemical parameters. Propolis, a natural product with numerous useful biological effects, was shown to a have protective as well as amelioration and normalizing effect on CP-induced damage. Therefore, future studies conducted with higher doses and a mechanism of action are needed to substantiate our findings.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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Review Article

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Coronavirus (COVID-19) infection and antenatal care in pregnancy

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Abstract

In this review, we evaluated the protection of healthy pregnant women from the COVID-19 infection caused by the new type of coronavirus SARS-CoV-2 and the antenatal care with suspected or diagnosed COVID-19 in the light of current literature.

Keywords: antenatal care, COVID-19, pregnancy, infection

1. Introduction

The new Coronavirus infection (COVID-19), also called SARS-CoV-2, rapidly expanded to the remainder of China and beyond and turned into a pandemic after being reported as an urgent global public health problem for the first time in December 2019 in Wuhan (1-3).

Coronavirus is a kind of enveloped, non-segmented, positive sense ribonucleic acid (RNA) virus and coronaviridae belongs to the nidovirales family (4). It is known that pregnant women are disproportionately affected by respiratory diseases due to increased morbidity of infection and maternal mortality. Although most of the coronavirus infections have few symptoms, two β-coronavirus epidemics caused by SARS-CoV and MERS-CoV led to severe acute respiratory syndrome and over ten thousand patients in the past 20 years. Mortality has been reported at the rate of 10% for SARS-CoV and 37% for MERS-CoV (5-10). COVID-19 is a member of the identical β-coronavirus subgroup and this virus shows approximately 80% and 50% genomic similarity for SARS-CoV and MERS-CoV, respectively (11). COVID-19 is effused by direct contact and/or droplets (body secretions, somebody's eye, nose, or mouth, or an open incision, contact with injury or abrasion). The universal mortality proportion of COVID-19 infection was reported as 3.4% (12).

Pregnancy is a special condition which makes women suitable for respiratory complications of viral infections. Respiratory tract of pregnant women infected with viruses brings the risk of developing more severe disease due to immunological changes and cardio-pulmonary systems during pregnancy. In 2009, 1% of the patients infected with H1N1 were pregnant women and 5% of all deaths related to H1N1 occurred in pregnant women (13). Both SARS-CoV and MERS-CoV infections are responsible for endotracheal intubation, acceptance to the intensive care unit (ICU), serious complications during pregnancy, including hospitalization, renal failure and death (9, 10).

Mortality rate of pregnant women infected by SARS-CoV is about 25% (9). There is no certain and clear information regarding that if pregnant women with COVID-19 infection are more sensitive than other healthy population. There is no evidence found about COVID-19 infection causing intrauterine and congenital infection. Also, it is difficult to make a final decision about this clinical condition because the COVID-19 cases in pregnant women are low (14, 15).

2. Transition types of COVID-19 infection

The disease is mainly transmitted by droplets. It is spread by the droplets emitted by ill people through coughing and sneezing with the hands of other people, and then by touching and touching the mouth, nose or eye mucosa (16). Disease transmission through droplets does not occur at a distance of more than 2 meters. It can be contagious because viruses can be identified in respiratory tract excretions of asymptomatic people (17). The incubation period is thought to be five days (2-14 days) on average. However, it has been reported that among the recent cases, there are also those without any symptoms of contact with infected people (18).

3. Clinical findings of COVID-19 infection in pregnant women

Widespread symptoms of infection are respiratory symptoms, pyrexia, cough and dyspnea. However, less common clinical findings have also been reported. Pneumonia, severe acute respiratory infection, kidney failure, and likewise death may occur in some people with more severe illness. The most common symptoms in COVID-19 patients during pregnancy

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are prexia (87.9%), dry cough (67.7%), weakness (38.1%), inability to smell and taste (34%), sputum (33.4%), dyspnea (18.6%), myalgia or joint pain (14.8%) (17).

4. Laboratory findings

The white blood cell count may change in patients with COVID-19. Leukopenia and leukocytosis have been described; nevertheless, lymphopenia is still the widespread finding. Lactate dehydrogenase (LDH) and ferritin levels elevation are frequent and furthermore, elevated aminotransferase levels are defined. Numerous patients with pneumonia have normal serum procalcitonin levels at first diagnosis. On the other hand, higher levels may be possible in patients who require ICU. Elevated D-dimer levels and severe lymphopenia have been correlated with mortality (1, 19).

5. Precautions to take during pregnancy

Pregnant women ought to come after the identical recommendations as non-pregnant people to prevent exposure to the virus.

6. Suspected or diagnosed infection during pregnancy

Clinical symptoms, laboratory and radiological findings have the same characteristics as non-pregnant individuals. The clinical course of the disease can be mild (no symptoms or flu-like symptoms), moderate-severe (dyspnea, hypoxia, or lung involvement >50% in imaging), or critical (respiratory distress, shock, and multiorgan failure) (20). Available data show that pregnancy and delivery do not worsen the clinical process of Coronavirus infection and most infected mothers recover before giving birth (21, 22). Severe inflammatory response and cytokine storm are observed in critically ill COVID-19 cases; It is not known whether the physiological immunosuppressive state of pregnancy affects the formation and effect of this response. In addition, it is advantageous for pregnant patients to be in the young age group, the risk of severe course increases in pregnant women with comorbid diseases as in other adults. In pregnant women who develop pneumonia with COVID-19 infection, although the need for intensive care unit is at the same rate as their same age women, preterm delivery and cesarean rates seem to be increased (22, 23).

7. Pregnancy complications

There are data showing that third trimester pregnancies are affected more, rather than early-term pregnancies. The frequency of cesarean section seems to be increased due to preterm labor, premature rupture of membranes, preterm delivery, preeclampsia, and fetal distress, especially in pregnant women who develop pneumonia after COVID-19 infection (24-26). In 41 pregnant patients who had COVID-19 infection, 41.1% had preterm labor at <37 weeks, premature rupture of the membrane in 18.8%, preeclampsia in 13.6% and 91.1% were delivered by cesarean section (26). A birth decision made with the thought that it will improve the mother's symptoms related to the lungs may worsen the current situation, and there is no clear information on this issue yet.

Miscarriage has not been reported for early pregnancy. Theoretically, high fever in the first trimester may affect organogenesis and lead to congenital anomalies. Since it is not yet known whether there will be an increase in the frequency of congenital anomalies with COVID-19 infection, it may be recommended to patients postpone planned pregnancies. There is no termination in patients who had COVID-19 infection in early pregnancy.

8. Vertical transition

In a review in which 38 pregnant women with COVID-19 infection and their newborns were evaluated, no vertical transition was found (27). Second day-onset pneumonia which has been associated with postnatal contact in 3 newborns are reported (28). According to the clinical data available so far, regarding the pregnancies with COVID-19 infection in the late period, there are no proof of vertical transition. Up to the present, there is not enough data on the safety of breastfeeding and separation of mother and baby. Isolation seems the best option in the presence of severe maternal infection. If the patient is asymptomatic or mildly affected, breastfeeding can be performed with protection rules.

9. COVID-19 diagnosis during pregnancy

Submit for testing in the entity of respiratory symptoms for instance fever or dyspnea, cough, or in the presence of suspected contact history; the place where the test will be performed should not be an obstetrics outpatient clinic or delivery room.

Chest radiography contains very low radiation (0.0005 to 0.01 mGy). If indicated, thorax CT can also be carried out because it contains low dose radiation (0.01 to 0.66 mGy) that does not cause fetal anomaly or pregnancy loss. Pulmonary ultrasonography is also an option to show pneumonia in pregnant women (29).

10. How should routine antenatal care be in asymptomatic pregnant?

During the examination of pregnant women without symptoms in line with the recommendations of ACOG and SMFM, it is appropriate to limit the number of people in the polyclinic room, widen the antenatal follow-up intervals, and limit obstetric ultrasonography with accurate indications such as the need for fetal anomaly screening, fetal development and placental monitoring (30, 31). In this period, applying glucose load tests to the risky group and using a cell free DNA test instead of a combined test will shorten the period of pregnant women in the hospital. If possible, pregnancy follow-up by teleconference method, home blood pressure measurements, and fetal movement follow-up are among the recommendations (32). Our recommendation is to limit the antenatal follow-ups to weeks 12, 20, 28, and 36 of gestation when ultrasound and laboratory follow-ups can be performed

in asymptomatic pregnant women without perinatal risk factors. Healthcare professionals must wear a surgical mask during outpatient clinic examination. Relatives of the patient should not be allowed inside, but video shooting may be supported. It should also be taken into account that pregnant women experience social anxiety during this period.

11. Approach to COVID-19 infected pregnant

Fetal monitoring should be arranged according to gestational age, patient's comorbid diseases, and obstetric history. Maternal oxygen saturation (SaO_2) should be kept above >95%, if SaO_2 is <95%, PaO_2 should be measured in arterial blood gas. PaO_2 should be >70 mm-Hg to provide adequate oxygen support from mother to fetus. LMWH is recommended for these pregnant women as venous thromboembolism prophylaxis (eg, enoxaparin 40 mg subcutaneously daily or 1 mg/kg [commonly rounded to the nearest 10 mg] subcutaneously every 24 hours). If delivery occurs, postpartum 10 days prophylaxis should be continued.

Hydroxychloroquine and chloroquine are used in pregnant women for treatment in moderate and severe cases. Although fetal ocular toxicity has been reported with these agents in animal studies, no such effect has been observed in humans and is also available in pregnant women with SLE disease. It can be combined with azithromycin. Since both have a risk of prolonged cardiac repolarization and QT interval, ECG and electrolyte control are required before starting. Remdesivir can also be used in pregnancy. Ribavirin and baricitinib are considered teratogenic, their use is not recommended in pregnant women. Lopinavir can be used in pregnancy; however, its effectiveness against COVID-19 has become controversial in new publications. In the use of Oseltamivir in influenza cases in pregnant women, no risk has been detected for the fetus (33). Favipravir is contraindicated in pregnant women (16).

12. Classification of severity of disease

The National Institutes of Health (NIH) classified degrees of disease severity in five groups in non-pregnant people as follows respectively (34):

-Asymptomatic or presymptomatic infection-Positive test for SARS-CoV-2 but there are no signs of illness.

-Mild illness-The presence of any clinical findings or symptoms (for instance, fever, cough, sore throat, exhaustion, headache, myalgia) without shortness of breath, dyspnea, or abnormal chest imaging.

-Moderate illness-Finding of lower respiratory disease by clinical evaluation or imaging and a saturation of oxygen (SaO_2) ≥94% on room air at sea level.

-Severe illness-Periodicity of respiration >30 breaths per minute, SaO_2 <94% on room air at sea level, rate of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300, or lung infiltrates >50%.

-Serious illness-İnsufficiency of respiration, septic shock, and/or multiple organ dysfunction.

13. Medical treatments we use to manage pregnancy complications

13.1. Antenatal betamethasone

CDC reports that glucocorticoid use should be avoided in MERS-CoV positive patients, as it may delay viral clearance and increase mortality. However, there is no explanation about the use of antenatal steroids in COVID-19 positive pregnant women with preterm birth risk.

ACOG reports that in COVID-19 positive pregnancies up to 24 + 0 to 33 + 6 weeks if it is predicted that delivery may occur within seven days, betamethasone can be used to reduce neonatal morbidity and mortality. However, since the benefit of antenatal steroids between 34 + 0 and 36 + 6 weeks is not clear, it is not recommended for COVID-19 positive pregnant women for now.

13.2. Low-dose aspirin

ACOG recommends the continuation of low-dose aspirin, which was started with an indication such as prevention of preeclampsia (35).

13.3. Tocolysis

Nifedipine is recommended as the first choice if there is an indication to initiate tocolysis to a pregnant woman with suspected or infected COVID-19. Although there are various speculations about ibuprofen, WHO emphasized that NSAIDs should not be avoided (36). Therefore, indomethacin, an NSAID, can also be used as tocolysis.

13.4. Magnesium sulfate usage

If $MgSO_4$ is intended to be used due to its fetal neuroprotective effect or for eclampsia prophylaxis, it should be kept in mind that it may cause respiratory depression and close monitoring is recommended.

13.5. Vaccination

Numerous vaccines are being evaluated for prevention of COVID-19, but pregnant/lactating people have been excluded from these trials. The first vaccines to become clinically available are based on mRNA or protein subunits and do not contain infectious virus (either SARS-CoV-2 or a vector virus). There is no routine vaccination schedule to protect pregnant women from COVID-19 infection, and there is recently no clear consensus on vaccination during pregnancy. If a person conceives of after implementation the first dose of the COVID-19 vaccine series, the second dose ought to be performed when there is an indication.

Vaccination should be timed so that patients do not receive a COVID-19 vaccine within 14 days of administration of a routine vaccination, such as the Tdap or influenza. However, anti-D immunoglobin does not interfere with the immune response to vaccines, so timing of administration for prevention of alloimmunization is based on standard clinical protocols.

As a result, although the fetal, newborn, and maternal effects of available vaccines have not been studied in

preclinical trials, experts believe they are unlikely to pose a risk for pregnant people or breastfeeding newborns based on how mRNA vaccines work, while observational studies have demonstrated that pregnant people who become infected with SARS-CoV-2 are at increased risk of severe maternal disease and adverse pregnancy outcome.

14.Approach to pregnant women recovered from COVID-19

It is important to follow up fetal development a few weeks later, especially in patients who recovered from severe infections (26). It is not known whether it will cause intrauterine growth retardation because of limited data. Fetal growth retardation and thrombotic vasculopathy in the placenta have been reported in patients with previous SARS (37).

14.1. Delivery timing in pregnant women infected with COVID-19

Delivery is not recommended for pregnant women with mild COVID-19 infection without obstetric or medical indication for preterm delivery; The possibility of transmission to the newborn is minimized by giving birth after a negative test result is acquired or after the isolation is eliminated (30).

In serious patients, many aspects should be considered while making the decision to give birth. There is insufficient information about whether the mother's respiratory symptoms will improve with birth and that delivery, when maternal symptoms are present, may increase postnatal transmission to the newborn. Also, maternal antibodies will not have time to develop passive neonatal immunity (38).

For pregnant women with COVID-19 who were hospitalized for pneumonia but were not intubated after the 32nd week, delivery can be planned. In this case, the aim is to decide to perform the birth before the mother's respiratory symptoms deepen and without maternal hypoxemia risking the fetus. However, it is not recommended before the 32nd week of gestation.

In intubated pregnant women, if the patient is stable after 32 weeks of gestation, delivery is recommended. However, this may aggravate the birth symptoms. In pregnancies less than 32 weeks, which has exceeded the viability limit, the decision should be individualized, taking into account the fetal monitorization and the mother's condition (39). The timing and type of delivery ought to be individualized according to the clinical condition of the pregnant, gestational week and the status of the fetus.

15.Negative pressure isolation chambers

Negative pressure isolation chambers are necessary for safe labor and care of newborn. Women with critical illness should be followed in isolation rooms of intensive care units that have negative pressure. Antenatal examination and delivery of pregnant patients infected with COVID-19 ought to be in the negative pressure isolation room of the delivery room (40). When managing infected pregnant women, personal preventive equipment ought to be available for healthcare professionals.

16.Conclusion

To conclude, there are some key points to consider in the management pregnant women infected with COVID 19. Management of infected pregnant woman ought to be carried out multidisciplinary with obstetrician, primatologist, intensive care specialist, anesthesiologist, midwife, virologist, neonatologist in tertiary hospitals. These women ought to be told about the possible risk of morbidities and mortalities during pregnancy.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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Treatment of Alzheimer's disease by natural products

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Abstract

Alzheimer's disease is a neurological disorder that progressively affects the brain cells. It is sometimes accompanied with dementia and commonly seen in elderly people. It ultimately leads to impaired cognitive functions and loss of memory. Widely used drugs such as donepezil and galantamine successfully abate mild to moderate symptoms of the disease though they have profound side effects. Natural products have been a source of medicine for over thousands of years. Plant secondary metabolites such as alkaloids, flavonoids, terpenoids, etc. are major sources of such medicines. In recent times, the use of natural products to treat various diseases has gained a lot of popularity. Extensive research is being undertaken to identify and isolate various natural products that can be used to not only reduce the disease symptoms but also as a permanent cure. The review article aims to summarize the effects of various natural products and their neuroprotective mechanisms that can be helpful to cure Alzheimer's disease.

Keywords: AChE, AD, Aβ aggregation, resveratrol, plant secondary metabolites

1. Introduction

Earth's biodiversity is a major natural resource. The variation in the climatic conditions of the earth is what makes the living system so diverse. Biodiversity is considered as a major natural resource because of the various products obtained from it. Out of these products the ones that have the highest importance to us are the ones with medicinal properties. Such natural products form an important form of treatment and healthcare. Nature has been a continuous source of biological agents and natural products used as traditional medicines. These come from aquatic or terrestrial plants, animals, macro- and micro-organisms (1).

Herbal medicines were first used in China several thousands of years ago according to the records. Likewise, Ayurvedic medicine of India is also thought to be 5000 years old (2). There are many Islamic Hadiths that mention the curative properties of *Nigella sativa* also known as black cumin seeds. It specifically mentions that *N. sativa* has cure to every illness except death (3-5). World Health Organization (WHO) report suggests 75% of the global population still depends on herbal medicines for health care (2).

Natural products have therapeutic effects on many chronic as well as acute diseases (6). The plants as well as animal derived products have served as the base of many drug discoveries for many diseases. They have not only been used for the treatment of common diseases, but recent studies have shown that certain natural compounds extracted from plants may have the ability to treat chronic diseases like Alzheimer's Disease (AD) (1).

AD is progressive disorder that leads to the degeneration of brain cells and affects majority of the aged people globally. Most people affected with this disease lie in the age group of 65 to 75 years. Neuroprotective effects of biological agents against AD have been widely studied and further studies related to the mechanism of action may help us to gain an insight as to how these products can be used as drugs for efficient therapies. The knowledge of natural products as well the details regarding the onset of AD may open new vistas in the world of drug development using natural products (7).

2. Alzheimer's disease and its treatment

AD is related to aging. The progressive neuro-degeneration results in loss of memory and impaired cognitive functions. According to 2020 report of Alzheimer's Association, nearly 508 million people affected by AD in USA alone and the numbers are expected to increase with coming years. So far there is no satisfactory cure to this disease. The mechanism of the disease is completely unknown yet however, overproduction of β-amyloid proteins, involvement of oxidative stress and hyperphosphorylation of tau proteins are supposedly the main reasons. This causes disruption of synapsis and neurons in the hippocampus and cerebral cortex,

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decline in cognitive functions and loss of memory. Apart from these, many hypotheses have been postulated that aim at explaining the cause of AD (8).

The current drugs available in market for AD treatment are mainly designed based on the cholinergic hypothesis and the oxidative stress hypothesis. Donepezil, rivastigmine, galantamine, memantine are the FDA approved drugs for the treatment of this disease. These drugs alleviate the symptoms caused by the degeneration of cholinergic neurons and disrupted transmission but do not delay the onset of AD. Apart from memantine, all the other is mainly Alzheimer's disease (AChE) inhibitors. The efficacy of these drugs is poor to moderate and drug response varies individually based on various genetic factors (7, 9).

Metabolism of galantamine and donepezil takes place in the liver. Main enzymes involved in the metabolism are CYP3A4, CYP2D6 and CYPA12. Rivastigmine is bio transformed through carbamylation. Donepezil and galantamine primarily inhibit AChE but also act on BChE, whereas rivastigmine inhibits both equally. Memantine on the other is an antagonist of the NMDA (N-methyl-D-aspartate) receptor and inhibits the effect of increased glutamate levels which lead to neuronal cell death (9).

According to a study reported by Santamaria et al. (9), certain mild to moderate side effects of these drugs were observed over a certain period. It was noted that patients administered with donepezil developed nausea, bradycardia, abdominal pain, diarrhea, dizziness, orthostatic hypotension accompanied with headache, rhinitis, muscle cramps and cholinergic symptoms. Donepezil also causes syncopal episodes. Also due to enhanced activation of visual association cortex, donepezil has been known to cause nightmares during REM sleep (10).

Administration of rivastigmine led to anorexia, diarrhea, nausea and vomiting after about 26 weeks. Furthermore, people treated with a combination of donepezil, rivastigmine and galantamine suffered from epigastric pain, diarrhea, irritability, vertigo, dizziness, abdominal pain, nausea, cardiovascular events, rash, depression, perspiration, hallucination, falls, agitation, and insomnia. When administered with a combination of just donepezil and rivastigmine, patients suffered from vomiting, diarrhea, nausea, anorexia, abdominal pain, falls, anxiety, agitation, dizziness, headache, and urinary tract infection (9).

3. Natural products for the treatment of AD

As discussed earlier, the FDA approved drugs for AD cause a lot of side effects thus, researchers have undertaken studies to discover natural remedies that may not only alleviate the disease but have the potential to completely cure it. A promising cure for AD has not yet been found, but a number of natural compounds extracted from various plants have successfully been used to reduce the symptoms of AD. These products belong to various classes of secondary metabolites produces by different plants. A brief classification of the products according to the class is given below.

3.1. Flavonoids

Flavonoids (Fig. 1) are plant secondary metabolites, which contain 15-carbons, two phenyl rings and a heterocyclic ring (11). All types of flavonoids have a capacity to act as antioxidant. They have an additive effect to endogenous scavenging compounds. In addition, flavonoids contain anti-AChE activity. Both of these properties can be harvested for the treatment of AD. Following flavonoids have been found to be effective against AD (12).

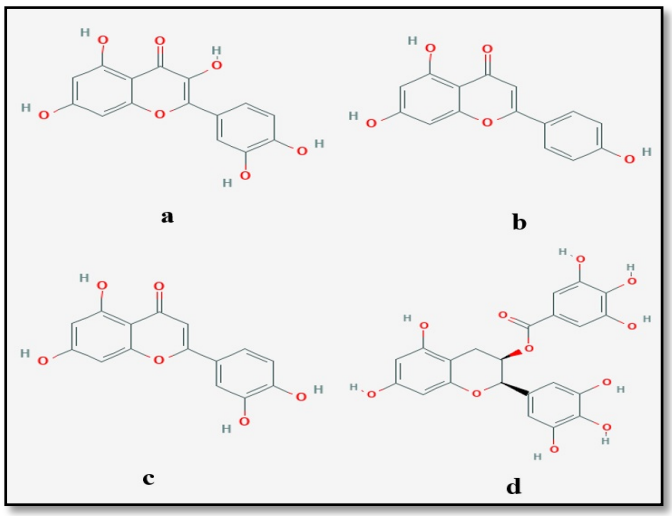

Fig. 1. Structures of flavonoids, (a) Quercetin (National Center for Biotechnology Information. PubChem Compound Database; CID=5280343), (b) Apigenin (National Center for Biotechnology Information. PubChem Compound Database; CID=5280443), (c) Luteolin (National Center for Biotechnology Information. PubChem Compound Database; CID=5280445), (d) Epigallocatechin gallate (National Center for Biotechnology Information. PubChem Compound Database; CID=65064)

3.2. Quercetin

Quercetin (Fig. 1a) is present in a variety of foods such as apples, onions, berries, tea, etc. It is a flavone type flavonoid that possesses antioxidant properties and scavenges ROS. This free radical scavenging property could inhibit lipid peroxidation (7). It shows strong inhibition of 5-lipoxygenase and cyclooxygenase-2 (COX-2), which are involved in the production of arachidonic acid and eicosanoids. It also inhibits prostaglandins production and nuclear factor κB (NF) activation (13).

In the brains of Sprague-Dawley rats, quercetin inhibited manganese (Mn) induced apoptosis and inflammatory response by the activation of nuclear factor E2-related factor 2 (Nrf2) and hem oxygenase (HO-1) and inhibits NF-κB activity (13, 14). Quercetin doses up to 10 μM showed anti-amyloidogenic effects thus inhibiting Aβ fibrils. It also reduces the apoptosis in neuronal cells due to Aβ, however induces cytotoxicity at levels higher than 40 μM. Recently, solid lipid nanoparticles of quercetin have shown better

penetration in the blood-brain barrier (15). The nano-encapsulated quercetin shows enhanced neuroprotective effect. The elimination of quercetin is exceptionally low which might lead to its accumulation in the body (16). Though neuroprotective in nature, increase in the levels of quercetin may lead to neurotoxicity and carcinogenicity. Therefore, further toxicology research is required to derive a successful drug from this compound (7).

3.3. Apigenin

Apigenin (Fig. 1b) is a yellow crystalline non mutagenic flavone obtained from *Apium graveolen* (celery), parsley, artichoke, and basil. When ingested, apigenin showed high intestinal permeability (7). It is absorbed in the duodenum. Apigenin exhibits strong potent chelating and antioxidant properties (15). It reduces the toxicity of β amyloid (Aβ) induced by copper by binding with transition metals (17). Thus, it controls free radical formation by preventing metal ion participation in the radical reaction. It is found to be effective in scavenging free radicals such as oxygen, nitric oxide, and superoxide anion (15). Apigenin also shows actions like that of estradiol because of which, apoptosis of human neuroblastoma cells induced by oxidative stress in controlled. It is also a potent inhibitor of CYP450 (17). In an experimental model, APP/PS1 double transgenic AD mice when treated with 40mg/kg apigenin for three months, mice showed improved memory retention and learning deficits which was tested using Morris Water Maze. Thioflavin-T (THT) staining proved that the mice showed reduced levels of amyloid deposits (18,19). A reduction of glutamate induced toxicity was seen in rat hippocampal brain and increased intracellular Ca^{+2} by extracellular-signal-regulated kinase/cAMP-response element binding protein/brain derived neurotrophic factor (ERK/BDNF/CREB) pathway (17). 10-50 μM apigenin was able to reduce apoptosis induced by ER stress inducer thapsigargin and brefeldin A. This was achieved by scavenging ROS and inhibiting the activation of caspase-12 (7).

3.4. Luteolin

Luteolin (Fig. 1c) is a yellow crystalline flavonoid found in many plants of bryophyte, pteridophyta, pinophyta and magnoliophyte families. Among dietary products, it is found in celery, oregano, carrots, peppers, olive oil, thyme, etc. (20). It has antioxidant, anti-inflammatory, and antimicrobial activities (7). It can cross the blood brain barrier. HepG2 cells when treated with 50 μM luteolin, showed reduced levels of tumor necrosis factor α (TNF-α) induced inflammation. This was done by suppression of NF-κ light chain enhancer of B cells (20). In several *in vitro* and in vivo models luteolin has been seen to regulate various cytokines (21). Results of an experiment conducted by Ali et al. (22) on Drosophila revealed that luteolin bound to AChE and Aβ peptides which led to the inhibition of AChE and prevented Aβ aggregation. Through THT staining, they also reported decreased Aβ aggregation. Luteolin action also partly includes inducible nitric oxide synthase (iNOS) function, iNOS expression and NO production (22). Reports suggest that 20-100 μM luteolin reduces zinc induced hyperphosphorylation of tau proteins by its antioxidant activity and regulation of tau phosphatase/kinase system. Moreover, luteolin can control the expression of amyloid precursor protein (APP), reduce the formation of Aβ proteins (15), inhibits caspase-dependent apoptosis (23). It relieves the learning and understanding problems, strengthens the antioxidant system, decreases the lipid peroxidation and inflammation of the brain tissue (16).

3.5. Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (Fig. 1d) is a flavonoid-type catechin found in *Camellia sinensis*, carob flour. Apples, berries like blackberries, strawberries and raspberries, nuts, plums, peaches, avocados, onions have lower amounts of epigallocatechin-3-gallate (7, 15). The strong antioxidant effects of catechins have been studied in cancer, and AD. Activation of antioxidant enzyme, protein survival genes and APP processing are some of its biological roles. Several *in vitro* studies have reported neuronal protection from Aβ induced damages (13). Certain reports indicate an enhanced synaptic plasticity in brain due to epigallocatechin-3-gallate's interaction with AChE receptors and its direct binding with Aβ protein inhibits β sheet formation (17). Epigallocatechin-3-gallate is absorbed at the small intestine by passive diffusion; however, high concentrations saturate these tissues. Important roles of Epigallocatechin-3-gallate include:

- Enhancement of glutathione peroxidase activity
- Inhibition of AChE activity, NO metabolite formation and ROS generation (7)
- Inhibition of γ-secretase enzyme activity
- Prevention of lipopolysaccharide induced memory loss and apoptosis
- Reduction of the expression of inflammatory factors; TNF-α, IL 1β, IL-6, inducible nitric oxide synthase (iNOS), COX-2
- Prevention of astrocyte activation in neuronal cells (15)
- Enhancement of neurite outgrowth (24)
- Cholinergic transmission enhancement (24)
- Cognitive function improvement (24)

3.6. Alkaloids

Alkaloids (Fig. 2) are naturally occurring organic compounds containing basic nitrogen atom. They can be extracted from a several organisms such as bacteria, fungi, plants, and animals. The pharmacological properties of alkaloids include antiasthma, anticancer, antimalarial, analgesic, etc. Alkaloids exert anti-AD effect by activating the cholinergic system and exciting the central nervous system (25). Alkaloids that can be used in treatment if AD are:

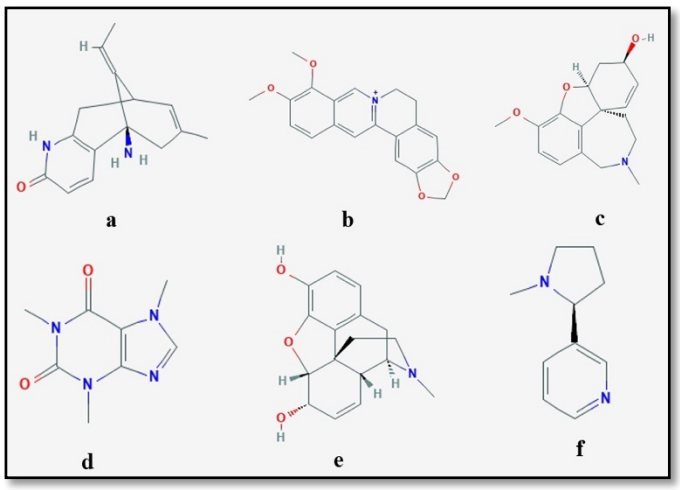

Fig. 2. Structures of alkaloids, (a) Huperzine A (National Center for Biotechnology Information. PubChem Compound Database; CID=44461111), (b) Berberine (National Center for Biotechnology Information. PubChem Compound Database; CID=2353), (c) Galantamine (National Center for Biotechnology Information. PubChem Compound Database; CID=9651), (d) Caffeine (National Center for Biotechnology Information. PubChem Compound Database; CID=2519), (e) Morphine (National Center for Biotechnology Information. PubChem Compound Database; CID=44461111), (f) Nicotine (National Center for Biotechnology Information. PubChem Compound Database; CID=86594)

3.7. Huperzine A

Huperzine A (Fig. 2a) is a sesquiterpene alkaloid isolated from the moss *Huperzia serrata* belonging to the family Lycopodiaceace (17). The *H. serrate* extracts are used for improving the memory (7). It is a reversible and selective cholinesterase inhibitor. It is highly non-polar and hence passes through the blood-brain barrier easily (16). It reduces inflammatory loss induced D-galactose-induced in rat hippocampus via NF-kB and inhibiting neurovascular damage and blood brain barrier impairment. In neonatal rats having hypoxia-ischemia induced brain injury, huperzine A prevented cognitive decline and brain damage. In rats with transient focal cerebral ischemia huperzine A is involved in protection through cholinergic anti-inflammatory pathway (13). In streptozotocin induced diabetic rats, it protects full stop is not required against cognitive damage (23). Via activation of Wnt/β-catenin and increasing phosphorylation of GSK-3β and PKC/MAPK pathways by triggering β secretases. Huperzine A (0.1 mg/kg) enhances cognitive impairment (17).

