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Risk factors for lymph node involvement in early-stage cervical cancer: A retrospective cohort study

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Abstract

One of the most important prognostic factors in cervical cancer is lymph node involvement that affects disease-free survival and plays an important role in the treatment. This study aimed to determine the factors affecting lymph node involvement in early stage cervical cancer. A total of 169 cervical cancer patients with stage IA2-IIA2 were enrolled. Age, histologic subtype, deep stromal invasion (DSI), largest tumor diameter (LTD), lymphovascular space invasion (LVSI), vaginal surgical margin, ovarian metastasis status, parametrial involvement, lymph node count and presence of metastasis were retrospectively reviewed. All of these parameters are divided into two groups: LNM positive (group 1) and negative (group 2). The median age of the patients was 52 (26-77) years. In the univariate regression analysis; LVSI ($p < 0.001$), DSI ($p=0.018$), parametrial involvement ($p = 0.001$), and vaginal surgical margin positivity ($p = 0.020$) were in statistically significant correlations with lymph node involvement. In multivariate regression analysis, LVSI [51/118 (43.2%) vs 7/51 (13.7%), OR = 3.9, $p = 0.003$] and parametrial involvement [(16/24 (66.6%) vs 42/145 (28.9%), OR = 3.9, $p = 0.009$] were the independent risk factors for lymph node metastasis. LVSI and parametrial involvement are two crucial risk factors for lymph node metastasis in patients diagnosed with early-stage cervical cancer.

Keywords: cervical cancer, lymph-vascular space invasion, lymph node metastasis, risk factors

1. Introduction

Cervical cancer is an important public health issue, ranking fourth leading cause of cancer-related deaths among women globally (1). The treatment options for cervical cancer vary based on the stage of the cancer, with radical hysterectomy and pelvic lymph node dissection serving as the standard approach in early-stage cases (2).

Lymph node involvement emerges as a critical prognostic factor in early-stage cervical cancer that affects disease-free and overall survival. Lymph node metastasis (LNM) is a vital determinant of survival (3). The incidence of LNM ranges between 4.3% and 19.6% in early-stage cervical cancer (4). Surgical staging of lymph nodes represents the preferred method both for management and prognostication in early-stage cervical cancer (5). Debates persist on whether lymphadenectomy should be done for all patients and the extent of the radicality of the surgery (6,7). The therapeutic benefit of lymphadenectomy in early-stage cervical cancer remains controversial (8).

Sentinel Lymph Node (SLN) mapping and biopsy have recently gained popularity in gynecological oncology, particularly in vulvar cancer and cervical cancer, as an alternative method to identify the status of lymph node metastasis (9). However, this technique requires specific equipment and trained personnel making it unfeasible in various settings, particularly in developing nations with high cervical cancer prevalence.

This study aims to identify factors influencing lymph node involvement in early-stage cervical cancer and to propose avenues for future research to identify low-risk cervical cancer cases that may not require lymphadenectomy.

2. Materials and Methods

This retrospective cohort study was carried out between September 2008 and January 2018, on 169 patients with stage IA2-IIA2 cervical cancer who underwent radical hysterectomy and systematic bilateral pelvic-paraaortic lymph node dissection at a tertiary hospital. The study was approved by the

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Board of Medical Speciality Education at the University of Health Sciences, Zekai Tahir Burak Women's Research and Training Hospital and was in agreement with Declaration of Helsinki (Approval number:8, date:05.12.2017). This study included patients who had complete medical records and regular post-operative follow-up Patients who did not have systematic lymph node dissection, those with incomplete medical records, those without regular follow-up, those who underwent only simple hysterectomy (stage IA1), and those with advanced cervical cancer (IIB-IVB) were excluded.

In order to determine the factors that influence lymph node metastasis, an analysis was conducted on the demographic and clinicopathological characteristics of patients. We classified patients into two groups: group 1 had lymph node metastasis, while group 2 did not. The study analyzed parameters such as age, histology, International Federation of Gynecology and Obstetrics (FIGO) stage (2009), deep stromal invasion (DSI), largest tumor diameter (LTD), lymphovascular space invasion (LVSI), positive surgical margins in the vagina, status of ovarian metastasis, parametrial involvement, quantity of lymph nodes, and the status of metastasis. The clinical stage and histological classification of the patients were assessed according to the FIGO 2009 guidelines (10). All procedures were conducted by surgeons specializing in gynecological oncology. The pelvic lymphadenectomy involved extracting the lymph nodes of iliac and obturator arteries. Similarly, the para-aortic lymphadenectomy involved eliminating lymph nodes above the inferior vena cava and aorta up to renal vein. Systematic lymphadenectomy was characterized by the removal of a minimum of 15 lymph nodes. For a thorough pelvic and para-aortic lymph node dissections, the removal of at least 10 and 5 lymph nodes were considered adequate. The study accounted for the largest of the three diameters of the tumor observed macroscopically by the gynecopathologist. The presence of tumor cells in the endothelium of lymphatic or vascular structures supplying the tumor defined LVSI, while DSI was described as tumor invasion into the outer third of the cervical stroma.

2.1. Statistical Analysis

To determine the distribution of data, the Kolmogorov-Smirnov normality test was conducted. Descriptive statistics were used to obtain the median, minimum, and maximum values. The categorical variables were compared between groups via the $\chi 2$ test. The Independent T test was utilized to examine parametric data. For pairwise comparisons, numerical variables were evaluated using the Mann-Whitney U test. Univariate and multivariate regression analyses identified risk factors for LNM and to calculate relative risks. All variables were expressed with 95% confidence intervals (CI). Data were processed using the Statistical Package for Social Sciences (SPSS) version 21 (IBM Corp., Armonk, N.Y.; USA). A p value of less than 0.05 was accepted as statistically significant.

3. Results

A total of 169 patients were involved in the research and were split into two groups: non-LNM (group 2, n: 111, 65.7%) and LNM (group 1, n: 58, 34.3%). Age, the number of lymph nodes, stage, and histological subtype did not differ between the groups (p>0.05) (Table 1).

Table 1. Comparison of patients according to the lymph node metastasis

		LNM (+) *n*: 58	LNM (-) *n*: 111	*p-value*
Age		49.5 (32-70)	52 (26-77)	0.120*
Number of pelvic lymph nodes		43 (14-97)	39 (14-92)	0.471*
Number of paraaortic lymph nodes		12.5 (5-41)	12 (5-45)	0.130*
Total number of lymph nodes		58 (20-138)	51 (24-119)	0.252*
Pelvic lymph node metastasis		2 (1-43)	NA	NA
Paraaortic lymph node metastasis		0 (0-33)	NA	NA
Total number of lymph node metastases		2 (1-73)	NA	NA
Histology	SCC	44	0.306**	0.306
	Adenocarcinoma	7	20	
	Adenosquamous carcinoma	7	7	

SCC: Squamous cell carcinoma LNM: Lymph node metastasis NA: Not applicable, *:Mann-Whitney U, **:$\chi 2$ test
P<0.05 was considered statistically significant.

In univariate analysis, tumor size (*OR*=1.02, 95% *CI*: 1.01–1.04, P=0.004), LVSI (*OR*=8.3, 95% *CI*: 3.3–20.6, P=0.001), DSI (*OR*=3.6, 95% *CI*: 1.4–9.2, P=0.007), LTD ≥4 cm (*OR*=4.3, 95% *CI*: 1.5–12.2, P=0.007), parametrial involvement (*OR*=1.06, 95% *CI*: 1.00–1.12, P=0.021), and positive vaginal margins (*OR*=3.6, 95% *CI*: 1.7–7.8, P=0.0001) were significantly associated with lymph node metastases (Table 2).

In binary multivariate logistic regression analysis, LVSI (*OR*=6.3, 95% *CI*: 2.4–16.5, P=0.0001) and parametrial involvement (*OR*=2.6, 95% *CI*: 1.6–6.0, P=0.020) were significantly associated with lymph node metastases (Table 3).

4. Discussion

Our study sought to identify risk factors for lymph node metastasis in stage IA2-IIA2 cervical cancer. We found that LVSI and parametrial involvement were significant factors linked to lymph node metastasis.

Lymph node metastasis had prognostic significance in

early-stage cervical cancer. Several clinicopathological parameters, including advanced stage (11), large tumor size (3, 11), LVSI (3,11,12), DSI (13-15) and parametrial involvement (3), have been identified as potential indicators of LNM in cervical cancer. Our analysis found that LVSI and parametrial involvement were significantly related to LNM. However, we observed a higher rate of LNM than previously reported in the literature. One possible explanation is that most of our patients had tumors larger than 2 cm. Moreover, the FIGO staging system was revised in 2018, taking into account any imaging modality or pathological findings to allocate the stage. As a result, some of our patients previously classified in lower stages have now been upstaged to stage IIIC.

Table 2. Univariate regression analysis for Lymph node metastasis (LNM)

	Estimate	SE	Z	OR (95% CI)	P-value
Age	-0.0157	0.0130	-1.204	0.98 (0.96-1.01)	0.229
Tumor size	0.0250	0.00879	2.84	1.02 (1.01-1.04)	**0.004***
Total number of lymph nodes	0.00231	0.00665	0.347	1.0 (0.99-1.0)	0.729
Age >52	-	-	-	0.674 (0.421-1.08)	0.101
LVSI (+)	-	-	-	8.3 (3.3-20.6)	**0.0001***
DSI (+)	-	-	-	3.6 (1.4-9.2)	**0.007***
LTD ≥2 <4 cm ≥4 cm	-	-	-	 2.5 (0.8-7.3) 4.3 (1.5-12.2)	 0.094 **0.007***
Parametrial Involvement (+)	-	-	-	3.6 (1.7-7.8)	**0.0001***
Vaginal Surgical Margin	-	-	-	2.0 (0.8-5.2)	0.156

*LTD: Largest tumour diameter. DSI: Deep stromal invasion. LVSI: Lymphovascular space invasion. OR: Odds Ratio SE: Standart Error (SE), CI:C*onfidence Interval, **:P<0.05 was considered statistically significant*

Table 3. Multivariate regression analysis for Lymph node metastasis (LNM)

Independent variables	OR (95% CI)	P-value
Age >52	0.84 (0.42-1.67)	0.617
LVSI (+)	6.3 (2.4-16.5)	**0.0001***
DSI (+)	0.6 (0.2-1.9)	0.421
LTD ≥2 <4 cm ≥4 cm	 1.7 (0.5-5.7) 3.0 (0.9-10.0)	 0.407 0.078
Parametrial Involvement (+)	2.6 (1.6-6.0)	**0.020***
Vaginal Surgical Margin	1.2 (0.4-3.6)	0.681

SE: Standart Error (SE), OR:Odds Ratio, CI:Confidence Interval, LTD: Largest tumour diameter. DSI: Deep stromal invasion. LVSI: Lymphovascular space invasion, +: positive;
*Reference for age : ≤52, reference for LTD: <2 cm, reference for LVSI, DSI, Parametrial Involvement, and Surgical margin: negative; *:P<0.05*

Roman et al. conducted a study that revealed none of the patients with negative LVSI had pelvic LNM, indicating a significant correlation (p=0.0001) (16). Similarly, Sakuragi et al. analyzed LVSI and LNM correlation in their study and found that lymphatic and blood vessel invasion separately caused an increase in LNM positivity from 9.4% when LVSI was negative to 43% when LVSI was positive (p<0.0001) (17). Our findings align with those of Sakuragi et al., with 43.2% (51/118) LNM in LVSI-positive and 13.7% (7/51) in LVSI-negative patients.

In a study of 399 cases of early cervical cancer, 32 patients (12.4%) were found to have parametrial invasion, the majority of which were squamous cell carcinoma (18). This finding is consistent with our study, in which 14.2% (24/169) of patients were found to have parametrial invasion.

Although this study has a few drawbacks, including its retrospective design, limited sample size, and absence of central histopathological examination, it has several strengths. All patients underwent surgery by a gynecological oncologist, and all pathological examinations were conducted by an experienced gynecopathologist.

Our study found that LVSI and parametrial invasion are significant risk factors for lymph node metastasis in early-stage cervical cancer.

Conflict of interest

The authors have no conflicts of interest.

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The data that support the findings of this study are available on reasonable request from the corresponding author (EK).

Authors' contributions

Concept: U.E., V.K., B.Ö., Design: U.E., V.K., K.A., B.Ö., Data Collection or Processing: U.E., V.K., K.A., B.Ö., Analysis or Interpretation: U.E., V.K., E.K., K.A., B.Ö., A.B., Literature Search: U.E., V.K., E.K., K.A., B.Ö., Writing: U.E., V.K., E.K., K.A., B.Ö.

Ethical Statement

The database management in accordance with privacy legislation and the presented study in accordance with the ethical principle of the Declaration of Helsinki. Ethical approval for this study was obtained by the the Board of Medical Speciality Education at the University of Health Sciences, Zekai Tahir Burak Women's Research and Training Hospital (Approval number:8, 05.12.2017). The work has not been published previously and it is not under consideration for publication elsewhere.

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Clinical characteristics and outcomes of chronic limb threatening ischemia patients after treated with endovascular treatment during COVID-19 outbreak in Vajira Hospital

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Abstract

To study clinical characteristics and outcomes of chronic limb threatening ischemia patients after treated with endovascular treatment during COVID-19 Outbreak. This research is a retrospective descriptive study through electronic medical records. Samples are all patients that had been treated at Vajira Hospital during the COVID-19 outbreak in Thailand between January 2020 to December 2022. Results shown that out of 180 patients with chronic limb threatening ischemia, 106 are male (58.9%) while 74 are females (41.1%). The average age of the patients is 77.01 years old. Sixty-seven patients (37.2%) have history of smoking. Seventy-four patients (41.1%) were found with Wifi Classification state. One hundred and seventy-five patients (97.2%) have comorbidity including Hypertension 24.5%, Diabetes mellitus 23.1%, Dyslipidemia 19.2%. 38.7% of the patients receive Dual Antiplatelet 38.7%. It is found that low Serum Albumin (3.26 g/dL) and Wifi Classification are having significant correlation with occurrences of complications after surgeries ($p<.05$). Patients infected with COVID-19 who have chronic limb threatening ischemia have lower Serum Albumin than patients who do not have COVID-19 which becomes a variable that increases risks of complications after surgeries, losses of limbs, deaths and numbers of days admitted in hospital which leads to increased treatment expenditures.