The important roles of Huperzine A in the body include:

- Inhibition of several apoptotic factors, including caspase 3, Bax, and p53.
- Regulation of the production and secretion of the nerve growth factor.
- Restoration of the activity of respiratory chain complexes and prevention of Aβ-induced ATP reduction and mitochondrial swelling (7).
- Reduction of the clumping of β-amyloid and oligomeric A amount in the cortex and hippocampus.
- Inhibition of the NMDA receptor and potassium channel in the brain (15).

Clinical trials of Huperzine A gave good results without any side effects such as dizziness, nausea, gastroenteric problems, headaches, and low heart rate which gives an advantage to Huperzine A compared to other AChE inhibitors for the treatment of AD (16).

3.8. Berberine

Berberine (Fig. 2b) is a chemical found in plants. It is an isoquinoline alkaloid and the main sources are *Coptis chinensis*, *Berberis aquifolium, Berberis vulgaris, Berberis aristata,* etc (8). It is used as a natural yellow dye due to its characteristic yellow fluorescence upon UV activation (15). Berberine is mainly isolated from the *Coptis* rhizome. It mainly has anti-inflammatory, cardio-protective, neuroprotective and antioxidant effects. Berberine in male Wistar rats with focal cerebral ischemia reduced infarct volume and brain edema by acting as an anti-inflammatory agent (26, 27). In rats with memory impairments induced by scopolamine, berberine reduced the production of TNF-α, COX-2, and IL-1β. It also restored the levels of CREB and BDNF (13). Other major roles of berberine include:

- Potent AChE inhibitor, butyrylcholinesterase and monoamine oxygenase.
- Prevention of Aβ plaques formation and aggregation in neuronal cells.
- Inhibition of the production of pro-inflammatory cytokines such as IL-6 in Aβ-activated primary microglia and murine microglial cell line (7).
- Reduction of the expressions of COX-2 and iNOS.
- Prevention of inflammation via by inhibiting NF-κB, phosphoinositide 3-kinase and MAPK signaling pathways.
- Scavenging free radicals such as nitric oxide, peronitrite (ONOO-), hydrogen peroxide, and 1, 2-diphenyl-2-picryl hydrazyl radicals.
- Reducing lipid peroxidation (28).
- Activation of anti-oxidative enzymes such as superoxide dismutase and glutathione peroxidase to protect against oxidative stress (7).

3.9. Galantamine

Galantamine (Fig. 2c) is an alkaloid obtained from *Galanthus* and *Narcissus* species of the family Amaryllidaceae. The structure of galantamine suggests it is a phenylalanine and tyrosine derivative. This compound exhibits many types of pharmacological activities, particularly on central nervous system. It is a potent AChE inhibitor and is thus useful for symptomatic treatment of AD. Galantamine controls the oxidative neuronal damage by scavenging ROS through: (1)

inactivation of P2X7 receptors, (2) protecting mitochondrial membrane potential, and (3) preventing changes in the membrane fluidity (29).

3.10. Caffeine

Caffeine (Fig. 2d) is a common dietary product found abundantly in tea, coffee, cola, and cocoa. It has been reported as a selective non-competitive inhibitor of AChE (24). It is a strong stimulant of the central nervous system via its ability to antagonize adenosine A2A receptors (30). Depletion of AChE from the cerebral cortex is also reduced by caffeine (31). It promotes behavioral functions such as alertness, attention, mood as well as improved learning. All these behavioral functions are stimulated at a low dose. High doses cause anxiety, insomnia, restlessness, and increased heart rate. At low doses, caffeine reduced levels of Aβ and Aβ induced toxicity by reducing caspase-3 expression which was seen in neuroblastoma 2a cells expressing Swedish Mutant APP and protected basal forebrain neurons and cerebellar granule neurons from neurotoxicity due to Aβ. It reduces β-secretase levels, presenilin 1 and controls the level and deposition of Aβ in the hippocampus and entorhinal cortex (30). It scavenges hydroxide and methoxy free radicals thus control the oxidative stress. In addition, caffeine reduces hippocampal tau phosphorylation and the respective proteolytic products (25). It also increases the activity of Protein Kinase A (PKA) and pCREB levels by stimulating the survival pathway which leads to the blocking of p-ERK and p-JNK expression (25).

3.11. Morphine

Morphine (Fig. 2e), type of opioid, widely used as reliable analgesic. Opioids act through G protein-coupled receptors named μ, δ, κ, and the nociceptin orphanin peptide receptor. Heroin, morphine, and oxycodone target the μ-opioid receptors to exert the analgesic effects. Reports on the therapeutic effect of morphine for treatment of AD suggest that morphine protects against microglia-mediated neuroinflammation and oxidative stress. Morphine plays an important role in controlling the intracellular amyloid toxicity by inducing estradiol release and the activation of heat shock protein. Activation of μ-opioid receptor weakens the Aβ oligomer-induced neurotoxicity. Hence, opioid receptors are potential therapeutic targets for AD. However, the use of opioids as therapeutics is strictly restricted due to its addictive property (25).

3.12. Nicotine

Nicotine (Fig. 2f) has been investigated extensively for its therapeutic value as it supports cholinergic functions of the body. It binds to and prevents the aggregation of Aβ, exerting a neuroprotective effect. Therapeutic effect of nicotine in animal models has shown encouraging results. Unlike the effect on memory nicotine demonstrated promising result on attention in AD patients. Cotinine, metabolite of nicotine, is also considered as an effective alternative to nicotine. Cotinine possesses similar effects as nicotine, without the negative side-effects. Results with AD mouse model have shown that it prevents working and reference memory loss. This is achieved by preventing the aggregation of Aβ peptides both *in vitro* and *in vivo*, inhibiting GSK-3β activity, and activating the pro-survival enzyme Akt. Thus, cotinine may become a promising compound for AD treatment (25).

3.13. Terpenoids

Terpenoids (Fig. 3) are a diverse class of secondary metabolites which are derived from terpenes. They are multicyclic with oxygen containing functional groups. Terpenoids are responsible for imparting scent, colour, flavor etc. to different plants. Terpenoids that can be used for the treatment of AD are.

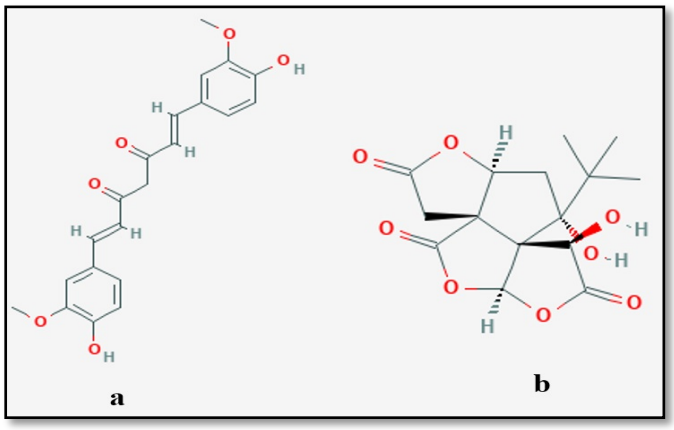

Fig. 3. Structures of Terpenoids, (a) Curcumin (National Center for Biotechnology Information. PubChem Compound Database; CID=969516), (b) Bilobilade (National Center for Biotechnology Information. PubChem Compound Database; CID=73581)

3.14. Curcumin

Curcumin (Fig. 3a) is a principal diarylheptanoid isolated from the rhizome of *Curcuma longa* which belongs to the Zingiberaceae family (7). The other curcuminoids present are demethoxycurcumin and bis-demethoxycurcumin. Curcumin imparts the characteristics yellow color to the rhizome. It is useful in the prevention and treatment of several diseases such as cystic fibrosis, inflammatory diseases, neurodegenerative diseases, etc. (15). It is an efficient antioxidant, antibacterial and antitumor agent. Curcumin suppresses the formation of amyloid plaques. It not only interferes with Aβ aggregation which leads to formation of Aβ fibrils, but also destabilizes preformed Aβ fibrils. It suppresses the formation of APP and β-secretase mRNA (16). In mice models, intragastric administration of curcumin showed reduced Aβ formation. This was done by downregulating the BACE1 expression which is responsible for cleaving APP to Aβ (32). Curcumin at a concentration range of 12.5-2 μM reduces the expression of cytokines TNF-α and IL-1β and chemokines. Curcumin inhibits AChE activity (28). The distance between the two aromatic rings of curcumin molecule allows it to favorably interact with quaternary and peripheral sites of AChE (24). Curcumin posseses strong antioxidant, anti-inflammatory and free

radical scavenging properties which help to control some AD symptoms. In vitro curcumin competes with vitamin E in controlling lipid peroxidation and ROS levels which reduces amyloid accumulation and neurotoxicity (33). Curcumin at a dose of 5–10 μM protects cells against Aβ-induced neurotoxicity by inhibiting oxidative damage and tau hyperphosphorylation (7). It is also involved in suppressing Presinilin-1 activity stimulated by GSK-3β and thus inhibits further Aβ formation (33). Though curcumin does not show any major side effects, however, excess use of it may damage the gut microbiota, and disturb the immune functions (16).

3.15. Bilobalide

Bilobalide (Fig. 3b) is a principle terpenoid obtained from the leaves of Ginko biloba. It is a sesquiterpene trilactone which gives strong protection to neurons and Schwann cells (16). It reduces the expression of p53, Bax, and caspase-3 proteins as well as inhibits ROS-induced apoptosis. It blocks the β-secretase activity of cathepsin B and in turn brings down the production of two β-secretase cleavage products of APP, Aβ and soluble APP, via PI3K dependent pathway (15). To achieve this bilobalide acts through GSK-3 signaling as a downstream target of the activated PI3K pathway. In hippocampal neurons, BB activates neurogenesis and synaptogenesis by increasing the levels of cAMP-response element binding protein (pCREB) and brain derived neurotrophic factor (BDNF) (7). Overdose of BB may initiate adverse effects on the recovery of regenerated nerves (28).

3.16. Phenylpropanoids

Phenylpropanoids are a vast class of compounds which are synthesized from amino acids tyrosine and phenylalanine. They are found throughout the plant kingdom and provide protection to the plant from ultraviolet light and pathogens. Stilbenoids, derivatives of stilbenes, is a class of phenylpropanoids. The stilbenoids involved in the treatment of AD are:

3.17. Resveratrol

Resveratrol (Fig. 4) is a compound found in red wine, nuts, skin of grapes and various plants belonging to the class Vitaceae (28). It has good anti-cancer, anti-inflammatory, antioxidant and neuroprotective properties (7). It helps in lowering the blood pressure and blood glucose levels. Resveratrol is taken up effectively in gastrointestinal lumen, but it does not remain in the body due to rapid metabolism and clearance. To overcome this problem Bui and Nguyen reported the use of resveratrol loaded lipid core nano-capsules to increase its concentration in brain tissue, compared to free resveratrol (15). The strong antioxidant activity of resveratrol increases glutathione as well as antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase levels and improves the levels of endogenous antioxidants (34). It inhibits lipopolysaccharide induced inflammatory mediators such as NO, TNF-α, variety of interleukins, C-reactive protein and chemokine monocyte chemotactic protein-1 in astrocytes (34). Resveratrol can also lower the level of β-amyloids by promoting the cleavage of APP and its rapid elimination. It also enhances the binding of transthyretin, a transporter protein to Aβ oligomers by structure stabilization, and thus prevents Aβ aggregation (35). It inhibits AChE activity in neuronal cells. In a helminth parasite *Raillietina echinobothrida*, after treatment with 135μg resveratrol, it showed about 46% inhibition of AChE (24). Resveratrol prevents hyperphosphorylation and mediates dephosphorylation of tau proteins. It is a strong antioxidant and scavenges ROS, increases GSH level and enhance overall antioxidant capacity of the cell (7). Resveratrol increases intracellular Ca^{2+} in cortical neurons via modulating second messengers, cGMP, cAMP, and NO. This rise in Ca^{2+} enhances the cellular glucose utilization by calcium dependent AMP-activated protein kinase (28). It is also an activator of various sirtuins such as SIRT1, SIRT2 and SIRT3. Sirtuins are involved in neuronal cell survival and longevity. Thus, resveratrol reduces Aβ aggregation via activation of sirtuins (36).

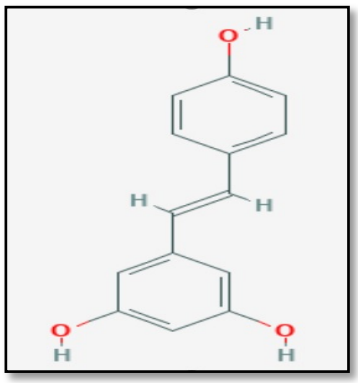

Fig. 4. Structure of Resveratrol (National Center for Biotechnology Information. PubChem Compound Database; CID=445154)

3.18. Carboxylic acids

Carboxylic acids are organic acids containing a hydroxyl group. As the name suggests, these are acidic in nature and are generally weaker than mineral acids. These acids occur widely in nature. They are found in milk, citrus fruits and also in the bodies of many animals such as ants. Carboxylic acids useful in treatment of AD are:

3.19. Rosmarinic acid

Rosmarinic acid (Fig. 5) is a polyphenol carboxylic acid found in many Lamiaceae herbs which are commonly used in food such as *Mellisa officinalis* (Lemon Balm), *Rosmarinus officinalis* (Rosemary), *Origanum vulgare* (Oregano), *Salvia officinalis* (Sage), etc. It is an ester of caffeic acid and dihydroxyphenyl lactic acid (37). Taguchi et al. (38) conducted an experiment using docking simulation and direct binding studies and showed that the catechol functional group on caffeic acid is important for binding. It is used in many pharmacological activities. Rosmarinic acid is a strong antioxidant, antibacterial, anti-inflammatory, anticancer, antiviral, and neuroprotective. 10 μM of rosmarinic acid suppressed Aβ cytotoxicity, ROS generation, caspase3 activity, DNA fragmentation, lipid peroxidation and tau

hyperphosphorylation in cultured PC12 cells (39). It has been observed to prevent β-amyloid induced memory loss. This is because of its ability to inhibit NF-κB and TNF-α expressions (7). It protects neuronal cells by protecting against cytotoxicity induced by β-amyloid. It reduces the hyperphosphorylation of tau proteins. Rosmarinic acid inhibits apoptotic pathways by preventing ROS formation, caspase-3 activation, and DNA fragmentation. Rosmarinic acid by controlling lipid peroxidation and inflammation is able to prevent locomotor activity, short-term spatial memory and alterations of brain tissue found in a rat model of AD. These results further need to be supported by clinical trials to demonstrate the effect of rosmarinic acid against AD (16). Rosmarinic acid also showed 28% inhibition of AChE and 80% inhibition of BChE at a concentration of 10 µg/ml (39, 40).

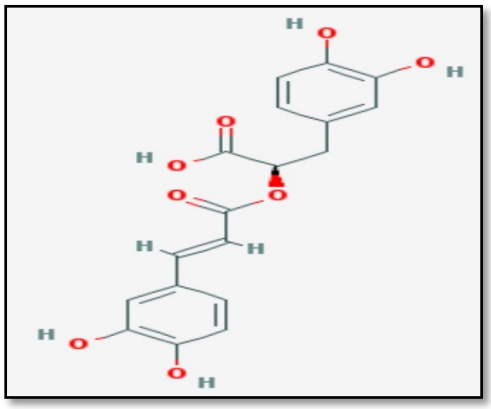

Fig. 5. Structure of Rosmarininc Acid (National Center for Biotechnology Information. PubChem Compound Database, CID-5281792)

3.20. Polysaccharides

Polysaccharides are long chain of monomers joined together by glycosidic bonds. These are basically sugar molecules bound to each other. Polysaccharides can be linear or branched depending upon the monomers that make them up. Polysaccharides that are active against Alzheimer's Disease are:

3.21. Chitosan

Chitosan (Fig. 6) is a polysaccharide of D-glucosamine and N-acetyl-D-glucosamine. It is produced by deacetylation of chitin, component of the exoskeleton of crustaceans and cell wall of fungi (7, 41). It is a potent coagulant and is used in making of bandages. Chitosan nanoparticles have been studied for the delivery of rivastigmine and tacrine to CNS for the treatment of AD (7, 42). More interestingly, a chitosan nanocarrier is designed as a nano-vaccine to deliver Aβ into brain (43). Recent studies suggest that chitosan itself could protect neurons against H_2O_2-induced apoptosis by preventing Aβ formation and blocking intrinsic apoptosis pathway (44). It upregulates Nrf2 and inhibits NF-κB in neural cells. Chitosan protects the cells by reducing the intracellular ROS and Ca^{+2} levels and suppressing apoptosis. It inhibits Aβ induced AChE activity as well as the phosphorylation of MAPK whose aberrant phosphorylation has been implicated in AD. Water soluble chitosan (10 µg/ml) was able to prevent inflammation in Aβ-stimulated human astrocytoma cells by reducing the TNF-α and IL-6 levels, and suppressing iNOS expression (45). However, the clinical studies did not report any serious side effects of chitosan (7).

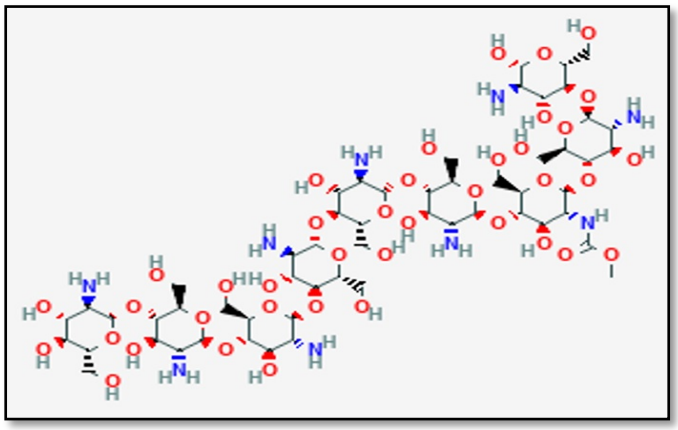

Fig. 6. Structure of Chitosan (National Center for Biotechnology Information. PubChem Compound Database; CID-71853)

3.22. Other Plants

Apart from these above-mentioned compounds, there are other plants which have been identified to have potential to cure AD. These plants belong to various families and the compounds that possess the capability to treat AD has not been classified or tested. These plants are:

3.23. *Zingiber officinale* (Ginger)

Zingiber officinale (Fig. 7a) has been widely used in food supplements and beverages. Principal compounds present in *Z. officinale* are gingerols, shagaols, bisabolene, zingiberene (Fig. 7b) and monoterpenes (46). The main biological effect includes inhibiting the AChE enzyme resulting in higher ACh release in synapses, higher activity of cholinergic pathways, and thereby improved cognitive functions in AD patients. Grzanna et al. (47) reported that treatment of human monocytic THP-1 cells with ginger prevents pro inflammatory cytokines and chemokines (47, 48). It also reduces NF-κβ and IL-1β levels, thus attenuating Aβ induced neuronal cell death and behavioral dysfunction (48). Shagaol, a constituent of ginger inhibits release of NO and the expression of iNOS (46). 6-gingerol treatment protects neuronal cells from Aβ induced cytotoxicity (48). Gingerol treatment led to reduction of oxidative stress and inflammation while it enhanced learning and memory in rat model (49, 50). Furthermore, *Z. officinale* can inhibit lipid peroxidation, reducing the overstimulation of NMDA receptors and prevent the formation of oxidative free radicals (15). In human chondrocyte cells with IL-1 induced oxidative stress, treatment with ginger decreased ROS and lipid peroxidation and increased the levels of catalase, superoxide dismutase. This activity protects against AD.

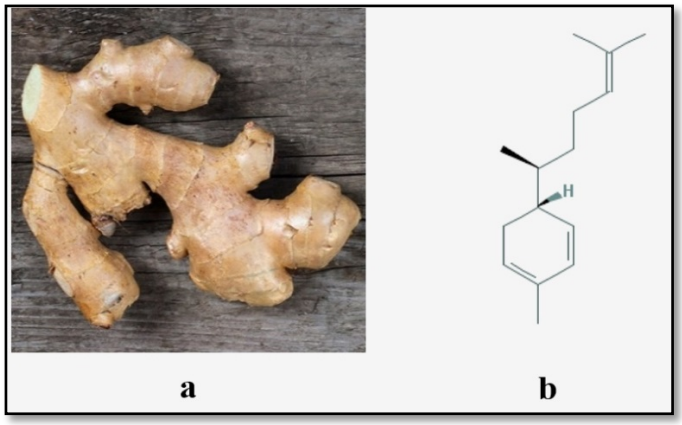

Fig. 7. (a) Rhizome of *Zingiber officinale* (National Center for Complementary and Integrative Health), (b) Structure of Zingiberene (National Center for Biotechnology Information. PubChem Compound Database, CID-71853)

3.24. *Crocus sativus* (Saffron)

Croccus sativus (Fig. 8a) or saffron is a small perennial plant cultivated in Afghanistan, Iran, Spain, and Turkey. It is traditionally used as antispasmodic, stimulant, expectorant, etc. The stigmas (Fig. 8b) of the flowers are a rich source of many compounds such as lycopene, α-crocetin, safranal, etc. These compounds show antioxidant properties as well as inhibit the Aβ fibrilogenesis. It shows moderate AChE inhibitory activity thus inhibiting the ACh breakdown. It also inhibits nitric oxide induced astrocyte cytotoxicity (29). In a study conducted by Akhondzadeh et al. (51), a group was given 15 mg saffron capsules for 16 weeks twice a day. This group showed improved cognitive functions as compared to the placebo group (51, 52). In a time and concentration dependent manner, saffron is also involved in inhibition of Aβ fibrillogenesis. Crocin a component of C. sativus reduced Bax/Bcl-2 ratio and cleaves Caspase-3 and thus inhibits apoptosis (53). By inhibition of LPS induced NO release in rat brain microglial cells, crocin and crocetin provide neuroprotection (46). Reduced levels LPS induced TNF-α, IL-1β and ROS, as well as NF-κβ activation was also observed because of these compounds (53).

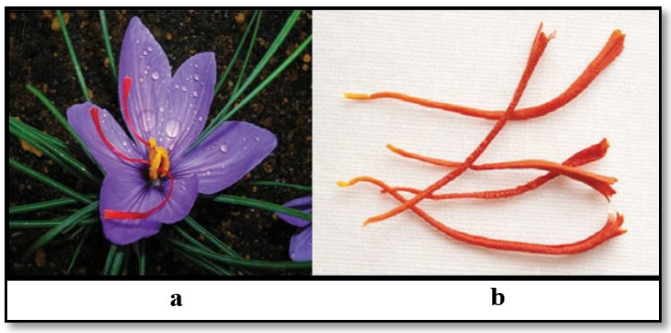

Fig. 8. (a) Flower of *Croccus sativus*, (b) Stigma of *Croccus sativus* (Adapted from Abd-Razak et al., 2017)

3.25. *Allium sativum* (Garlic)

Allium sativum (Fig. 9) belonging to the Amaryllidaceae family is extensively used in food and medicines (53). The components of *A. sativum* are active free radical scavengers. They increase the levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase; glutathione levels; inhibit lipid peroxidation; and reduce inflammatory prostaglandins. S-allyl cysteine, an organo-sulphur component of garlic protects PC12 cells against Aβ toxicities in a dose dependent manner (54). Aged garlic extracts inhibit 3-hydroxy-3-methylglutaryl-CoA reductase and reduce cholesterol synthesis. They also protect neurons from β-amyloid neurotoxicity and death; prevent cognitive damage, ischemia, and enhance learning and memory (15). Neuronal cultures treated with aged garlic extract and S-allyl cysteine resulted in protection against H2O2 induced oxidative stress (54). This treatment also decreases GSK-3β activity thereby reducing hyperphosphorylated tau in brain of APP-Tg mice. It can also increase insulin and insulin like growth factor in brain which in turn can help decrease Aβ burden in the brain (55).

Fig. 9. Bulb of *Allium sativum* (National Center for Complementary and Integrative Health)

3.26. *Bacopa monnieri* (Brahmy)

Bacopa monnieri plant (Fig. 10) is commonly used in traditional medicine as a nerve tonic, diuretic, cardiotonic agent and for the treatment of asthma and rheumatism (56). Compounds found in *B. monnieiri* are bacopasides III, bacopasides IV, bacopasides V, bacosides A, bacosides B, bacosaponins A, B, C, D, E and F. Saponin glycosides such as jujubogenin and bisdesmosides are also found. It is also applied as paste for epilepsy and insomnia treatment. Saponins and triterpenoids in *B. monnieri* have antioxidant properties thus reducing the oxidative stress in brain (15). It is used as a nootropic drug, i.e., it is used to enhance memory and cognitive functions. It augments protein kinase activity in the hippocampal region to induce the nootropic effect. Consumption of *B. monnieri* decreases cholinergic degradation and enhances cognition. It increases the ACh levels by inhibiting AChE. *B. monnieri* extracts also protect neuronal cells from damage caused by Aβ fibrils. It is known to inhibit lipid peroxidation in hippocampus, striatum areas and frontal cortex of rat brain (57). It also enhances the

expression of SOD and GSH as well as quenches H_2O_2 mediated oxidative stress along with downregulation of lipoxygenase and thus rescues cells from oxidative stress (58). In a study carried out by Nemetchek et al. (59) in cell free assay system, complete inhibition of caspase 1 and 3 was seen while, significant inhibition of TNF-α and IL-6 release from LPS-activated microglia was observed (59).

Fig. 10. Plant of *Bacopa monnieri* (Adapted from Pant et al., 2015)

3.27. Centella asiatica

Centella asiatica (Fig. 11) is a plant which has been used traditionally for rejuvenating neuronal cells. It is shown to increase intelligence, longevity, and memory. Asiatic acid and asiaticoside are the bioactive compounds found in this plant. They can control H_2O_2-induced cytotoxicity, reduce free radicals and prevent Aβ induced cell damage. These compounds reduce β amyloid pathology and the oxidative stress response in brain. *C. asiatica* can also reduce oxygen radical production and strengthen the antioxidative defense system by upregulating the activities of antioxidative enzymes and levels of glutathione and glutathione disulfide. Hence, *C. asiatica* can serve important role in the prevention and treatment of AD (15). Asiatic acid, a component of *C. asiatica* demonstrated neuroprotective effects in AlCl3 induced rat model of Alzheimer's disease (60). Asiatic acid also demonstrated improved memory as well as learning in male Sprague-Dawley rats (61). Certain studies have also reported that this plant shows excellent neuritogenic ability by stimulation of neurite outgrowth in human neuroblastoma cells (56).

Fig. 11. Leaves of *Centella asiatica* (Adapted from Tripathi et al., 2015)

3.28. *Olea europaea* (Olives)

Olea europaea is found mainly in the Mediterranean region. The fruits are mainly cultivated to obtain extra virgin olive oil and olive oil. Olive oil alleviates cognitive decline resulting from neurodegeneration. It enhances the activity of glutathione reductase and superoxide dismutase. High doses of extra virgin olive oil reduce the Aβ burden in brain as well as increases Aβ clearance in the blood brain barrier. Oleocanthal, a compound found in extra virgin olive oil removes fibrillation of tau proteins. At low concentrations, it has the capacity to depolymerize Aβ oligomers and protect the neurons (29).

4. Conclusion

Recently, there has been an increase in the cases of neurodegenerative diseases. With synthetic drugs having their side effects, natural products have become the key to the treatment. Several natural products are used alone or in combination to improve memory, alertness and learning in AD patients. The therapeutic effect of herbs and medicinal plants has drawn researchers' attention to study natural products as potentially promising compounds for drug discovery to replace chemically synthesized drugs. This review aims at listing the natural products that can be a cure for AD. Various alkaloids and flavonoids have properties such as anti-AChE, degradation if Aβ fibrils, reduction of hyperphosphorylation of Tau proteins etc. Due to these properties, such compounds have become top candidates for treatment of AD. The major advantage of these products is that they have minimum side effects with high efficacy. Though they are still not fully capable of treatment, they can be used to reduce the symptoms and pathology of the disease.

The increase in the cases of people affected with AD put into sharp focus an urgent need to find a cure for the disease. The many synthetic drugs available in market do a good job of mitigating the effects of the disease, but synthetic drugs are known to have adverse effects on health of a person. The side effects of these drugs are the driving force that majority of the population is looking for an alternative to allopathic medicines. Thus, to avoid these adverse effects, natural products are desirable candidates. Right now, none of the mentioned natural products can fully cure the disease, but in coming years, they can form base for designing a drug which has the potential of eradicating AD. Further research and advanced technological knowledge will surely pave a path for creating such a drug which will be highly efficient with minimum side effects.

Conflict of interest

Authors have no conflict of interest.

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Review Article

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NSAIDs medication for headache as the presenting symptom with migraine and COVID-19

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Abstract

Headache is one of the most prevalent disorders of the nervous system. Headache is also the most common symptom of a variety of diseases, including migraine, COVID-19. International Classification of Headache Disorders (ICHD) lists over a thousand different types of headaches. Migraine is a widely known type of primary headache. Much research supports that the enhancement in migraine intensity related to chronic migraine such as neurogenic neuroinflammation, possibly leading to increased cytokine expression via activation of protein kinases in neurons and glial cells of the trigeminovascular system like some of the other headache diseases. No currently drug class available, either specific (triptans, ergots) or non-specific (opioids, paracetamol, NSAIDs), is effective in all types of headaches, in all patients and all attacks of the same patient. However, non-steroidal anti-inflammatory drugs (NSAIDs) minimize prostaglandin synthesis by blocking cyclooxygenase, which is included in the pathophysiology of migraine headaches. We searched the employed source was The Journal of Headache and Pain database by using NSAIDs with Headache, Migraine, and COVID-19 keywords. The search was performed from April 2021 and included 2017-2018-2019-2020-2021 (last five years) the studies and reviews from the Journal of Headache and Face Pain Sites. Additionally, we noted the published or on-going studies, eight of these, about NSAIDs information contain searches that exist in the 12th European Headache Federation Congress (jointly with 32nd National Congress of the Italian Society) Study of Headaches' book. Also, we included relationship migraine with COVID-19 studies to highlight the connection between the headache, which is one of the most common symptoms of both migraine and COVID-19, and the importance of managing migraine pain with NSAIDs during corona processing.

Keywords: headache, migraine, neuroinflammation, non-steroid anti-inflammatory drugs (NSAIDS)

1. Introduction

Headache disorders remain one of the prevalent medical problems, most people experience them at a stage in their life regardless of age, gender, and race. Additionally, while COVID-19 viruses embracing the world are mainly a respiratory illness characterized by symptoms of cough, fever, and respiratory distress; headache is one of the most common neurological symptoms in COVID-19 (1). The International Classification of Headache Disorders (ICHD) explained more than 150 different versions of headaches. However, they fall into two subtitles, primary headaches, and secondary headaches. Primary headaches are headaches that are stand-alone disorders caused by independent pathomechanism and not by other disorders whereas secondary headaches are underlying other causes such as severe illness, high blood pressure (2). As migraines, tension headaches, cluster headaches, exertional headaches, and hypnic headaches are fallen under primary headaches while medication-overuse headaches, airplane headaches, and pregnant-related headaches, post-traumatic headaches are included by secondary headaches. Identification of the patient subgroups with different sensitization scales and clinical characteristics can help identify at-risk groups and help to develop better therapeutic strategies/tactical approaches (3).

A systematic searching about headache from 495 papers published in 2018, analyzed that common analgesics such as acetaminophen/paracetamol or metamizole, non-steroidal anti-inflammatory drugs (NSAIDs), triptans, anti-emetics (if employed for pain relief), opioids, ergot derivatives, caffeine, magnesium, oxygen, devices that could be easily procured by patients at home for headaches. Moreover, the result of the search showed that NSAIDs are the most common participants in this many studies like the research (4-7).

No currently drug class available, either specific (triptans, ergots) or non-specific (opioids, paracetamol, NSAIDs), is effective in all types of headaches, in all patients and also in all attacks of the same patient (8-12). Besides, a healing process of any headaches with any drug can be affected by many reasons as well as for the formation of pain, the pregnant term, caffeine, cannabis use, over-weight obesity, drug-drug interaction, types of administration (topical or injection or different form of the drugs such as nimesulide gel form, etc.) (6, 13-18). And a correctly characterized headache is of great importance to take precautions against these reasons. For example, if a migraine diagnosed patient puts down the daily coffee, pay attention to what he/she eats; he/she can make better his/her life with migraine. NSAIDs in the treatment of migraine, COVID-19, and COVID-19

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migraine patients Migraine is the primary type of headache characterized by persistent headaches that are moderate to severe. Migraine has been proclaimed to be the third prevalent disorder and the second cause of disability by WHO (21). The exact pathophysiology of migraine is still not clear. However, some theories can be explaining how migraine is produced. The *vascular theory* is vascular disturbances (vasodilatation–vasoconstriction) causing migraine symptoms associated with inflammation and the serotonin system; *the neural theory* is hyperexcitability in the form of cortical spreading depression leads to migraine through a cascade involving inflammation and sympathetic nervous system whereas *the alternative theory* that may overlap with a mechanism which activates neural inflammation and it leads to migraine pain (22). Even if the evidence of a direct role of COX-2 on the face and headache pain is still controversial and inhibited with cyclooxygenase inhibition by NSAIDs, which avoids the conversion of arachidonic acid into the inactive prostaglandin precursor, prostaglandin H_2 (PGH_2), is a basis of the acute migraine attack, thus implicating prostaglandins in migraine pain (20, 23-25). But, NSAIDs are not one way to treatment for migraine diseases (26). For example, if headaches are mild to moderate in intensity, generally, it is favorable to initiate with a NSAID (acetylsalicylic acid used in medicines like Aspirin, diclofenac, ibuprofen) or acetaminophen (paracetamol) to be started as a first. This is the most appropriate strategy that has been found unless the patient has already tried over-the-counter medicines. If this proves ineffective, the triptan option or combinations of the drugs can be attempted/followed the next (5). Many pieces of the research mention that the drugs' effectiveness, differences from NSAIDs, and the effect of their combination treatment with NSAIDs on migraine or other headaches (7, 11, 14, 16, 19, 27-34). But the usage of the drugs has to be controlled by doctors or pharmacists. Because mindfulness medication is dangerous and might cause some risks such as overuse headache or GI comorbidity like side effects with or without drugs used for other diseases (20, 35). For example, analysis shows a correlation with sociodemographic variables and indices of symptom severity by professional healthcare providers (HCPs). And the analyses show that medication-overuse headache consulted most (87.0%), followed by those with migraine (67.2%) and those with tension-type headache (48.6%; $p < 0.001$) (13, 27, 34, 36-40). Moreover, several reports emerged on March 14, 2020, raising fears that NSAIDs, e.g., ibuprofen) may worsen symptoms of COVID-19. A substantial body of evidence suggests that NSAIDs can alter the course of bacterial pneumonia, resulting in more invasive disease and complications, as well as higher rates of hospitalization and intensive care unit admission (41-43). There is reasonable evidence for an association between NSAIDs and respiratory and cardiovascular side effects in a variety of settings, but no evidence directly related to people with COVID-19 (44). However, before administering any drug, physicians can thoroughly test for COVID-19 symptoms and notify patients. NSAIDs should be stayed away from medication (45).

In addition to the current pharmacological treatment with the drugs, some point of pain area is important to take as a target with drugs or physical techniques. Such REN technique and cervical myofascial point targeting with drugs etc. exist (20). Moreover, preventive therapies such as Yoga, relaxation therapies might shorten the treatment period (11, 46).

In this study, we reached the following results, which we conducted that much researches about effectiveness and cost were the main criteria to choose drugs, conclude that national stakeholders in cooperation with scientific organizations should be implicated in continuous clinical practice and education concerning appropriate counseling processes and that, as with any disease, its interaction with drugs used for other diseases should be considered (32, 36).

2. Conclusion

Headaches are one of the most common health issues, affecting almost everyone at some stage in their lives, regardless of age, gender, or race. Migraine is a much more severe form of headache and also a very prevalent neurological disease that affects people all over the world. It is also the most common symptom for the COVID-19 epidemic that affects the world. NSAIDs are most effective on migraine or many other headaches. However, in the treatment of both migraine disease with COVID-19 and headaches due to COVID -19, regardless of this observation, we agree that paracetamol (acetaminophen) should be used first in headache management before starting to NSAIDs because of paracetamol's greater tolerability (47, 48). As the result, developing better therapeutic strategies is mostly related to an accurate diagnosis of headache types and also to its interaction with drugs used for other diseases that occur at the same time with migraine such as COVID-19 (5). And also, price, effectiveness, and safety are the main things to choose medication by the patients (49-51).

Conflict of interest

The authors have no conflict of interest to declare. The authors alone are responsible for the content and writing of this review.

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Review Article

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Epidemiology, virology, clinical features, diagnosis, and treatment of SARS-CoV-2 infection

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Abstract

Since December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged and spread quickly worldwide. The disease is generally mild in adult people but in any with comorbidities may proceed to acute respiratory distress syndrome (ARDS), pneumonia, and multi-organ dysfunction. By performing molecular tests on respiratory secretions can diagnose the virus. Elevated C-reactive protein (CRP) and normal/low white cell counts are common laboratory diagnoses of COVID-19 while the tomographic chest scan is usually irregular for many infected people. Some patients progress to respiratory failure, pneumonia, and finally death by the end of the first week of illness because of the sharp rise in inflammatory cytokines such as IL7, IL2, GCSF, IL10, MIP1A, MCP1, IP10, and TNFα. Various approaches to the COVID-19 are being performed by scientists. Use of chemical medical drugs that are effective for other viral infections. Among them, remdesivir was approved by FDA on 1th May 2020 because of its impact to treat patients. Also, several studies have revealed that many Chinese herbal remedies have a remarkable impact on the healing process when simultaneously were used along with pharmacological drugs. In the meantime, many efforts have been made to produce an effective vaccine, and so far, the Ad5-vectored COVID-19 vaccine has been successful and has entered phase 2 in the human trial. The current review focus on epidemiology, virology, clinical features, diagnosis, and available treatment of coronavirus that might assist researchers and clinicians in establishing action options for timely against this infection.

Keywords: coronavirus, COVID-19, SARS-CoV-2, severe acute respiratory syndrome, treatment

1. Introduction

Coronaviridae is a family of positive-single-stranded RNA viruses that its length is in the range 26–32 kilobases and has a 5 cap structure and 3 polyadenylation tract (1). On the surface of these viruses, there is a crown-like protein and this is the reason that they were named Coronaviruses. As can be seen in Fig.1, the family Coronaviridae is classified into two subfamilies: Orthocoronavirinae and Letovirinae. The subfamily Orthocoronavirinae contains four genera, including Gammacoronavirus, Alphacoronavirus, Betacoronavirus, and Deltacoronavirus (2). The betacoronavirus genus is divided into five sub-genres of ancestors (3). Genomic studies have shown that the gene sources of alphaCoVs and betaCoVs are from rodents and bats, further, deltaCoVs, and gammaCoVs are from avian species (4). Among the species of coronaviruses, seven of them are related to humans, including HCoV-OC43, MERS-CoV, HCoV-HKU1, and SARS-CoV, which are from betacoronavirus genus, plus, HCoV-229E and HCoV-NL63 that are from Alphacoronavirus genus (5). Genome sequencing of viruses isolated from patients with SARS-Cov-2 has shown that most of the sequences are similar. The SARS-Cov-2 genome sequence is about 96.2% similar to the Bat SARS-like CoVs, 79.5% similar to the SARS-CoV, and 50% similar to the MERS-CoV. Bat SARS-like CoVs include bat-SL-CoVZXC21 and bat-SL-CoVZC45 (6). Because of these similarities, the novel coronavirus was called SARS-CoV-2. The length of SARS-CoV-2 RNA is exactly 29891 nucleotides that encode 9860 amino acids (4). The SARS-CoV-2 genome is generally similar to Bat-SARS-CoV, but the SARS-CoV-2 proteins are similar to SARS-CoV (5). One study discovered that the SARS-CoV-2 genome can mutate in different patients, but the mutation was much less than the changing of H7N9 avian influenza (7). The frame of most coronaviruses is the same size, except a floating part (6–10) of open reading frames (ORFs). There are transcription

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regulatory sequences between the ORF, which are templates for the production of sub-genomic mRNAs. Sixteen non-structural proteins are encoded by the first part of the ORF and accessory proteins are encoded by the rest of the ORF (8). Nucleocapsid protein (N), small envelope protein (E), spike glycoprotein (S), and matrix protein (M) are four essential structural proteins. These proteins interact with the human innate immune response (9).

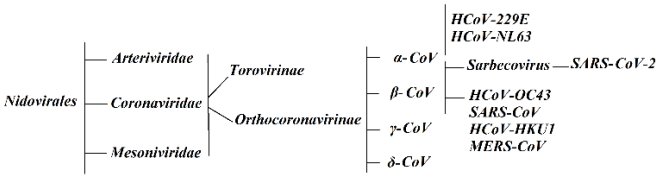

Fig. 1. Human Coronavirus classification

These viruses in animals and humans lead to enteric, intestinal, and respiratory diseases, and in some cases to diseases of the nervous system (10). Most kinds of human coronaviruses have been associated with diarrhea, colds, and in some cases, occurring multiple sclerosis (11). Coronaviruses were first discovered in the mid-1960s (12) and they were not very popular before 2003 because they just had been causing nearly 10 to 15 percent of colds, and rarely caused serious lower respiratory region disease until Severe Acute Respiratory Syndrome was distinguished in China. SARS-CoV could have arisen as a mutant of a human coronavirus (13). In 2012, another type of coronavirus called Middle East Respiratory Syndrome (MERS) was first diagnosed in the Middle East. This strain was another mutant type of coronavirus, which was isolated from a person with flu-like symptoms (14). Finally, a novel coronavirus has appeared in early December 2019 (15). The coronavirus disease 2019 is called abbreviation COVID-19 and leads to an acute respiratory disease (16).

The first report of acute respiratory infection was revealed on December 12, 2019, in the seafood market in Wuhan, China. It is supposed that the animal-to-human transmission was the first mechanism to transmit the SARS-CoV-2. Yet, some of the following cases did not deal with this market at all, so it is obvious that human-to-human delivery has occurred. Patients with symptoms are serious carriers of the virus (17). COVID-19 is an acute infectious disease that is first transmitted through direct contact with the SARS-CoV-2 or respiratory droplets of people infected with the virus (18) or exposed to high aerosol concentrations in restricted spaces (4). On January 7, 2020, Chinese scientists isolated the virus from infected people and sequenced it. Since the phylogenetic structure of SARS-Cov-2 has a high correlation to Bat SARS-like CoVs (19), it has been suggested that an animal has played the important role of intermediate host to transfer the virus in the market (20,21). There is a unique peptide (PRRA) in batCOV and human SARS-CoV-2 virus but pangolins do not have this RRAR motif, thus, SARS-CoV-2 did not come directly from pangolins (22). Fig. 2 represents the schematic image of SARS-CoV-2 structure and transmission into human.

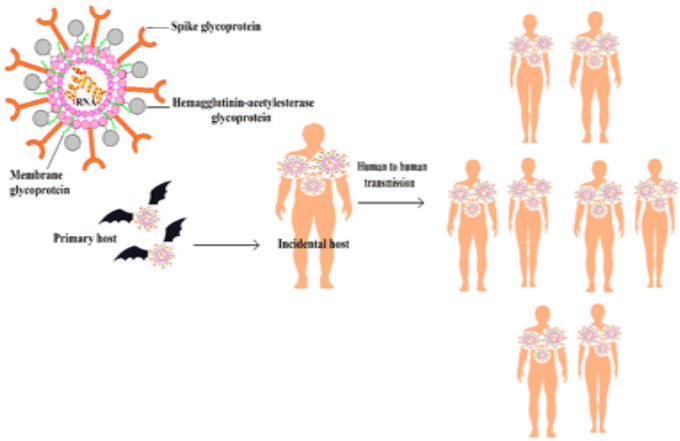

Fig. 2. Schematic representation of SARS-CoV-2 transmission into human

However, the emergency needs to control infected people with the virus has not yet allowed for a more detailed survey about the intermediate host. On January 12, 2020, the virus was named 2019- novel coronavirus, and subsequently on February 11, 2020, the virus and the disease it caused were called SARS-CoV-2 and coronavirus disease 2019 (COVID-19). Now, the transmission of SARS-CoV-2 occurs mainly between human-to-human, while nosocomial transmission occurs for SARS-CoV and MERS-CoV (23,24). The World Health Organization (WHO) stated the SARS-CoV-2 as a new epidemic on February 28, 2020. Finally, the novel epidemic intensified about every seven days (4) until, 11th March, COVID-19 was officially announced a pandemic by the WHO and as of August 2020, SARS-CoV-2 has infected nearly 23.000,000 people and caused more than 790,000 deaths (https://www.worldometers.info/coronavirus/). COVID- 19 is a late and long illness thus, People who are infected these days will start showing patient symptoms soon. A study presented that if the pandemic is left unchecked, for every possible critical-care bed, there will be nearly 15 COVID-19 patients by the end of June. The first possibility is that every country head to simultaneously make the virus to heel, as the SARS in 2003. The second possibility is that the virus passes through the population around the world and finally leaves people who have strong immune systems (25).

2. Clinical characteristics, symptoms and laboratory diagnostic

The incubation time of COVID-19 is on average 3-14 days, during this time the person would be disease carrier. The first detection of the virus in China was performed by electron microscopy using a morphological examination of SARS-CoV-2 (26). The risk of this disease is higher for the elderly or people with immunodeficiency. Men are also more likely to get it with 56% than women with 41.9 to 45.7% (27). In a study by Zhang et al, it is reported that the virus is presented

in the blood and fecal swabs of the infected person (28). Symptoms in patients include cough and fever, and most people indicate mild flu-like symptoms. In people with this severe disease, symptoms of shortness of breath, failure of some organs, and even death are observed (29).

Older people show more severe symptoms than others when they become infected, also, contrary to the first impression, many children can get the disease (30). The frequent clinical symptoms include headache, sore throat, shortness of breath, sputum production, fatigue, cough, and fever with about 13.6%, 13.9%, 18.6%, 33.4%, 38.1%, 67.8%, and 88.7%, respectively (31). Some patients express that in addition to mentioned symptoms, they have gastrointestinal symptoms such as vomiting and diarrhea with nearly 5.0% and 3.8%. Of all the symptoms, fever and cough are the most common (29). In many patients, the number of white blood cells and lymphocytopenia is reduced (32), but severe symptoms of the respiratory syndrome is decreased. Plus, creatinine levels, blood urea, neutrophil levels, and inflammatory factors increase in these people (29). Generally, the procalcitonin value is normal in most patients. CT imaging reveals pneumonia. There are a bilateral patchy shadowing and ground-glass opacity on the chest. Also, a peripheral lung distribution and a rounded morphology can be seen (33). The disease is divided into three groups based on the symptoms; about 85% of infected people undergo mild to modest infection and nearly 10% have vigorous infections and 5% experience critical cases half of which die (34).

Currently, the main method for diagnosis is real-time PCR, which makes a double-stranded DNA from the viral RNA. This method is performed on samples taken from a throat swab, nasal, and other respiratory tract samplings (35) that should be immediately stored at 4ºC after collection. If the result of RT-PCR from blood or respiratory samples would be positive, it means that the person has been infected (30). The following are disease division based on the symptoms:

1. **Mild Illness;** People with the disease show mild symptoms of the respiratory virus, including dry cough, mild fever, muscle pain, etc. Shortness of breath does not exist in these people at all.
2. **Modest Pneumonia;** There are no signs of severe pneumonia, but the shortness of breath and cough is obvious.
3. **Severe Pneumonia;** People with acute pneumonia have much more severe symptoms, such as shortness of breath and tachypnea, so they need artificial oxygen. But the degree of fever varies from person to person.
4. **Acute Respiratory Distress Syndrome (ARDS);** This syndrome leads to severe respiratory failure. The occurrence of hypoxia is one of the obvious symptoms. ARDS is divided into three categories based on the amount of hypoxia; mild ARDS, moderate ARDS, and severe ARDS.

Sepsis, The dysfunction of vital human organs due to infection is called sepsis (36). Very serious and dangerous symptoms can be seen in a person with sepsis. These symptoms include tachycardia, renal impairment, and severe hypoxemia and dyspnea (4).

3. Pathogenesis and mechanism of action

The effects of SARS-CoV-2 infection varies in different people and the foundation of differences is not still apparent. By sequencing the SARS-CoV-2, it was identified that there are several similarities between SARS-CoV-2 and SARS-CoV-1, for example, the 3D structure of S protein on the viral surface and the kind of receptors that it has an affinity to it (37). Cellular responses to the SARS-CoV-1 are different. The cellular tropism might be related to the differential expression level of membrane receptors for the virus. The most obvious receptor that the SARS-CoV uses to enter is Angiotensin-converting enzyme 2 (ACE2). Through COVID-19 infection, the S protein of the virus is split into subunits; S1 and S2. The receptor-binding domain (RBD) is the site that binds to host cell receptors. RBD is situated in the S1 subunit. Membrane fusion is performed by S2 (38). This receptor prevents lung fibrosis by reducing angiotensin one and two during some processes. But when the receptor is involved by the virus, lung damage occurs (39). Fig. 3 shows the schematic representation of the SARS-CoV-2 replication in human respiratory system.

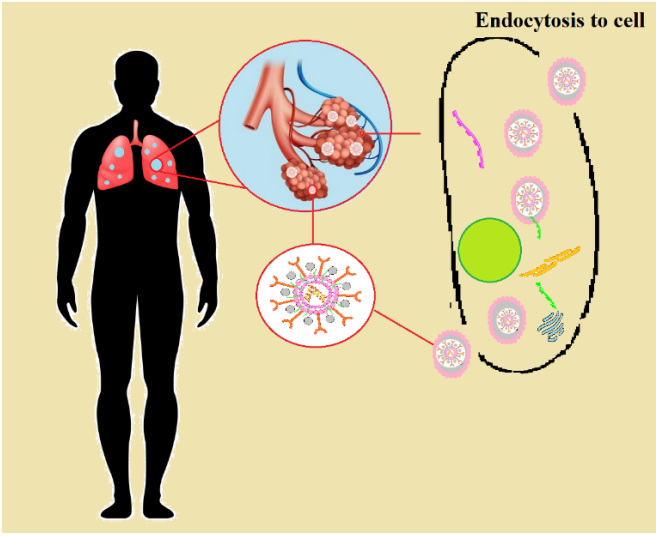

Fig. 3. Schematic representation of the SARS-CoV-2 replication in human respiratory system

The virus uses another receptor called transmembrane protease serine 2 (TMPRSS2) while interacting with the ACE2 receiver. TMPRSS2 is essential for priming and the viral spread of SARS-CoV-2. In other words, it was shown that in vitro studies, TMPRSS2 activates the S protein, which in turn, induces virus-cell membrane fusion at the host cell surface. Although this role of TMPRSS2 during coronavirus infection in vivo has still some unclear aspects, we think it

may be the same in COVID-19 infection (40). It has been shown that the greatest damage to lung tissue in all severe cases is caused by severe inflammation, not the direct effect of the SARS-CoV-2 itself (41). Excessive immune response is responsible for severe pneumonia and respiratory failure. In some cases, immune systems are not able to stop their inflammatory response and lead immune cells into the lungs known as the cytokine storm (42), elevating IL-6, lymphopenia, ferritin, D-dimers, and raised neutrophil to lymphocyte ratio (42,43). It is worth noting that this pathogenic outcome is similar to SARS CoV-1. In patients with severe respiratory syndrome three stages occur; a) escape from the immune system and suppress IFN, which causes the virus to multiply easily, b) cytokine storm, and c) manifestation of severe symptoms due to the unregulated inflammation. In patients with the mild disease experience a robust adaptive immune response in the first seven days, then, the titers of helper T cells, activated CD8 T, and CD4 T cells are enhanced (44) ACE2 is a well-known receptor of SARS-CoV involves in pathogenesis (45). Using the same receptor by the SARS-CoV-2 for infecting the host has been approved by earlier research (34). As soon as the virus attaches to the cell, the viral +ssRNA enters to the human cell cytoplasm and is translated, then, two proteins (named pp1ab and pp1a) are encoded. These non-structural protein construct a replication-transcription complex (RTC) in double-membrane vesicles (46). The role of RTC is making sub-genomic RNAs that encode structural viral proteins (47). Another receptor that is involved in entry SARS-CoV-2 is TMPRSS2 (48). There are several possible causes for the virus to spread so quickly, such as: happening right at the height of the crowd at the Chinese Spring Festival, Lack of accurate knowledge of virus mechanisms to enter host cells (35).

The following biochemical events related to the viral replication cycle occur as soon as the viral RNA genome is released into the cells. RNA-dependent RNA polymerase is in charge of starting biosynthetic events of SARS-CoV-2 replication in the cytoplasm of infected cells. Coronavirus replication requires to continue protein synthesis from the very start of viral infections. Transcription is done through a replication-transcription complex (RCT) that is organized into two-membrane vesicles by synthesizing the sequence of subgenomic RNAs (sgRNAs). ORFs act as templates for generating subgenomic mRNAs. Atypical CoV genomes can have at least six ORFs (ORF3a, ORF7a, orf1ab, ORF8, ORF6, and ORF10). Amongst these, a frame change between ORF1a and ORF1b makes the output of pp1a and pp1ab polypeptides, which are processed by encrypted proteases such as chymotrypsin (3CLpro) or the main protease (Mpro). Besides, one or two papain-like proteins are processed to produce 16 non-structural proteins (nsps) (49). The functions of the nsps are not completely obvious; but they probably participate in viral RNA synthesis, virus assembly, closing the host macromolecular synthesis, disable the host's innate immune response. The structural proteins such as N protein interact with the virion genomic RNA, then assembly of virus particles happens, finally, the nucleocapsid is formed. The nucleoid capsule grows inside the perinuclear membranes inside the endoplasmic reticulum. The mature virus particles are moved across the Golgi complex and get out of the infected cell through budding (11). Therefore, SARS-CoV-2 infects host cells, but conversely, nonenveloped viruses do not lyse cells personally (6). The cell-mediated immune arm of the adaptive immune system attacks contaminated cells and eliminates these cells; it is termly called a cytokine storm. The humeral immune arm hits the available viruses outside cells and locks them from invading other healthy cells. It is named neutralization. The innate and humeral adaptive immunity prevents from happening severe infections and it is the cell-mediated immunity system that destroys any lung cells infected with the virus (50). Heme is an essential part of hemoglobin. It is a porphyrin including iron. Firstly, the ORF8 binds to the porphyrin, then, ORF10, orf1ab, and ORF3a hit the heme that is on the 1-beta chain of hemoglobin, resulting in dissociating the iron to form the porphyrin. Gradually, as these attacks increase, hemoglobin decreases. Eventually, less oxygen and carbon dioxide are transferred into the bloodstream, which in turn contributes to the symptoms of suffocation (51). Other mechanisms by which the virus is involved have not yet been fully elucidated, and research is underway to find out.

There is a link between coronavirus infection and hypertension, there seems to be a direct link to Type 2 diabetes mellitus (T2DM). In the respiratory tract, ACE2 has the function of destroying angiotensin II to angiotensin 1-7 and acts to regulate the angiotensin system. By increasing ACE1 activity and inhibiting ACE2, angiotensin II binds to the angiotensin 1 (AT1R) or AT2R receptor, leading to inflammatory responses and stimulation of aldosterone secretion. As a result, not only does blood pressure rise but the risk of respiratory distress syndrome increases. In contrast, angiotensin 1-7 leads to anti-inflammatory responses that are desirable for the recovery of patients with COVID-19. People with COVID-19 have an imbalance in the activation of these pathways due to high blood pressure and insulin resistance and T2DM (52).

4. Prevention and current pharmacological treatments

No specific treatment is yet available for SARS-CoV-2, so it is important to prevent further spread of the new virus. When the R0 is from a disease above one, it means it can be transmitted very quickly and easily. While that of COVID-19 is about 2.2. Therefore, due to the limitations of medical and health facilities, the focus should be on prevention. The WHO has released some general recommendations to prevent infection, including avoiding touching the nose, mouth, and eyes by your hands, regular handwashing with soap for about 20 seconds, avoiding contact with people, disposable masks should completely cover the mouth and nose of people for

prevention and be replaced every day, using latex gloves outdoors (53). Two meters should be kept away from people who have suspicious symptoms. Keys, cell phones, doors, and car handles should be disinfected regularly with an alcohol pad or disinfectant solution. SARS-CoV-2 is sensible to heat and ultraviolet rays. Moreover, this virus can be significantly inoperative by lipid solvents including ether 75%, chlorine-containing disinfectant, ethanol, chloroform except, and peroxyacetic acid. National Institutes of Health in the USA has stated that it is a steady virus for several days on surfaces (copper, cardboard, steel, plastic). So far various studies have looked at the persistence of the virus on different inanimate surfaces; it has revealed that the virus is persistent for at least up to 9 days on surfaces such as plastic and glass. The hopeful thing is that the virus will be inactivated by using disinfection substances to clean the surfaces, including 0.5% hydrogen peroxide, 62 to 71% ethanol, and 0.1% sodium hypochlorite. Other biocidal substances that have less effect are 0.02% chlorhexidine digluconate and 0.05 to 0.2% benzalkonium chloride (54). However, strengthening the immune system is effective by consuming citrus fruits, getting enough sleep and exercising, as well as eating fluids. Vitamin C with the role of antioxidant activity can probably decrease inflammation. Vitamin C improves immune cell function, vasopressor synthesis, endovascular function, and epigenetic immunologic modifications, thus, using it can help to prevent infection (55).

There is currently no precise treatment for the SARS-CoV-2, but research is ongoing. Since the virus is spreading, researchers are conducting several experiments on several drugs. Most of the medications and methods used during this period and clinical trials have been effective drugs against SARS, HIV, MERS, and influenza viruses, which it is hoped these drugs are effective in COVID-19, too. Oxygen therapy, antibiotics combination, antivirals, corticosteroids, and convalescent plasma are some types of conventional treatment for patients infected by SARS-CoV-2 (56). No specific treatment is yet available for SARS-CoV-2, so it is important to prevent further spread of the new virus. When the R0 of a disease is above one, it means it can be transmitted very quickly and easily. While that of COVID-19 is about 2.2. Therefore, due to the limitations of medical and health facilities, the focus should be on prevention. The WHO has released some general recommendations to prevent infection, including avoiding touching the nose, mouth, and eyes by your hands, regular handwashing with soap for about 20 seconds, avoiding contact with people, disposable masks should completely cover the mouth and nose of people for prevention and be replaced every day, using latex gloves outdoors. Two meters should be kept away from people who have suspicious symptoms. Keys, cell phones, doors, and car handles should be disinfected regularly with an alcohol pad or disinfectant solution. SARS-CoV-2 is sensible to heat and ultraviolet rays. Moreover, this virus can be significantly inoperative by lipid solvents including ether 75%, chlorine-containing disinfectant, ethanol, chloroform except, and peroxyacetic acid. National Institutes of Health in the USA has stated that it is a steady virus for several days on surfaces (copper, cardboard, steel, plastic). So far various studies have looked at the persistence of the virus on different inanimate surfaces; it has revealed that the virus is persistent for at least up to 9 days on surfaces such as plastic and glass. The hopeful thing is that the virus will be inactivated by using disinfection substances to clean the surfaces, including 0.5% hydrogen peroxide, 62 to 71% ethanol, and 0.1% sodium hypochlorite. Other biocidal substances that have less effect are 0.02% chlorhexidine digluconate and 0.05 to 0.2% benzalkonium chloride (54).

5. Effective chemical drugs

1. **Remdesivir (GS-5734):** The Function of this drug is blocking RNA-dependent RNA polymerases. Recent research on animal models and cell cultures has shown that Remdesivir can prevent the proliferation of coronaviruses, but it is not clear how it does so. The research surveyed the effect of this drug on the virus that leads to MERS. It was discovered that remdesivir blocks a specific enzyme needed for viral replication. Coronaviruses are increased by replicating their genetic material with an enzyme known as RNA-dependent RNA polymerase (57). Remdesivir is still searching in experimental cases. An investigation revealed some information showing that remdesivir is effective against the SARS-CoV-2 in Vero E6 cells (58). Based on the available scientific evidence to the FDA, it is sensible to consider remdesivir as an effective treatment for COVID-19 (59).

2. **Chloroquine Phosphate**: It is widely used as antimalarial medicine that has a significant antiviral effect (60). The action mechanism of this drug may include viral protein glycosylation, virus assembly, suppressing the viral RNA polymerase, new virus particle transport, and virus release (58, 61–64). It has been observed that very low concentrations have been very effective in dealing with SARS-CoV-2.

3. **Hydroxychloroquine**: this drug is being used as a clinical trial on AIDS treatment (61). In an up-to-date trial with patients on COVID-19 remedy has demonstrated antiviral activity in composition with the antibiotic azithromycin against SARS–CoV-2 in vitro and unchecked clinical studies (65).

4. **Lopinavir-Ritonavir**: This combination is approved for AIDS that can be utilized for adults and infants over 14 days of age (66). In fact, both medicines are HIV protease inhibitors. But they are also undergoing phase 2 tests for MERS (67). SARS-CoV-2 has a key enzyme for replication called Mpro. Lopinavir-Ritonavir may bind to this enzyme and hope to suppress coronavirus activity

(68).