Keywords: Chronic limb-threatening ischemia, COVID-19, Outcome, Serum Albumin

1. Introduction

In the present, health problems related to cardiovascular diseases can increasingly been found in urban society which was caused by daily lifestyles and changing environment that leans toward western lifestyles including inappropriate food consumptions, lacking of exercises, sleep deprivation, crowded housing and workplace. These factors have created more risks towards cardiovascular and other non-communicable diseases for people living in Bangkok more than people in other regions (1).

Chronic limb-threatening ischemia (CLTI) is cardiovascular disease in the end-stage of peripheral artery disease (PAD) which can cause effects to patients' health in which it creates risk of disabilities, high healthcare expenditures and increases rate of morbidity and mortality. It is found that ten percent of patients with peripheral artery disease will be worsen to the point of chronic limb-threatening ischemia within five years, and 80 percent of amputated patients were having chronic limb-threatening ischemia. Patients with chronic limb-threatening ischemia and were amputated are at risk of premature death (2). In addition, patients will have higher healthcare expenditures. It is found that the average treatment fees for these group of patients is 337,873.66 Thai baht with 21 days being admitted in the hospital. As a result, it is a complex disease with high healthcare expenditures (3).

Chronic limb-threatening ischemia happens with distal legs which causes ischemic pain in the foot while a person is at rest. Pain is caused to the foot, and severe pain will occur while a person is at rest or when raising the foot. The pain can last more than two weeks, and when there is a wound, tissues will start to be in shortage of blood and lead to gangrene. In chronic ischemic ulcer patients, they will be classified with Fontaine classification (stage IV) and Rutherford classification (stage 5-6). Moreover, the patients may be admitted with other complications such as foot infection which can commonly be found in diabetes patients with chronic limb-threatening ischemia due to decreased blood circulation. The number of white blood cells and oxygen circulating to the foot were decreased. Variables that can lead to chronic limb-threatening ischemia are Diabetes Mellitus. Patients with Diabetes Mellitus will be twice at-risk of chronic limb-threatening

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ischemia. Elderly people in each 10-year span is found 1.5 time more at-risk of having chronic limb-threatening ischemia while patients with hypertension is found 1.5 time more at-risk of having chronic limb-threatening ischemia. Patients with Dyslipidemia, especially having high cholesterol will be 1.25 times at-risk while patients with smoking habits are twice more at-risk. In addition, coronary artery disease which is an important factor that increases primary amputation rate to 30 percent and mortality rate at 25 percent within 1 year after diagnosis of COVID-19 will increase the mortality rate to 40 percent and amputation rate at 23.5 percent (4, 5). Treatment of chronic limb-threatening ischemia has the main target to reduce pain from the lack of blood circulation and prevent losses of legs. If the patient has ischemic wound, treatment should aim at removing severe inflamed tissues such as having extensive soft tissue infection or sepsis. This type of patient must go through surgeries. There are several types of treatment for chronic limb-threatening ischemia, but the treatment is more likely towards endovascular type because of the advanced equipment and methods of treatment. However, there is still limitations in terms of the treatment expenses. For patients with severe conditions which has several levels of clogged arteries, it is necessary to use mixed treatments between endovascular treatment and open bypass surgery, or what is called as Hybrid procedure (6).

Chronic limb-threatening ischemia affects patients in many aspects including that the infection around ischemic wounds can lead to longer length of stays and higher healthcare expenses. Moreover, after surgeries to increase blood flow to the legs or toes, patients will need continuous treatment on ischemic wounds which affects the rehabilitation after surgery. Patients are likely to be dependent on others when returning home (7). The COVID-19 outbreak has changed the way patients around the world are being treated which is similar to what happened at Vajira Hospital where it needs to control the infections and prevent patients from COVID-19. Chronic limb-threatening ischemia patients who are not emergency cases will not go through surgeries, while hospital referrals are ceased to operate. According to the Ministry of Public Health's policy, all incoming patients must be screened in each and every services post pf the outpatient clinic. Risk group will be referred to OPD ARI. Before chronic limb-threatening ischemia patients be treated as inpatient, they must go through COVID-19 or SARS-CoV-2 test with RT-PCR which will provide result within 4-10 hours depending on the time of receiving samples of test rounds. While waiting for the results, patients will be referred to the Buffer Ward which widen treatment time. Chronic limb-threatening ischemia patients that are infected with COVID-19 will be in worsening condition especially with the patients with cardiovascular diseases, diabetes or being elderly. This group of patients are in risk of being dead. Patients with COVID-19 will experience the release of Cytokine which can lead to organ failure and increase repeated blood clogged in peripheral blood vessels with higher risk of amputation. In the same time, lungs and respiratory system fail to operate leading to less oxygen risking more losses of blood circulation to legs (8). Factors supporting recovery of wounds, reducing of ischemic pains and prevent relapse of diseases of the chronic limb-threatening ischemia patients are knowledge towards the diseases and risk factors, knowledge and behaviors on how to take care of oneself and also knowledge of families or relatives of the patients (9, 10). Methods to preventing relapse of disease after treatment is to reduce risk factors including stop smoking, taking antiplatelet, exercise, control comorbid diseases including diabetes, hypertension and dyslipidemia while monitoring abnormalities of arteries. Literature reviews suggest that there are no studies towards the outcomes of chronic limb threatening ischemia patients' endovascular treatment during the COVID-19 outbreak in Thailand in which the situation has affected patients in every aspect, causing changes in livelihood and changes in hospital services to be more adaptive to new normal medical service which can cause impact to the time in accessing necessary and urgent healthcare services and the outcomes of treatment accordingly. Researchers would like to study clinical characteristics and outcomes of chronic limb threatening ischemia patients during the COVID-19 outbreak which have been treated with endovascular treatment in order to evaluate the outcomes of treatment resulted from the research which will become preliminary data to improve treatment of the hospital and the whole country, and to apply these knowledge in treatment plan in order to increase quality of treatment or adjust chronic limb threatening ischemia patient treatment system, reducing the likelihood of amputation, suffering from ischemia and mortality. Literature reviews suggest that important clinical characteristics of chronic limb threatening ischemia patient which have been treated with endovascular treatment are the general profile of the patients, genre, comorbidity diseases and its control, smoking habit, COVID-19 infection, outcomes of important treatments including complication after surgeries such as Myocardial infarction or Ischemic stroke (major adverse cardiovascular; MACE) Major Amputation (major adverse limb events; MALE) and Limb salvage shown in fig. 1 (11).

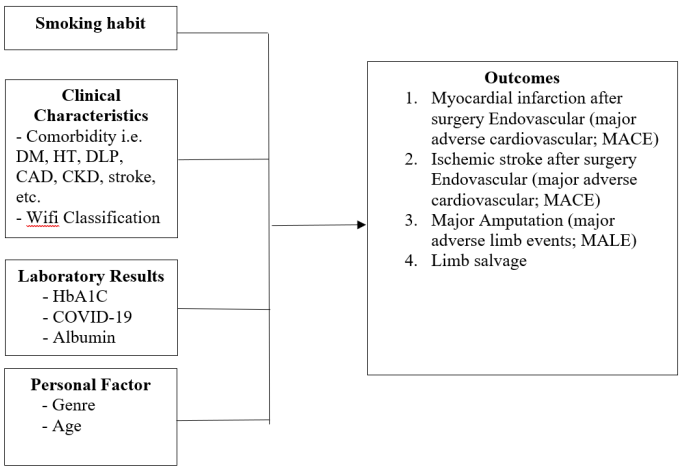

Fig. 1. Conceptual Framework

2. Materials and Methods

This research is a Retrospective descriptive study to study clinical characteristics and outcomes of chronic limb threatening ischemia patients during the COVID-19 outbreak which have been treated with endovascular treatment at Vajira Hospital. Samples are medical records of all chronic limb threatening ischemia patients by searching from disease code ICD 10 (I7020-29) which has gone through medical procedures ICD 9 (0045, 0041, 0047, 0023, 3990, 0055, 3950) and have received treatment at Vajira Hospital during COVID-19 outbreak in Thailand from January 2020 to December 2022.

2.1. Research Tools

There are five sets of research tools to collect data and implement the study including patients' basic information and clinical symptoms record form, medical procedures record form, complication after surgery record form and number of hospital admission days, laboratory testing results record form and medication record form. These tools must be examined of its content validity by three vascular surgery physician and nursing instructors. The tools got CVI=0.84. For other issues that receive additional recommendations, researchers have modified the tools according to the recommendations of experts.

2.2. Patients' confidentiality

Researchers are tasked to access the data, copy and record from medical records. The research presents the results in general. Moreover, the researchers keep patients' data in confidential. Names of the patients will not be mentioned but rather use codes instead.

2.3. Data Collection

Researchers review electronic medical records and record them into the developed tools. Researchers collect data from the medical records since January 2020 to December 2022 in which researchers are the one who collect and record the data themselves. Researchers are careful of how the data is kept to protect patients' confidentiality by using codes. In analyzing the data, researchers use SPSS version 28 to process and analyze the data, then present it in two parts following the type of data and with frequency, percentage and identify $p< 0.05$ of having statistically significant difference.

3. Results

3.1. General information and clinical symptoms of patients

From 180 electronic medical records of chronic limb threatening ischemia patients, 106 patients are male (58.9%) while 74 patients are female (41.1%). The average age of the patients is 67.01 years old. Sixty-seven patients (37.2%) have history of smoking. Seventy-four patients (41.1%) are at Wifi Classification clinical state 4. In terms of comorbid diseases, 175 patients (97.2%) have comorbid diseases with 157 patients (87.2%) have Hypertension, 148 patients (82.2%) have Diabetes mellitus, 123 patients (68.3%) have Dyslipidemia, 62 patients (34.4%) have Coronary artery disease, CAD), 84 patients (46.7%) have Chronic kidney disease, CKD) and 18 patients (10%) have Ischemic stroke). In terms of medication that the patients receive, 14 patients (7.8%) got single Antiplatelet, 143 patients (97.4%) got dual Antiplatelet and 146 patients (81.1%) got Statin. It is also found that Wifi Classification are having significant correlation with occurrences of complications after surgeries ($p<.05$) as shown in table 1.

Table 1. General Information and Clinical Symptoms of patients

Characteristic		Total N=180 (%)	CLTI + COVID-19	CLTI non COVID-19	*p*
Genre	Male	106 (58.9)	2	104	.282
	Female	74 (41.1)	1	73	
Age (years) mean ± SD		67.01 ± 11.46	67.33	67	
	Less than 40 years old	1 (0.6)	-	1	.926
	41-60 years old	49 (27.1)	1	48	
	61-70 years old	68 (37.8)	-	68	
	71-80 years old	41 (22.8)	2	39	
	More than 80 years old	21 (11.7)	-	21	
History of smoking	Yes	67 (37.2)	-	67	.821
	No	113 (62.8)	3	110	
Comorbid diseases	Diabetes Mellitus	148 (23.1)	2	146	.893
	Hypertension	157 (24.5)	3	154	.111
	Dyslipidemia	123 (19.2)	3	120	.567
	CAD	62 (9.7)	-	62	.964
	CKD	84 (13.1)	1	83	.109
	Ischemic stroke	18 (2.8)	-	18	.251
	other	49 (7.6)	2	47	.094
Clinical symptoms Wifi classification	State 1	9 (5.0)	-	9	.003*
	State 2	32 (17.8)	1	31	
	State 3	65 (36.1)	1	64	
	State 4	74 (41.1)	1	73	
Medication	Single antiplatelet therapy/Aspirin or Clopidogrel	14 (3.7)	1	13	-
	Dual antiplatelet therapy/Aspirin and Clopidogrel	143 (38.0)	2	141	
	Aspirin and Statin	22 (5.8)	-	22	
	Statin	146 (38.7)	3	143	
	Other	52 (13.8)	1	51	

3.2. Medical Procedures

Medical procedures that the patients received are PTA constituting for 90 cases (50%), PTA together with stent constituting for 84 cases (46.7%), Endarterectomy constituting for 4 cases (2.2%), Hybrid procedure (By Pass together with Stent, PTA together with Stent and Endarterectomy) constituting for 7 cases (3.9%). After medical procedures taken, complications found include 9 cases (5%) of acute kidney Injury, 2 cases (1.1%) of acute limb ischemia, 1 case (0.6%) of Myocardial Infarction, 1 case (0.6%) of Ischemic stroke, 1 case (0.6%) of major amputation and 5 cases (2.8%) of Peri-operative death. The average length of stay is 18.77 days with the shortest of 2 days and longest of 127 days. The median is at 18.23 days. For patients infected with COVID-19, the average length of stay is 50.33 days, as shown in table 2.