5. **Favipiravir:** This is an RNA-dependent RNA polymerase inhibitor for novel influenza. In addition to the influenza virus, favipiravir can control other RNA viruses such as flavi-, alpha-, filo-, bunya-, arena-, noroviruses (69). It inhibits viral RNA polymerase and prevents the replication of viral genes in the cell. The drug appears to have an antiviral effect on SARS–CoV-2 and other viruses (70). In an investigation in China, favipiravir had even more potent antiviral effect than that of lopinavir. Right now, this drug is under more investigation.
6. **Ganciclovir:** Cytomegalovirus infections are targeted by ganciclovir. In fact, it is a potent inhibitor for the cytomegalovirus (71).
7. **Acyclovir/Penciclovir:** Herpes simplex virus and Varicella-zoster virus are the main targets for acyclovir. It is a synthetic acyclic derivative that results in chain termination (72).
8. **Ribavirin:** Ribavirin is also called Umifenovir has a broad-spectrum of antiviral effects, but the main use of this drug is for influenza virus infections. Also, it has not shown noticeable adverse effects (73). Ribavirin is used only as an intravenous injection (74).
9. **Methylprednisolone:** It has already been used in COVID-19 patients in combination with oseltamivir and antibiotics. But there is still no conclusive evidence that methylprednisolone is effective, and more research is needed (29).
10. **Nitazoxanide:** Its role is to modulate the proliferation of bacteria and viruses. It is approved to use for treating the animal coronaviruses but is still under investigation for humans (75).
11. **Nafamostat:** This is a synthetic serine protease inhibitor, which is prescribed for Influenza, MERS, and Ebola. The main action of this drug is to prevent membrane fusion by decreasing the release of cathepsin B (76,77). This drug is right now under investigation.
12. **Oseltamivir:** It is another remedy for influenza A and B. Oseltamivir decreases the spread in the respiratory tract by preventing the viral enzymes and blocking the release of viral particles from human cells (78). In a clinical trial for curing COVID-19, Oseltamivir is using with Chloroquine and Favipiravir right now(79).
13. **Arbidol:** It is also known umifenovir. Arbidol is applied to treat the influenza B and A viruses; it inhibits viral fusion with the host cell membrane, so the virus cannot enter into the cell. The only way to use this medicine is orally and considerably suppress SARS-CoV-2 (73).
14. **Bevacizumab:** It is a monoclonal antibody and its target is vascular endothelial growth factor (VEGF)(79). Bevacizumab may decrease the levels of VEGF caused by hypoxia of the infected respiratory tract epithelium, therefore, it might overcome the edema in COVID-19 patients (80).
15. **Darunavir: S**ome surveys illustrated that darunavir in combination with cobicistat (81) Actually, darunavir is an HIV protease inhibitor, and cobicistat is a booster for enhancing the pharmacodynamics and pharmacokinetics of Darunavir (82). darunavir can inhibit SARS-CoV-2 infection in vitro by repressing viral replication (83).
16. **Type II transmembrane serine protease (TMPRSS2) inhibitors:** Because this receptor is one of the receptors involved in preparing and entering the virus into the host cell (84), it may be effective to block it (70).
17. **Recombinant human interferon α2β:** Due to its good effect on SARS and MERS diseases, it seems to be effective in treating patients with COVID-19. However, it needs to investigate in terms of its efficacy and safety (79).
18. **Thalidomide:** Antiinflammatory action, destroying mRNAs in blood cells, and enhancing interleukins secretion is the main role of thalidomide. Activating interleukins such as IL-12 can activate natural killer cells (85). It is supposed that thalidomide is used to evaluate its effect on COVID-19 (79).
19. **Pirfenidone:** It is used in curing patients with idiopathic pulmonary fibrosis diseases. This drug by IL-1β and IL-4 inhibition could play an important role in anti-inflammatory and anti-oxidant effects. It also appears to have a significant effect on exposure to the SARS-CoV-2 (79).
20. **Anakinra:** It is a recombinant protein medication, which is approved for curing patients with Cryopyrin Associated Periodic Syndrome, Still's disease, and Rheumatoid Arthritis. This drug harnesses the IL-1 that expressed in different organs and tissues (86).
21. **Danoprevir:** It is a protease inhibitor for the treatment of hepatitis C and used in combination with ribavirin, peginterferon-α, and ritonavir (87).
22. **Fingolimod:** it is an immunology modulator that is helpful in multiple sclerosis. It has been shown that using it with ventilator support could be healful for patients with ARDS, so, its efficacy is determining for COVID-19.
23. **Bromhexine:** It is a transmembrane protease serine inhibitor for inhibition of the S-glycoprotein of MERS-CoV and SARS-CoVfor viral entry. It is evaluating in terms of the efficacy with standard treatment in patients with COVID-19.

24. **Baricitinib:** It is an enzyme inhibitor that is suggesting to use for COVID-19 treatment, but no clinical data is still revealed (88). This drug may cooperate to reduce combat cytokine release syndrome (CRS), viral entry, and virus particle assembly (89).
25. **Brilacidin:** It is an Innovation protein made similar to host defense peptide. It has begun to test against SARS-CoV-2 in the middle of March 2020 (90).
26. **Disulfiram:** It seems that it can prevent the papain-like proteases of SARS; however, but no clinical and in vitro data exist for COVID-19 yet.
27. **Eculizumab:** It is a kind of monoclonal IgG antibody that attaches to complement protein C5, so it suppresses membrane attack complex. It has been evaluated in a clinical examination for COVID-19, but yet no data exist to support utilization(91).
28. **Galidesivir (BCX4430):** It is used to cure Ebola and other hemorrhagic fever virus infections. In fact, Galidesivir inhibits RNA polymerase.
29. **Griffithsin:** This is a potent HIV entry inhibitor that displayed effectively in vitro activity against SARS (92).
30. **Nelfinavir**: it is an HIV-1 protease inhibitor, so it may be effective against SARS-CoV-2, but no clinical information exists yet (93).
31. **Teicoplanin:** It is an antiviral for the staphylococcal infection that also has inhibitory effects on the MERS. Therefore, it is considered as a potential remedy to control the SARS-CoV-2 (94).
32. **Baloxavir:** This antiviral is active against influenza viruses and could be a potential drug. There is no published clinical trial in terms of its safety and effectiveness in the treatment of COVID-19 (95).
33. **Atazanavir:** This drug with ritonavir or alone is usually utilized for treating HIV. It has seen that azanatavir has activity against SARS-CoV-2 in Vero E6 cells in vitro (96).
34. **Azithromycin:** It belongs to the category of macrolides and has in vitro activity against viruses such as Zika and influenza. Although no clinical data currently exist, it can be used in combination with other drugs as its anti-inflammatory and immunomodulatory effects (97).
35. **Colchicine:** This drug may fight the cytokine storm of COVID-19 by suppressing proinflammatory cytokines and chemokines. NOD-like receptor protein 3 (NLRP3) is responsible for the release of interleukins, in particular IL- 1β. 37 in acute respiratory distress syndrome. Colchicine can inhibit NLRP3 (98).

Drugs Tocilizumab, Corticosteroids, Leronlimab, and Sarilumab have also been used as adjunctive therapy (99–101).

6. **Effective biological treatments**
    1. **Convalescent plasma therapy (CPT):** Immunoglobulins are helpful in several diseases (102). In this way, the body prepares the necessary antibodies to fight the virus in advance. Although, some viruses such as influenza viruses, Ebola virus (EBOV), HIV-1, and hepatitis C virus could mutate their superficial glycoproteins to avoid the antibody responses. Thus, using this kind of remedy has its obstacles (103). Currently, one of the ways to save people with COVID-19 is using blood plasma from those who have been cured of the disease. In an FDA-approved treatment plan, this method has been used, and doctors hope to use the plasma of patients who have recovered from COVID-19 to patients who are still in critical condition to help improve current patients (104). However, the amount of plasma available is less than the number of patients, so this method is currently used to treat critically ill patients (105). It has been observed that RNA of SARS-CoV-2 disappears completely by CPT therapy and neutralizing antibody titers rise, so mortality rate in severe COVID-19 patients reduces (106).
    2. **Interferons (IFNs):** IFNs are a broad class of cytokines divided into type I, type II, and type III that release by the immune cells when subjected to infections, autoinflammatory, and autoimmune diseases (107). IFNs have antiviral activities initiated by their interactions with cognate receptors (108). Type I IFNs could effectively inhibit virus replication and activate immune cell subsets. Type I IFNs can be used alone or in combination with other therapies to treat chronic and acute viral infections due to its antiviral and immunomodulatory effects (109). Among the different types of type I IFNs, IFN-α is generally used to treat hepatitis B and C viruses. It binds to cellular surfaces' receptors and initiates signaling cascades, and finally, could inhibit the replication of vires. This antivirus has been used as an inhaler and injector for the treatment of COVID-19 (110).
    3. **Vaccines:** Vaccines are effective weapons against infectious diseases caused by viruses. The isolation of the new coronavirus has led scientists to think about produceing a new vaccine, but the chances of success due to various processes in the development and final approval of a vaccine are low (111). Five technical routes including recombinant genetically engineered vaccines, nucleic acid vaccines, inactivated vaccines, vaccines made from attenuated influenza virus vaccine vectors, and adenovirus vector vaccines for the development of SARS–CoV-2 vaccines have developed by scientists (112). At present, there are 10 candidates for COVID-19 vaccines that are examining;

ChAdOx1 nCoV-19 (Moderna), mRNA-1273 (University of Oxford), bacTRL-Spike (Symvivo Corporation), LV-SMENP-DC (Shenzhen Geno-Immune Medical Institute), Adenovirus Type 5 Vector (CanSino Biologics Inc), Pathogen-specific aAPC (Shenzhen Geno-Immune Medical Institute), INO-4800 (Inovio Pharmaceuticals), BNT162 (BioNTech), and two vaccines developed by Wuhan Institute of Biological Products and Sinovac Biotech. on May 22, 2020, the first-in-human trial of a recombinant adenovirus type-5 (Ad5) vector COVID-19 vaccine was reported and the results revealed that The Ad5 vectored COVID-19 vaccine was well immunogenic at 28 days after vaccination (113). Also, on August 15, 2020, The Lancet journal published the results of randomized, double-blind, placebo-controlled, phase two trial of the Ad5-vectored COVID-19 vaccine to determine the appropriate dose of this vaccine, indicating that the vaccine at 5 × 1010 viral particles was safe and induced the immune responses (114).

4. **CD24Fc:** CD24 is a glycosylated membrane protein expressing in granulocytes, immature B and T cells, some epithelial cells, and macrophages. CD24 can play a regulatory role in the homeostasis of B and T cells (115) and decrease the immune response of the host against proteins released by damaged cells. Thus, it is another molecule that can control the inflammatory response related to SARS-CoV-2 infection. CD24Fc is a recombinant fusion protein consisting of CD24 attached to the Fc region of human IgG1. Preclinical studies have demonstrated that CD24Fc relieves the graft-versus-host disease by decreasing TNFα, IL1β, and IL6 release (116). Therefore, according to these data, a biological reason has been provided for conducting a CD24Fc clinical trial on COVID-19, and at present, a randomized, double-blind, placebo-controlled, phase 3 study is performing on severely ill infected patients [NCT04317040] (117).

5. **Mesenchymal stem cells:** It is a cell-based approach to moderate the damage resulting from inflammation in COVID-19 and is represented by allogeneic mesenchymal stem cells (MSCs). MSCs are multipotent cells with reparative that have immunomodulatory properties, they are isolated from different tissue types (118). MSCs have protective effects by secretion of multiple paracrine factors such as anti-inflammatory cytokines (119). Based on A clinical trial, it was indicated that allogenic MSCs transplantation was safe in 7 COVID-19 patients (120). Also, another study examined exosomes efficacy and safety resulted from allogeneic bone marrow MSCs in moderate-to severe acute COVID19 patients. Exosomes contain a variety of growth factors, cytokines, microRNAs, and mRNAs; they mediate regenerative, anti-inflammatory, and immunomodulatory properties of MSCs. Based on this study, the survival rate was 83%, oxygenation was increased, and coagulation parameters, inflammatory, and leukocyte count were changed. All these results approved that MSCs had a positive impact on the cytokine storm of SARS-CoV-2 (121).

7. Effective natural herbs

China recommends using herbal treatments for the SARS–CoV-2, but not all scientists agree. Dr. Edzard Ernest of United Kingdom's University warns that these drugs pose two direct and indirect risks to patients. According to the National Health Commission of China, 90% of people with COVID-19 use herbal remedies for their treatment. Traditional Chinese medicine (TCM) uses phytochemical compounds in different forms such as pills, teas, tinctures, powders, and so on (83). Luo et al., analyzing the frequency of TCM in 23 provinces, concluded that 10 main and important drugs in traditional Chinese medicine are helpful to treat SARS–CoV-2, including Glycyrrhizae uralensis, Astragalus membranaceus, Rhizoma Atractylodis Macrocephalae, Saposhnikoviae divaricate, Fructus forsythia, Lonicerae Japonicae Flos, Radix platycodonis, Atractylodis Rhizoma, Cyrtomium fortune J. Sm and Agastache rugose (122). According to research by Xu et al, Yu Ping Feng and Astragalus membranaceus have been used in coronavirus-2 prevention programs. These drugs strengthen the immune system. Medicinal plants used in northern China include Agastache rugosa and Atractylodis Rhizoma. Medicines used in southern China included medicinal plants with aromatic dehumidification properties (123). Liu has stated that the most important medicinal plants for the treatment of COVID-19 include: qinggan capsules, lianhua qingwen capsules, and shufeng jiedu capsules. Also, qingfei touxie fuzheng, gancaoganjiang decoction, sheganmahuang decoction, and qingfei paidu decoction (QPD) are some effective recipes of TCM. QPD has been one of the most important medical prescriptions used during last few months, which consisted of Glycyrrhizae Radix et Rhizoma Praeprata cum Melle, Ephedrae Herba, Armeniacae Semen Amarum, Cinnamomi Ramulus, Gypsum Fibrosum, Alismatis Rhizoma, Poria, Bupleuri Radix, Polyporus, Atractylodis Macrocephalae Rhizoma, Scutellariae Radix, Zingiberis Rhizoma Recens, Pinelliae Rhizoma Praepratum cum Zingibere et Alumine, Asteris Radix et Rhizoma, Farfarae Flos, Dioscoreae Rhizoma, Belamcandae Rhizoma, Asari Radix et Rhizoma, Aurantii Fructus Immaturus, Pogostemonis Herba, and Citri Reticulatae Pericarpium (124).

Several Chinese herbs have been also suggested to be used by some patients to prevent SARS–CoV-2, including Shu Feng Jie Du Capsule, Huo Xiang Zheng Qi Shui, and Jin Hua Qing Gan Granule. Drugs used during clinical therapy include: Re Du Ning Injection, Xue Bi Jing injection, Qing Fei Pai Du Tang, Xing Nao Jing Injection, Xi Yan Ping Injection, Tan Re Qing Injection and some formulas of

traditional Chinese medicine should be taken with these drugs (56). Sheng Mai Injection, Gong Niu Huang Pill, Shen Mai Injection, Shen Fu Injection, and a Su He Xiang Pill are also used in patients in critical condition. Some other medicinal herbs suggesting to test in order to prevent and treatment of COVID-19 are dried rhizome of Curcuma longa L. This herb has antiviral activities against herpes simplex virus and HIV, therefore, it can also have inhibitory effects in terms of SARS-CoV-2 entry into the host cell (125).

8. Pharmacological and natural combination treatment

Dr. Lei Guan Shi, head of the Chinese Medicine Department at Corona's Guan Man Hospital in Beijing, explained the symptoms of the disease from the perspective of Chinese medicine, noting that most patients benefit from a combination of conventional medicine and traditional medicine in China. It is important to control the disease in the first week and prevent it from entering a critical phase. In this regard, the combined use of chemical and herbal medicines has had a significant impact, and a high percentage of patients undergoing combination therapy with traditional medicine and they are common medicine, they have improved. He said that herbal medicines also help to increase the effectiveness of chemical medicines, adding that there are several herbal medicines for the treatment of corona in this country and specified: Herbal medicines have been used to boost the immune system and reduce inflammation and edema in patients' lungs, and in some cases, acupuncture has been used.

Previous experiments to treat SARS and MERS have been successful with the simultaneous use of common medicine and herbs. As a result, the Chinese scientists took the first steps to treat the COVID-19 with these two methods. In some surveys, TCM and conventional medicine are combined for assessing the effect on patients with COVID-19. Drugs that are using include antiviral drugs such as IFNα-2b and ribavirin a-interferon, antibiotics such as azithromycin and moxifloxacin, and human immunoglobulin. Chinese herbs using include mixtures of different herbs, single herbs, and Chinese proprietary medicines. Each of the three types can be combined with western medicines, separately. The mixture of herbs prescribed to combination with drugs depends on the patient's symptoms (126).

9. *In Vitro* studies

The most important IVD test for SAS-CoV-2 is RT-PCR that takes a few hours to perform. It takes 45 minutes for an IVD assay with a Cepheid. Interestingly, POC molecular assay by Abbott was reduced the assay time to 5 minutes. Most of the molecular tests by the FDA, EUA, and CE have been affirmed. Many serological assessment methods, along with molecular methods, are used to diagnose SAS-CoV-2. Many serological assessment methods, along with molecular methods, are used to diagnose the virus. The most significant serological methods are manual ELISA, automated chemiluminescent IA, and rapid lateral flow IA. These methods detect IgG and IgM produced in people infected with coronavirus-2. Three teams of investigators are developing serologic tests to answer key questions about SARS-CoV-2. Guo and colleagues looked at the kinetics of immunoglobulin M, (IgM), IgA, and IgG antibody response in infected patients using an ELISA based on COVID-19 viral nucleocapsid protein. The researchers assessed 208 plasma samples from 82 confirmed and 58 probable COVID-19 cases (127). Antibodies were found as early as 1 day after the start of symptoms. IgM ELISA detected more cases than polymerase chain reaction (PCR) on day 5.5 of sickness. The combination of IgM ELISA plus PCR detected 98.6% of cases versus 51.9% with a single PCR. During the first 5.5 days, PCR had a higher positivity rate than IgM; the reverse was true after day 5.5. No cross-reactivity was found with common coronaviruses that cause upper respiratory infections. In a family cluster, PCR-negative family contacts of COVID-19 cases had positive serologic assay, confirming the presence of antibodies in asymptomatic infection. Zhao and colleagues assessed the IgM antibody, IgG antibody, and total antibody against SARS-CoV-2 on serial blood samples obtained from 173 patients (median age, 48 years) with PCR-confirmed COVID-19 in Shenzhen, China. Plasma samples were tested using ELISA kits prepared by Beijing Wantai Biological Pharmacy Enterprise Co in China (128). In samples collected during the first 7 days after illness onset, positive rates were 66.7% for PCR and 38.3% for antibody assays. During the second week after illness onset, positive rates were 54.0% for PCR and 89.6% for antibody assays. The combined use of PCR and antibody testing improved the identification of positivity through various phases of illness. Increases in antibody levels were not associated with RNA clearance, including in three patients with a critical illness. A strong correlation was found between antibody titer and clinical severity for more than two weeks following illness onset. The total antibody was more sensitive than IgG or IgM antibody. Li and colleagues developed a lateral flow immunoassay that detects IgM and IgG antibodies simultaneously and can test finger-prick blood, plasma, and serum. The point-of-care test provides results within 15 minutes and requires no special equipment. The assay was tested in six provinces in China on blood samples from 397 COVID-19 patients which were confirmed by PCR and on 128 negative cases (129). However, in COVID-19 patients, the number of white blood cells may fluctuate. Leukocytosis, Leukopenia, and lymphopenia have been notified, though the most typical manifestation is lymphopenia. High levels of aminotransferase have also been reported in these patients. At the time of hospitalization, most pneumonia patients have standard serum procalcitonin levels; although, their levels are likely to be higher in people who need intensive care (ICU). In one study, high levels of D-dimer and further severe lymphopenia were associated with mortality (130).

There is evidence for the ability of Chloroquine and

Hydroxychloroquine to inhibit SARS-CoV-2 in vitro. Liu et al. obtained a similar 50% cytotoxic concentration (CC_{50}) for both drugs. Irrespective of the multiplicity of infection (MOI), the 50% maximal effective concentration (EC_{50}) of Chloroquine (CQ) is lower than Hydroxychloroquine (HCQ) (131). According to research by Yao et al. (1) in vitro, it was identified that HCQ was more effective than CQ in eliminating coronavirus-2 (the HCQ EC_{50} of 0.72 μM and 5.47 μM for CQ, and is MOI = 0.01). In a study conducted by Wang et al., The antiviral activity of chloroquine was identified in vitro. The CC_{50} >100μM at an MOI of 0.05 and EC_{50} of 1.13μM. In this study, they ascertained that chloroquine had high selectivity for coronavirus-2 instead of host cells (132). Lopinavir has shown in vitro activity against SARS-CoV, and a study of lopinavir plus the protease inhibitor ritonavir demonstrated clinical efficacy for human SARS. To evaluate whether lopinavir-ritonavir would be effective for COVID-19, investigators conducted an open-label randomized trial at a single hospital in Wuhan, China, beginning on January 18, 2020. They assigned 199 adult patients with COVID-19 infection, radiographically confirmed pneumonia and oxygen saturation of <94% or partial pressure of oxygen <300 mm Hg to receive standard care alone or with oral lopinavir-ritonavir (400 mg–100 mg) twice a day for 14 days. Lopinavir-Ritonavir and standard caregivers do not differ significantly in clinical improvement over time (16 days), duration of intensive care unit stay, days of mechanical ventilation, or days of oxygen support. Patients who received lopinavir-ritonavir had lower 28-day mortality (19% vs. 25%), but the between-group difference was not significant. SARS-CoV-2 RNA concentrations in throat swabs obtained over time did not differ between the two groups (133). The Lopinavir-Ritonavir test was performed in adults hospitalized with severe COVID 19. 199 patients were randomly selected for this study. 99 were in the Lupinavir-Ritonavir treatment group and 100 were in the standard care group. There was no difference between patients taking lopinavir and ritonavir and patients in the standard care group. People who used Lupinavir-Ritonavir recovered one day earlier. Gastrointestinal side effects were more frequent in patients taking lopinavir-ritonavir, but serious side effects were more prevalent in cases in the standard care group (133). In one study, SARS-CoV-2 inhibition was investigated by Remdesivir and Chloroquine in vitro. The study found that the drugs were effective in controlling COVID-19. These drugs are used in patients with a safety track record and be effective against various diseases. Therefore, it is recommended that these drugs be used to treat patients with coronavirus-2 (134). Another study examined the effects of Lopinavir/ritonavir versus Favipiravir on the treatment of coronavirus-2 in vitro. Several patients received oral Favipiravir plus (IFN)-α by aerosol inhalation and others treated with Lopinavir/ritonavir plus IFN-α by aerosol inhalation. The study found that Favipiravir was more effective in treating coronavirus-2 (135).

However, another suggested therapy is immunopathogenesis that needs to be more surveyed. Any action to help the immunomodulatory effect of T-helper2 may help ill patients to reduce the damaging consequence of inflammatory pneumonia. Interleukin-10 inhibits IFN-gamma TNF-alpha, and IL-12 so the immune system polarizes towards T-helper2 and away from T-helper-1 (136).

10. *In Vivo* studies

Experimental evidence regarding the effect of CQ/HCQ on the treatment of COVID-19 is currently very limited. The first clinical results were released from the Chinese government in February 2020 that reported treatment of more than 100 patients with chloroquine phosphate in China led to significant improvements in pneumonia and lung imaging and a reduction in disease duration. No side effects have been stated. These findings came from several pooled continuous trials utilizing a variety of study patterns. So far, no experimental data have been published to support these findings. In the clinical trial by Gautret and colleagues in France On the 17th of March 2020 (65), 36 patients with COVID-19 were examined. Six of them had no symptoms while 22 patients had symptoms of upper respiratory tract infection and eight people had symptoms of a lower respiratory tract infection. For 10 days, twenty patients received 200mg of HCQ three times a day. For six people, azithromycin was also prescribed to prevent bacterial superinfections. The control group had normal care. The principal result of the test was the SARS-CoV-2 carrier on Day 6, experimented using the PCR technique on nasopharyngeal swabs. The results of the treatment group of patients tested on the sixth day were noticeably negative compared to those in the control group. The test of six patients who had received simultaneously azithromycin and HCQ was negative on Day 6. The researchers believe that the combined treatment of HCQ and azithromycin has remarkable effects on the treatment of patients. According to the positive results of the first clinical trials, official guidelines for the treatment of coronavirus-2 were published using CQ and HCQ drugs.

The National Health Commission of the People's Republic of China announced in mid-February that it would recommend that the patients use 500 mg of chloroquine phosphate (300 mg for CQ) twice a day be treated for a maximum of 10 days (70). On the 17th of March, the L. Spallanzani National Institute in Italy suggested treatment for coronavirus-2. The recommended treatment was 400 mg HCQ per day or 500 mg CQ per day with another antiviral drug (137). Controlling cytopathy at a concentration of 1 (mg/l) Lupinavir with ribavirin at 6.25 (μg / ml) and collected information suggest that the compound may be synergistic against coronavirus-2 in vivo (74). An in vivo investigation of MERS revealed that EC_{50} values made for lopinavir were 11.6, CC_{50} values > 50 μM, the SI was > 4.3, and EC_{50} values generated for ritonavir 24.9 μM with CC_{50} values > 50 μM,

the SI > 2 (138). The study of Sheahan et al found that both prophylaxis and treatment with Remdesivir had protective effects against MERS-CoV proliferation and associated pathology. In general, they suffer less lung damage than the control group, and their pulmonary function is better. Among the mice tested, those who took Remdesivir the day before the infection had a six-day better survival than those who did not receive Remdesivir (138).

11. In Silico studies

Regarding the fact that SARS-CoV-2 has caused a pandemic and lack of efficacious vaccine/drug, in silico studies play an important role in testing whether existing drug-like compounds are effective against viral infections or not and it can help health workers to use the best treatment against this virus. Therefore, the in silico studies were collected and the effective herbal and chemical drugs against this virus discussed. SARS-CoV-2 genome is translated into polyproteins that have been processed by its protease enzymes. These proteins are prime antiviral targets. In fact, the crucial role of 3CLprotease or Mpro makes this enzyme as the most promising target. In silico and genomic structural features of novel coronavirus showed that it is closely like the SARS coronavirus and it suggests that traditional Chinese medicine (TCM) may be useful in the current outbreak (5) because treating patients with the severe acute respiratory syndrome (SARS) who were treated with TCM in 2003, resulted in the shorter length of hospitalization, decreased steroid-related side effects, and improvement of symptoms (139). In this issue of the Journal of Integrative Medicine, Zhang *et al.,* provided in silico methods to narrow down TCM remedies that may directly inhibit the coronaviral reproduction. In this study, integrative model of absorption, distribution, metabolism, and excretion (ADME) was used to screen natural compounds that may be bioactive via being administrated orally and also molecular docking software AutoDock4 was used to perform protein compound docking analysis and finally it was shown that 13 natural compounds have potential anti-2019-nCoV activity and 125 Chinese herbs contain 2 or more of these compounds (140). Moreover, a library of 100 FDA approved antiviral compounds and 1000 active phytochemicals have been screened through PyRx and autodock-Vina virtual screening tools in which Nelfinavir displayed the highest binding affinity -8.4 Kcal/mol and strong and stable interactions with the amino acid residues present on the active site of COVID-19 Main Protease. Besides, drugs including Rhein, Withanolide D, Withaferin A, Enoxacin, and Aloe-emodi also showed a good binding affinity with favorable ADME properties respectively (141).

The biological activity of seven popular anti-malarial compounds as a ligand of COVID-19 protease including mepacrine, quinine, chloroquine, hydroxychloroquine, phomarin, artemisinin, and proguanil have been assessed and compared by log P and log S values and based on the binding affinity of these drugs, it has been revealed that epacrine appears as the most powerful inhibitor among other six compounds. Other potential inhibitors of COVID-19 protease are chloroquine, hydroxychloroquine, and phomarin (142). In addition, using the SwissDock web-based on the EADock ESS docking algorithm and ALOGPS 2.1 program which is based on the electro-topological state indices and associative neural network modeling, it has shown that 11 different species of Indian medicinal plants, more particularly those with anti-viral, anti-malarial or other similar activities have inhibition properties against COVID-19 protease. On the other hand, lipophilicity, aqueous solubility, and binding affinity of the extracted compounds suggest the most inhibition potentials in harsingar, aloe vera, giloy, turmeric, neem, ashwagandha, red onion, tulsi, cannabis, and black pepper respectively. Notably, inhibition potentials of the extracts of harsingar, aloe vera, and giloy are very encouraging in comparison with binding affinity with hydroxychloroquine (143). In another docking and molecular dynamics study, it has been revealed that Flaviolin which is a fungal metabolite can interact with one of the important target proteins of SAR-CoV2, 3CLpro, and block its function (144). Based on a study conducted by Rimanshee Arya et al., in which the catalytic domain of PLpro has been delineated by comparing it with the known sequences of other coronaviruses., sixteen FDA approved drugs, namely formoterol and chloroquine were found to bind the target enzyme with significant affinity and good geometry, signifying their potential to be applied against 2019 novel coronavirus (145). Using the CASTp server, the active site pocket in the 'SARS-CoV2 E' protein was calculated (146). Active pocket with the highest volume as well as area was considered for molecular docking studies with phytochemicals having 250 conformations (147, 148) via the AutoDock (149) tool. After binding human 'SARA-CoV E' protein with three phytochemicals namely Belachinal, Macaflavanone E, and Vibsanol B, the random motion of the human 'SARS-CoV2 E' protein gets decreased; this, in turn, stops the human "SARS-CoV2 E" protein function. As Belachinal, Macaflavanone E & Vibsanol B, have passed the ADMET test and 'Lipinski's Rule of 5s', they may be consumed in controlling disease caused via SARSCoV2, after further in vitro and in vivo investigations (150).

Using PyRx 0.8, virtual screening of pharmacologically active compounds has been performed in order to recognize new potential antiviral drugs against 3CLprotease or main protease (Mpro) of SARS-CoV-2 which is the major proteolytic enzyme of SARS-CoV-2 and cleaves nsp4-nsp16. All the drug-like molecules have been gained from LOPAC1280 drug library (Library of Pharmacologically Active Compounds, Sigma-Aldrich, St. Louis, MO). Among 1280 compounds, the top 10 molecules including Etoposide, BMS_195614, KT185, Idarubicin, GSK 121015A, WIN_62577, Eptifibatide_acetate, KT203, SB_202190, and GR_127935 were showing good binding efficacy. Molecular

docking of the top 5 selected screened compounds was carried out using AutoDock 4.2.6. finally, it was shown that all identified molecules were capable of interacting with His41 residue which is the key site for catalytic activity (151). In addition, it was found that KT185, KT203, BMS_195614, GSK1210151A, Eptifibatide acetate, and GR127935 hydrochloride hydrate drug molecules were able to inhibit the spike-ACE2 interaction at virus entry step (152). Therefore, these molecules can be better antiviral candidates because of their inhibitory action at both virus-host cell receptor interaction and post-translation step of non-structural proteins. However, a new drug made up of the morpholino based drug coupled with physcion was designed by using Chemsketch free software and further selected for molecular docking study. Notably, designed ligand structures are closely similar to the structure of Remdesivir with slight modification i.e., nucleoside analog has been replaced with morpholino analog. The in silico study revealed that this drug has a high potential of virus RNA Dependent - RNA polymerase inhibition with minimum binding energy (153).

In a study, an online tool "ZINC-pharmer" from ZINC database was used for pharmacophore-based virtual screening of around 1500 drug-like compounds (154). For further evaluation of these drug-like compounds, all the retrieved compounds were screened by molecular docking-base and it was docked with Mpro of SARS-CoV-2. Then, using the Molecular Operating Environment (MOE) system, the selection of screening ligand complex was done with Mpro based on Score (higher than reference inhibitor) and root-mean-square deviation (RMSD) value. Among near 200 compounds which were identified having strong interaction with Mpro of SARS-CoV-2, three compounds (ZINC20291569, ZINC90403206, and ZINC95480156) showed highest binding energy with Mpro of SARS-CoV-2 and strong inhibition effect than the reference inhibitor which can suggest greater potential to stop the replication of SARS-CoV and curing COVID-19 subsequently (155). In another pre-printed in silico study, flavonoids have been screened against novel drug target, Mpro, of SARS-CoV-2 for the identification of Mpro inhibitors to provide natural scaffolds for drug development. Performing virtual screening-based molecular docking, it was shown that binding affinity of flavonoids such as hesperidin and rutin to Mpro of COVID-19 is better than Nelfinavir. Potent of flavonoids can be ranked based on moldock binding score. According to this score, hesperidin, rutin, diosmin, apiin, diacetylcurcumin have the most affinity respectively (156).

In another pre-printed article, as Hepatitis-C virus (HCV) and SARS-CoV-2 are both +ssRNA viruses, molecular interactions between FDA-approved antiviral drugs against the HCV (including beclabuvir, tegobuvir, dasabuvir, deleobuvir, setrobuvir, radalbuvir, lomibuvir, remdesivir, uprifosbuvir and favipiravir) and RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 have been investigated. using InterPro Scanner, the amino acid sequence of the viral RdRp was scanned for the identification of known functional domains/motifs (157). Homology modeling of the RdRp was carried out through SwissModel and quality was checked using PROCHECK. Then, Rigid-body molecular docking between several established antiviral drugs and RdRpSARS-CoV-2 was carried out using AutoDock (v4.2). Molecular docking studies were visualized by PyMol, (158). Finally, it was shown that antiviral drugs for HCV could be utilized as anti-SARS-CoV-2 drugs and Beclabuvir which is a non-nucleoside inhibitor of the RdRpHCV is the best one among other ones because it can efficiently bind to RdRp SARS-CoV-2 and also showed better binding free energy (159). Another study has been designed to predict a protein-based drug for SARS CoV 2. The RNA sequence of that virus was extracted from NCBI. Also, the serface glycoprotein sequences were retrieved from NCBI and genbank. Then, the protein structure was predicted with swiss model and phyre 2 protein structure prediction tools. The predicted spike glycoprotein had a docking function with the human ACE2 receptor which was shwon by using Hex 8.0 docking tool. The proposed amino acid sequence of FC region IgG1 and human ACE2 receptor are taken and fused as amino acid sequence. Fc region IgG1 has a vital immunological response and helps curing the disease. SARS CoV 2 SPIKE GLYCOPROTEIN and ACE2-FC region IgG1 both have a bonding and docking ability. This protein complex can be considered as a potential neutralization drug against SARS CoV 2 virus (160).

In a study conducted by Chi Xu et al. in 2020, 28 putative viral proteins encoded in its genome were listed in order to use the best available structures for screening. Then, small peptides and other 10 proteins were removed from the list as there was no structure for either SARS or SARS-CoV-2. Among other remained proteins, S protein and nsp5 of SARS-CoV-2 have structures deposited in the protein data bank (PDB) with PDB ID 6CS2 and 6LU7, respectively. The remaining viral proteins shared high sequence identities with their SARS counterparts, ranging from 76.60% in nsp3 to 99.84% in nsp13, except nsp4. Finally, 14 structural models were built and followed by molecular dynamics refinement and simulation for optimized protein structures. In addition, FDA-approved drugs and drugs currently in clinical trials in DrugBank were selected for virtual screening. A list of active sites from structures of the 16 viral proteins and ACE2 protein (PDB ID: 6CS2) as the ligand targets for screening were selected. In fact, this study evaluates current candidate drugs based on structures of viral proteins and human ACE2 receptor. Besides, the binding energies for nsp5 (Mpro), nsp14, and nsp15 were generally low as the surface geometry and hydrophobicity of the active sites make them more suitable as a drug target. The drugs introduced on the top of the list were anti-HIV drugs, anti-HCV drugs, influenza virus antagonists, chemotherapeutic drugs, and asthma drugs. Anti-

HIV drugs divided into enzyme inhibitors and dideoxynucleoside. Nucleoside reverse transcriptase inhibitors (NRTIs), including Tenofovir and Emtricitabine, may work insufficiently in coronaviruses because coronavirus is positive-sense single-stranded RNA virus which lacks nucleoside reverse transcriptase, and this is also reflected in the docking of this study as most of them ranked at the bottom with low binding affinity. Among the enzyme inhibitors of HIV in docking results of this study, Lopinavir, Dolutegravir, and Raltegravir showed a strong binding affinity with multiple target sites, especially at the catalytic sites of main protease and exonuclease which suggests their potential of clinical drugs in the treatment of COVID-19. Saquinavir, a multi-target inhibitor of SARS-CoV-2, shows a strong affinity with the main protease of SARS-CoV-2. Based on the results of this study, Beclabuvir, Saquinavir, Bictegravir, and Dolutegravir were recommended to be tested in clinical trials. Beclabuvir, used in the treatment of HCV infection, was one of the drugs which performed the best in the docking with low binding energy of-10.4 to nsp5. Also, strong hydrogen bonding, π–π stacking and hydrophobic interactions implies that this drug may be a stronger exonuclease inhibitor of nsp15 rather than Lopinavir. Saquinavir, Bictegravir, and Dolutegravir are antiretroviral drugs used for the treatment of HIV infection. Saquinavir has a binding energy of-9.9 kcal/mol to nsp5. The binding energy of Dolutegravir and Bictegravir to nsp5 are very similar (-8.9 and -9.5 respectively). Surprisingly, all these three drugs are in the category of protease inhibitors. These resemblances make it hopeful to consider them as a potential treatment of COVID-19. Interestingly, other enzyme inhibitors of HIV like Ritonavir, Tipranavir, Elvitegravir, Nelfinavir, Darunavir, and Fosamprenavir have a relatively low binding affinity with the chosen targets in the docking of this study. Six anti-HCV drugs including five RdRp (NS5B of HCV) inhibitors, including Filibuvir, Bictegravir, Ribavirin-monophosphate, Sofosbuvir, and one protease (NS3/4B) inhibitor - Bictegravir are also among best-performed drugs. It is noteworthy that Bictegravir has a profound strong affinity with Mpro (binding energy-10.4 kcal/mol), nsp13 (binding energy -9.8 kcal/mol), nsp14 (binding energy -8.8 kcal/mol) and nsp15 (binding energy -8.3 kcal/mol) and it makes this drug as one of the best-performed drugs in the docking of this study. The comprehensive score of filibuvir does not fall far behind the score of Bictegravir and even exceeds in some docking sites. Therefore, anti-HCV drugs should be tested for fighting against SARS-CoV-2. Tivantinib, Lifirafenib, Entrectinib, Nilotinib, and Radotinib, the chemotherapeutic drugs and Montelukast and Zafirlukast which are used in the treatment of asthma are also on the top of the list. The docking result also reveals the low binding energies of oseltamivir with different targeted proteins of SARS-CoV-2. Moreover, it was shown that Lopinavir, an anti-HIV drug in the category of a protease inhibitor, maybe a potent drug through inhibiting nsp15. Also, it has been predicted that Remdesivir, a nucleotide analog used for the antiviral purpose, binds to nsp14, nsp5, and nsp13 with low binding energy (-8.3 kcal/mol, -7.4 kcal/mol and -7.2 kcal/mol, respectively). It is noteworthy that strong Hydrogen bonds, π–π stacking and hydrophobic interactions between the ligand and protein make Remdesivir as a potent inhibitor. On the other hand, the binding affinity of Remdesivir with RdRp (binding energy-6.3 kcal/mol) is lower than that with endonuclease (binding energy-8.3 kcal/mol), likely to the difficulty to choose the right key residues in this interaction. Finally, the effect of two natural products (Quinine and Doconexent) has been observed in this study. Quinine, an anti-malarial drug, had binding energy of-7.5 kcal/mol against nsp13, which is comparable to some of the drugs used in clinical trials. It can be a potential inhibitor due to its interaction with nsp13 including hydrogen bonding, π–π stacking food supplement, and hydrophobic interactions. Doconexent, a mixture of fish oil and primrose oil which has minor anti-inflammatory effects, is ranked at the bottom of the half against all active sites and it can be due to lack of π–π stacking while it has low binding energy with nsp14 (-6.7 kcal/mol) (161).

In an in silico study, after investigating the inhibitory effect of 21 selected compounds on Chymotrypsin-like protease (3CLpro) called also the main protease (Mpro) (12) by blind and specific docking experiments and performing molecular docking by AutoDock Vina program on molecules obtained from PubChem database, it has been revealed that three molecules (hispidin, lepidine E and folic acid) could bind tightly with the enzyme (162). Another study examined the tendency of 1033 medicinal herbal compounds to ACE2, TMPRSS2, GRP78, and AT1R receptors, and found berbamine, hypericin, and hesperidin had the highest affinity. This study also examined hsa-miR-1307-3p and its tendency to inhibit the virus genome (163) . It is worth noting that His41, Cys145, and Glu166 are important residues in the substrate-binding subsite S1 for the proteolytic activity and the involvement of these residues is effective in forming hydrogen bonds which is important for the inhibitory impact of the Mpro. The best molecule is hispidin because the blind docking shows that 100% of poses are in the active site, it forms a strong hydrogen bond network with nCoV-2019 protease, six hydrogen bonds have been observed in both blind and specific docking and the binding affinity to the 2019-nCoV protease is-7.2 Kcal/mol. The second molecule is lepidine E. 76% of poses are in the active site. Lepidine E bind tightly with the 2019-nCoV protease residues with a binding affinity-7.8 kcal/mol. A very strong and short hydrogen bond has been observed between hydroxyl groups of lepidine E and Leu141 with length of 1.64Å and 1.74Å in blind docking and specific docking respectively. The third best molecule is folic acid. The blind docking implies that 90% of poses are in the active site with a binding affinity of-7.6 kcal/mol and different positions have been verified in specific docking but all positions contain hydrogen bonds.

Briefly, it has been suggested that Hispidin, Lepidine E, and folic acid prevent the spreading of viral infection by stopping the COVID-19's lifecycle through inhibiting the activity of 3CL enzyme strongly (164). In a study conducted by Andre Fischer et al. in 2020, a total of 606 million compounds were extracted from the ZINC database, and all compounds in three-dimensional form were screened with respect to their shape similarity versus a pre-selected set of known and co-crystallized inhibitors of SARS-CoV-1 and SARS-CoV-2. Then, the high number of initial hits were refined to 14240 compounds by selecting the best hits regarding shape overlap. The remaining compounds were docked into the active site of five representative structures of the protease using the smina docking protocol. Among these compounds, 5490 potential hits were selected based on a score below the defined threshold of-7.0 kcal/mol. Glide SP was used as the second docking protocol to evaluate the interaction of the remaining 5490 compounds with the binding site of the protease, in order to increase confidence in ligand ranking based on docking scores. Then, compounds were clustered according to the Tanimoto coefficient. The two compounds with the best Glide score were selected and evaluated regarding their pharmacokinetic properties. This selection process resulted in 144 compounds used for final MD simulations and free-energy calculations. Compounds with a predicted binding free energy better than the cocrystallized ligand were finally selected and 29 compounds were characterized by their potential toxicity, assessed by the VirtualToxLab. The final 29 compounds included 13 compounds with a toxic potential above 0.5 that were discarded from the final set. The final compound selection was measuring ligand efficiency. Concisely, a virtual screening workflow consisting of seven individual steps have been applied to ultimately determine 12 potential binders. In order to provide immediate advice to ongoing clinical treatment options, existing protease inhibitors were evaluated in this study and an additional list of nine compounds including apixaban and nelfinavir as top hits have been reported. Furthermore, two natural compounds with the lowest predicted binding free energy namely taxifolin and rhamnetin have been evaluated as potential inhibitors of Mpro. The Persian walnut (Juglans regia), Agrimonia pilosa, and Japanese cypress (Chamaecyparis obtusa) are natural sources for the extraction of taxofolin (165). In a pre-printed study conducted in 2020, GeneCard17 was used to select mutated genes of SARS-CoV and MERS-CoV, Tumor Necrosis Factor (TNF), and Angiotensin-Converting Enzyme 2 (ACE2). Then, around 150 drug compounds that were effective against SARS-CoV and MERS-CoV were selected and then screened through ZINC15 Database, and the Lipinski rule of five was used to optimize active drug development structure based on the pharmacokinetics of drugs in human beings' body (166). Thence force, clustering-based drug-drug interaction (DDI) networks, and drug repositioning approach were used based on modularity in order to prevent receptor binding capacity and membrane fusion of 2019-nCov. GEPHI was used to make the intensely interacted cluster. Then, promising drug candidates were filtered by the toxicity indicator via ProTox web server (167). For molecular docking, the PDB RCSB server was utilized to recover post-fusion core of 2019-nCoV S2 subunit protein; afterward, complexes of protein-ligand were predicted through submitting ligands (drug compounds) and prepared protein Pdb files. Analysis of molecular docking showed that ZINC000029038525 and ZINC000029129064 drug compounds have the lowest binding energies which means significant potential binding with active sites of post fusion core of 2019-nCov 'S2'. As S2 subunit of 2019-nCov is a main element in fusion of 2019-nCov with host cell membrane which leads to inoculation of its DNA in to the host cell, these compounds may be able to prevent membrane fusion and receptor binding capacity of 2019-nCov and it makes them strong effective therapeutic candidates against 2019-nCov infections (168).

In conclusion, Since the first detection of the 2019-nCoV in Wuhan, China, the COVID-19 is spreading worldwide. There is an urgent need to find efficient therapeutic strategies against it. The development of new drugs has shown some progress but no specific treatment against this new virus is still available. Therefore, the current insightful review evaluates the proposed in vitro, in vivo and in silico therapeutic protocols and earlier records that affords comprehensive information about the contemporary situation of COVID-19 and depicts a picture of the current status of the art with regards to epidemiology, virology, clinical features, diagnosis, and available treatments.

Conflicts of Interest

The authors declare no conflicts of interest.

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Coexistence of human immuno deficiency virus, diabetes mellitus, epididymal cysts, and Fournier's gangrene: A case report

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Abstract

Fournier's gangrene (FG) is a form of necrotizing fasciitis that is localized in the external genital organs and perianal region and causes skin and subcutaneous tissue gangrene. The clinical picture may vary depending on the patient's comorbidities and the extent of infection; Many predisposing conditions such as immunodeficiency, diabetes, alcoholism encourage the spread of the infection. In this case report, we highlighted the importance of emergency debridement in patients with multiple comorbidities by presenting the Fournier's Gangrene case in a 57-year-old immunosuppressive male patient with cystic lesions in the epididymis, with a history of hypertension, coronary artery disease, diabetes, HIV (human immunodeficiency virus) and a history of bipolar disorder. The patient, who was operated on for debridement by the urology, was given 1x500mg daptomycin, 3x1g meropenem, 3x450mg clindamycin IV treatment. The patient was discharged with full recovery after 17 days of hospitalization. Clinical suspicion in Fournier's gangrene cases, early surgical debridement, and extended-spectrum anti biotherapy are important. with rapid diagnosis and treatment in patients with improvement can also be seen in patients with comorbidities.

Keywords: Fournier's gangrene, debridement, HIV, epididymal cysts

1. Introduction

Fournier's gangrene (FG) is a specific form of necrotizing fasciitis that is localized in the external genital organs and perianal region, causing skin and subcutaneous tissue gangrene (1). It is usually polymicrobial (2). The most common microorganisms isolated are *Escherichia coli*, *Klebsiella pneumoniae, Bacteroides fragilis* and *Staphylococcus aureus* (3).

The clinical characteristics may vary depending on the patient's comorbidities and the extent of infection; many predisposing conditions such as immunodeficiency, diabetes, and alcoholism encourage the spread of the infection, and systemic infection symptoms such as high fever and chills can be seen as well as septic shock and multiple organ failure. It is a life-threatening disease and has a high mortality rate (2). Its treatment consists of hemodynamic resuscitation, aggressive surgical debridement, and broad-spectrum antibiotics (3). In this case report, it is aimed to present a patient with epididymal cysts, diabetes mellitus, and human immune deficiency virus (HIV) infection who was admitted to the emergency department with Fournier gangrene.

2. Case report

A 57-year-old male patient was admitted to the emergency room with complaints of swelling and pain in the testicles. He had a history of swelling, pain, and discharge in the testicles for 5 days. It was learned that the patient, who applied to another hospital earlier, was evaluated as a scrotal abscess, and applied to us. It was learned that there were cystic lesions (2 cm on the right and 42 mm on the left) in the previously known bilateral epididymis.

It was learned that he had diabetes mellitus, hypertension, coronary artery disease, HIV (human immunodeficiency virus) history and bipolar disorder in his medical history. The drugs he used were olanzapine 2.5 mg, metoprolol 50 mg, sertraline 50 mg, quetiapine 50 mg, trimetazidine dihydrochloride 35 mg, metformin 1000 mg, insulin glargine 10U, acetylsalicylic acid 100 mg, elvitegravir + cobicistat + emtricitabine + tenofovir combination. The admission fever was: 36.2° C, pulse: 85/min, blood pressure: 85/45 mmhg, respiratory rate: 20/min, SpO_2: 97 %. The patient was conscious, cooperatively oriented, GCS (Glasgow coma scale) was 15, and no pathological finding was found in his neurological examination. In scrotal examination, there was subcutaneous edema, redness, and warmth, as well as tenderness with palpation in the left epididymis and testicle (Fig. 1). In ultrasonographic imaging, testicular parenchymal echo structures and blood supply were homogeneous, and an anechoic cystic lesion with a diameter of 4.5 mm at the left rete level and a 51x37 mm blob thin septation in the left scrotal sac at the epididymis level were present. Left epididymis increased in size, parenchyma echo was heterogeneous, scrotum skin and subcutaneous fatty tissue were edematous. There was an 18x22 mm anechoic cyst in the right epididymis.

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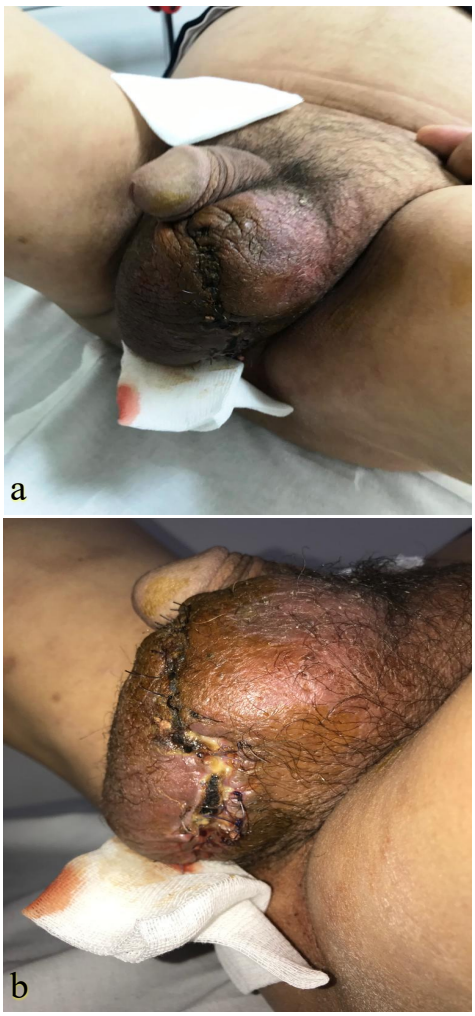

Fig. 1. Subcutaneous edema and redness in the scrotum (a, b)

In laboratory tests, hemoglobin was: 12.6 g/dl, htc (hematocrit): 38.1%, thrombocyte: 212.000 u/L, leukocyte:14910 u/L (Cutoff: 4.000-10.000), neutrophil:11890 u / L (Cut off: 2.000-7.000), neutrophil (%): 79.8%, glucose: 629 mg / dl, Crp (C-Reactive Protein): 27.5 mg/dL (Cut off: 0.0-0.5), and liver and kidney function tests were normal. During the follow-up of the patient, blood glucose regulation was provided in consultation with internal medicine. (Control blood glucose was: 183 mg/dl.) The patient, who was operated on for debridement by the urology, was given 1x500mg daptomycin, 3x1g meropenem, 3x450mg clindamycin IV treatment. The patient was discharged with full recovery after 17 days of hospitalization.

3. Discussion

Fournier's gangrene (FG), polymicrobial necrotizing fasciitis, is a rare, life-threatening soft tissue infection that affects the reproductive organs and the perineum with a high mortality rate (4). The incidence of the disease is approximately 1.6-3 /100,000, varying according to various studies, and it is 10 times more common in males (5). Most patient populations have an underlying systemic disease, such as diabetes mellitus, alcoholism, obesity, peripheral vascular disease, perianal disease, urethral stricture, trauma, and immunosuppression, which increase susceptibility to necrotic fasciitis (4). Smith et al. determined that chronic alcoholism is the most common accompanying disease to Fournier gangrene (6). HIV is the most important concomitant disease in some studies in Africa (7, 8). In a study conducted by Sockkalingam et al., It was observed that FG significantly accompanied HIV infection. (9). In the study of Başoğlu et al., the most related comorbidity with FG was seen as diabetes mellitus (10). Our patient had both diabetes mellitus and HIV infection.

In addition to comorbidities, accompanying diseases have also taken their place in the etiology of Fournier gangrene. Unalp et al., found in their study that anorectal abscess and primary scrotal abscess accompany Fournier gangrene (11). In a case report reported by Cook et al., epididymal cysts were found to be present along with epididymis (12). In addition, there is an epididymal cyst without epididymitis in the case reported by Islam et al. (5). While more than one epididymal cyst was detected in our study; there was no sign of epididymitis. Since epidermal cysts on the scrotum are painless, they are ignored by patients. It is very difficult to determine the relationship between epididymal cysts and treatment response or mortality, considering that they can be detected when the diagnosis of scrotal abscess or gangrene and that comorbidities are more prominent in patients with FG. The presence of epididymal cysts in our patient is insufficient in determining prognosis due to the presence of concomitant HIV infection and diabetes mellitus. When the scrotal skin is infected, the infected part should be debrided extensively to prevent Fournier gangrene and septicemia (13). Recently, while a decrease in mortality rates can be expected due to advances in Fournier etiology and management, a meta-analysis showed that there was no decrease in mortality, but also comorbidities and increasing age were found to be effective in mortality. While the most common cause of death was sepsis; the second reason has been recorded as multiple organ failure (3), mortality was reported at a rate of 11.8% (9), 8.8% (14). Radcliffe et al. reported that FG mortality is 20% (15). The aggressive use of extended-spectrum antibiotics can improve outcomes and reduce mortality. Surgical debridement is the key point in FG management (3). The effect of treatment on mortality in FG has also taken its place in the literature. Yoshino et al. In his study, a patient with Fournier gangrene diagnosed with rectal cancer underwent emergency diverting colostomy and intensive debridement, and the recovery period reached 108 days (16). Even with extended-spectrum antibiotics and wide exudation debridement, the mortality rate reaches up to 45%. (15). In addition, according to the study of Carrell et al., mortality was 100% in patients who did not receive debridement; in debridement patients, mortality is 6% (16). Although it is not known exactly whether debridement or broad-spectrum antibiotic treatment is more effective in determining the prognosis, our patient was discharged after 17 days with early debridement and treatment follow-up. The low incidence of

FG has led to more retrospective study preferences. Thus, it should be kept in mind that the clinical follow-up and history of the patient may be insufficient. In addition, most of the patients are elderly. In addition, we think that case reports will have an important place in the follow-up of recovery times and patient prognosis in rare cases. The effect of treatment on mortality should be evaluated together with comorbidities. We think that our patient, who had cystic lesions in the epididymis in addition to diabetes mellitus and HIV and recovered, will contribute to the literature.

With rapid diagnosis and treatment in Fournier's gangrene cases, patients with multiple comorbidities can also improve. In addition, epidermal emergency physicians should be aware that infections in the perianal region can rapidly progress to the fournier gangrene, especially in elderly patients with comorbidities.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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Expanding the discussion on fibrinolytic contraindications

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Abstract
The European Resuscitation Council Guidelines recommend the administration of fibrinolytic therapy when acute pulmonary embolism is a known or suspected cause of cardiac arrest. However, contraindications that limit the use of fibrinolytics are sometimes challenged by clinicians, including head trauma in the previous three weeks. We report on the successful use of rescue fibrinolytic therapy on a patient with acute head trauma who had a cardiac arrest in the emergency department as a result of a pulmonary embolism (PE). To the best of our knowledge, this is the first case of successful fibrinolytic therapy for a patient with acute head trauma in the literature.

Keywords: cardiopulmonary resuscitation, pulmonary embolism, resuscitation, thrombolytic therapy

1. Introduction

Pulmonary embolism (PE) is a pulmonary emergency that is often difficult to diagnose and sometimes fatal within a short time. In the treatment of patients with PE-related cardiac arrest or near to cardiac arrest, 50mg IV tissue plasminogen activator (Alteplase) bolus can be given for two minutes. The dose can be repeated after 15 minutes if the return of spontaneous circulation (ROSC) is not achieved (1).

Potentially fetal bleeding complications of systemic fibrinolytic therapy in acute PE are an important limiting factor. One of the absolute contraindications is recent head trauma (<3 weeks) (2). However, for a patient who has undergone cardiopulmonary resuscitation (CPR), fibrinolytic therapy may be the only option.

We present a patient with head trauma who had recurrent cardiopulmonary arrest associated with PE and was successfully resuscitated with rescue fibrinolytic therapy, followed by discharge from the hospital without complications. To our knowledge, this is the first case of successful fibrinolytic therapy in a PE-induced cardiopulmonary arrest patient with severe acute head trauma.

2. Case

A 72-year-old female patient was admitted to the emergency department with syncope, chest pain, and head trauma. She fell from her height (158cm tall) uncontrollably and hit her head on the concrete floor, creating a 2-cm incision on her right eyebrow. Her Glasgow Coma Scale was 14, vital signs were unstable (blood pressure: 85/47 mmHg; pulse: 112 beats/min. rhythmic; O_2 saturation: 78%). When we did a point-of-care ultrasound, the right ventricle was seen dilated with tricuspid regurgitation. She had a right branch block, an s1q3t3 pattern, and sinus tachycardia on her electrocardiogram (Fig. 1).

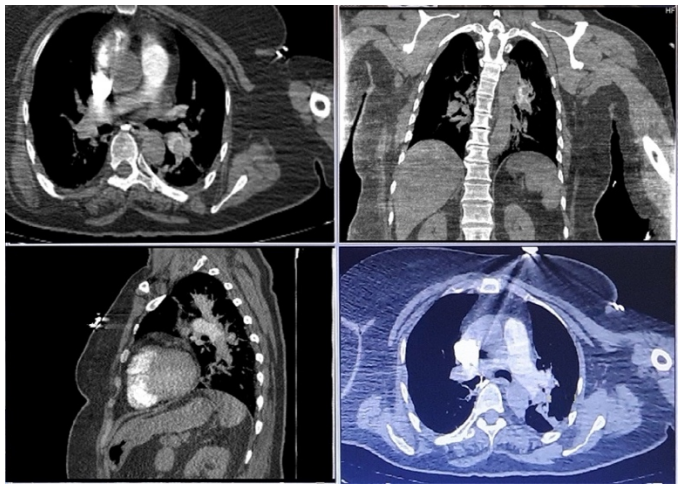

Fig. 1. Arrows show the PE settling on the main pulmonary artery branches

We excluded other causes of hypotension with ultrasonography (USG). However, due to the lack of deep vein thrombosis and the patient's acute head trauma, we did not want to give fibrinolytic therapy without brain and pulmonary computerized tomography (CT) angiography scanning. Moreover, the emergency department had an easily accessible CT. After the diagnosis was made, the patient suffered sudden cardiac arrest. The ROSC was achieved after two cycles of CPR. We stabilized the patient with a positive

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inotropic agent and conducted CT scanning. The patient went into cardiac arrest again, and we started CPR. There was no bleeding, contusion, or similar pathology in the brain tomography, but there was a massive pulmonary embolism in the chest tomography (Fig. 2).

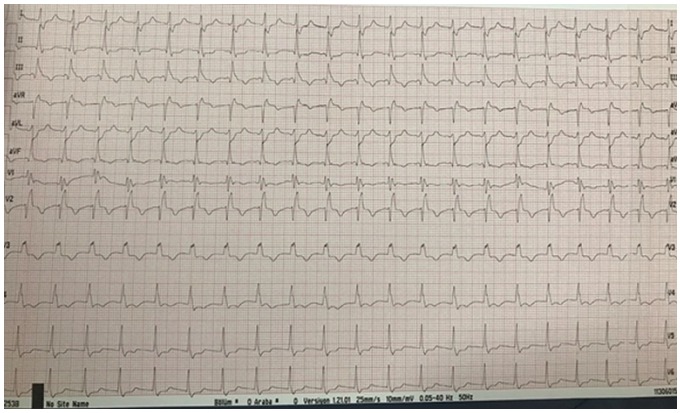

Fig. 2. This ECG with the finding of s1q3t3 is extremely specific for pulmonary embolism

We applied an Alteplase 50-mg bolus therapy for 15 minutes and gave her a 50-mg infusion for 1.5 hours. The ROSC was achieved in the third cycle. After 15 minutes of the Alteplase bolus, her blood pressure increased to a normal level. We saw no intracerebral hemorrhage as a complication. She was discharged with warfarin treatment six days later without any neurological sequelae. A detailed statement that written patient consent/next of kin is present.

3. Discussion

PE is the cause of 2–9% of out-of-hospital and 5–6% of in-hospital cardiac arrests (1-3). Different methods are chosen according to the clinical conditions and risk factors of the patients at the stage of diagnosis. Differential diagnoses in clinical probability high-risk PE include acute valve dysfunction, cardiac tamponade, acute coronary syndrome, and aortic dissection. In these situations, the most useful initial test is bedside transthoracic echocardiography, which will provide evidence of acute pulmonary hypertension and right ventricular (RV) dysfunction if acute PE is the cause of the patient's hemodynamic decompensation. In a hemodynamically unstable patient, echocardiographic evidence of RV dysfunction is sufficient to order reperfusion therapy immediately without further testing. As soon as the patient can be stabilized with supportive therapy, the diagnosis should be confirmed with CT angiography (2).

A trained emergency department clinician can easily identify findings suggestive of PE, such as deep venous thrombosis, right heart thrombus, increased pulmonary artery or right ventricle pressures, RV dilatation, tricuspid regurgitation, and interventricular septal deviation, with the help of point-of-care ultrasound (3, 4). Early relief of pulmonary obstruction leads to a rapid decrease in pulmonary artery pressure/resistance and a concomitant improvement in RV function (2).

Meta-analyses have shown that RV dysfunction, when detected by echocardiography, is associated with increased short-term mortality, even in those who are not hemodynamically unstable (5). In our case, we excluded the other causes of hypotension with an echocardiographic examination. The RV cavity was dilated with the absence of diastolic paradox wall motion, and the bilateral lower extremity doppler USG was negative. The patient's acute head trauma caused concern about giving fibrinolytic therapy without brain and pulmonary CT angiography scanning. However, there was an easily accessible CT in the emergency department.

Primary reperfusion therapy, especially in systemic fibrinolysis, is the preferred treatment for patients with high-risk PE. In-hospital mortality associated with PE is lower for unstable patients receiving fibrinolytic therapy compared to those who do not (5). Those who are unsuitable for fibrinolysis can be treated with surgical embolectomy or percutaneous catheter-directed therapy (2). However, systemic fibrinolysis may be the only option in CPR patients or in hospitals where such treatment options are not available, despite its contraindications. Although our hospital has a cardiovascular surgery team that can perform mechanical thrombectomy, we did not want to waste time, as it would take time to prepare the team, and we preferred systemic fibrinolysis. The European Society of Cardiology's 2014 PE guidelines (5) suggest that many fibrinolytic contraindications should be considered relative in life-threatening high-risk PE patients, this recommendation is not in their 2019 guidelines (2).

Absolute contraindications to systemic fibrinolytic therapy in acute PE include hemorrhagic stroke or a history of unknown stroke, ischemic stroke in the previous six months, major trauma, surgery, head trauma in the previous three weeks, bleeding diathesis, central nervous system neoplasm, and active bleeding (6). Fibrinolytic therapy has a risk of major bleeding, including intracranial hemorrhage. An analysis of the collected data from studies using various fibrinolytic agents and regimens showed that intracranial bleeding rates were reported between 1.9% and 2.2% (6). Advanced age and the presence of comorbidities are associated with the risk of bleeding complications (7). The use of low-dose recombinant tissue plasminogen activator was proven safe in a moderate PE setting in one study, and similar results were reported in another study in 118 patients with hemodynamic instability with massive PE (8,9).

Geriatric Triage Criteria (age ≥ 70 years) consider falling from any height, including standing, with evidence of traumatic brain injury severe head trauma (10). The patient had complained of syncope with serious head trauma, and she was of advanced age. No complications developed either during or after fibrinolytic therapy.