Table 2. Medical Procedures, Complications and Treatment Results

Medical Procedures/ Complications/ Treatment Results		Total N=180 (%)	CLTI + COVID-19	CLTI non COVID-19	*p*
Medical Procedures	PTA	90 (48.6)	1	89	-
	PTA with stent	84 (45.4)	2	82	
	Endarterectomy	4 (2.2)	-	4	
	Hybrid procedure	7 (3.8)	-	7	
Complications	Myocardial Infarction	1 (2.3)	-	1	-
	Acute Kidney Injury	9 (21.0)	-	9	
	Ischemic stroke	1 (2.3)	-	1	
	Acute limb ischemia	2 (4.7)	-	2	
	Major Amputation	1 (2.3)	1	-	
	Peri-operative death	5 (11.6)	-	5	
	Other	24 (55.8)	-	24	
Length of stay (Days) mean ± SD		18.77 ± 21.69	50.33	18.23	-

3.3. Laboratory Test Results

Laboratory testing results show HbA1C at average of 6.93% and Serum Albumin at 3.26 g/dL. COVID-19 test results found 3 patients (1.70%) detected with COVID-19 in which the level of Serum Albumin has a statistically significant correlation with complications after surgery at p<.001 as shown in Table 3.

Table 3. Laboratory Testing Results

Laboratory Testing Results	Total N = 180 (%)	CLTI + COVID-19	CLTI non COVID-19	*p*
HbA1C mean ± SD	6.93 ± 1.49	7.40	6.93	.761
Albumin mean ± SD	3.26 ± 0.68	2.70	3.27	<.001*
COVID-19 (Detected)	3 (1.7)	-	-	.542

4. Discussion

The outbreak of COVID-19 has affected the time to access treatment due to increasing procedures taken to screen for COVID-19 before being accepted as inpatient. In the early stage of COVID-19 infection, technologies to detect and report COVID-19 infections were taking much time while infected patients would experience the release of Cytokine which can lead to organ failures and increasing blood clogged in the same peripheral artery risking of higher chance of amputation (8). As this research aims to study clinical characteristics and outcomes of chronic limb-threatening ischemia patients which were treated with Endovascular treatment during COVID-19 outbreak, it is found that the majority of patients are male and have history of smoking which is in accordance to several researches being studied (12). Most of the patients are elderly and since Thai society is moving towards Aging-society, there are higher chances to find more chronic limb-threatening ischemia patients (13). Older patients treated with Endovascular Treatment can experience higher risk of complications and high mortality rate of 20 percent especially in patients whose age is more than 75 years old (14). Wifi Classification state IV which can lead to higher risk of amputation can refer to the level of advanced tertiary care hospital that can treat patients with chronic and complex conditions and that it can receive referral patients from sub-urban hospitals and adjacent regions. Most patients have comorbid diseases especially hypertension and diabetes at 87.2 percent and 82.2 percent in consequence. These diseases are risk factors that can cause chronic limb-threatening ischemia and complications after surgeries more than patients with no comorbid disease (3). Medication that most of the patients received is Dual Antiplatelet at 38 percent which explains that after the revascularization to reduce blood clogged, relapse of arterial disease and reduce risk of amputation, patients should continue receiving Dual Antiplatelet for 1-6 months after going through medical procedures. However, the efficacy of medication might be reduced depending on types of medical procedures taken, anatomy and other personal factors such as the complexity of disease or record of failed revascularization (2). One male chronic limb-threatening ischemia patients infected with COVID-19 were found experiencing complication after surgery due to the pathology of the patients with five clogged spots and Wifi Classification state 3. It is also found that the average length of stay is 2.68 times longer than

the patients who are not infected, which might be caused by the procedures taken during COVID-19 outbreak where infected patients must be separated to Isolation Ward for 2 weeks. After that, they will receive treatment of the main disease. Patients infected with COVID-19 has lower level of Serum Albumin than the patients who were not infected. The Wifi Classification at state 2-4 explains that chronic limb-threatening ischemia patients that were infected with COVID-19 has more risk to have complications and amputation because Serum Albumin was low (2.70 g/dL) and the level of Serum Albumin has statically significant correlation with complications after surgery ($p<.001$). Rates of losing legs are also associated with many factors both internal and external which related to the management of each hospital during COVID-19 outbreak (15).

Chronic limb-threatening ischemia is a complex and chronic disease which can be found commonly in elderly, smokers and patients with comorbid diseases. This group of patients is in risk of being disabled from losing legs, and the disease could be more severe and lead to death. Patients with COVID-19 infection and lower Serum Albumin are in risk of losing legs, deaths and wider length of stay in hospitals which eventually lead to higher treatment expenses. Thus, medical personnel and nurses should give more attention to the management of Nutrition status to solve low level of Serum Albumin because the patients will have higher state of Wifi classification which lead to higher risk of amputation.

Ethical Statement

This research has been certified by the ethical committee of the Faculty of Medicine, Vajira Hospital (COA 038/2566) on February 20, 2023, by collecting data based on the respect for persons.

Conflict of interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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Authors' contributions

Concept: P.K., S.W., Design: S.C., P.W., Data collection or Processing: P.K., S.C., Analysis or Interpretation: S.C., Literature Search: T.S., K.O., N.K., Writing: P.K., S.C.

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Current comments on upper gastrointestinal system bleeding data from a research hospital

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Abstract

Upper gastrointestinal system (GIS) bleeding is one of the most common causes of hospitalization. This study aims to investigate the etiology of bleeding in patients admitted to Adana Numune Training and Research Hospital with the diagnosis of upper GIS bleeding. The study was conducted with 135 patients who applied for Upper GIS bleeding and underwent endoscopy between April and August 2005. This was a prospective study and all consecutive patients with upper GIS bleeding were included. Data were presented as 'n, (%)' ,' mean ± standard deviation'. Of the patients 65.2% (n=88) were male, and the mean age was 56.7. The most common presenting symptoms were melena 44%(n=60), hematemesis 33% (n=45) and hematemesis+melena 16.3%(n=22). The most common etiologies were duodenal ulcer (35%, n=48), gastric ulcer (17%, n=23) and acute mucosal lesion (11%, n=15). Hp+ cases were 62.2% (n=84). Smoking was 28.1%(n=38), alcohol use was 13.3%(n=18). Of the patients 33% (n=45) had a history of previous Upper (GIS) bleeding, 52% (n=70) had ulcer-dyspepsia and 43% (n=58) had a history of NSAID use. Acetyl salicylic acid (ASA) was most commonly used as NSAID in 68.9%(n=40), flurbibrofen sodium in 39.6%(n=23), diclofenac in 29.3% (n=17) and multiple drugs in 39.6%(n=23). The reasons for taking ASA were cardiovascular protection in 55%(n=22).The use of NSAID's accompanies upper GİS bleeding with ASA use is the most common. When using these drugs, their effects on upper GI bleeding should be taken into consideration.

Keywords: nonsteroidal anti-inflammatory drug, acetylsalicylic acid, upper gastrointestinal system (gis) bleeding, COVID-19

1. Introduction

Upper gastrointestinal system (GIS) bleeding occurs in the GI tract above the Treitz ligament with the frequency of approximately 100/100,000 cases. It accounts for approximately 1% of hospitalizations (1). It has a high morbidity and mortality with mortality rates being 2-10% and it's an important cause of emergency hospitalization. Causes of GI bleeding include peptic ulcer disease, anticoagulant and anti-inflammatory drug use, cancers, gastrointestinal malformations and varicose veins caused by cirrhosis. Smoking and alcohol use, H. Pylori infection increase the risk of ulcers, cancer and bleeding. Peptic ulcer is usually caused by mucosal breakdown. Mucosal breakdown can occur due to various reasons acid, pepsin, bile, infection, drugs etc. infection with H pylori causes mucosal inflammation and injury. NSAIDs blocks cyclooxygenase-1 (COX-1) pathway. Hence inhibits prostaglandin synthesis which is important for maintaining mucosal barrier. Anticoagulants worsen bleeding by inhibiting clotting (1,2).

The color of the blood and its appearance provide important clues about the location, amount, duration and severity of bleeding. Melena is black, runny and strong-smelling stool mostly (90%) originating from the upper gastrointestinal tract. Its color is the result of the breakdown of blood by digestive fluids. Rarely, in cases where the passage time from the intestine is prolonged, it may originate from the small intestine or the right colon. If a person vomits black blood, it is melenamesis and indicates that hydrochloric acid has interacted with the blood. Hematemesis can be described as vomiting blood and should not be confused with hemoptysis. Red blood coming from the rectum indicates that the bleeding is from the lower GI tract. In the clinic, GI tract bleeding can be seen with symptoms of hypotension, orthostatic hypotension, tachycardia, confusion, dizziness, cold and clammy extremities, angina pectoris and palpitations. Severe symptoms may indicate excessive bleeding and deterioration of hemodynamics (2,3).

2. Materials and Methods

2.1. Study Design

This study was conducted with all patients who applied to the Gastroenterology Clinic of Adana Numune Research Hospital due to upper GIS bleeding and underwent endoscopy between April and August 2005. This study was a prospective study and all consecutive patients with upper GIS bleeding were included. Inclusion criteria were consecutive applications with GIS bleeding, etiologies determined by endoscopic intervention and voluntary participation in the study. Exclusion criteria were being under 18 years of age and patients who could not undergo endoscopy for various reasons.

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Panendoscopy was performed on the patients within an average of 12 hours (range, 1-48 hours) from the time of admission, and Helicobacter pylori was tested with a urease test in the biopsy sample in those without active bleeding, and with antigen in the stool or antibody in the blood in those with active bleeding.

2.2. Statistical analyses

Data were analyzed with SPSS 13.0 version PC for Windows (SPSS Inc, Chicago, IL) and shown as 'n, (%)', 'mean ± standard deviation'. In addition to the demographic data of the patients, the application symptoms, nonsteroidal anti-inflammatory drug and other drug use, ulcer-dyspepsia and previous bleeding history, comorbidities, smoking and alcohol use data were examined.

3. Results

Of the patients (n=135) 65.2% (n=88) were male, 34.8% (n=47) were female, and the mean age was 56.7±15.2 (22-95). While 80% of the bleeding occurred at the age of 40-80, the most frequent bleeding was seen in the age group of 50-59. The most frequent presenting symptoms were melena 44% (n=60), hematemesis 33% (n=45) and hematemesis+melena 16.3% (n=22). The most frequent etiology was duodenal ulcer 35% (n=48), gastric ulcer 17% (n=23) and acute mucosal lesion 11% (n=15). Of the cases 62.2% were Hp+ (n=84), 37.8% Hp(-) (n=51). The mean hospital stay was 4.1 days. Of the patients 92.6% (n=125) were followed up with medical and endoscopic treatment, 5.2% (n=7) were referred to other centers for various reasons and 2.2% (n=3) died. Smoking was 28.1% (n=38), alcohol use was 13.3% (n=18). Of the patients 33% (n=45) had a history of previous Upper gastrointestinal system (GIS) bleeding, 52% (n=70) had ulcer-dyspepsia, 43% (n=58) had a history of NSAID use. Acetylsalicylic acid was 68.9% (n=40), flurbibrofen sodium was 39.6% (n=23), diclofenac was 29.3% (n=17) and polypharmacy was 39.6% (n=23). Reasons for taking aspirin were cardiovascular protection in 55% (n=22) and pain in 47.5% (n=19). Of the patients 16.2% (approximately 1/6) with Upper gastrointestinal system (GIS) bleeding were using aspirin for cardiovascular protection. Comorbidities included hypertension in 21.5% (n=29), diabetes in 14.1% (n=19), coronary heart disease in 8.1% (n=11), heart failure in 4.4% (n=6), and renal failure in 4.4% (n=6) (4).

4. Discussion

This study is one of the rare GIS bleeding studies in which family medicine has been involved by conducting research. At first glance, it may not seem directly related to the primary care. However, the most commonly prescribed drugs in family medicine are ASA and NSAIDs. A family physician who knows his patient and risk factors well can take precautions against the risk of GI bleeding and give prophylaxis.

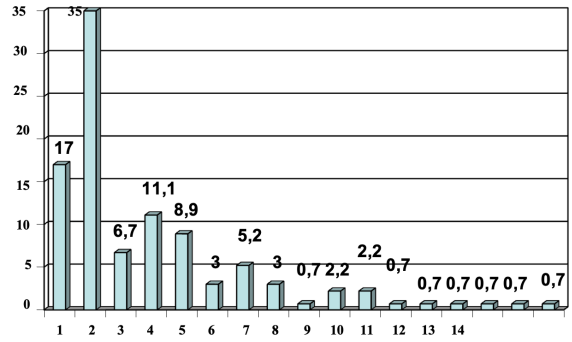

Fig. 1. Endoscopic Findings in Upper GIS Bleeding (%) 1.Gastric Ulcer (GU) 17% (n=23) 2. Duodenal Ulcer (DU) 35% (n=48) 3. Gastritis 6.7% (n=9) 4. Acute Mucosal Lesion 11% (n=15) 5. Varicose vein 8.9% (n=12) 6. Malignancy 3% (n=4) 7. Other 5.2% (n=7) 8. GU+DU 3% (n=4) 9. GU+Varicose vein 0.7% (n=1) 10. GU+Other 2.22%(n=3) 11.GU+Gastritis 2.22%(n=3) 12. GU+Acute Mucosal lesion 0.7%(n=1) 13. GU+Varicose 0.7%(n=1) 14. GU+Other 0.7%(n=1)

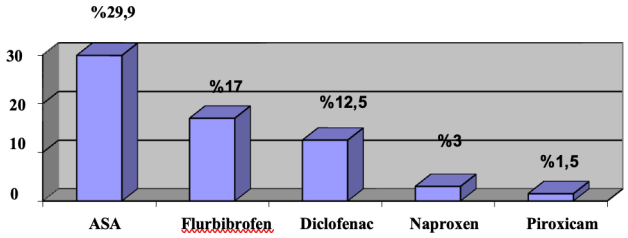

Fig. 2. NSAIDs used by patients with upper GI bleeding

In the literature, the most common cause of bleeding is duodenal ulcer (5) and our data is consistent with the literature. Tielleman et al. reported the use of anticoagulants and NSAIDs as important risk factors for GIS bleeding (6). It is consistent with our data. Coleman et al. reported that ASA is most commonly prescribed for cardiovascular protection and that ASA increases the risk of GIS bleeding by 37% (7). In our study, 29.8% of our patients used ASA.

In short, nonsteroidal anti-inflammatory drug use accompanied bleeding at a significant rate in this study as it is the case all over the world. The most common nonsteroidal anti-inflammatory drugs in Upper gastrointestinal system (GIS) bleeding is ASA. In addition, the use of heparin, warfarin and other anticoagulants increases the risk (8).

Although it is not a direct result of the research, we find it necessary to address a topic in the discussion. In the chaos that occurred during the COVID-19 pandemic, drugs associated with GI bleeding were used freely. Seeing this, based on this research, we emphasized the risks of this behavior at an international meeting before most articles in the literature (Causes of Acute Upper gastrointestinal system (GIS) bleeding and the Role of NSAIDS in the Light of Recent Corona Epidemic, 6th IMedHSC 27 - 29 DECEMBER 2020, Paris).

In 2023, a systemic review examining GI bleeding in COVID-19 patients included drugs such as Warfarin, NSAIDs, aspirin and corticosteroids at the top of the risk factors in line with our findings (9). The mechanism proposed for the relationship between GIS bleeding and COVID-19 was that the virus enters easier through ACE-2 receptors, which are abundant in gastrointestinal tract tissues, and may cause bleeding (10-11). In one study, the prevalence of GIS bleeding in COVID-19 patients was given as 3% (12). During COVID, not only NSAIDs and other anticoagulants, but also other drugs that disrupt the integrity of the duodenum and stomach and thus increase the risk of GIS bleeding have been used to increase survival. Examples include colchicine (13), which was used in the first months of the pandemic, Tocilizumab (14), which reduces the systemic inflammatory response by inhibiting IL-6, and dexamethasone (15).

In addition to the increased use of drugs that increase the risk of GIS bleeding in life-threatening infections such as COVID-19, diagnostic and therapeutic interventions such as endoscopy, are also decreased leading to decreasing survival (16).

The limitations of this study include the short data collection period and the fact that the study was not conducted during the pandemic. Therefore, since the data is from 2005 there is no direct corelation with COVİD-19. However, there are important lessons to be learned. This includes adopting a calmer disease management with a multidisciplinary approach in the next possible pandemic according to the guidelines (17).

Rational use of nonsteroidal anti-inflammatory drugs, providing preventive treatment in the gastrointestinal system in line with guidelines for risky patients, and eradicating Hp will reduce bleeding in the upper gastrointestinal system. It is clear that this will make significant contributions to the patient management and the country's economy.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.Ü, S.S., Design: M.Ü., S.S., Data Collection or Processing: M.Ü., S.S., Analysis or Interpretation: M.Ü., S.S., Literature Search: M.Ü., S.S., Writing: M.Ü., S.S.

Ethical Statement

This study is not required ethics approval.

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C7 pedicle vs. lateral mass screws in cervical spondylotic myelopathy: A retrospective analysis of cervical alignment parameters

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Abstract

Cervical spondylotic myelopathy (CSM) frequently requires surgical intervention at C7, with the choice between pedicle and lateral mass screws influencing outcomes. Pedicle screws offer superior biomechanical stability but carry higher neurovascular risks, while lateral mass screws are safer but less stable. Limited data compare these techniques in CSM treatment, particularly without O-arm navigation. To compare the safety, efficacy, and spinal alignment outcomes of C7 pedicle screws versus lateral mass screws in CSM patients, especially in settings lacking advanced imaging technologies. This retrospective cohort study analyzed 23 patients (13 with lateral mass screws, 10 with pedicle screws) who underwent posterior fusion surgery for CSM between 2013 and 2022. Preoperative and postoperative CT scans and a minimum one-year follow-up were required. Radiological parameters, including C2 slope, T1 slope, C2-7 Cobb angle, and sagittal vertical axis (SVA), were assessed, along with complications such as screw loosening, breakage, and revision surgeries. No significant differences were found between the two groups in screw loosening (69.2% vs. 60%), breakage (7% vs. 20%), or distal junctional kyphosis rates (7.7% vs. 0%). Both groups demonstrated similar improvements in spinal alignment parameters at postoperative and one- year follow-ups. One patient in the pedicle group required revision for a major foraminal breach, while three patients in the lateral mass group underwent revision for proximal junctional kyphosis. Both pedicle and lateral mass screws provided comparable safety, efficacy, and alignment outcomes at C7 in CSM patients. The choice of screw type should depend on patient anatomy, surgeon preference, and the availability of imaging technology.

Keywords: cervical vertebrae, bone screws, spinal fusion, tomography

1. Introduction

Cervical spondylotic myelopathy (CSM) is a widespread and disabling degenerative disease of the cervical spine, which usually results in the progressive compression of the spinal cord, thus causing severe neurological deficits and significantly affecting the quality of life.

The C7 vertebra, situated at the cervicothoracic junction, is a special case in surgical planning because spine surgeons have to take into account both the inclusion of C7 in the spinal construct and the choice between pedicle and lateral mass screws. This choice is based on the different biomechanical properties of each screw type and their possible effect on the surgery results. Pedicle screws are more biomechanically stable but have a higher risk of neurovascular complications (1,2). Lateral mass screws, although they may be less stable, have a safer profile (3–5).

This research is intended to contribute to the discussion through the comparison of safety, treatment success, and cervical alignment outcomes of the pedicle versus the lateral mass screw fixation in CSM patients.

2. Materials and Methods

2.1. Study design and patient selection

We conducted a 10-year retrospective analysis of patients with cervical spondylotic myelopathy (CSM) treated surgically at a single tertiary center by a single surgeon between January 2013 and April 2023. The choice to review patients starting in 2013 was driven by a major system change in our patient management: a transition to all-electronic storage, which most likely improved the consistency and reliability of the data from that time on.

Patients included in this study met the following criteria: diagnosis of cervical spondylotic myelopathy (CSM), age over 18 years, surgical intervention at our tertiary center by the designated surgeon, availability of one-year follow-up data, and preoperative and postoperative computed tomography (CT) scans. Also, only patients who had C7 as their final vertebrae incorporated into the fusion construct were included.

Patients were excluded for any of the following reasons: lack of one-year follow-up, incomplete preoperative or postoperative imaging (CT), surgery performed due to trauma

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or malignancy, or had no C7 screw or didn't have C7 as the final construct level.

The study was conducted in accordance with the 1964 Helsinki Declaration and was approved by our university's ethics committee (Decision no: 24-3T/35, Date: 07.03.2024). Consent for publication was obtained using our institutional consent form.

2.2. Clinical variables

The following data was collected from eligible patients' medical records: demographic data (age, sex, comorbidities), survival status, clinical scales (American Society of Anesthesiologists (ASA) score, Modified Rankin Scale (mRS), Nurick scale), radiological parameters (proximal junctional kyphosis (PJK), distal junctional kyphosis (DJK)), and complications (foraminal/spinal canal breach, screw loosening or breakage, revision surgery). Preoperative Goutalier index of paraspinal musculature at the level of C5-6 and Hounsfield units of the C4 vertebra corpus at the midline were also measured. PJK and DJK were defined as 10-degree changes in the relevant vertebrae in the one-year follow-up compared to post- operative CT scans (6).

The patients were also evaluated for their preoperative, postoperative, and one-year follow-up measurements of C2 slope, C2-7 Cobb angle, C2-7 sagittal vertical axis (SVA), T1 slope, neck tilt, and thoracic inlet angle (calculated as the sum of T1 slope and neck tilt) (Fig. 1.).

While all revision surgeries were recorded, only surgery due to DJK was considered relevant to construct failure in the context of C7 instrumentation, as we are comparing the effectiveness and safety of the C7 pedicle and lateral mass screws.

The Goutalier classification system for muscles, previously used for the cervical spine (7–9), was used to assess the posterior muscular support of the construct preoperatively. The multifidus muscle was measured at the C5-6 level.

The Hounsfield units were measured from preoperative CT scans of the C4 vertebra corpus at the midline sagittal view, as this was previously associated with construct failure and may be a predictor of osteoporosis (10,11).

2.3. Statistical Analysis

Descriptive statistics (means, standard deviations, frequencies, etc.) were calculated for demographic data, clinical scores, and radiological parameters. Comparative analyses between patients receiving pedicle screws and those receiving lateral mass screws were conducted using appropriate statistical tests, such as independent samples t-tests for continuous variables and chi-square tests for categorical variables.

The spinal alignment parameters and their change with treatment and time were subject to a repeated ANOVA test. Cox regression analysis and Kaplan Meier survival analysis were performed for the outcome measures.

A p value of <0.05 was considered statistically significant. Data analysis was performed using SPSS version 27 (IBM Corp., Armonk, NY, USA).

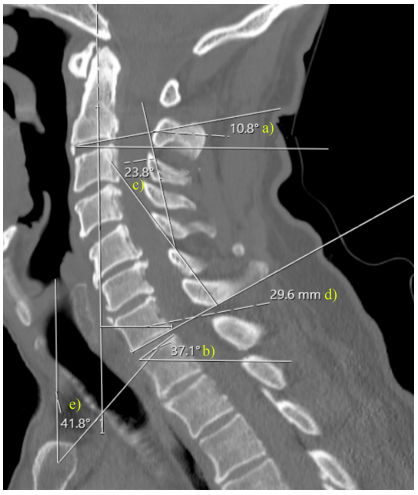

Fig. 1. Figure 1 presents an example of a sagittal cervical spine CT scan, illustrating the key sagittal alignment parameters assessed in this study. These parameters include (a) the C2 slope, defined as the angle between the inferior endplate of C2 and a horizontal line; (b) the T1 slope, the angle between the superior endplate of T1 and a horizontal line; (c) the C2-7 Cobb angle, representing the overall cervical lordosis, measured as the angle between the inferior endplate of C2 and the superior endplate of C7; (d) the C2-7 sagittal vertical axis (SVA), indicating the overall sagittal balance of the cervical spine, measured as the horizontal distance between the posteroinferior corner of C7 and a plumb line dropped from the center of C2; and (e) the neck tilt, the angle between the vertical line (plumb line) and a line connecting the center of C2 to the center of C7.

3. Results

Of 97 patients who underwent posterior fusion surgery, 23 were eligible for analysis, and the exclusion process may be seen in Fig. 2. Both groups were similar in age, sex, ASA score, mRs score, Nurick scale, preoperative Goutalier index at C5-6, and Hounsfield units of C4. These values can be seen in (Table 1).

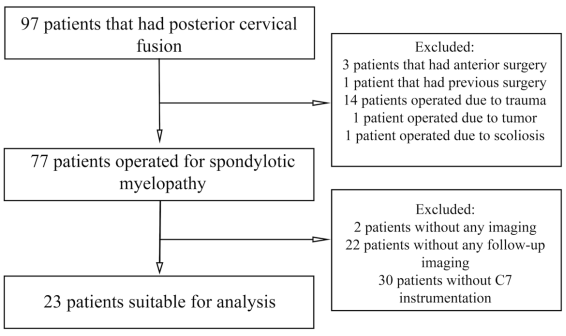

Fig. 2. This flowchart illustrates the step-wise process of identifying the final study cohort (n=23) from the initial pool of 97 patients who underwent posterior cervical fusion. Exclusion criteria were applied sequentially, resulting in the selection of patients with spondylotic myelopathy who had C7 instrumentation and available follow-up imaging.

Table 1. Comparison of baseline patient characteristics between lateral mass and pedicle screw groups

		Lateral mass (n=13)	Pedicle (n=10)	*P*
Age, years	Median (IQR, range)	55 (13.5, 41-60)	59.5 (19, 42-77)	0.089
Sex				0.768
Female	n (%)	6 (46.2%)	4 (40.0%)	
Male	n (%)	7 (53.8%)	6 (60.0%)	
Length of hospital stay (days)	Median (IQR, range)	3 (1.5, 2-53)	3 (1, 2-10)	0.459
Follow-up duration (months)	Median (IQR, range)	31 (57, 12-106)	(27.75, 13-63)	0.252
ASA score	Median (IQR, range)	2 (1, 1-3)	1.5 (1, 1-3)	0.642
Preoperative mRs	Median (IQR, range)	1.5 (1, 1-5)	1 (0.75, 1-3)	0.079
One year follow-up mRs	Median (IQR, range)	1 (1, 0-5)	1 (1, 1-3)	0.118
Preoperative Nurick scale	Median (IQR, range)	1 (1.5, 0-5)	1 (1.25, 0-2)	0.728
Myelopathy in preoperative MRI	n (%)	5 (45.5%)	5 (50%)	0.835
C5-6 Goutalier classification	Median (IQR, range)	1 (1, 1-3)	1.5 (1, 1-3)	0.514
C4 Hounsfield units	Mean (Standard deviation)	348.58 (70.58)	338.20 (96.89)	0.774

Patients were then assessed for outcome parameters, namely, foraminal/spinal canal breach, screw loosening and breakage at the C7 level and other levels, DJK, PJK, and revision surgery (overall and for DJK only). We found out that both groups were similar in terms of outcome variables, suggesting that lateral mass screws might be as effective as pedicle screws in posterior cervical spine surgery for spondylotic myelopathy (Table 2). It should be noted that one of the patients in the pedicle group had early revision due to a major foraminal breach (causing neurological deficit) at the C7 level. At the same time, none did in the lateral mass group. While all three follow-up revisions were in the lateral mass group, only one was due to DJK, and the other two were due to PJK. Due to the low number of revisions, no statistical analysis could be made within the two groups for DJK/PJK surgery.