In one case report, a cardiopulmonary arrest because of

massive PE occurred in a patient who had been operated on for glioblastoma multiforme 20 days prior. Treatment included administering a 50-mg Alteplase bolus after 10 minutes of CPR and within minutes, and the ROSC was achieved. No bleeding complication developed in the patient (11).

As a result of the reported data and an analysis of the available literature, it is emphasized that the last surgery should be evaluated as a relative rather than an absolute contraindication for thrombolysis (12).

We believe that fibrinolytic contraindications in acute trauma patients merit further discussion because it seems that some absolute contraindications may be ignored in certain cases, and some patients may be treated without complications. However, because few complicated cases have been published, it is difficult at this time to reach a definitive interpretation of this situation. Nevertheless, it may be reasonable to ignore complication risks in high-risk patients.

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Case Report

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Descending necrotizing mediastinitis due to an odontogenic infection: A case report

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Abstract

Descending necrotizing mediastinitis a clinical entity formed by the spreading of cervical infection from the dental and oropharyngeal structures through the deep cavities between the deep fascia on the neck to the mediastinum, pleural and pericardial spaces with necrosis of soft tissue and has a high mortality. Herein we present the case of a 73-year-old admitted to emergency department with septic clinic. The patient was diagnosed with descending necrotizing mediastinitis due to odontogenic infection. Although sternal irrigation, sternal debridement and mediastinal drainage were performed patient was died postoperative third day.

Keywords: case report, descending necrotizing mediastinitis, mediastinitis, mediastinum,

1. Introduction

Mediastinum is the part between the two lungs that is located medially in the thorax. Mediastinitis is an inflammation of the mediastinal tissue, which can be quite fatal. Most acute mediastinitis can develop secondary to esophageal perforation or chest trauma. Descending necrotizing mediastinitis (DNM) is a polymicrobial infection disease caused by the spread of oropharyngeal flora to the mediastinum. The infection spreads through the deep fascia around the neck into the mediastinum, the pleural and pericardial cavities, and even into the abdomen. It can cause empyema, pericardial and pleural effusion, peritonitis, cardiac tamponade, and intrathoracic bleeding. Mortality has been reported to be between 4.3-40% (1). In this case report, we aimed to present a case of DNM which is rare and has high mortality.

2. Case Report

A 73 years old male patient admitted to our department with fever and altered mental status for five days. He had undergone extractions of the right mandibular second molar teeth 20 days before presentation. He had history of an untreated infection on left mandibular third molar teeth for a week. He had a sinus tachycardia of 110 /minute, blood pressure 70/40 mmHg, and fever 39 °C. Glasgow Coma Scale score of the patient was 13 (V:4, E:3, M:6). He had severe submandibular and neck (extending to chest) non-fluctuant swelling, that was warm to palpation and erythematous. On examination, cervical subcutaneous crackling, crunching sound could be heard with stethoscope over the precordium during systole were noted (Fig. 1). His complete blood count revealed leukocyte of 27.8 K/uL, hemoglobin of 12.6 g/dl, hematocrit of 36.2%, and platelets of 120,000 nl. Biochemical parameters were within the normal ranges. CT scan was obtained, which showed free air in the pericardial space, anterior mediastinum, and subcutaneous tissue of the neck, bilateral pleural effusion and compressive atelectasis (Fig. 2). From the clinical examination and CT findings it was noted compatible with necrotizing mediastinitis.

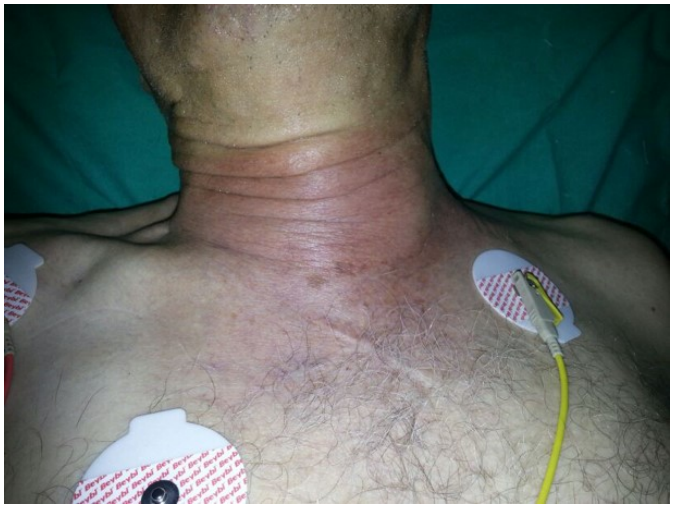

Fig. 1. Erythematous appearance of submandibular area and neck

Empirically, he was given parenteral antibiotic therapy that consisted of intravenously (IV) administered metronidazole 500 mg eight hourly and IV administered ceftriaxone 1 gm 12 hourly). Endotracheal intubation was performed and, severe edema of the vocal cords was noted, on direct laryngoscopy. He was transferred to operating room urgently where he underwent sternal irrigation, sternal debridement and mediastinal drainage. Patient was admitted

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to surgical intensive care unit (ICU) post operatively while hemodynamically unstable (blood pressure 62/47) mmHg, and afebrile (36.7 °C). He was sedated, mechanically ventilated in ICU. Vancomycin 1000 mg and levofloxacin 750 mg were added to antibiotherapy by intensive care unit team. His postoperative period was essentially unremarkable without showing significant relief of symptoms and vital parameters. During ICU follow-up, he was hypotensive and tachycardic despite inotrope support. On the third postoperative day he developed sudden onset of cardiac arrest from which he could not be resuscitated.

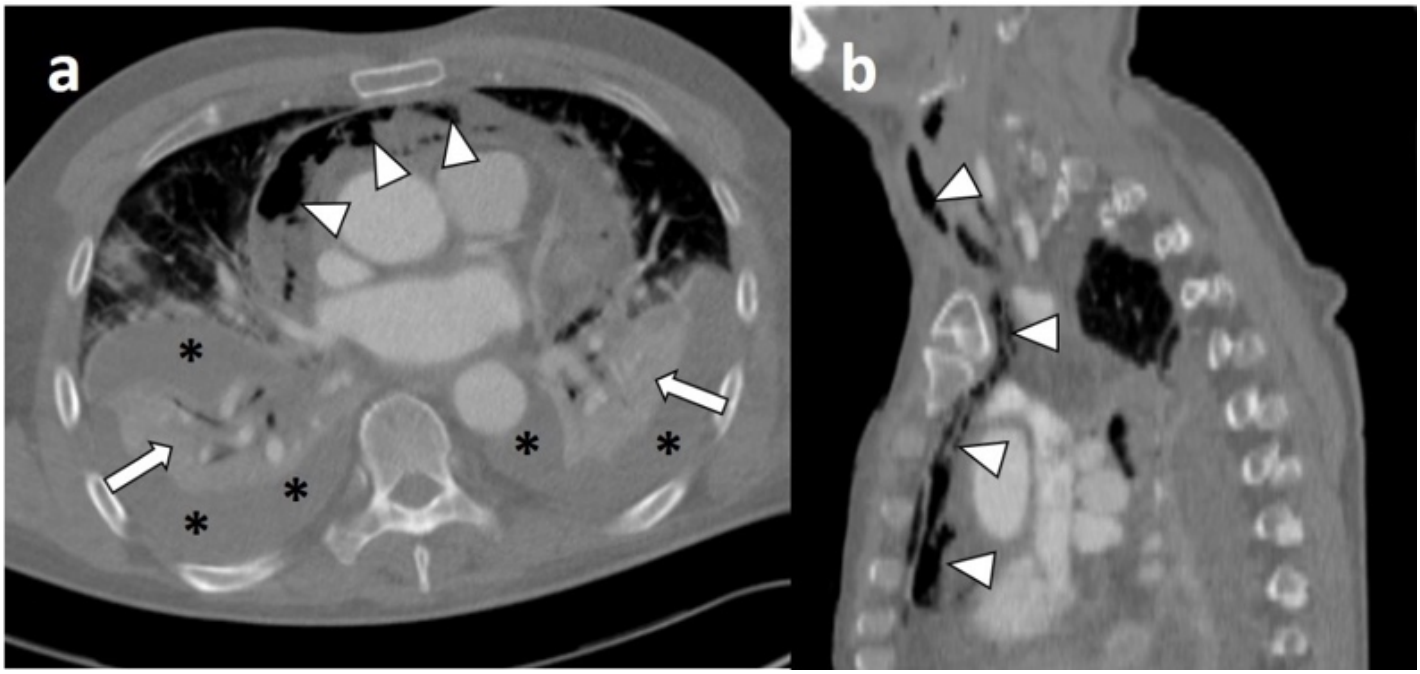

Fig. 2. The transverse and sagittal reformatted contrast enhanced thorax CT images of the patient. Bilateral pleural effusion (asterisks) and compressive atelectasis (arrows) are observed. Free air is seen in the pericardial space, anterior mediastinum, and subcutaneous tissue of the neck (arrowheads) are noted compatible with necrotizing mediastinitis

3. Discussion

Mediastinitis is a rare but severe infection of the mediastinum. The most common cause of acute mediastinitis is esophageal perforation (90%). Secondary causes are spread of lung and pleural infections into the mediastinum and lymphohematogenous spread. The reaching of the oropharyngeal or cervical infection to the mediastinum through the deep facial plan is a rare cause of acute mediastinitis and is called descending mediastinitis (2). DNM is a clinical entity formed by the spreading of cervical infection from the dental and oropharyngeal structures through the deep cavities between the deep fascia on the neck to the mediastinum, pleural and pericardial spaces with necrosis of soft tissue and has a high mortality of 4.3-40% (1). In order of frequency, odontogenic, pharyngeal and cervical infections can be the causes of DNM (2). Pathogenesis of DNM was first described in the literature by Pearse in 1938 (3). DNM is classified in three types; Type I, Type IIa and Type IIb. Type I is local infection, Type IIa is diffuse mediastinitis, spreading into the lower anterior mediastinum and Type IIb is diffuse mediastinitis, spreading into both the anterior and posterior lower mediastinum (4). Our case was Type IIa DNM because mediastinitis was diffuse and extending into the lower anterior mediastinum not posterior lower mediastinum.

Symptoms of DNM aren't specific. Symptoms depend on the etiology of disease and consist of infective process causing tachycardia and fever also altered mental status in late cases as in our case. Sepsis and septic shock often develop abruptly, and symptoms may be related to it. There is no specific laboratory finding. Infection markers such as procalcitonin, leucocyte count, and C-reactive protein are often increased. Thrombocytopenia could be predictor of severe sepsis.

The preferred radiological imaging method contrast-enhanced CT of the neck and thorax. High-resolution computed tomography (HRCT) can assist in diagnosis and can be used for diagnosis. Contrast-enhanced CT help identifying the etiology of the mediastinitis and planning potential surgical interventions (5,6).

Management of DNM depends on management of severe infection, sepsis and septic shock and the underlying reason. Patients with DNM should manage in ICU, because there is relationship between mortality and late ICU admission and the high ICU severity scores on admission to ICU (7).

DNM is typically polymicrobial infection. Empirical antimicrobial therapy of DNM, should cover anaerobic and aerobic bacteria commonly associated with odontogenic and pharyngeal infections. A combination of a third-generation cephalosporin such as ceftriaxone with metronidazole or a combination of clindamycin and piperacillin/tazobactam are

recommended for empirical antimicrobial therapy in DNM patients (8,9).

Surgical treatment is important to control infection and debridement of affected tissue. Cervical and mediastinal drainage is classically required for DNM (2). In our case sternal irrigation, sternal debridement and mediastinal drainage was performed.

In conclusion, DNM, is rare entity and a life-threatening infection. Symptoms and laboratory findings are non-specific. Early empirical antimicrobial treatment and surgical treatment significantly associated morbidity and mortality.

Conflict of interest

We declare no conflict of interest.

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We received no financial support for the research, authorship, or publication of this article. We asked the patient to help us to publish the case report in an international journal for discussion, including disease symptoms, diagnosis, and image related content. The patient agreed us to use his medical records and signed the consent form.

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Prospective observational study of the endotracheal intubation complications in Emergency Department

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Abstract

Endotracheal intubation is the gold standard intervention for emergency airway management. Complications related to endotracheal intubation are numerous and frequent. Complications were identified as being related to endotracheal intubation in our study: hypoxia, hypotension, dysrhythmia, cardiac arrest, hypertension, tachycardia, bradycardia, regurgitation and aspiration of stomach contents, endobronchial intubation, and incorrect positioning of the endotracheal tube in either the esophagus or hypopharynx. The study included 186 patients that were over 18 and intubated. The complication rate associated with endotracheal intubation was found to be over 50%. Patients included in our prospective, observational study were all initially evaluated in our ED. A survey was filled out at a time as soon as possible after intubation to record the personnel in charge of intubation, details of the procedure, and hemodynamic changes and complications. Our study found that the following factors were associated with increased rates of complication in intubated patients: history of acute renal failure, history of cancer, GCS < 8, midazolam use during intubation, history of trauma, crash intubation, history of shock, history of cardiac arrest, resident with <1 year of experience carrying out the intubation, residents with 2+ years of experience and specialists carrying out the intubation, history of respiratory failure, and patient age <65. To better understand which patients were likely to be affected by complications associated with intubation, as well as to understand which precautions to take, this study aims to investigate the aggravating factors and complication rates of endotracheal intubation.

Keywords: airway management, intubation, rapid sequence induction and intubation

1. Introduction

Endotracheal intubation is frequently utilized in the emergency department (ED) as a life-saving intervention (1). Although alternative strategies may be used in emergencies, endotracheal intubation is the gold standard intervention for emergency airway management (2). In existing studies, the complication rate associated with endotracheal intubation was found to be over 50% (3). Patients requiring emergency intubation often have underlying conditions which may complicate the procedure. Factors such as the practice of intubating patients prior to premedicating them provide an explanation for this high complication rate (4). Complications related to endotracheal intubation are numerous and frequent. In one study, severe hypoxia and hypotension were reported with rates of 25% and 10 - 25%, respectively (5). The causes of a decrease in oxygen saturation can work in isolation or in combination and may include esophageal intubation, vomiting, aspiration, multiple attempts at intubation, and endobronchial intubation (6). Alongside this, unsuccessful intubation risks many emergency complications such as soft tissue damage, arrhythmia, and/or cardiac arrest (7, 2). Many variables independent of the patient also affect the rate of complications of intubation. The experience level of the doctor carrying out the intubation, selection and dosage of neuromuscular blockers, and treatment prior to induction are all important factors (5).

Despite being a frequently used intervention in emergency airway management, few studies have investigated the factors associated with emergency endotracheal intubation success rates. To better understand which patients were likely to be affected by complications associated with intubation, as well as to understand which precautions to take, this study aims to investigate the aggravating factors and complication rates of endotracheal intubation.

2. Materials and methods

The study included 186 patients that were over 18, intubated, and admitted to ED of University of Health Sciences İstanbul Haydarpasa Numune Training and Research Hospital between January 01 and June 30. Patients included in our prospective, observational study were all initially evaluated in our ED. All

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patients were intubated in accordance with Rapid Sequence Intubation [RSI] guidelines.

The University of Health Sciences İstanbul Haydarpasa Numune Training and Research Hospital Ethics Committee approved the study on the 22nd of June 2015 (HNEAH-KAEK 2015/KK/51). The patients' data set was used for follow-up with patients and tracking potential complications. Traditional straight and curved laryngoscope blades were used for intubation. Most patients in need of intubation were intubated using RSI. Patients intubated during cardiopulmonary resuscitation were intubated according to crash intubation guidelines. Vecuronium and rocuronium were used as neuromuscular blocking agents. Etomidate, midazolam, and ketamine were used as sedative-hypnotics. All intubated patients were supervised using heart monitors and automatic, non-invasive blood pressure monitors. All intubated patients' oxygen saturation levels were tracked using pulse oximeters. Before intubation, patients were routinely preoxygenated. Endotracheal intubation success was evaluated using colorimetric end-tidal CO_2 detection. To locate the end of the endotracheal tube, chest auscultation was performed. After intubation, chest x-rays were taken.

A survey was filled out at a time as soon as possible after intubation to record the personnel in charge of intubation, details of the procedure, and hemodynamic changes and complications. The following parameters were analyzed: patient age, sex, date of admission, weight, Glasgow Coma Scale (GCS) score at admission, indication for intubation, intubation method, sedative and paralytic medicines administered, prevailing year of medical residency of the doctor carrying out the intubation, comorbid diseases, duration of the intubation procedure, and complications and mortality associated with intubation.

Furthermore, the following complications were identified as being related to endotracheal intubation in our study: hypoxia (defined as less than 80% oxygen saturation as measured on a pulse oximeter at any time), hypotension, dysrhythmia, cardiac arrest, hypertension, tachycardia, bradycardia, regurgitation and aspiration of stomach contents, endobronchial intubation, and incorrect positioning of the endotracheal tube in either the esophagus or hypopharynx (defined as either negative end-tidal CO_2, no chest sounds on auscultation, or endotracheal tube fogging). Patients whose file was unavailable or inadequately filled in, patients who died before the relevant tests were carried out, and patients transferred to other facilities were not included in the study.

All data was stored using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Statistical analysis of the data was carried out using SPSS Statistics for Windows, version 17.0 (SPSS, Inc., Chicago, IL, USA). The threshold for statistical significance used was $p<0.05$. Definitive statistical assays were carried out using the Student's t-test or Chi-squared test for parametric and nonparametric data, respectively.

2. Results

Of patients included in the study, 112 were male while 72 were female. The average age of male and female patients was 73.04 and 70.28, respectively. Altogether, 38 male patients (34%) and 20 female patients (28%) experienced complications. Male gender was associated with increased development of complications (p:0.0419, as was age under 65 years old (p=0.045) (Table 1).

Table 1. Sociodemographic characteristics of patients

	Patients with Complications	Total Patients	p
Male	38	112	0.0419
Female	20	72	
<65 years old	17	33	0.045
>65 years old	41	124	

Upon investigation, patients admitted to the study with chronic illnesses were found to suffer from chronic obstructive pulmonary disease, coronary artery disease, hypertension, diabetes mellitus, acute kidney failure, chronic kidney failure, cerebrovascular accidents, congestive heart failure, and cancer. Patients with a history of acute kidney failure, cerebrovascular accidents, or cancer were found to have an increased incidence of intubation-related complications (p=0.032, 0.07, 0.002, respectively) (Table 2).

Table 2. Chronic illnesses and complication development of patients admitted to the study

Comorbidities	Total Patients	Patients with Complication	p
Chronic Obstructive Pulmonary Disease	31	10	> 0.05
Coronary Artery Disease	22	7	> 0.05
Hypertension	48	18	0.370
Diabetes Mellitus	33	15	> 0.05
Acute Kidney Failure	3	3	0.032
Chronic Kidney Failure	14	8	0.070
Cerebrovascular Accident	7	1	0.0433
Congestive Heart Failure	31	7	0.292
Cancer	12	9	0.002
Cirrhosis	8	2	> 0.05

The conditions necessitating intubation in patients included in our study were respiratory failure, shock, intoxication, intracranial hemorrhage, stroke, sepsis, trauma, and GCS ≤ 8. Patients presenting with shock, trauma, or GCS ≤ 8 were found to have a statistically significant increase in the rate of complications (Table 3).

Table 3. Conditions necessitating intubation

Condition	Total Patients	Patients with Complications	p
Respiratory Failure	73	12	0.00034
Shock	7	7	0.00026
Intoxication	10	1	0.173
Intracranial hemorrhage	5	2	0.655
Sepsis	15	3	0.394
Trauma	6	5	0.013
GCS≤8	57	30	0.000082

Midazolam and ketamine were used as anesthetic agents while carrying out the RSI. Vecuronium and rocuronium were used as neuromuscular blockers. Upon analysis of the relationship between complication rate and agents used, the use of midazolam and non-drug assisted intubation were both found to be associated with increased complication rates (p: 0.013 and p: 00008, respectively) (Table 4).

Table 4. Administered anesthetic and neuromuscular agents

Anesthetic/ Neuromuscular agents	Total Patients	Patients with Complications	p
Midazolam	67	13	0.013
Ketamine	83	27	0.875
Rocuronium	3	0	0.552
Vecuronium	138	40	0.152
Non-drug assisted intubation	23	16	0.00008

Complications that occurred during RSI are displayed in Table 5. The three most common complications were, in order, cardiac arrest, hypotension, and hypoxia (Table 5). The relationship between the experience level (in years) of the resident carrying out the procedure and complication rates was investigated. Residents with less than a year of experience and residents with more than two years of experience were each associated with an increased risk of complications in patients (Table 6). The duration of the intubation procedure was not associated with a significantly increased rate of complications (Table 7).

Table 5. Complications observed during intubation

Total (n:185)	Patients with Complications
Cardiac arrest	22
Hypotension	21
Hypertension	0
Bradycardia	3
Tachycardia	2
Dysrhythmia	6
Aspiration	4
Death	11
Soft tissue trauma	3
Difficult Intubation	4
Hypoxia	18

Table 6. Relationship between the resident carrying out the procedure's experience in years and complication rate

Experience (Years)	Total Patients	Patients with complications	p
0-1	44	26	0.00003
1-2	37	9	0.327
>2	100	23	0.0007

Table 7. Relationship between duration of the intubation procedure and complication risk

Duration	Total Patients	Patients with Complications	p
0-5	112	28	0.167
5-30	42	11	0.453
30-120	32	11	0.835
<120	36	9	0.426

4. Discussion

Due to inadequate preparation of patients in the ED, complications are frequent occurrences. In this study, we aimed to identify these complications and investigate the associated factors to facilitate the development of solutions to reduce the incidence of complications. For this purpose, we prospectively analyzed 186 intubation procedures in the ED. A statistically significant relationship was found between male gender and an increased rate of complications. Similarly, a statistically significant increase in complications was identified in patients under 65 years old.

Intubation-associated complication risk was also found to be significantly increased in patients with respiratory failure. An existing study found respiratory failure to be associated with an increase in the rate of intubation-related complications. Our study replicated these results. In this area, inadequate preoxygenation, hypoxia occurring during intubation, and time pressure on doctors may be causative factors for complications (8, 9).

Midazolam is a slow (2-3 minutes without opioid action) and long-acting agent for inducing anesthesia. According to a study by Sagar et al., conventional midazolam dosage was insufficient for use in RSI (10). This is generally due to a fear of associated hypotension. Intravenous midazolam dosage for use in RSI is 0.2 - 0.3 mg/kg. For a 70 kg patient, this is a 15-20 mg induction dose. Recently, physicians have been dissuaded from using midazolam to induce anesthesia (10). This is due to its association with delayed induction, unpredictable hypotension, and prolonged effects. In this study, midazolam was identified as an important factor that influenced the complication rates of endotracheal intubation. Patients who were administered midazolam in our study experienced a high rate of hypotension. Neither ketamine nor propofol were associated with a significant increase in complication rates. For this reason, ketamin or propofol should be recommended for use in RSI.

Our study identified a statistically significant increase in intubation-associated complications when non-drug assisted intubation was applied. Crash intubation is applied in patients undergoing cardiopulmonary resuscitation. Due to the urgency with which this group of patients must be intubated, an increased rate of complications is observed. According to one study, the most frequent complications in this group of patients were soft tissue injuries, hypotension, and esophageal intubation. In this study, crash intubation was associated with a higher risk of tissue damage when compared to RSI. Another available study suggests that crash intubation increases the risk of complications including hypotension, repeated intubation attempts, esophageal intubation, cardiac arrest, and oxygen desaturation (11). Our study identified an association between increased intubation-related complication rate and crash intubation. Cardiac arrest, oxygen desaturation, and hypotension rates were significantly increased.

Furthermore, our study found a significantly increased rate of complications if the intubations were performed by residents in their first year of specialty training. Similarly, increased rates of complications were associated with intubations performed by those with more than two years of experience, a category that includes both residents and fully trained specialists. Intubation procedures are lifesaving, extremely important procedures that must be performed with utmost care. Residents with inadequate experience may cause tissue damage, repeatedly fail to intubate patients, or increase the risk of oxygen desaturation prolonging the interval before successful intubation. Difficult intubations were associated with residents that have completed their second year of training and fully trained specialists. Difficult intubations were also, predictably, associated with high complication rates. Due to this, these intubations may be better categorized as distinct from RSI. One study concluded that residents that have completed their first year of training had a 50% success rate in intubating patients compared to a 70% success rate for residents in their third year of training. Similarly, a past study found that intubation success rates were directly correlated with resident or specialist experience (9). Our study produced similar results.

Our study found that the following factors were associated with increased rates of complication in intubated patients: history of acute renal failure, history of cancer, GCS <8, midazolam use during intubation, history of trauma, crash intubation, history of shock, history of cardiac arrest, resident with <1 year of experience carrying out the intubation, residents with 2+ years of experience and specialists carrying out the intubation, history of respiratory failure, and patient age <65. Our patients experienced the following complications: hypoxia, dysrhythmia, cardiac arrest, soft tissue trauma, unsuccessful intubation, tachycardia, bradycardia, and aspiration. Paying extra attention to the above factors may result in reduced complication rates.

Conflict of interest

There is no conflict of interest between authors.

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A rare case: Covid-19 infection diagnosed by transthoracic fine needle aspiration biopsy

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Abstract

Covid 19 is an acute respiratory disease caused by a novel type of Coronavirus (SARS-CoV-2) that was first detected in Wuhan, Hubei Province of China. Its most common symptoms are fever, cough, and weakness; and in the early stages, there may not be findings on chest computed tomography. In pulmonary involvement, peripheral ground-glass areas, a cobblestone appearance, consolidated areas, and interlobular septal thickening, which are usually prominent in the lower lobes may be seen in thorax computed tomography. Consolidation, solid nodules, halo sign, inverted halo sign, vascular enlargement, subpleural line, air bronchograms, and bronchiectasis are other less common findings. In our case, we aimed to present our 69 years old female patient with bilateral nodular densities on thoracic CT and transthoracic fine-needle aspiration biopsy result was reported as lesions compatible with interstitial fibrosis due to Covid-19 inflammation.

Keywords: Covid-19, interstitial fibrosis, nodule, thorax computed tomography, transthoracic fine needle aspiration biopsy

1. Introduction

Covid 19 is an acute respiratory disease caused by a novel type of Coronavirus (SARS-CoV-2) that was first detected in Wuhan, Hubei Province of China. Its most common symptoms are fever, cough, and weakness; and in the early stages, there may not be findings on chest computed tomography (1). In pulmonary involvement, peripheral ground-glass areas, a cobblestone appearance, consolidated areas, and interlobular septal thickening, which are usually prominent in the lower lobes may be seen in thorax computed tomography (2). Consolidation, solid nodules, halo sign, inverted halo sign, vascular enlargement, subpleural line, air bronchograms, and bronchiectasis are other less common findings (3).

For the diagnosis of Covid-19, the real-time reverse transcription-polymerase chain reaction (RT-PCR) of viral nucleic acid is accepted as the reference standard, however, thoracic CT examination is important in Covid-19 patients with false-negative RT-PCR results, and recent studies have reported the CT sensitivity as 98% (4-6).

In our case, we aimed to present our female patient who had bilateral nodular densities on thoracic CT and transthoracic fine-needle aspiration biopsy (TTFNAB) was reported as lesions compatible with interstitial fibrosis due to Covid-19 inflammation.

2. Case report

The sixty-nine-year-old female patient attended an external center with the complaint of cough and tested negative for Covid PCR test. Positron emission tomography / computed tomography (PET/CT) was performed due to the presence of the bilateral consolidated, solid nodular appearance in the thorax CT of the patient (Fig. 1), whose Covid PCR test was negative twice. In PET/CT; low-moderate increased metabolic activity involvement (left SUVmax: 4.94, right SUVmax: 3.58) in bilateral lower paratracheal, subcarinal and bilateral hilar lymph nodes in the mediastinum, subsegmental collapse/consolidation areas showing nodularity in both lungs especially in the basal area, nodular-reticulonodular densities of maximum 2 cm in size again in both lungs, and pathologically increased metabolic activity involvements (SUVmax: 7.36) were observed. The TTFNAB performed with a pre-diagnosis of malignancy was reported as "Lung parenchyma compatible with fibrosis that develops after Covid-19 inflammation". Covid PCR sample of the patient, taken for the third time was detected positive and favipiravir treatment was initiated. The patient was transferred to our service for further examination and treatment. In the chest radiography of the patient, there were bilateral nodular infiltrations (Fig. 1). Her saturation at room air was 96%. The laboratory findings were reported as; the leukocyte count: 7300 µL, ferritin value: 432 ng/ml, D-dimer: 0.62 mg/L,

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lymphocyte count: 1450 µL, C-reactive protein (CRP): 75 mg/L, Troponin: 14.4 ng/L. In addition to favipiravir treatment, methylprednisolone 40 mg, a prophylactic dose of low molecular weight heparin, and broad-spectrum antibiotic treatment were initiated in the patient who previously used empirical antibiotics. During the follow-up of the patient, hypoxia developed on the 7th day of hospitalization and Covid PCR positivity. The patient was provided oxygen support with a mask with a reservoir, whose saturation value was below 90% despite the 10 L/min oxygen therapy with a nasal cannula. Progression was observed in bilateral infiltrations on chest radiography. The laboratory values of the patient were CRP: 180 mg/L, ferritin: 1650 ng/ml, D-dimer: 2.19 mg/L, as lymphocyte count: 510 µL. The patient was considered to have entered a cytokine storm, and 250 mg methylprednisolone pulse therapy was administered for three days. The patient, who also developed bilateral hearing loss, was consulted to the Neurology Department.

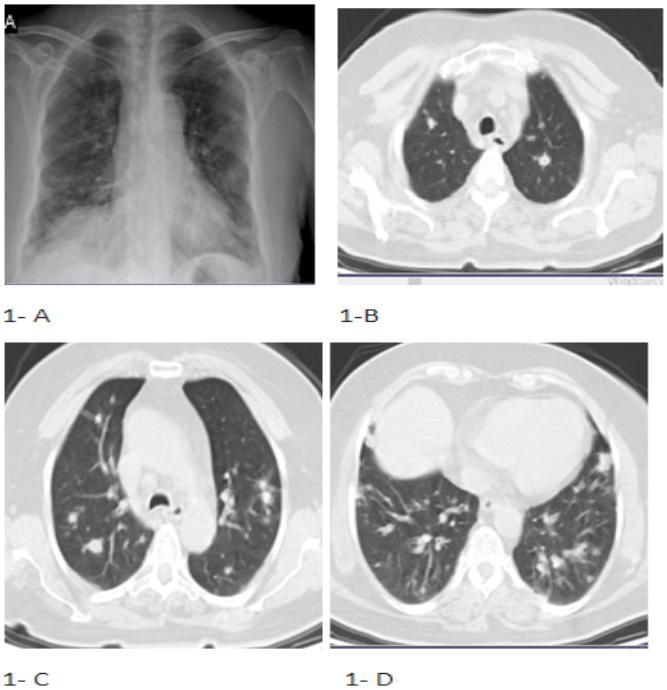

Fig. 1. A: Initial chest X- ray; bilateral nodular lesions. B: Thorax computed tomography (CT), bilateral nodular lesions at upper lobes of lungs. C: Thorax CT, bilateral nodular lesions at upper and middle zones of lungs. D: Thorax CT, bilateral nodular lesions at lower lobes of lungs

Neurological pathology was not detected patient. The patient was consulted to the otorhinolaryngology department and hearing loss was thought to be due to serous otitis which was secondary to Covid-19. Continuing the steroid therapy was recommended. Clinical improvement was observed in the follow-up. Oxygen saturation in room air increased to 96%. Regression was observed in the lesions on chest radiography. Steroid therapy was tapered and ended. The patient was discharged with full recovery. On the control visit, it was learned that the hearing loss improved. The patient was recommended for clinical and radiological follow-up.