Cox regression analysis was used to assess the time to failure of DJK, PJK, and revision surgery. None of the investigated factors (i.e., Age, sex, ASA score, preoperative mRs, C5-6 Goutalier grade, C4 Hounsfield units) affected the outcome parameters. In the Cox regression analysis for time to posterior junctional kyphosis (PJK), 69.6% of cases were excluded due to missing values, and the model showed no significant difference in survival distributions between lateral mass and pedicle groups with a hazard ratio of 1.143 (P = 0.872). In the analysis of time to distal junctional kyphosis (DJK), 82.6% of cases were dropped due to missing values, and the model, comparing lateral mass and pedicle, showed no significant difference but with a highly unstable estimated hazard ratio of 434.450 (P = 0.097).

Analysis of the spinal alignment parameters over time suggested that lateral mass screws had a similar effect on C2 slope, T1 slope, C2-7 Cobb angle, C2-7 SVA, neck tilt, and thoracic inlet angle as the pedicle screws, showing that lateral mass screws might be effective as pedicle screws in preserving cervical alignment in spondylotic myelopathy surgery (Table 3).

4. Discussion

The study found no significant differences in the demographics and baseline clinical parameters between the lateral mass and pedicle screw groups. This similarity ensures that the comparison between the two surgical techniques is not biased by age, sex, ASA score, mRs score, Nurick scale, preoperative Goutalier index at C5-6, and Hounsfield units of C4. Both groups had the same frequency of screw loosening (69.2% for the lateral mass screws and 60% for pedicle screws) and breakage (7% vs. 20%). These results indicate that neither technique is better in terms of screw durability. The literature that exists already confirms these findings, which show that both types of screws perform well under physiological loads (1,12), even though pedicle screws are intrinsically more resistant to pull-out forces (13,14).

The need for revision surgery was the same in both groups. One patient in the pedicle screw group had to be revised early because of a major foraminal breach that caused a neurological deficit. In contrast, three patients in the lateral mass screw group were revised during the follow-up, mainly because of the proximal junctional kyphosis (PJK). This indicates that while

pedicle screws may carry a higher risk of acute complications, lateral mass screws might be associated with longer-term alignment issues (15–17).

The spinal alignment parameters, such as C2 slope, T1 slope, C2-7 Cobb angle, C2-7 sagittal vertical axis (SVA), neck tilt, and thoracic inlet angle, were found to be the same in the two groups at the preoperative, postoperative, and one-year follow-up time points. This result means that both techniques are as reliable as the other in keeping the cervical alignment, which is a significant factor for the success of cervical spine surgeries (17–20).

Table 2. Comparison of surgical outcomes between lateral mass and pedicle screw techniques: Chi-Square analysis and Kaplan-Meier Survival estimates for time to PJK and DJK

		Lateral mass (n=13)	Pedicle (n=10)	*P*
Foraminal breach	n (%)	6 (46.2%)	3 (30%)	0.428
C7 screw failure	n (%)	9 (69.2%)	7 (70%)	0.968
Screw breakage	n (%)	1 (7%)	2 (20%)	0.386
Screw loosening	n (%)	9 (69.2%)	6 (60%)	0.645
Other level screw failure	n (%)	8 (61.5%)	6 (60%)	0.940
PJK	n (%)	4 (30.8%)	3 (30%)	0.968
Time to PJK (months)	Mean (SD)	22 (15.56)	26.6 (9.13)	0.872
DJK	n (%)	1 (7.7%)	3 (30%)	0.159
Time to DJK (months)	Mean (SD)	8	23.67 (10.17)	0.083
Early revision surgery	n (%)	0	1 (10%)	-
Revision surgery in follow-up	n (%)	3 (23.1%)	0	0.052

Pedicle screws, although they provide better biomechanical stability, are connected with a higher risk of neurovascular complications. Research has shown that neurovascular injury is more common with pedicle screws because of the closeness of the screw trajectory to the vertebral artery and spinal cord (19). Lateral mass screws, being technically more straightforward to place and associated with fewer complications, offer a safer alternative, especially in anatomically challenging cases (15,16,18).

The rates of both proximal junctional kyphosis (PJK) and distal junctional kyphosis (DJK) were the same in the two groups, which means that the screw type at C7 does not have a significant impact on the occurrence of junctional kyphosis, which is an essential factor for the long-term sagittal balance (13,17,21). Advanced imaging techniques, such as O-arm-based navigation, enhance the accuracy of screw placement, particularly for pedicle screws, reducing the risk of misplacement and related complications (22–26). Considering the safety and strength of the pedicle screws placed using navigation, they may be preferred where a more stable construct is necessary. But lateral mass screws might be safer when no navigation systems are available.

Our study has limitations: Having only 23 patients who met the inclusion criteria out of the 97 we initially assessed, the study may have needed to be more powerful to detect the subtle but possibly significant differences between the two groups. The study's retrospective nature brings inherent limitations, like the possibility of missing data and the inability to control all confounding variables. The absence of random assignment to the treatment groups may result in unknown biases, which can, in turn, affect the allocation of surgical techniques and, hence, the outcomes. The findings might not apply to the entire population because of the one-center, one-surgeon setup, as the surgical outcomes can differ with different surgeons and institutions.

This study supports the use of both C7 pedicle and lateral mass screws in the surgical treatment of cervical spondylotic myelopathy, with no significant differences in complication rates or spinal alignment outcomes. Patient-specific anatomical considerations, surgeon expertise, and the availability of advanced intraoperative imaging technologies should guide the choice between these techniques. Future research with more extensive multicenter studies is needed to refine surgical guidelines further and improve patient care in cervical spondylotic myelopathy.

Table 3. Longitudinal comparison of spinal alignment parameters in patients treated with lateral mass vs. pedicle screws

		Lateral mass (n=13)	Pedicle (n=10)	*P*
C2 Slope				0.365
Preoperative	Mean (SD)	17.278 (10.4049)	14.800 (13.1734)	
Postoperative	Mean (SD)	19.411 (7.2713)	17.111 (7.474)	
One year Follow-up	Mean (SD)	22.767 (7.9398)	17.389 (11.9416)	
T2 Slope				0.962
Preoperative	Mean (SD)	25.400 (12.4199)	25.978 (15.4339)	
Postoperative	Mean (SD)	26.033 (7.2834)	24.890 (10.741)	
One year Follow-up	Mean (SD)	25.467 (9.2145)	23.756 (13.6184)	
C2-7 Cobb Angle				0.841
Preoperative	Mean (SD)	10.356 (6.1746)	12.278 (9.6475)	
Postoperative	Mean (SD)	9.344 (7.4913)	8.220 (8.212)	
One year Follow-up	Mean (SD)	10.856 (5.2515)	11.467 (9.9212)	
C2-7 SVA				0.540
Preoperative	Mean (SD)	25.511 (4.5925)	18.000 (8.689)	
Postoperative	Mean (SD)	27.167 (6.7755)	24.330 (6.819)	
One year Follow-up	Mean (SD)	30.267 (11.8755)	28.560 (11.609)	
Neck Tilt				0.240
Preoperative	Mean (SD)	60.200 (8.0343)	54.971 (7.9554)	
Postoperative	Mean (SD)	59.343 (9.6764)	46.290 (10.719)	
One year Follow-up	Mean (SD)	57.986 (6.8837)	50.100 (11.1692)	
Thoracic Inlet Angle				0.549
Preoperative	Mean (SD)	87.717 (14.0862)	83.233 (10.3241)	
Postoperative	Mean (SD)	83.383 (9.2687)	70.000 (12.946)	
One year Follow-up	Mean (SD)	79.967 (7.6813)	75.067 (11.7294)	

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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None to declare.

Authors' contributions

Concept: M.S.B., H.B., T.Y., Design: M.S.B., H.B., T.Y., Data Collection or Processing: B.B.A., E.C., Analysis or Interpretation: B.B.A., E.C., Literature Search: B.B.A., M.S.B., Writing: B.B.A., M.S.B, H.B., T.Y.

Ethical statement

The study was approved by Ege University's ethics committee (Decision no: 24-3T/35, Date: 07.03.2024).

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Should we try closed reduction and casting treatment first for cases with developmental dysplasia of the hip beyond 18 months of age?

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Abstract

This study aims to evaluate the clinical and radiological outcomes of closed reduction and casting in patients older than 18 months with developmental dysplasia of hip (DDH) and to assess the success of this technique. We retrospectively analyzed medical records of DDH patients treated between March 2011 and June 2014. A total of 20 hips from 13 patients (2 boys, 11 girls) aged 18 months or older were included. Among them, 4 had right unilateral involvement, 2 had left unilateral involvement, and 7 had bilateral involvement. The mean age at treatment initiation was 19.4 months. Radiographic evaluations included the acetabular index (AI) using the Hilgenreiner method, Wiberg's center-edge (CE) angle, acetabular angle (AA), and femoral neck-shaft angle. Hips were classified as normal, slightly dysplastic, or severely dysplastic based on Tönnis' acetabular index table. Stable reduction was achieved in 12 hips (60%) of 8 patients with closed reduction and casting. However, 8 hips (40%) of 5 patients required acetabular osteotomy due to persistent dysplasia. Our findings suggest that closed reduction and hip spica casting should be considered for DDH in patients older than 18 months based on radiographic outcomes. However, our study is limited by a short follow-up period and a relatively small sample size. Further studies with larger cohorts and long-term follow-ups are necessary for more definitive conclusions.

Keywords: developmental dysplasia of the hip, late-presenting DDH, closed reduction, hip spica cast

1. Introduction

Developmental dysplasia of the hip (DDH) is one of the most common orthopedic conditions, characterized by excessive laxity of the hip capsule and failure of concentric reduction of the femoral head and acetabulum (1). The incidence of DDH varies between 1 and 34 per 1000 live births, depending on diagnostic criteria and screening methods used in different populations (2). It is a neonatal condition, but it can remain undetected and manifest at later ages. The main purpose of DDH treatment is concentric reduction of the femoral head in the acetabulum and the prevention of possible degenerative hip-joint development. The regeneration potential of the hip joint is highest in the early stages of life, so it is recommended that treatment starts as early as possible, and the effectiveness of treatment decreases in delayed cases (3–6). The closed reduction procedure involves a dislocated or subluxated hip reduction under general anesthesia and a pelvipedal cast application. Traditionally, closed reduction and cast therapy are applied in an early period (7,8,9), but some have also tried it in selected cases in the post-walking period with no open reduction (10,11,12). This study aims to evaluate the efficacy of closed reduction and casting in DDH patients older than 18 months and assess radiological and clinical outcomes in this patient population.

2. Materials and Methods

Following approval from the local ethics committee under protocol number 10840098-604.01.01-E.15430, we retrospectively evaluated the medical records of patients with DDH between March 2011 and June 2014. Informed consent was obtained from all patients before undergoing treatment. We excluded patients if they were younger than 18 months of age, had undergone open reduction, had undergone prior closed reduction and casting treatment, or had cerebral palsy, myelomeningocele, hypoxic encephalopathy, muscular dystrophy, or leukoencephalopathy accompanying teratological hip dislocation. We included 20 hips of 13 patients (2 boys, 11 girls) who were 18 months and older. There were four patients who had right unilateral involvement, 2 patients with left unilateral involvement, and 7 patients with bilateral involvement (Table 1). The mean age at the start of treatment with a closed reduction and cast was 19.4 months

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(18-28 months).

We evaluated the acetabular index (AI) using the Hilgenreiner method, Wiberg's CE angle, acetabular angle (AA), and femoral neck-body angles in direct radiographs. Using Tönnis' acetabular index table for various age groups, the hips were classified as normal, slightly dysplastic, and severely dysplastic (13). Values between one and two standard deviations from the mean were considered mild dysplasia, and values of 2 standard deviations above the mean were considered severe dysplasia.

Table 1. Demographic features of dysplastic hips

	Unilateral Right	Unilateral Left	Bilateral	Total
Male	1	0	1	3
Female	3	2	6	17
Total	4	2	7	20

2.1. Closed reduction and pelvipedal casting procedure

Under general anesthesia, a gentle closed reduction was attempted for each patient. In cases of adductor tightness, an adductor tenotomy was performed. The adequacy of the reduction was assessed based on Ramsey's safe zone criteria (10). In arthrographies performed with a sub-adductor approach, we evaluated the reduction achieved in hips with contrast material pooling of 2 mm or less as good, and reduction with pooling over 7 mm and soft tissue interposition was considered poor (Figs. 1A, 1B).

After confirming reduction under fluoroscopy, a pelvipedal cast was applied. Rolled cotton was placed over the abdomen to allow breathing space, and additional padding was applied in a figure-eight configuration through the groin and around the lower extremities. The hips were positioned in 90–110° flexion and 45–60° abduction.

Patients underwent closed reduction and pelvipedal cast application after arthrography up to three times at eight-week intervals. After the first eight weeks of this procedure, we removed the cast under anesthesia and examined the hip-joint stability. If the hip was stable, we did not force dislocation and performed a second cast in the human position. We removed the cast again under anesthesia and examined the hip stability after a second eight-week period. Then, we applied a third cast in the Ferguson position with less hip flexion and abduction (10-20 degrees of flexion, 30 degrees of abduction). We confirmed the perioperative reduction by intra-articular injection of contrast medium (Omnipaque, GE Healthcare, Ireland) in casting procedures under fluoroscopy (Ziehm Vision R, Ziehm Imaging GmbH, Nürnberg, Deutschland). We did not apply traction to any of the patients before reduction. We evaluated the efficacy of the therapy with anteroposterior (AP) radiographs of the pelvis after the end of the casting treatment.

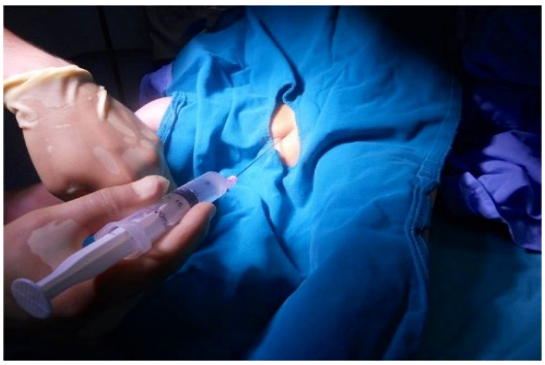


Fig. 1. (A) Sub-adductor arthrography procedure. In sub-adductor approach for arthrography of the hip that extravasated contrast material runs through the adductor space and is not superimposed on the hip joint.

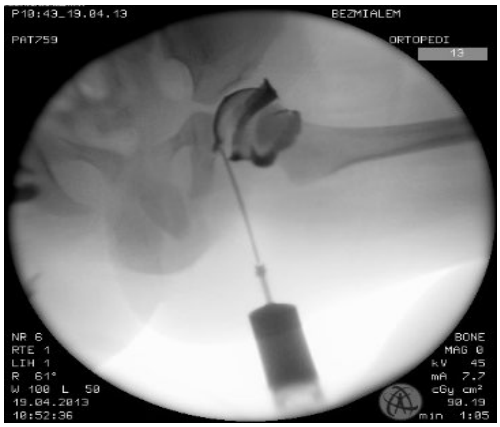


Fig. 1. (B) Scope view of sub-adductor arthography procedure. Arthrography is used to confirm reduction after closed reduction under anesthesia and help identify possible blocks to reduction. The reduction in scope view was evaluated contrast material pooling of 2 mm or less as good, and reduction with pooling over 7 mm and soft tissue interposition was considered poor.

2.2. Statistical analysis

We formed two different groups comprising those who benefited from closed reduction and cast treatment and those who needed osteotomy despite these treatments. We analyzed the data and treatment efficacy of hips in both groups. Fisher's exact test was used to analyze data such as gender, side, and tenotomy, and the Mann-Whitney test was used for age, follow-up time, number of castings, acetabular index, acetabular angle, femoral neck-body angle, and Tönnis criteria. We evaluated the results with the software SPSS (Statistical Package for the Social Sciences) (IBM SPSS Statistics for Windows, version 22.0, IBM Corp., Armonk, NY, USA). Significance was determined using $p < 0.05$, and the 95% confidence intervals were calculated.

3. Results

The mean follow-up period for the patients was 41.5 months (7-90 months). The mean age at the time of the first pelvic cast treatment was 19.4 months (18-28 months). Eight of the patients were 18 months old, three were 20 months old, one

was 21 months old, and the eldest was 28 months old.

Two of the 20 hips were Tönnis 1, six were Tönnis 2, seven were Tönnis 3, and five were Tönnis 4 in pre-reduction pelvic AP radiographs corresponding to the Tönnis displacement criterion. We detected adductor contracture in six hips of five patients and performed an adductor tenotomy. Two of the patients who underwent adductor tenotomy were 18 months old, two were 20 months old, one was 28 months old, and all were female (Table 2).

Table 2. Demographic features of patients who underwent adductor tenotomy

	18 Months	20 Months	28 Months	Total
Right	1	2	0	3
Left	1	1	1	3
Total	2	3	1	6

There was no distinction between the groups in terms of gender, side, and need for tenotomy because of adductor tension (Fischer's exact test, p=1.000). We could not find a significant distinction between the groups when age, follow-up period, number of casts, acetabular index value at first admission, acetabular angle, femoral neck-stem angle and Tonnis displacement criteria (Mann-Whitney Test) were compared (Tables 3, 4).

We detected mild dysplasia in one hip and severe dysplasia in three hips according to the Tönnis grading performed on X-rays after a closed reduction and cast treatment. The three patients who underwent Dega osteotomy were girls and first had casts at 18, 20, and 21 months. We performed Salter's osteotomy in two other patients who were normal in follow-ups but unstable after the removal of the third cast (Table 5).

Table 3. Results of patients requiring and not requiring osteotomy according to the Mann-Whitney test

Patients		Age (Month)	Follow-up Time (Months)	Casting Period	AI (Right)	AI (Left)	AA (Right)	AA (Left)	FBCA (Right)	FBCA (Left)	Tonnis (Right)	Tonnis (Left)
Only closed reduction	Standard Deviation	3.197	13.284	0.467	7.270	8.226	5.574	4.650	8.426	4.413	0.924	0.924
Osteotomy	Standard Deviation	2.702	12.818	0.548	10.714	9.423	6.164	5.899	14.241	15.620	1.342	1.414

Table 4. Significance of differences between the groups according to the Mann-Whitney test

	Age (Month)	Follow-up Time (Months)	Casting Period	AI (Right)	AI (Left)	AA (Right)	AA (Left)	FBCA (Right)	FBCA (Left)	Tonnis (Right)	Tonnis (Left)
p	0.270	0.450	0.225	0.819	0.362	0.646	0.954	0.909	0.955	0.952	0.209

Among the hips of patients who underwent closed reduction and pelvipedal casting, which was evaluated as reduced, one was Tönnis stage 4, three were Tönnis stage 3, five were Tönnis stage 2, and one was Tönnis stage 1. We evaluated 10 hips as normal, and one patient with Tönnis stage 3 developed mild dysplasia, but we performed osteotomy in one patient with Tönnis stage 3 who developed severe dysplasia. Dysplasia did not develop in two patients with Tönnis stage 2 and stage 3 who underwent Salter osteotomy, while in three patients who underwent Dega osteotomy, we noted severe dysplasia in one hip with Tönnis stage 3, and the other hips were normal. We did not find a significant relationship between the Tönnis staging and need for osteotomy (right hip: p=0.952; left hip: p=0.209).

We calculated the mean age of the patients who underwent osteotomy because of dysplasia in the follow-up period as 23.4 months. The youngest was 18 months old, while the oldest was 28 months old. The interval between performing the surgical procedure after the closed reduction and pelvic plaster was 5.2 months (4-6 months).

Table 5. Demographic features of patients who underwent osteotomy

Osteotomy	Dega	Salter
Casting Period	3	2
Mean Osteotomy Time (Months)	23	21,5

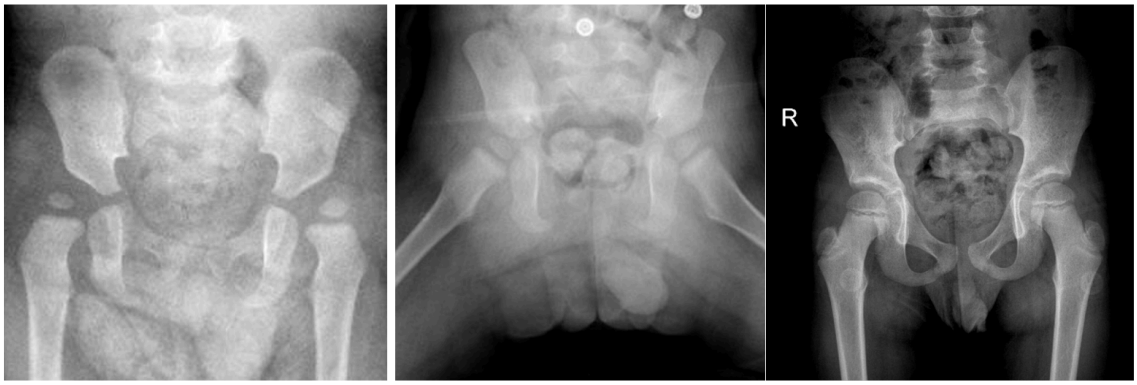
The mean AI of the right hips of patients treated with closed reduction and pelvipedal casting was 35.5 degrees before reduction, while the mean AI value at the time of the last cast removal was 18.2 degrees. The mean AI of the left hips before reduction was 33.4 degrees, but we calculated the mean AI value at the time of the last cast removal as 21.2 degrees. In patients who required surgery because of dysplasia, the mean

AI value of the right hip before closed reduction was 34.5 degrees, while the mean AI value after the last cast removal was 23.5 degrees. Thus, the left hip mean AI value decreased from 40.5 degrees to 24 degrees (Table 6).

In this study, the mean CE angles of the right and left hips of the patients treated with closed reduction and pelvipedal casting on the last pelvic AP radiographs were 18.4 (min 7 - max 32, SD 8.46) and 19.2 (min 9 - max 28, SD 5.87), respectively. Those of patients who underwent osteotomy were 18.25 (min 12 - max 26, SD 5.22) and 15.5 (min 14 - max 25, SD 7.23), respectively. In the 20-month-old patient treated with closed reduction and pelvipedal cast, dysplasia regressed in follow-up radiographs at 96 months (Figs. 2A–C).

Table 6. Pre-reduction and last mean AI values of patients who underwent closed reduction and osteotomy

AI	RIGHT	LEFT
The mean AI values before closed reduction	35.5° (20 - 42°)	33.4° (21 - 44°)
The mean AI values since the last cast removal	18.2° (12 - 24°)	21.2° (12 - 29°)
The mean AI values before closed reduction in osteotomy patients	34.5° (20 - 42°)	40.5° (21 - 46°)
The mean AI values of the patients who underwent osteotomy after the last cast removal	23.5° (16 - 29°)	24.0° (15 - 30°)



A **B** **C**

Fig. 2. (A) Pre-reduction pelvic AP radiograph (20th month), **(B)** Post-reduction pelvic AP radiograph (24th month), **(C)** Post-reduction pelvic AP radiograph (96th month). A single AP radiograph is the most appropriate examination in children with DDH where femoral head ossification has occurred. And the frog leg lateral view is a special radiograph of the pelvis to evaluate the hip to reduce exposure and maintain high diagnostic accuracy.

4. Discussion

In this study, we aimed to find the efficacy and success-related criteria of closed reduction and cast therapy as the first treatment for patients with DDH aged 18 months and older. We achieved stable reduction with closed reduction and casting treatment in 12 hips (60%) of 8 patients, while we performed acetabular osteotomy on 8 hips (40%) of 5 patients because of the development of dysplasia. We may consider closed reduction and cast therapy in patients with low dysplasia at the beginning of treatment, and we found no evidence that delaying osteotomy in patients with severe dysplasia might be a waste of time.

Closed reduction and cast treatment are sufficient for DDH treatment up to 18 months of age, while open reduction is performed after 18 months of age (14–18). Avascular necrosis, joint stiffness, and re-dislocation rates are high with closed reduction therapy in patients older than 18 months (19,20). The guidelines recommend direct open reduction in cases older than 24 months (12,13,21,22), although some authors suggest that closed reduction may be sufficient as the first-line treatment for patients older than 18 months (10–12,23–27).

Tachdjian applied closed reduction and casting treatment up to 30 months of age, depending on the case (11,12). Ponseti achieved successful results with closed reduction and cast treatment in patients treated after walking age and suggested an open reduction in patients under the age of 3 years (11). In this patient series, we noted that children with DDH older than 18 months benefited from closed reduction and cast treatment before osteotomy, independent of dysplasia. According to Murray et al., closed reduction and casting treatment fails in up to 30% of advanced-age patients (28). Other studies show that 66% of patients treated with closed reduction and cast plaster may need surgical procedures (29,30). Cases of residual dysplasia have a chance of spontaneous recovery without surgery (31,32). In our study, only 40% of the patients required osteotomy because of residual dysplasia.

The mean time before osteotomy was 5.2 months, which is

acceptable according to the literature, demonstrating the feasibility of closed reduction and cast treatment in patients with DDH older than 18 months. In our study, we performed acetabular osteotomy 23.8 months after closed reduction and pelvipedal cast treatment in five patients aged between 18 and 21 months after the detection of dysplasia because of inadequate treatment. There was no distinction in age at presentation between patients treated with closed reduction and cast treatment and those requiring osteotomy (p=0.270).

There are two different opinions on the adequacy of closed reduction and cast treatment. Absolute anatomical reduction is prominent in the first opinion (12). In the second classical opinion if the hip is immobilized in a stable and unforced position, there will not be negative effect on the results from the soft tissue interposition between the femoral head and the medial wall of the acetabulum and the femoral head not being in full contact with the acetabular medial wall oriented towards the triradiate cartilage (23–25). If the inverted labrum is not large or fibrotic, it can be resorbed (33).

Hattori et al. reported that the prominent soft tissue interposition seen in arthrography disappears in 71% of cases over time, and the long-term follow-up results of these hips were the same as those of hips with anatomical concentric reduction (34). Another MRI study found similar results and reported that pulvinar could be resorbed if concentric reduction is achieved (35). As we practiced in our patients, we think that an acceptable non-absolute reduction is sufficiently provided by arthrographic closed reduction with confirmation under fluoroscopy.

The CE angle is most often used for examining hip-joint development in direct radiographs. Values less than 15 degrees are abnormal in children (36). We noted that the mean CE angles of the hips that underwent osteotomy were low as we evaluated the radiographs of the hips that underwent osteotomy after closed reduction because of acetabular dysplasia and the last radiographs of the hips that underwent closed reduction and pelvipedal cast. However, this result was not significant.

Avascular necrosis (AVN) is the most significant complication leading to joint deformity, length inequality, and late osteoarthritis in the long term after treatment (32). Thomas et al. found a 2.5-fold increase in the probability of developing AVN after open reduction compared with closed reduction (37). In our study, the mean follow-up period was 41.5 months (7-90 months), and we did not recognize AVN or joint stiffness in any patient during this period.

Our study has several limitations. First, the follow-up period was short, and we only had early results. We could not discuss our mid- and long-term results. Second, we had a limited number of cases. More precise data could be obtained with further case series and long-term follow-ups.