3. Discussion

The Covid-19 pandemic, which started in China in December 2019 and spread all over the world, is a serious health problem today that concerns all countries (7). In the course of the disease, although fever and cough are the first common signs, cases with nonspecific findings such as weakness, loss of appetite, muscle pain, diarrhea, nausea, vomiting, abdominal pain, and headache are also reported (8). In our case, there was no fever, but weakness and cough complaints were present.

In the literature, the presence of ground-glass opacities with or without consolidation in bilateral, peripheral, and posterior areas on thoracic CT in the lungs has been identified as the most important feature of Covid-19. However, it was observed that there were also findings such as a cobblestone view, airway changes, and inverted halo sign in further and more investigations made with the increased number of cases. (9, 10). It has been stated in the literature that multifocal, solid, irregularly circumscribed nodular structures in thorax CT are one of the findings were seen in viral pneumonia cases, which can also be seen in 3-13% of Covid-19 cases which are accompanied by minimal ground glass appearance around them (11-13).

In our case, there were bilateral solid nodular appearances on thorax CT. The possibility of the presence of lymphadenopathy (LAP) larger than 1 cm is 4-8% and it is generally an unexpected finding, and it may be seen in serious cases. It has been reported that 17% of patients healed with fibrotic bands, which was considered as a good prognosis because of being stable disease finding and could indicate a poor prognosis for the risk of development of fibrotic lung disease in the future (11). Pulmonary nodules can be encountered in many clinical conditions, from infections to malignancies, and lung tissue biopsy is needed for differential diagnosis. PET/CT is useful in the differentiation of benign pulmonary nodules and malignant nodules. TTFNAB is usually the first preferred interventional procedure in the evaluation of peripheral lesions suspected of being malignant.

In our case, TTFNAB was performed due to the presence of mediastinal lymphadenopathies and high bilateral nodular SUVmax values in PET/CT performed with a pre-diagnosis of malignancy; and the pathology was reported as lung tissue compatible with fibrosis after Covid-19 pneumonia. In the literature, the mainly reported pathological findings are diffuse alveolar damage, organized pneumonia, reactive type II pneumocytes, and chronic interstitial pneumonia (14, 15). In the pathology report of our patient, it was stated that interstitial fibrosis was detected, which could be due to Covid -19. It was seen that the chest radiography of the patient was normal in the previous radiological images from the records and this new situation was believed to develop due to Covid-19.

In the literature, it has been stated that otitis findings may

be the first sign for Covid-19 infection in some cases, and case series are reported (16). In our case, the hearing loss that developed while our patient was receiving Covid-19 pneumonia treatment was thought to be due to, serous otitis, a complication of Covid-19.

Covid 19 continues to manifest itself with different clinical and radiological findings every day and threaten the whole world. Although bilateral solid nodular consolidations are not common in Covid-19, it should be kept in mind in differential diagnosis despite the negatively detected Covid PCR.

Conflict of interest

We declare that there is no conflict of interest, in particular no financial funding potentially relevant to the contents of manuscript.

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Case Report

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Headache can be the only symptom of COVID-19: A case series

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Abstract

Headache is the fourth most common cause among the total applications to emergency services; it constitutes 5% of the applications to hospitals every year. Five-fold increase was detected in the incidence of headache in the regions affected by coronavirus disease-2019 (COVID-19) pandemic. While the symptoms of respiratory systems have been frequently observed, the occurrence of symptoms and complications in peripheral and central nervous system has become increasingly prevalent in the cases of COVID-19 disease. In this case series, we highlight that the patients with isolated headache may be diagnosed with COVID-19 infection. Three female patients (forty, sixty and sixty-two years of old) were admitted to the emergency service with complaints of severe headache. Their headache did not respond to the paracetamol and the nonsteroidal anti-inflammatory drug (NSAID) they used prior to their application to the hospital. No pathologic finding was detected in cerebral imaging. All patients were diagnosed with COVID-19 by their clinical status and history. The headache was the isolated symptom of the COVID-19 in all the three cases. The possibility of COVID-19 infection must be considered in the evaluation of the patients admitted to hospital with complaints of headache, one of the most frequent reasons for hospital assistance requests.

Keywords: COVID-19, headache, neurologic manifestations, SARS-CoV-2

1. Introduction

Coronavirus disease-2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in 63, 965, 092 confirmed cases and 1,488,120 deaths all around the world as of December 3, 2020 (1). Respiratory system symptoms are frequently found in COVID-19 disease with complications in the peripheral and the central nervous systems are increasingly found (2). Headache is the fourth most common reason for total applications to emergency services; it represents 5% of the applications to hospital (3). Five-fold increase was detected in the incidence of headache in the regions affected by COVID-19 pandemic (4).

Only neurologic symptoms were found as the initial symptoms in some patients diagnosed with COVID-19 such as headache, anosmia, ageusia, languidness, unstable walking, cerebral hemorrhage, cerebral infarction, and other neurological diseases (5). These complications are possibly caused by direct viral injury, immunological mechanisms and by hypoxia (6). SARS-CoV-2 uses the transmembrane ACE2 receptor to enter the mammalian host cells. The ACE2 receptor was detected in various cells in humans including the airway lung epithelial cells, vascular endothelial, pericytes, and smooth muscle cells, and neuronal cells in the trigeminal ganglia, olfactory bulb, and other cortical and subcortical areas (7, 8). Headache is in close temporal relationship with the symptoms, and it is frequently accompanied by phonophobia developed in patients with anosmia and ageusia (9). Headache was found as an early finding of COVID-19 infection. Additionally, it was observed that symptoms involving other systems or apparatus arise in the later stages of the disease, following the headache. Cases of isolated headache are less common than the cases where headache is combined to other symptoms (10).

In this case series, we aim to draw attention to the fact that the patients with isolated headache may be diagnosed with COVID-19 infection.

2. Case report

2.1. Case 1

A 62-year-old female patient applied to the emergency service with complaints of severe headache. She reported that she had had the headache continuously for three days and it became intolerable. She did not respond to the paracetamol and the nonsteroidal anti-inflammatory drug (NSAID) she used at home. She described the headache as the most severe one in her life. She had an intense pain in both sides of her head as if something were compressing her head. She had no cough, shortness of breath and fever history associated with COVID-19 for several days. She reported that she had not been in close contact with a person with COVID-19 infection to her knowledge. She stated that she had hypertension and type-2 diabetes as additional chronic diseases. On arrival, her

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temperature was 36.4°C, her pulse rate was 96 beats/min (regular rhythm), her blood pressure was 150/90 mmHg, and her oxygen saturation was 97% in room air. Her physical examinations including lung auscultation did not show any apparent abnormal findings. Neurological examination revealed alerted consciousness and normal eye movements. Her gait was normal. Muscle weakness or abnormal tendon reflexes of extremities was not observed. CRP:88 mg/L and D-Dimer: 96 µg/L was found in the blood tests; other results are given in Table 1. Emergency unenhanced cerebral computed tomography (CT) was normal. Magnetic resonance imaging (T1, T2, and fluid attenuated inversion recovery) and intracranial magnetic resonance angiography (arterial and venous) were normal. As the patient's severe headache continued, she was planned to be hospitalized in the neurology service for a follow-up. Considering the patient's elevated levels of CRP and D-Dimer values and the fact that headache is a potential symptom of COVID-19 infection, a SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) swab test was taken to rule out possible COVID-19 infection. The RT-PCR test result was found to be positive. Chest CT scanning was performed on the patient and ground-glass opacities were found in her right lung basal (Fig. 1). The patient was admitted to the pandemic service for a close follow-up and her COVID-19 treatment was started. Her headache intensity decreased in the following day after the admission and no additional symptom and finding were found in the follow-up. The finding that the intensity of the patient's headache further decreased and disappeared after the COVID-19 treatment, shows that the headache developed due to COVID-19 infection. The patient was discharged from the service with full recovery after she was followed up in the service for five days.

2.2. Case 2

A 41-year-old female patient applied to the hospital with complaints of sudden severe headache. She had a throbbing pain in both sides of her head. The pain was particularly focused in the frontoparietal area. She had no chronic disease and no history of headache previously. She did not respond to the paracetamol and NSAI medication she received during the day of her hospital visit. In the detailed medical history of the patient, it was found that she was too often present in crowded places, and she was at close distance with a person with COVID-19 infection. On arrival, her vital signs were normal. Her respiratory rate was at normal range and physical examinations including lung auscultation did not show any apparent abnormal findings. Neurological examination was normal. Laboratory test values are summarized in Table 1. No pathologic finding was detected in emergency unenhanced cerebral CT. In chest CT scanning of the patient, peripheral ground-glass opacities were found in all lobes and segments in both of her lungs (Fig. 1). The patient was also tested positive for SARS-CoV-2 RT-PCR. Her COVID-19 treatment was arranged as ambulatory treatment. The patient was called on days 3 and 7 and she reported that she did not have additional symptoms or health complaints.

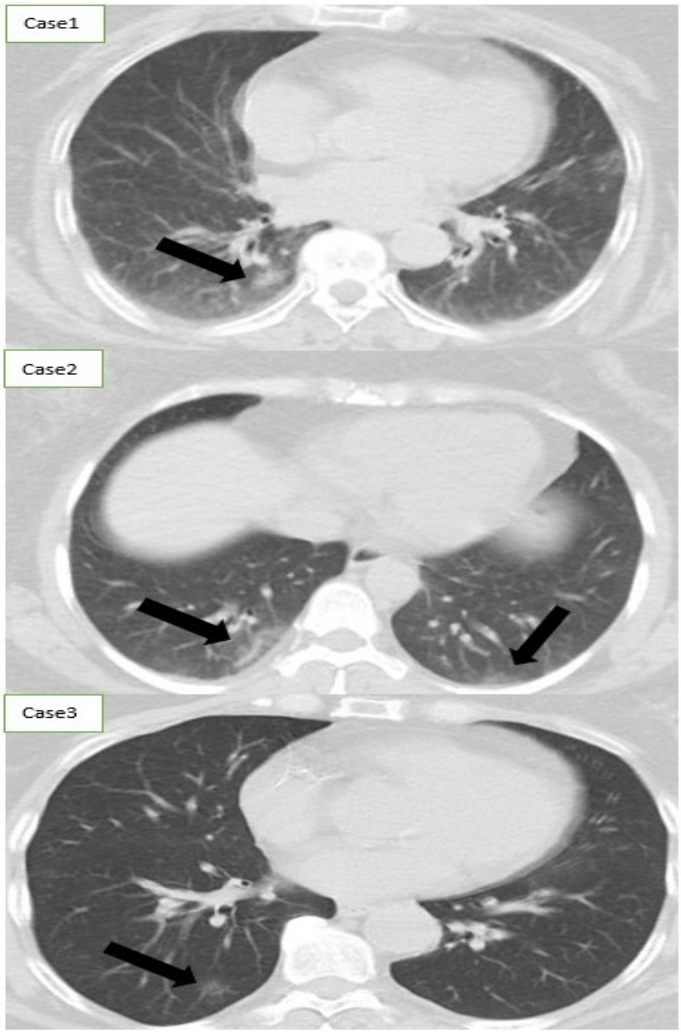

Fig 1. Chest CT images cases'

2.3. Case 3

A 60-year-old female patient had a severe headache for two days. She had a throbbing pain in both sides of her head. She reported that she also had complaints of headache and dizziness one month earlier; she applied to neurology clinic, but all test and examination results were normal. She did not have a history of migraine. She stated that she responded partially to paracetamol and NSAI medication in that period. She did not respond to any medical treatment for two days when she applied to the emergency service. She said that she entered in close contact with COVID-19 patients three days before the headache started. On arrival, her vital signs were normal. The physical examination was normal. Her neurological examination did not show pathological findings. Laboratory test values are summarized in Table 1. She was tested positive for SARS-CoV-2 RT-PCR in her nasopharyngeal swab sample. In chest CT scanning, ground-glass opacities were found in the periphery of the right lung (Fig. 1). Her COVID-19 treatment was arranged as ambulatory treatment. She was called on days 1, 3, and 7 and she said that she did not have any additional symptoms or health complaint.

Table 1. Laboratory test values of cases'

Parameters	Case-1	Case-2	Case-3
Leucocytes (x10^9/l; normal range 4.5-10.5)	5.0	6.1	5.8
Hemoglobin (g/dl; normal range 12-17.4)	13.9	12.9	12
Platelets (x10^9/l; normal range 142-424)	165	270	358
Lymphocytes (x10^9/l; normal range 0.6-3.4)	1.2	2.2	2.1
Neutrophils (x10^9/l; normal range 2-6.9)	3.4	3.3	3.2
Na^+ (mmol; 136-146)	**133**	137	140
K^+ (mmol; 3.5-5.1)	4.1	4.4	5.1
Cl^- (mmol; 101-109)	**96**	106	103
Glucose (mg/dl; normal range 74-106)	**262**	105	93
Serum creatinine (mg/dl; normal range 0.67-1.17)	0.9	0.5	0.6
Urea (mg/dl; normal range 17-43)	43	23	24
Aspartate aminotransferase (U/l; 5-50)	35	21	25
Alanine aminotransferase (U/l; 5-50)	23	15	17
C-reactive protein (mg/l; 0-5)	**88**	0.9	6.5
D-Dimer (µg/L; normal range; 0-0.55)	**96**	0.2	0.4
Troponin (ng/ml; normal range 0-0.16)	0.03	0.01	0.01

3. Discussion

While COVID-19 symptoms are mainly related to the respiratory system, the symptoms and complications in the central and peripheral nervous system including headache are increasingly reported. All the patients in this study applied to the emergency service with complaints of isolated headache. All the three patients had bilateral and severe headache. While two out of the three patients had a headache of migraine phenotype, one patient defined the pain as tension. The two patients did not have any diagnosed disease related to headache before and it was the most severe headache that the two patients had in their life. One patient occasionally had headache and dizziness before. All neurological and sensory examinations were normal in neurology polyclinic check one month earlier; no pathology was found in the examinations. The headache related to COVID-19 is generally found bilaterally (11). Headache like migraine was described with respect to various viral infections in previous studies (12, 13). In a study on 73 cases, it was found that the pain reported by the patients who had migraine previously is mostly considered to be the migraine phenotype during COVID-19 disease (9). Tension type headache was found more commonly in a study on the evaluation of neurological symptoms of the patients admitted to hospital due to COVID-19 (14).

No COVID-19 infection related disease or symptom in systems, including the respiratory system, were found in the follow-up of all the three patients in the study. As being one of the initial symptoms and early manifestation of the disease, headache seems not to be necessarily associated with the disease severity during the progression of the disease including lung involvement and vascular complications (7). Headache prevalence was found 10.9% in the metanalysis involving 21 studies on 6486 patients (15). Incidence of headache was found to be higher in the two studies on evaluation of neurological symptoms in the patients with COVID-19 (27% (11) and 43% (14)). As the symptoms related to respiratory system come into prominence with the highest hospitalization rate in the studies on COVID-19, the symptoms of headache may be underestimated. All the three patients applied to the hospital with the complaints of headache had higher intensity, rapid course, and resistance to usual analgesic medication. It indicates that the symptoms related to COVID-19 headache are probably related to peripheral infection mechanisms. It is rather improbable to link the isolated and early emergence of headache in the cases applying to hospital with complaints of isolated headache to the indirect effects of the virus by means of the circulating inflammatory mediators causing cytokine storm, viremia, endothelial and vascular invasion, metabolic disorder, or hypoxic damage (7). All the three cases were in good condition in terms COVID-19 infection. This points out the significance of headache for diagnosis of mild COVID-19 cases. Headache can be an isolated symptom of COVID-19 disease. The possibility of COVID-19 infection must be taken into consideration in evaluation of the patients with the complaints of headache, a common reason for the application to hospital.

Conflict of interest

We declare that there is no conflict of interests.

Acknowledgments

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Case Report

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A rare case: pulmonary thromboembolism and pneumothorax coexistence secondary to Covid-19 infection

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Abstract

Acute respiratory disease caused by a novel coronavirus (Serious Acute Respiratory Syndrome-Coronavirus-2) type that was first detected in Wuhan, Hubei Province of China, has turned into a pandemic. The disease commonly presents with fever, cough, and malaise, and typically bilateral peripheral ground-glass areas are seen in thorax computed tomography. While it may present as mild upper respiratory tract infection or pneumonia, patients may also develop pulmonary thromboembolism and pneumothorax. In our case, in a young male patient who was followed up for severe pneumonia due to COVİD-19 infection, pulmonary thromboembolism, parenchymal cysts, and pneumothorax were detected. Although pulmonary thromboembolism is a common complication, cyst formation and pneumothorax are rare. We aimed to present this case because of the coexistence of multiple pulmonary complications despite the young age of the patient.

Keywords: COVİD-19, pulmonary thromboembolism, pneumothorax, complication

1. Introduction

At the end of 2019, a new coronavirus subtype was identified in cases with pneumonia in Wuhan, Hubei Province of China, and the novel type of coronavirus infection, which turned into an epidemic in other provinces of China, was officially declared as an international public health emergency of concern by the World Health Organization (WHO) on January 30, 2020. In February 2020, the new type of coronavirus infection was named Serious Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), and the disease it caused as COVİD-19 (Corona Virus Disease 2019) by WHO (1). On thoracic computed tomography (CT), bilateral multilobar ground-glass opacities, consolidation, flagstone appearance, air cysts, nodules, halo and reverse halo sign, bronchial dilatation and pulmonary vascular enlargement can be seen, which are more significant in peripheral or posterior of the lungs (2). COVİD-19 infection may present as an upper respiratory tract infection, as well as a severe disease such as acute respiratory distress syndrome (ARDS) and septic shock. (3). We aimed to present our case who developed hydropneumothorax and pulmonary thromboembolism (PTE) after COVİD-19 pneumonia.

2. Case report

The thirty-five-year-old male patient was admitted to another hospital due to COVİD-19 pneumonia, and upon the development of pulmonary thromboembolism and hydropneumothorax, he was transferred to our center for further follow-up of thoracic diseases and thoracic surgery departments. The anamnesis of the patient revealed, he had complaints of cough and right flank pain that started three days before his admission to the hospital, he was a metal worker and he did not have a history of smoking or an additional disease. The patient's Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) test which was done upon admission was positive, there was bilateral pneumonia in chest radiography (Fig. 1) and the laboratory findings were as follows: lymphocyte count 1950/µl, ferritin 1381 ng/ml, C-reactive protein (CRP) 154 mg / L, D- dimer 9.94 mg/L, LDH 450 IU/L. Pulmonary thorax computed tomography angiography (CTA) and thorax CT were performed because of the development of chest pain with a stabbing character on the 2nd day of hospitalization while receiving prophylactic dose low molecular weight heparin (LMWH) and COVİD-19 pneumonia treatment (favipiravir and ceftriaxone). The CTA revealed appearance compatible with segmental pulmonary thromboembolism in the lower lobe of the right lung, bilateral multilobar ground glass, and consolidated areas, multiple increased nodular densities, unilateral pleural effusion on the right (Fig. 2). While the patient was receiving treatment for PTE and COVİD-19 pneumonia, on the 17th day, due to the development of severe dyspnea and deterioration in general condition, chest radiography was performed and an appearance compatible with hydropneumothorax was observed, so he was transferred to our hospital with the diagnosis of PTE, COVİD-19 pneumonia and hydropneumothorax.

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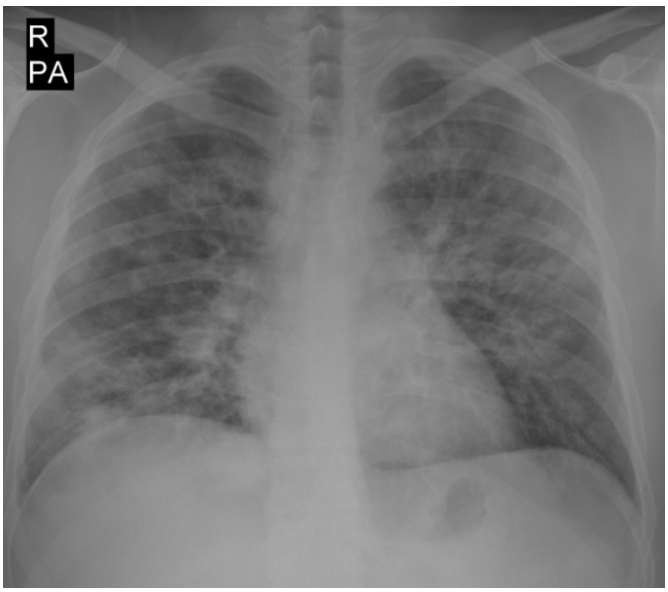

Fig. 1. Bilateral pneumonia in posteroanterior chest radiography

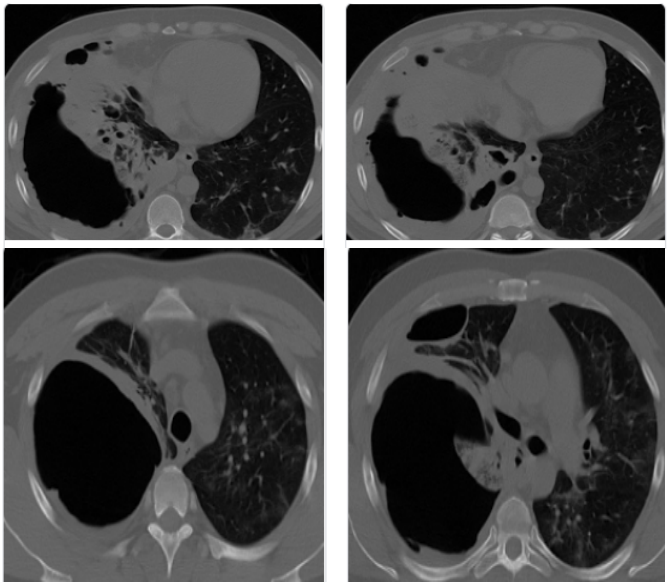

Fig. 2. Hydropneumothorax and COVİD-19 pneumonia

In the physical examination of the patient, the respiratory sound was missing in the right lung, and the saturation was measured as 95% under 5 L/min oxygen support with a nasal cannula. Other system examinations were normal. In the laboratory findings, lymphocyte count was 1850/µl, ferritin 1170 ng/ml, CRP 89 mg / L, D-dimer 7.82 m /L, and LDH 305 IU/L. Chest radiography revealed an increase in nonhomogeneous density in the upper-middle zone of the left lung and an appearance compatible with hydropneumothorax in the right lung. The patient who was consulted by the thoracic surgery department underwent tube thoracostomy through the mid-axillary right 7th intercostal space under local anesthesia. After one day of follow-up, subcutaneous emphysema developed in the patient whose lungs were not fully expanded. Thoracic surgery was consulted again. A second tube thoracostomy was performed on the patient through the 3rd intercostal space on the right midclavicular line and a pessary tube was placed under local anesthesia. Later, it was observed that the lung was expanded, however, the progression in subcutaneous emphysema was detected in the patient whose massive air leak continued. On the 4th day after the insertion of a chest tube, a sore throat and facial swelling developed in the patient. In the physical examination, crepitation was also taken in the neck and face. After COVİD-19 PCR test was detected negative twice, the patient was transferred to thoracic surgery for further treatment. During the follow-up in the thoracic surgery ward, the tube was terminated since there was no air discharge from the first chest tube inserted. Pleurodesis was performed in the patient whose air discharge continued through the pesser drain. During the 14-day follow-up in the thoracic surgery ward, the patient's subcutaneous emphysema and air discharge from the drain gradually decreased, and the antibiotic and LMWH treatments were continued. It was observed that the ground-glass infiltrates persisted but decreased, however, pleural thickening areas developed and partially consolidated areas in the lower lobes were present, in the thoracic tomography. The drain of the patient was taken to Heimlich Valve and discharged when his clinic stabilized, and his symptoms regressed. No complications were observed on the 3rd day after discharge in the outpatient clinic control.

3. Discussion

The COVİD-19 pandemic started in China in December 2019 and spread all over the world, is a serious health problem that concerns all countries. The COVİD-19 infection causes the development of many complications, especially in the respiratory and cardiovascular systems (4). Studies have reported that pulmonary thromboembolism is seen between 10% and 28% of the cases. The mean age range of patients with thromboembolism has been given as 57 to 61 years (5, 6). Besides, it been shown to cause spontaneous pneumothorax, and I BV F F`ts incidence is around 1% (7). There are also publications in the literature reporting that it is seen less frequently (8). Contrary to the literature, the age of our patient was younger, PTE and pneumothorax coexisted together. It has been observed that in these patients, pneumothorax developed after invasive mechanical ventilation, the patients were reported to have more than one pre-existing cardiopulmonary comorbidity (9). Studies have reported that prolonged mechanical ventilator therapy increases the incidence of pneumothorax (10). Our case didn't require mechanical ventilation and being followed up in the ward.

While spontaneous pneumothorax often develops secondary to cystic lung lesions, the mechanism in COVİD-19 infection is less understood. The known risk factors for spontaneous pneumothorax include male gender, thin and long body structure, smoking, trauma, and infection (11). In contradiction, our case did not have a history of smoking, and pneumothorax developed while being treated in the ward. In addition, no parenchymal lesions such as cyst or bulla were observed in the first thoracic CT of our patient. In the literature, a case that developed giant bulla as a result of

COVİD-19 infection which caused rupture and pneumothorax (12), and a case with cystic lung lesions developed due to COVİD-19 infection and caused pneumothorax was published by Liu k et al. were reported (13). In COVİD-19 infection, it is thought that the inflammatory response and ischemic damage in the lung parenchyma cause cell adhesion between type I and type II pneumocytes, alveolar damage, and predispose to subsequent rupture (13). In a report, in which autopsy was performed on two patients who developed pneumothorax and pneumomediastinum after COVİD-19 infection and subsequently died due to resistant hypoxemia, diffuse alveolar damage, active infarction, reactive pneumocytes, sparse lymphoplasmacytic inflammation, fibrin thrombus, and multinucleated giant cells were observed histopathologically. Since ventilation and perfusion were both affected concurrently, it is thought to lead to pulmonary tissue ischemia by significantly increasing the degree of hypoxemia and shunt, worsening the perfusion, and increasing the risk of air leak (14). Besides, it is believed that direct viral infiltration of the lung parenchyma, visceral and parietal pleura leads to the development of pneumothorax/pneumomediastinum by causing disruption of parenchymal and pleural integrity or alveolar rupture (14, 15). The severity of the symptoms may contribute to alveolar cystic rupture by increasing the respiratory effort due to the ventilation/perfusion imbalance and leading to coughing. Therefore, we think that the development of ischemic parenchymal damage and pulmonary thromboembolism may have caused the development of pneumothorax in our patient. Previous studies have shown that in COVİD-19 patients with the critical disease, pneumothorax, pneumomediastinum, and subcutaneous emphysema may contribute to deep hypoxemia, and it is reported as a poor prognostic factor (14, 16). It is thought that the most serious problem is pneumothorax and accompanying pneumomediastinum since chronic problems usually occur after the 10th day of the disease and cause a life-threatening risk (17). In the study of 3000 patients by Massa Zantah et al., the presence of lymphopenia and increased inflammatory markers including CRP, LDH, ferritin, D-Dimer, and IL-6 levels were reported in almost all patients who developed a spontaneous pneumothorax (8). In our case, inflammatory parameters were high when pneumothorax developed. The laboratory results of our patient were concurrent with the literature.

Our case had spontaneous pneumothorax and PTE which was caused by COVİD-19 pneumonia. It may occur at the beginning of the symptoms or in later stages. It should be kept in mind that complications such as pneumothorax or pulmonary thromboembolism may have developed in cases of acute worsening with rapid oxygen desaturation, newly developing dyspnea, and pleuritic chest pain.

Conflict of interest

We declare that there is no conflict of interest, in particular no financial funding potentially relevant to the contents of manuscript.

Acknowledgments

None to declare.

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Case Report

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A case report of atypical Ramsay-Hunt Syndrome presented with severe vertigo and vomiting

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Abstract

Ramsay-Hunt Syndrome (Herpes zoster oticus) is often characterized by severe ear pain, vesicles on external auditory canal or auricula and peripheral facial paralysis. However, the patient in this case presented to the clinic with dizziness, pain in the ear and vomiting. During the physical examination there was no vesicles and no evidence of skin changes found around the auricula that were typical for Ramsay Hunt syndrome. The patient did not have facial paralysis. If there is an unexpected severe ear pain accompanying vertigo, Ramsay Hunt syndrome should be considered in the differential diagnosis even if there is no rash or facial paralysis.

Keywords: acyclovir, Herpes zoster oticus, vertigo, vomiting

1. Introduction

Ramsay-Hunt Syndrome (Herpes zoster oticus) is often characterized by severe ear pain, vesicles on external auditory canal or auricula and peripheral facial paralysis. The syndrome is named after the American neurologist, James Ramsay Hunt; the first person to describe the relationship between facial paralysis and typical vesiculopathy in Ganglion's geniculitis, ear canal, and auricular concha (1). However, paralysis can be seen in other cranial and cervical nerves (due to anastomoses in between). Vesicles can be seen in various portions of the skin, inside the mouth or on the uvula (2, 3). Symptoms such as disfunctions in hearing and balance, defects in sense of taste, decreased secretion of tear and saliva, trouble in senses should come to mind with motor, autonomic and sensory nerve paralysis.

Although the clinical symptoms of primary Varicella infection had during and after early childhood completely disappears the virus settles into cranial and spinal nerve ganglions (4, 5). When the immune system weakens, the virus gets activated and reproduces. Vesicles occur-ring on the skin and nerves effected depends on the ganglion on which the virus settles into (6).

Facial paralysis and vesicles are the primary characterization of the Ramsay-Hunt Syndrome. The aim of this case report is to present an atypical case of Ramsay-Hunt Syndrome who was presented with severe vertigo, ear pain, vomiting and no facial paralysis.

2. Case report

2.1. Patient information

A 55-year-old female patient living in a rural area, in a family that works in agricultural and stock farming. Physical labor and fatigue were thought to explain the patient's vertigo. She had never consulted to a clinic from a similar complaint.

2.2. Clinical findings

Nystagmus, balance disorder, and positive Romberg test were found during the physical examination confirming vertigo. The patient vomited during the examination due to vertigo. Earache remained unexplained after the patient refused radiological imaging due to her economic condition.

2.3. Timeline

A 55-year-old female patient was presented to the clinic with nausea, vomiting, severe vertigo and pain in her left ear. Physical examination showed no vesicles in the ear canal or the auricular concha. Hearing loss was not present. A through anamnesis showed no history of a similar disease or an indication of a previous viral infection. The disease seemed to have an acute onset due to an increase in her physical workload.

2.4. Diagnostic assessment

Audiometry and tympanometry tests were normal. The patient re-fused radiological imaging (MRI to assess cranial nerve involvement for the earache) due to her economic condition. The lack of imaging which reveals the incidence of nerve edema typical of Ramsay Hunt syndrome caused a delay in the diagnosis of the syndrome until the rash-es developed. Facial paralysis improved without sequalae, but early treatment can be started with radiological imaging and better treatment can be provided. Otherwise, a delay in the an-ti-viral therapy could cause sequalae.

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2.5. Therapeutic intervention

The patient was hospitalized due to severe vertigo, nausea, and vomiting. After 2 days of hospitalization, vesicles in right auricular concha (Fig. 1a) and type 4 House-Brackmann peripheral facial paralysis on her right side (Fig. 1b and 1c) occurred. Acyclovir (5 mg/kg IV, 8-hour intervals), Prednisone (80 mg/day for 3 days, 30mg for the following 10 days) were added to the patient's anti-vertiginous and analgesic treatment. Superficial Rifocin and antibiotic pomade applications were performed to prevent secondary infections of vesicles on the skin. Patient's vertigo disappeared after day 5 of hospitalization. Facial paralysis started to recover on the 7th day. On day 10, the vesicles recovered completely (Fig. 2A). At the end of the second week, facial paralysis disappeared without any sequela (Fig. 2B and 2C).