We consider closed reduction and cast treatment as a first-line treatment in selected DDH cases with ages between 18 and 30 months because it is less invasive and it can give the patient a chance of recovery before open surgery. When closed reduction and casting treatment is unsuccessful, we think that a delay in treatment can be acceptable according to the current literature. Before starting treatment, parents should be warned that closed reduction and cast treatment may be insufficient and that osteotomy may be required.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: Ö.K., Design: Ö.K., Data Collection or Processing: N.D., Analysis or Interpretation: Ö.K., H.H.C., Literature Search: Ş.S., Writing: Ş.S., N.D., Ö.K., H.H.C., C.S.

Ethical Statement

The study protocol was approved by the Clinical Research Ethics Committee of Medipol University (Date: 21.05.2020, Protocol number: 10840098-604.01.01-E.15430).

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Investigation of the effect of sildenafil citrate on flap survival and SOD GPx antioxidant enzymes in random pattern skin flaps

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Abstract

In this study, the effect of sildenafil citrate, a phosphodiesterase type 5 (PDE 5) enzyme inhibitor, on the survival area of random pattern skin flaps and the effect of superoxide dismutase (SOD) and glutathione peoxidase (GPx) enzyme activities were investigated. Sildenafil citrate increases the level of cyclic guanosine monophosphate (cGMP) by inhibiting PDE 5. This causes dilation of the vessels and increased blood flow. In the study, 42 Wistar albino female rats weighing between 200-250 g were used. Rats were divided into 3 groups as sham group (S), experimental group (E) and control group (C). Subgroups were formed as day 1, day 4 and day 7 in groups E and C. In group E, 9 mg/kg/day sildenafil citrate was given intraperitoneally. Tissue samples were taken from the base, centre and the farthest living area of the flap of the sacrificed rats. SOD and GPx enzyme activity values were determined in the tissue samples. When SOD and GPx activities were compared between the groups, the difference was not significant ($p>0.05$). When the surviving flap areas were compared between the groups, an increase in necrotic area in the C7 group flaps was remarkable. However, there was no statistical difference between the groups in terms of flap survival area percentages ($p>0.05$).

Keywords: flap, sildenafil citrate, superoxide dismutase, glutathione peroxidase

1. Introduction

A flap refers to a section of tissue that can be repositioned from the donor area to the recipient area while maintaining its own blood supply. Flaps may be simple advances of skin and subcutaneous tissues or composite flaps consisting of any combination of skin, muscle, bone, fat, or fascia (1-5). The main reasons for flap necrosis are insufficient arterial blood flow and venous congestion, both of which decrease blood circulation through the flap (6). The flap elevation process disrupts the blood flow balance. Physical interruption of the internal flow vessels leads to acute ischemia of the peripheral parts of the flap (2). Increasing skin flap survival may depend on the preservation of the integrity of the circulatory system (7). Although blood flow continues in the basal part of the pedicled flap after flap elevation, blood flow at the tip of the flap decreases (8). When the length-to-width ratio of a flap surpasses 2:1, it can impair blood flow to the distal region. It results in extended ischemia, oxidative stress in tissues, and deficiencies in nutrition. These conditions increase the susceptibility to necrosis (9). The random pattern skin flap is susceptible to necrosis at its distal end because it lacks a defined arteriovenous system and blood supply, which restricts the size of the flap (10). Necrosis occurring in the distal regions of the flaps jeopardises flap use (11). In addition, reactive oxygen species (ROS) also trigger different cell death models in flap necrosis (12).

ROS consists of different types of reduced oxygen molecules, including superoxide anions, hydrogen peroxide, and hydroxyl radicals (13). ROS are produced by all cells (14). These reactive species originate from normal cellular processes and by-products of oxidative metabolism (15). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are essential antioxidant enzymes required to scavenge ROS in various cell compartments and to respond to stressful conditions (16). SOD is the enzyme that directly scavenges free radicals (17). SOD converts superoxide to oxygen (O_2) and hydrogen peroxide (H_2O_2) (18). The H_2O_2 component can easily cross cell membranes (19). Intracellular H_2O_2 concentration balance is important for cell viability and cell function. H_2O_2 is converted to H_2O and O_2 by GPx and CAT enzymes (20). GPx, as an important enzyme in the cell, converts H_2O_2 into water and lipid peroxides into alcohols (21). GPx protects cellular components from oxidative stress caused by ROS (22). This enzyme uses glutathione as a reducing agent (23).

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Sildenafil citrate is a PDE-5 enzyme inhibitor (24, 25). PDE-5 inhibitors often show strong vasodilatory properties (26). Sildenafil citrate promotes cyclic guanosine monophosphate accumulation, smooth muscle relaxation, and thus increased blood flow in target organs (27). Prevents thrombus formation and causes dilatation of arteries and veins (28). Flap necrosis can occur as a result of insufficient blood supply to the tissue, ischaemia-reperfusion injury, and inflammatory responses (29). In this study, the effect of sildenafil citrate application on the survival area of random patterned skin flaps and the effect of SOD and GPx enzyme activities were investigated.

2. Materials and Methods

All animals were obtained from Ondokuz Mayıs University Experimental Animals Application and Research Centre. Surgical procedures were also performed in this centre. The studies were carried out with permission numbered 2006/52, approved by the Ondokuz Mayıs University Animal Ethics Committee. In the experimental study, 42 Wistar albino female rats weighing between 200-250 g were used. Rats were kept at 22 ± 2 °C for 12 hours on a light/dark cycle. Rats were fed ad libitum with standard pellet rat chow and water.

2.1. Experimental groups

Rats were divided into 3 groups control group (C), experimental group (E), and sham group (S). Group E was subdivided into day 1 (E1), day 4 (E4) and day 7 (E7); and group C was subdivided into day 1 (C1), day 4 (C4) and day 7 (C7). A total of 42 rats were studied, six rats in each subgroup and S group. In group E, sildenafil citrate (Degra®) dissolved in saline was injected intraperitoneally at a dose of 9 mg/kg/day. In group E, a sildenafil dose was administered daily until the animals were sacrificed. Rats in group C were not administered any substance. In rats in groups E and C, flaps were sutured after elevation. Rats were sacrificed on day 1, day 4, and day 7, and tissue samples were taken. In the S group, rats were sacrificed after the flaps were sutured and tissue samples were taken.

2.2. Surgical Procedures

Surgical procedures were performed under sterile conditions and general anesthesia with adequate precautions to minimize pain or discomfort. General anesthesia was induced with 100 mg/kg Ketamine HCl (Ketalar®) intraperitoneally and 3 mg/kg Xylazine (Rompun®) intramuscularly. Rats were placed in the prone position, and all dorsal hair was shaved. Rats were sterilised with povidone-iodine before and after the surgical procedure. A 2.5x8 cm dorsal Mc Farlane (30) rat skin flap with a caudal pedicle was drawn with a skin pen. Following the drawing, the caudal pedicled flap was removed, including the panniculus carnosus, by taking the posterior iliac crest as the anatomical landmark (Fig. 1) and sutured with 4/0 silk.

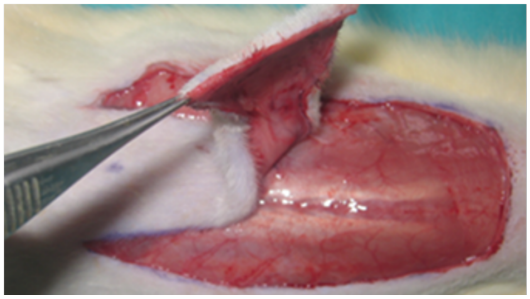

Fig. 1. Elevation of Mc Farlane rat skin flap with dorsal caudal pedicle

In the flaps of all groups, full-layer tissue samples were taken from the base, the middle, and the farthest living area of the flap, with the line assumed to pass through the midline as the center along the length of the flap after sacrifice. The samples were placed in labeled Ependorf tubes containing 0.25 M sucrose and stored in a -80ºC deep freezer.

2.3. Preparation of Tissues for Biochemical Analysis

Tissue samples were removed from -80 ºC and thawed at room temperature. Their weights were determined by weighing on a precision balance. 0.25 M sucrose solution (10 mg/ml) was added to the tissue samples taken in Eppendorf. Homogenisation + sonication was performed 6 times as a 20 s process - 10 s wait in ice medium. 15000 rpm 15 min. centrifugation was performed at + 4 ºC. Enzyme activity measurements were performed in the supernatants obtained after centrifugation.

2.4. SOD and GPx Activity Determination

SOD activity was determined using the methods of Mc Cord and Fridovich (31) and Flohe and Otting (32). A sample supernatant was added to the reaction solution containing xanthine and cytochrome c. The reaction was initiated by the addition of xanthine oxidase solution. The absorbance change was read at 550 nm after rapid stirring. Lawrence and Burk's method (33) was used to determine GPx activity. The reaction mixture and sample supernatant were mixed and incubated at 37 ºC for 5 min. Then H_2O_2 was added to the solution and absorbance change was read at 340 nm.

2.5. Calculation of flap survival areas

Digital photographs of the flaps were taken after suturing and before sacrifice in all groups. The living and necrosis areas of the flaps were calculated with the Image Tool programme. Measurement calibration was made on the ruler in the image. The total flap area was measured to include both living and necrotic areas. The necrotic tissue area was specifically measured, and the living tissue area was determined by subtracting the necrotic portion from the total flap area.

2.6. Statistical Analysis

For each animal, statistical analyses were performed by averaging the enzyme activity values obtained from tissue samples taken from the base, middle, and farthest living area of the flap. Statistical data were analysed with SPSS 14 software. Data were evaluated by ANOVA both in terms of

groups and days. The significance level was accepted as $p< 0.05$.

3. Results

When the antioxidant activities of the random pattern flaps were analysed, SOD activity was lower in the S group than in the E group and C group. However, the difference between the groups was not statistically significant ($p>0.05$). In group C, SOD activity was at its peak in group C1, decreased rapidly in group C4 and increased slightly in group C7. When SOD activity was compared between E1, E4, E7 groups and between C1, C4, C7 groups, the difference was not significant ($p>0.05$). GPx activity was measured as high in the S group compared to the E group and low compared to the C group. However, the difference between the groups was not statistically significant ($p>0.05$). In group E, GPx activity was below group C values on all days. When GPx activity was compared between groups E1, E4, and E7 and between groups C1, C4, and C7, the difference was also not significant ($p>0.05$) (Fig. 2).

When the living area measurements of the random pattern flaps were analysed, the living area was evaluated as 100% in the sham group because a digital photograph was taken immediately after the flap was lifted and sutured. In group E, which received intraperitoneal sildenafil citrate at a dose of 9 mg/kg/day, E1 was 92.8%, E4 85.9%, and E7 81.6%. In group C, C1 was 88.8%, C4 78.2%, C7 69.6%. (Fig. 3,4). Surviving flap area ratios of the E group were higher than those of the control group. This difference was especially evident in the E4 and E7 groups. Especially in the C7 group flaps, the increase in necrotic area was remarkable. However, there was no statistically significant difference between the E and C groups in terms of the percentages of survival flap area ($p>0.05$) (Fig. 5).

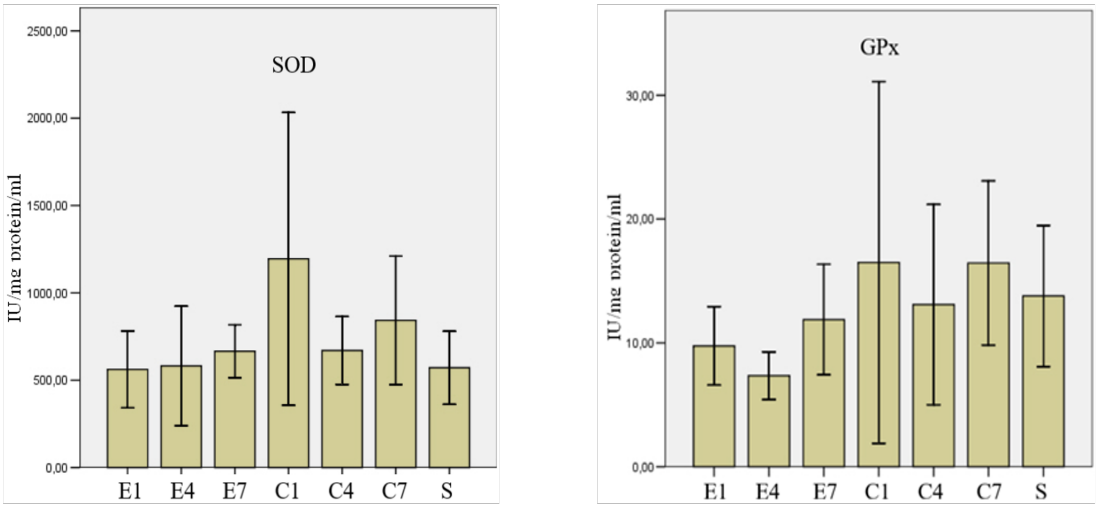

Fig. 2. The difference between SOD and GPx enzyme activity values in E, C and S groups was not statistically significant ($p>0.05$)