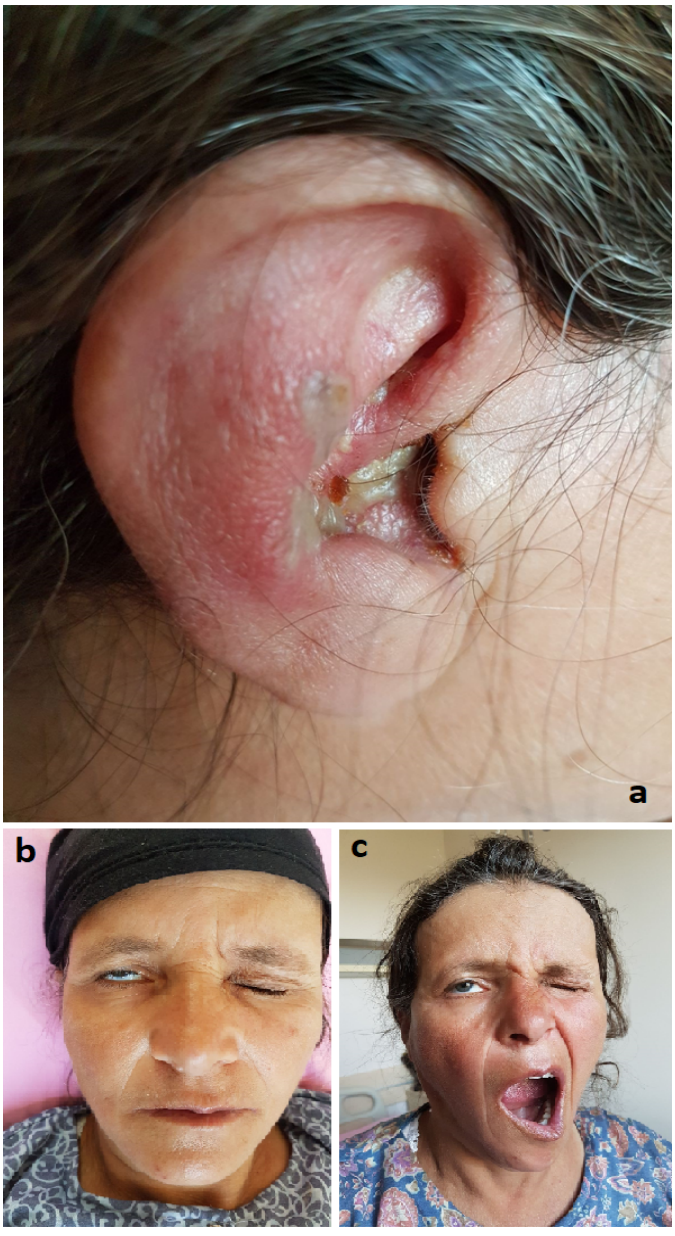

Fig. 1 (a) Vesicles in right auricular concha; **(b)** and **(c)** House-Brackmann peripheral facial paralysis on her right side

2.6. Follow-up and outcomes

Romberg test and other neurological examination showed normal results after the fifth day of hospitalization. Patient's complaints related to vertigo disappeared. Follow-up examination on the 15th day of discharge showed no sign of sequalae caused by facial paralysis and no skin abnormalities where the vesicles were located.

2.7. Patient perspective

The patient did not give any negative feedback about the drug use, hospitalization, and follow-up examinations. The patient was thankful for the total recovery without any sequela.

2.8. Informed consent

Informed consent was obtained from all individual participants included in the study.

Fig. 2 (a) Recovered vesicles **(b) and (c)** recovered facial paralysis

3. Discussion

After a previous Varicella zoster virus infection, the virus remains in the motor and sensory nerve ganglions inactively (7). The Virus gets activated following immune suppressing diseases and reproduces quickly. Ramsay-Hunt Syndrome occurs in 1% of the Varicella zoster infections. Primary characteristics of the syndrome include peripheric facial paralysis and typical, painful vesicles in the ear. However cranial polyneuropathy may be seen when the 5^{th}, 9^{th}, 10^{th} cranial nerves and the 2^{nd}, 3^{rd}, 4^{th} cervical nerves are infected (7). Pathological contrasting color may be seen at the facial nerve at the level of the internal acoustic canal during MRI with contrast. MRI with contrast could not be performed in this case due to patient's personal reasons. Ramsay Hunt Syndrome is the second most common cause of peripheric facial paralysis after Bell's palsy (7). The reason for presenting this case is contrary to typical symptoms the patient was presented with severe vertigo, nausea, and vomiting. Typical vesicle eruptions and facial paralysis developed later. Although facial paralysis developed from Ramsay Hunt syndrome has a lower rate of healing without any lesions of sequela compared to Bell's palsy, the patient has shown a full recovery without any lesions of sequela (3).

Virus eradication could not be reached. The biggest limitation was not being able to use radio-logical imaging. Although the literature indicates a high risk of sequalae in cases with late diagnosis the facial paralysis improved without sequalae in this case (8). Superficial Rifocin and antibiotic pomade applications were performed to prevent secondary infections of vesicles on the skin in addition to anti-viral therapy. This provided better results in the healing of skin lesions without any scars (9). Ramsay Hunt Syndrome should be considered in the differential diagnosis of vertigo cases with severe earache even if there is no rash or facial paralysis.

Conflict of interest

The author declared no conflict of interest.

Acknowledgments

None to declare.

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Demirtaş Erciyes-Mid urethral fibrin fixation technique (DE-MUFFT) for female stress urinary incontinence: A case series

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Abstract

Factors including suburethral blood flow impairment, collagen deficiency, and the lack of tissue healing factors are known to play a role in stress urinary incontinence (SUI). Autologous fibrin (AF) appears to be a viable material for the treatment of SUI. The aim of this study was to present the initial clinical outcomes of a novel technique named "Demirtaş Erciyes-Mid Urethral Fibrin Fixation Technique (DE-MUFFT)" that involved the placement of AF material in the suburethral space instead of sling material (mesh). In this study, the clinical outcomes of five women with pure SUI who underwent the placement of AF material in the suburethral space were examined retrospectively. The complaint of urine leakage during physical exertion and patients' quality of life were assessed using Incontinence Quality of Life Scale (I-QoL), International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form (ICIQ-UI SF), Incontinence Impact Questionnaire (IIQ-7), and 24-h pad test. Patient outcomes were evaluated preoperatively, at sixth week and third month postoperatively. Significant improvement was obtained in the quality-of-life tests and 24-h pad test. In all patients, the complaint of urine leakage disappeared almost completely. No adverse event or postoperative complication occurred in any of the patients. These results indicated that DE-MUFFT can be a promising procedure in the treatment of SUI due to its biocompatibility, minimally invasive nature, re-applicability, and cost-effectivity.

Keywords: autologous fibrin, platelet rich fibrin, stress urinary incontinence, sling

1. Introduction

Stress urinary incontinence (SUI) is a commonly seen health problem, affecting almost half of adult women with urinary incontinence (1). Despite their complications (erosion, infection, obstruction) and high costs, minimally invasive sling procedures such as transobturator tape (TOT) and transvaginal tape (TVT) are the most common surgical techniques used in the treatment of SUI (2). The primary aim in these techniques is to support the urethra and bladder neck with a synthetic mesh (3). In addition to the suspension mechanism formed by the sling, some researchers advocate that the inflammation, increased blood supply, and tissue reactions in the region where the sling is placed also support the urethra and thus provide clinical benefit (4-8).

Autologous Fibrin (AF) is a fibrin matrix frequently used in the treatment of clinical and urological diseases, consisting of cytokines, growth factors, and stem cell-like cells that are essential for immunomodulation and tissue healing (9, 10). To date, some other techniques such as stem cell injection, platelet rich plasma (PRP), and laser ablation have also been used for forming local inflammation and tissue response (4, 5, 11). However, to our knowledge, the use of AF in the treatment of SUI has not been reported in the literature.

The aim of this study is to describe a novel technique, which named as Demirtaş Erciyes-Mid Urethral Fibrin Fixation Technique (DE-MUFFT)", involved the placement of AF material in the suburethral space as the tissue support material without using a mesh-like foreign body and to present the clinical outcomes of the five patients with pure SUI that underwent this technique in our clinic.

2. Materials and Methods

2.1. Urethral preparation

In the lithotomy position, a 14-16 F Foley catheter is placed in the urethra. The balloon is inflated with 15 ml fluid and is placed against the bladder neck. The location of the bladder neck is determined and marked by moving the catheter in different directions. A 2-cm midline vertical incision is performed in the anterior vaginal wall, beginning 5 mm proximal to the urethral meatus, and extending to the bladder neck (Fig. 1A). Subsequently, sharp, and blunt dissection is used at the level of inferior pubic ramus, superior to the

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endopelvic fascia, to release the urethra (excluding the dorsal aspect) by preserving the periurethral tissue (Fig. 1B).

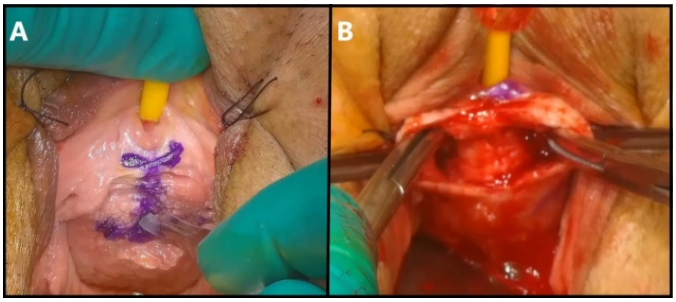

Fig. 1. Urethral preparation. **A.** 2-cm midline vertical incision, **B.** sharp and blunt dissection from the sides of the urethra

2.2. Acquisition of AF

A total of 20 ml of peripheral venous blood sample is placed into 4 biochemical tubes with separator gels, hence equally divided into 4 portions (Fig. 2A). The tubes are centrifuged at 2,700 rpm for 12 min (Fig. 2B) (12). The AF material that often forms in the middle of the tube is separated from the clot that forms at the bottom of the tube and then placed on a gauze pad (Fig. 3A). Afterwards, all the obtained AF materials are placed between two saline-soaked gauze pads either in combination or separately and transformed to membrane by minimal finger pressure (Fig. 3B).

2.3. Insertion of AF and finalization of the procedure

The AF membranes are inserted in the space previously formed below the urethra and then fixed to both ends of the external urethral meatus using 4-0 absorbable sutures (Fig. 4A and 4B). At this point, care should be taken to avoid the passage of the suture into the urethral lumen. The procedure is completed by closing the subcutaneous and skin layers with absorbable sutures. One of the four AF membranes is placed on the right, one on the left, and the other two in the middle suburethral space.

2.4. Data Collection

The clinical outcomes of five women with pure SUI who underwent DE-MUFFT were examined. The complaint of urine leakage during physical exertion and patients' quality of life were assessed using Incontinence Quality of Life Scale (I-QoL), International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form (ICIQ-UI SF), Incontinence Impact Questionnaire (IIQ-7), and 24-h pad test. Patient outcomes were evaluated preoperatively, at sixth week and third month postoperatively.

3. Results

The mean age of the patients included in the study was 53.2 ± 3.7 years. The mean duration of SUI complaints was 26.0 ± 4.5 months, and the mean postoperative follow-up time was 17.0 ± 3.9 months. All patients had previously applied pelvic floor exercises and used medical therapy (oral duloxetine) but did not benefit from these treatments. Urogenital examinations showed no clinically significant cystocele, rectocele, or descensus. The uroflowmetric parameters (maximum flow rate and post-voiding residual urine) of the patients were normal in pereoperatively and postoperatively.

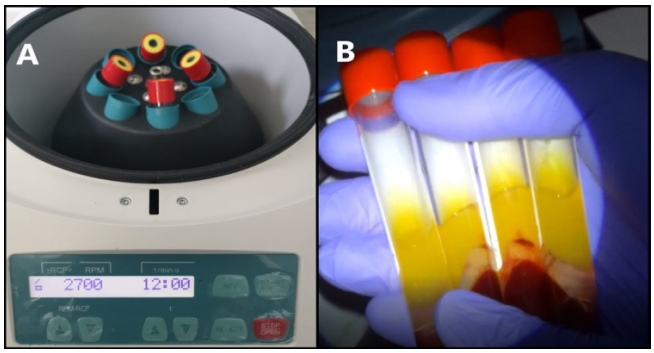

Fig. 2. Acquisition of AF (Part-1). **A.** The tubes are centrifuged at 2,700 rpm for 12 min, **B.** The AF material that forms in the middle of the tube

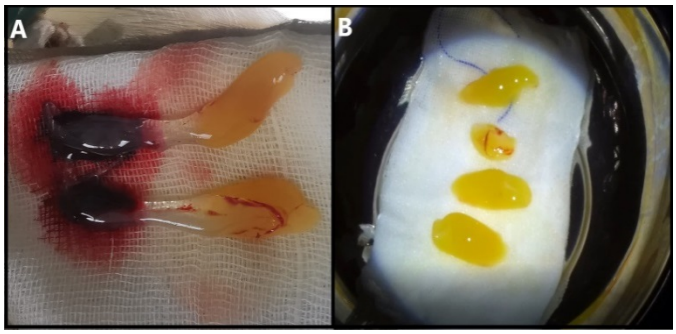

Fig. 3. Acquisition of AF (Part-2). A. The AF material is separated from the clot that forms at the bottom of the tube and then placed on a gauze pad. B. AF fragments separated from other cells and ready for processing

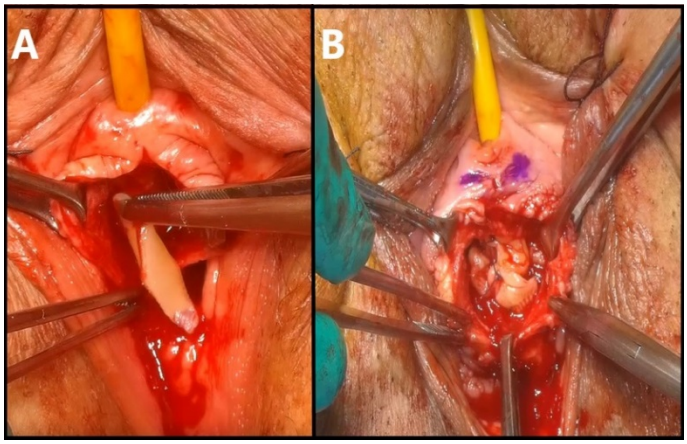

Fig.4. Insertion of AF. A. The AF membranes are inserted in the space previously formed, B. The AF membranes are fixed to both ends of the external urethral meatus using 4-0 absorbable sutures

In all patients, at six-week follow-up, urine leakage during sudden physical exertion and physical exercise reduced remarkably, I-QoL, ICIQ-UI/SF, IIQ-7 scores showed improvement, and the 24-h pad tests were negative.

In one of the five patients who completed their 12-week follow-up, it was observed that SUI symptoms started again at a very mild level. Despite this, the patient's satisfaction rate was 90%. The other patients did not have any SUI symptoms. Preoperatively, postoperative 6th and 12th week SUI tests of the patients are shown in Table 1, and their questionnaires are shown in Table 2.

Table 1. Demographics and SUI tests of the cases

Case	Age	Follow-up time (weeks)	ST-0	ST-6	ST-12	PT-0	PT-6	PT-12
#1	54	21	+	-	-	4 pads/day	0	0
#2	59	20	+	-	+	3 pads/day	0	**1**
#3	49	18	+	-	-	4 pads/day	0	0
#4	51	14	+	-	-	5 pads/day	0	0
#5	53	12	+	-	-	5 pads/day	0	0

ST-0: Stress test-preoperative, ST-6: Stress test-sixt week, ST-12: Stress test-third month, PT-0: 24 hours' pad test-preoperative, PT-6: 24 hours' pad test-sixt week, PT-12: 24 hours' pad test-third month

At 24th h after the AF procedure, the patients were discharged following catheter removal. None of the patients had early or late postoperative complications.

Table 2. Questionnaire results of the cases

Case	I-QoL-0	I-QoL-6	I-QoL-12	ICIG-UI-0	ICIG-UI-6	ICIG-UI-12	IIQ/7-0	IIQ/7-6	IIQ/7-12
#1	39	90	90	16	6	6	15	7	1
#2	42	85	75	18	4	10	16	5	5
#3	27	85	85	10	5	4	15	3	1
#4	41	90	88	14	4	4	18	4	4
#5	35	88	85	15	4	4	14	3	4

I-QoL-0: Incontinence Quality of Life-preoperative**, I-QoL-6:** Incontinence Quality of Life-sixth week**, I-QoL-12:** Incontinence Quality of Life-third month**, ICIG-UI-0:** Consultation on Incontinence Questionnaire Short Form-preoperative**, ICIG-UI-6:** Consultation on Incontinence Questionnaire Short Form-sixth week**, ICIG-UI-12:** Consultation on Incontinence Questionnaire Short Form-third month, **IIQ/7-0**: Incontinence Impact Questionnaire-preoperative, **IIQ/7-6:** Incontinence Impact Questionnaire-6th wk, **IIQ/7-6:** Incontinence Impact Questionnaire**-**12th wk**.**

4. Discussion

In this study, early postoperative outcomes of five women with pure SUI that underwent DE-MUFFT were highly promising. Although the sling and deep tissue fixation achieved in the technique achieved in the present study were not as substantial as those achieved by TVT and TOT, the patients obtained favorable clinical outcomes, which could be associated with the fibrosis that occurred secondary to suburethral incision and with the increased blood supply, collagen changes, and tissue healing resulting from the insertion of AF membrane.

This physiopathology has also been advocated by some other studies in the literature (8, 13, 14). In a previous study, Chen et al. evaluated the placement of a vaginal suburethral sling in rats and reported that the histologic changes caused by the insertion of the suburethral sling, such as inflammation, localized edema, and differential collagen remodeling, showed anti-incontinence effects despite the cutting of the polypropylene sling and the deactivation of the sling function (8). Klinge et al. reported that the collagen tissue dysfunction in the suburethral space results in SUI and the authors also confirmed the findings reported by Chen et al (13). Dobberfuhl et al. suggested that the obstruction formed by mid-urethral slings was not mandatory for obtaining successful outcomes (14). On the other hand, AF has been shown to be a viable material for tissue healing, collagen synthesis, fibrosis, and increased blood supply (15). Considering these notions, we consider that the favorable effects of suburethral placement of AF membrane on SUI could explain the physiopathology of the outcomes obtained in our study.

Both PRP and AF have been extensively used in urological diseases. Nikolopoulos et al. injected the PRP material in the pubourethral ligament (PUL) in SUI patients and found that the injection restored the ligament and provided successful outcomes (16). Similarly, Shirvan et al. reported that the closure of the fistula tract with AF membrane in vesicovaginal fistula repair provided favorable outcomes in the treatment of the fistula (17). A case report published in 2018 indicated that the injection of PRP material in the sphincter in a SUI patient led to 50% reduction in pad usage (11). The present case series, to our knowledge, is the first study reporting on the insertion of AF membrane in the suburethral region in SUI patients.

The technique administered in the present study provided several key advantages. First, the requirement of mesh, which is a foreign body for the patient, was eliminated. In the literature, mesh-related complications and the requirement of mesh revision surgery have been reported in up to 20% of the patients (18). Second, the technique was minimally invasive as it required a small suburethral incision and did not require deep tissue fixation as in TOT and TVT. In this way, the technique eliminated the risk of injury to the bladder, urethral, and bowel, which has been reported in up to 4% of the patients undergoing TVT and TOT procedures. Moreover, the risk of severe postoperative pain, which is a major complication of sling procedures, is remarkably low in the sub urethral AF procedure (18). Accordingly, we believe that this minimally invasive technique can be administered under local anesthesia in daily clinical practice in line with the accumulating experience, albeit not yet administered in our clinic. Third, this technique is highly cost-effective. A recent

report published in the UK indicated that mesh-related complications (infection, revision) lead to a significant increase in the costs of sling procedures (19). In contrast, the AF membrane is highly cost-effective since it is obtained from autologous blood sample. Additionally, it is also cost-effective when compared to other sling procedures as it does not require postoperative cystoscopy. Fourth, this technique is re-applicable as it solely consists of an autologous and biological material, can be inserted with a minimally invasive procedure, and is relatively cost-effective.

Our study was limited since the technique was performed in five patients only, the long-term outcomes of the patients remained unknown, and the immunohistochemical and histological changes were not analyzed.

In conclusions, DE-MUFFT can be a promising procedure in the treatment of stress urinary incontinence due to its biocompatibility, minimally invasive nature, re-applicability, and cost-effectivity. Further large-scale experimental and randomized-controlled studies based on histological analysis are needed to substantiate our findings.

Conflict of interest

None.

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Case Report

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Rare uterine didelphis and cervical pregnancy

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Abstract

Due to increased vaginal bleeding, a surgical operation was performed on a woman with cervical pregnancy. A diagnosis of uterine didelphis (UD) was made during surgery. The hypogastric arteries on both sides were ligated. After controlling the bleeding, the abdomen was closed. Uterine defects are a rare malformation that can cause a variety of obstetric problems in pregnant women, and they must be closely monitored and treated in terms of cervical pregnancy growth. As the pregnancy week continues, life-threatening bleeding may occur, and the patient may need a hysterectomy to stop the bleeding.

Keywords: cervical pregnancy, ectopic pregnancy, uterus didelphis, vaginal bleeding

1. Introduction

Genitourinary organ development begins in the 6th week of embryological life (1). Although the prevalence of Mullerian duct anomalies has been reported at different rates, among these anomalies, the frequency of uterine didelphis (UD) is estimated to be 5%. It is among the causes of infertility (2). The true incidence of congenital uterine anomalies is not known precisely, its prevalence in the general population is estimated to be between 0.001% and 10% (3). It is known that fertility and pregnancy outcomes of patients with Mullerian duct fusion anomaly are not good. The incidence of spontaneous abortion, premature birth, premature rupture of membranes and abnormal fetal presentation anomaly has increased in such patients (4).

Ectopic pregnancy is a disease seen in less than 2% of all pregnancies and is defined as implantation of the pregnancy product outside the uterine cavity. Although the most common type is tubal pregnancy (95%), we also encounter a rare type, cervical pregnancy. The incidence of cervical pregnancy, which constitutes 0.1-0.2% of all ectopic pregnancies, is as rare as one in 10000 pregnancies (5–7). Cervical pregnancy occurs because of abnormal implantation of the blastocyst into the endocervical canal. It is among the crucial causes of maternal mortality and morbidity due to massive bleeding outcomes. Early diagnosis and treatment of cervical pregnancy are lifesaving, essential in terms of preserving fertility. Systemic methotrexate (MTX) or intra-amniotic potassium chloride (KCl) is used in conservative treatment, but these conventional treatments can cause life-threatening bleeding during the procedure. In this presentation, we evaluated UD and cervical pregnancy's rare case with the literature findings' help.

2. Case Report

36-year-old pregnant admitted to our outpatient clinic with painless vaginal bleeding. In the anamnesis of the case, we have learned that she had been married for 16 years, her gravida was 5, parity 4, and living was 4. She gave birth to her first three children by standard spontaneous vaginal delivery and her last child by cesarean section. A bilateral tubal ligation was performed in the previous cesarean section. She got remarried after, and laparoscopic tube re-anastomosis was performed due to the request for a child. In the abdominal-obstetric ultrasonography (US) examination of the patient, uterus, double cervix, and double uterus, a 13 + 6-week pregnancy with a positive fetal heartbeat was observed cervical canal in the right uterus (Fig. 1). The patient was informed about the possibility of UD during the US examination in her first pregnancy.

In the patient's speculum examination, there was active vaginal bleeding that caused a decrease in the hemoglobin value from 10.2 to 7.3 gr/dl within 2 hours, and the patient was performed d / c. Since the patient continued to bleed after dilatation curettage (d/c) and the bleeding did not respond to medical treatment, a hysterectomy was planned. Intraop uterus didelphys were observed, the right hemiuterus cervix was observed to be significantly enlarged (Fig. 2). Total right hemihysterectomy was performed after bilateral hypogastric artery ligation (Fig. 3). Inspired by this case, we questioned the association of

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UD and cervical pregnancy with the help of the literature (Fig. 3).

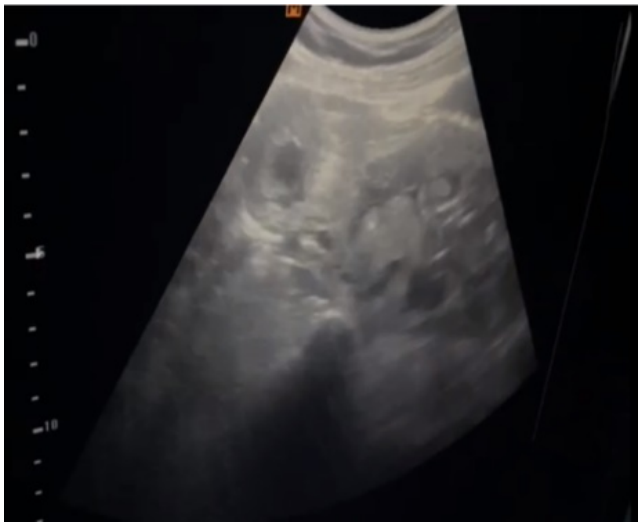

Fig. 1. USG evaluation of uterus to diagnose UD

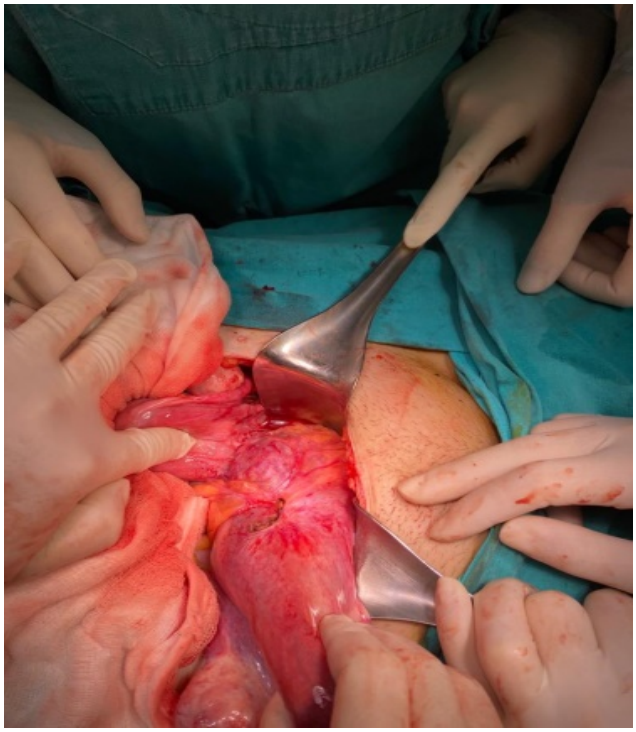

Fig. 2. The observation the right hemiuterus cervix

3. Discussion

The fusion of the two mullerian canals and the formation of the vaginal canal is completed between the 10th and 17th weeks (9, 10). Inadequate fusion of the Mullerian ducts causes the development of many uterine anomalies. Although the true prevalence of congenital uterine anomalies in the general population is not known exactly (11), the most common uterine anomaly is the uterine septus (90%), followed by the bicornuate uterus (5%) and UD (5%) (3).

Detection of reproductive tract anomalies in early pregnancy is necessary for clinical follow-up (10). The most common symptoms of cervical pregnancy are vaginal bleeding (91%) and groin pain (28%), as in our case (6). In addition to clinical findings, β-hCG measurement, ultrasonography (USG), and magnetic resonance imaging (MRI) are used. Diagnosis is made by observing the gestational sac in the cervix, trophoblastic invasion of the cervical wall, and typical uterus dimensions on USG (6).

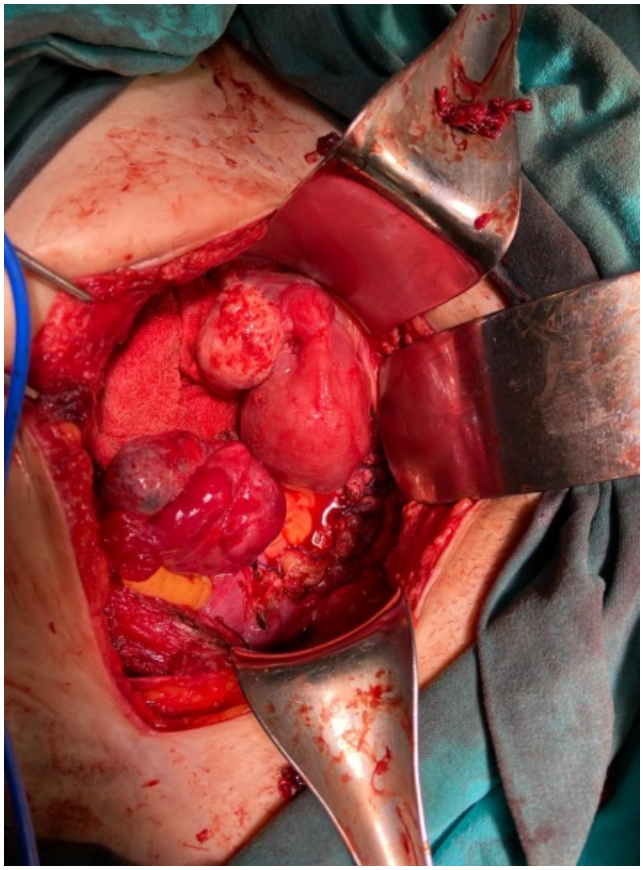

Fig. 3. The total right hemihysterectomy

In our case, uterine diadelphous and cervical ectopic pregnancy were observed together as a rare situation. Few cases have been reported on this subject in the literature (21-23). Patrick et al. managed a patient diagnosed with uterine diadelphous with a cervical pregnancy bHcg level of 6045 mIU / ml with vaginal bleeding at an early gestational week by administering methotrexate treatment. In our case, curettage was applied because methotrexate treatment was not appropriate due to the advanced gestational week (21). Another case study was reported by Chandrasekhar et al., two cases of UD, one cervical and the other cornual. Cervical located patient treated by applying intramuscular methotrexate after intraamniotic KCL application to 10 weeks of gestation. Cornual pregnancy resection was performed in the patient with 13 weeks of gestation in the cornual location (22). Like our case, a case of didelphus with advanced gestational week was reported by Haberal et al. In their case, supracervical hemihysterectomy was applied to the didelphus case with 18 weeks of placenta percrata (23).

Severe uterine bleeding may occur during conservative treatment in cervical ectopic pregnancies (13). Such a situation can be controlled by cervical curettage,

intracervical foley catheter application after curettage and cervical suture. (14, 15). If vaginal bleeding occurs after medical treatment and fertility are desired to be preserved, effective bleeding control can be achieved by applying selective uterine artery embolization (16, 17). In cases where bleeding cannot be controlled, radical treatments such as internal iliac artery ligation, vaginal or abdominal hysterectomy can be applied (18-20). Since the diagnosis of cervical pregnancy is made more complex than other ectopic pregnancies, there may be delays in treatment. Delayed intervention often causes massive bleeding and may lead to emergency hysterectomy in approximately 50% of the cases, as in our case, too (12).

Uterine anomalies are rare malformation that can cause many obstetric complications in pregnant women. When the pregnancy week progresses, life-threatening bleeding may develop, and as in our case, the patient may occasionally undergo a hysterectomy for bleeding control.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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