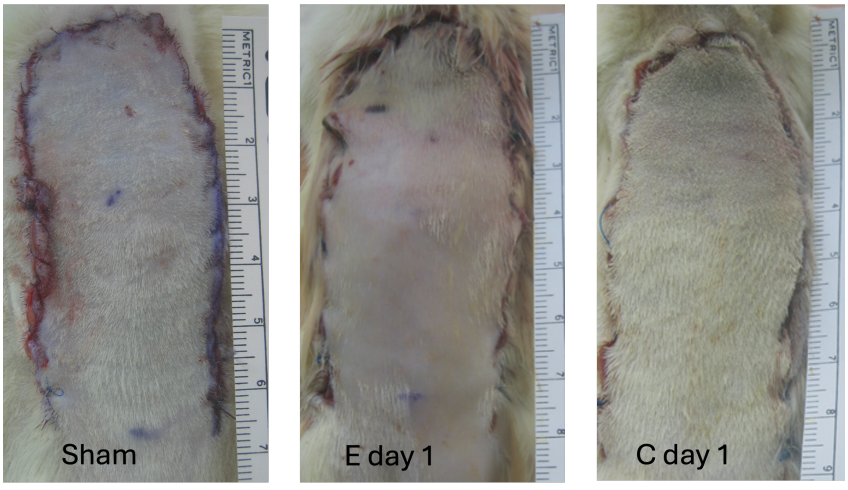

Fig. 3. Appearance of flaps in the S, E and C groups

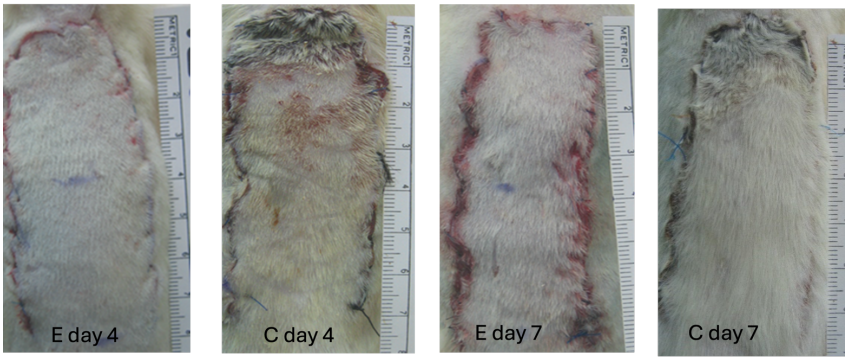

Fig. 4. Significant necrotic areas are seen in group C flaps

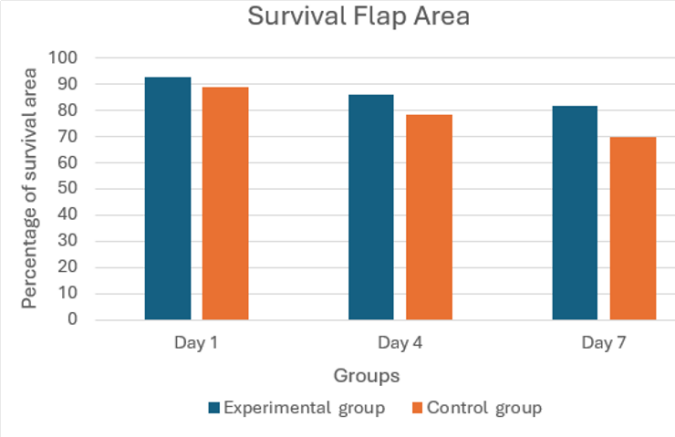

Fig. 5. Percentage of survival flap area in groups E and C ($p>0.05$)

4. Discussion

A flap is a tissue unit that can be transferred from donor to recipient sites and maintains blood supply during the procedure (27). It is widely used because flap elevation and transfer is simple and the colour is compatible with the tissues of the recipient site (34). These flaps are widely used for wound closure. Since the flap length is limited, flap viability is closely related to the aspect ratio (35). Complete or partial loss of tissue may be observed after flap application (36). Various pharmacological agents have been used to increase flap viability and prevent ischaemia. Sympatholytics, vasodilators, calcium channel blockers, antihemorrhagic agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free oxygen radical inhibitors have been tried, and successful results have been obtained to varying degrees (8, 28, 36).

After flap elevation, there is a significant increase in superoxide radicals due to anaerobic metabolism conversion (2). When the length-to-width ratio of a flap exceeds 2:1, distal perfusion is compromised, leading to prolonged ischaemia. Tissue oxidative stress and nutritional deficiencies increase susceptibility to necrosis (9). SOD is the primary scavenger of free radicals and can catalyse the rapid conversion of superoxide to hydrogen peroxide (11,13). GPx can reduce hydrogen peroxide to water (23).

Sarıfakioğlu et al. (35) reported that sildenafil citrate had an effect on the survival of flaps and 10 mg/kg dose (20mg/kg/day) given orally twice was as effective as 9mg/kg/day dose given intraperitoneally. Ayyıldız et al. (28) reported that locally applied sildenafil citrate increased flap viability and the most effective dose range was between 0.3 and 0.5 mg/kg. Ulusoy et al. (36) used sildenafil citrate in combination with fibrin glue to increase the flap survival rate. They reported that topical application of sildenafil favourably contributed to the survival of random patterned skin flaps.

Hart et al. (37) reported a significant decrease in flap necrosis and oedema on day 1 and day 3 in 3 × 10 cm flaps treated with 9 mg/kg intraperitoneal sildenafil daily. Similar to our study, 9 mg/kg intraperitoneal sildenafil citrate was given to the animals. In our study, no significant difference was found between the groups in flap surviving areas. Hart et al. (37) worked with 3x10 cm sized flaps in their study. In our study, the flap size was 2.5x8 cm. These results suggest that flap size is also an important factor in flap viability. In our study, antioxidant enzyme activities in flap tissue were also investigated.

Serin et al. (38), in their study, rats in the sildenafil group received daily subcutaneous injections of sildenafil for seven days before the 9x3 cm dorsal skin flap was removed. Similar to our study, they did not administer any substance to the control group. They reported that sildenafil sitrate provided a significant increase in the flap living area compared to the C group. Baykan et al.(39) evaluated the effect of sildenafil citrate on the viability of skin exposed to nicotine-induced ischaemia in rats. They formed 7 x 3 cm McFarlane flaps and applied 20 mg/kg/day sildenafil citrate subdermally for 7 days and reported that a significant improvement was observed in the skin vitality of the group. Kaya et al. (40), in their study with 3x9 cm flaps, gave saline to the control group 2 hours before flap removal and for 2 days after the operation. The sildenafil, tadalafil, and vardenafil groups received the respective medication. They indicated that although the flap necrosis area was lower in these groups compared to group C, there was no significant difference. Barral et al. (41) subdermally administered 0.5 mg/kg dosage and 5 ml/kg volume of sildenafil, citrate in the experimental group in 3x7 cm flaps in Wistar rats. In the control group, 0.9% saline

solution was applied subdermal. Macroscopically, there was no significant difference in the percentage of necrosis, ischaemia, and tissue viability areas when both groups were compared.

Hafez and El-Kazazaz (42) divided rats into 3 groups in their study. The groups received 0.5 ml 0.9% NaCl, 5 mg/kg, and 10 mg/kg sildanafil citrate intraperitoneally. In the 10 mg/kg sildenafil citrate treated group, hippocampal SOD concentration was significantly decreased compared to the other groups In our study, 9 mg/kg/day sildenafil citrate administration did not cause a significant difference between the groups in terms of SOD activities in skin tissue.

In conclusion, the application of sildenafil citrate has been investigated in various experimental designs to enhance the viability of random pattern flaps and to prevent necrosis or ischemia. We believe that the effect of sildenafil citrate application on flap viability varies depending on the daily dosage of sildenafil administered, the method of application, and the size of the flap.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: R.D., Design: R.D., E.D., Data Collection or Processing: R.D., E.D., Analysis or Interpretation: R.D., E.D., Literature Search: R.D., E.D., Writing: R.D.

Ethical statement

The studies were carried out with permission numbered 2006/52, approved by the Ondokuz Mayıs University Animal Ethics Committee.

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Research Article

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Effectiveness of instrument-assisted soft tissue mobilization and cupping applications in individuals with cervical disc herniation: A randomized controlled trial

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Abstract

This study investigated the effectiveness of instrument-assisted soft tissue mobilization (IASTM) and cupping therapy on neck awareness, grip strength, pain, balance, and sleep quality in patients with cervical disc herniation. This study was conducted with 27 individuals with cervical disc herniation aged between 18 and 65. The participants were randomly assigned to the intervention (n=13) or control group (n=14). While both groups received conservative treatment, the intervention group also received both IASTM and cupping therapy once a week on non-consecutive days. Intervention efficacy was evaluated by the Fremantle Neck Awareness Questionnaire to evaluate neck awareness, a Visual Analogue Scale to assess pain, a hand dynamometer to quantify grip strength, the Jenkins Sleep Scale to evaluate sleep quality, and the Single Leg Standing Test to determine balance. All assessments were made at baseline and the end of treatment. No significant discrepancy was identified between the intervention and control groups regarding pain, neck awareness, grip strength, sleep quality, and balance scores, both at rest and during activity. A significant difference was observed in all measures for the intervention group in the initial and final test comparisons. In the control group, a significant variation was detected in the VAS score for both rest and activity conditions and no significant difference was observed in the neck awareness, grip strength, sleep quality and balance scores. In the intervention group, neck pain decreased, neck awareness, grip strength and sleep quality increased, and balance improved after IASTM and cupping therapy. Alternative modalities such as IASTM and cupping therapy may be beneficial for individuals with cervical disc herniation; however, further research is recommended.

Keywords: Cupping therapy, Graston technique, pain, cervical disc herniation

1. Introduction

Neck pain resulting from a multitude of potential causes can lead to a range of adverse outcomes, including disability and dysfunction (1). Cervical disc herniation is a prevalent cause of neck discomfort. Cervical disc herniation is defined as the displacement of the nucleus pulposus from its position within the intervertebral disc. This can result in pressure being exerted on the spinal cord within the spinal canal or on the nerves passing through the neural foramen. It is more prevalent in women in their 30s and 40s, with an increasing prevalence with age (2). A variety of treatment options are currently available, although all of them entail some degree of disc excision (3). A minor alteration in volume results in a significant alteration in pressure, which releases the pressure on the compressed nerve roots or spinal cord (4). There is a definitive correlation between the presence of chronic pain and the functional status of individuals diagnosed with cervical disc herniation (5). Chronic neck pain has been demonstrated to result in the inhibition of deep flexor and extensor muscle groups, which in turn leads to a reduction in functional capacity (6). The impact of the reduced function may be observed in the context of individuals' basic and instrumental activities of daily living (7). It can be reasonably deduced that potential secondary psychosocial disorders, such as anxiety and depression, may have more concerning actual clinical consequences (8).

Various treatment modalities such as medication, invasive techniques, and conventional physiotherapy have been widely used to improve these common symptoms in patients with cervical disc herniation (9, 10). In recent years, Instrument Assisted Soft Tissue Mobilization (IASTM) has emerged as a popular technique with a favorable impact on soft tissue pathologies (11). IASTM is based on the principles of the Cyriax friction massage technique (12). Tools such as GuaSha, Graston, Ergon, Hawk Grips and Rock Tapes are used for IASTM. These instruments have different sizes, shapes and specific treatment and gripping sides (13, 14). Recent studies have demonstrated the impact of IASTM on a range of cervical symptoms, including cervicogenic pain, pain threshold, and disability (15-17). The IASTM technique has been demonstrated to be an efficacious and cost-effective approach for the management of low back and neck pain (11, 18). Nevertheless, the existing literature on the impact of IASTM in the context of neck pathologies remains scarce.

One of the forms of complementary therapy that may be employed in the treatment of neck pain is cupping. Cupping is a physical treatment, frequently employed by acupuncturists

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and other therapists specializing in complementary medicine. It involves the use of glass or plastic cups positioned on the skin over a painful area or acupuncture point to create negative pressure through suction. The rationale behind the use of cupping remains unclear. It is often described as a detoxification process, whereby waste matter and toxins are removed from the body. Additionally, it is believed to be a harmonization process, addressing the imbalance of Qi, which in traditional Chinese medicine is defined as 'vital energy' (19). The practice of cupping is currently employed as a holistic treatment modality for inpatients, as well as for the prevention and treatment of a range of ailments and the promotion of general health and well-being (20). There are two principal types of cupping: dry and wet. In dry cupping, cups are applied to the skin to create a vacuum for suction without drawing blood. In contrast, wet cupping involves drawing blood by scraping the skin before the cups are applied for suction. Cupping therapy is employed for post-stroke rehabilitation and hypertension, and has been demonstrated to be efficacious in the treatment of pain and musculoskeletal disorders (21, 22). A previously published systematic review of the literature on cupping for neck pain concluded that cupping is an effective method for reducing pain and improving function (23).

To the best of our knowledge, no comprehensive study has yet been conducted on the physical and psychosocial effects of IASTM and cupping on cervical disc herniation. A holistic approach to addressing the psychosocial status in conjunction with fundamental physical clinical parameters, such as pain, strength, and balance, can facilitate the attainment of significant clinical and practical outcomes (24). Therefore, it was hypothesized that IASTM and cupping would result in increased neck awareness, grip strength, balance, and sleep quality, and a reduction in pain in patients with cervical disc herniation.

The objective of this study was to examine the efficacy of IASTM and cupping on neck awareness, grip strength, pain, balance, and sleep quality in patients with cervical disc herniation.

2. Materials and Methods

This study is a prospective, randomized, controlled trial with a parallel group design conducted at a single center. The study was conducted in Kadıköy Medicana Hospital between September 2023 and December 2023. Each individual participating in the study was given a detailed explanation of the purpose, procedures, and measurements of the study, and written informed consent was obtained before participation.

2.1. Sample Size and Randomization

G*Power (v3.1) program was used to determine the sample size. The minimum sample size required for a significant difference between the groups was obtained as 24 under conditions where the effect level was 1.09 (large effect), the error level (a) was 0.05, and the power of the test (1-B) was 0.80 (16).

A total of 30 participants were included in the study, with 15 participants for each group. These groups were randomly assigned using a simple randomization method. The participants were randomly allocated to two groups using the 'Research Randomizer' website (https://www.randomizer.org/). The trial was ultimately concluded with 27 participants. A total of three patients were excluded from the study on the grounds of discontinuation of treatment. Ultimately, the study was concluded with 13 participants in the intervention group and 14 participants in the control group. Fig. 1 illustrates the flow of participants through the study.

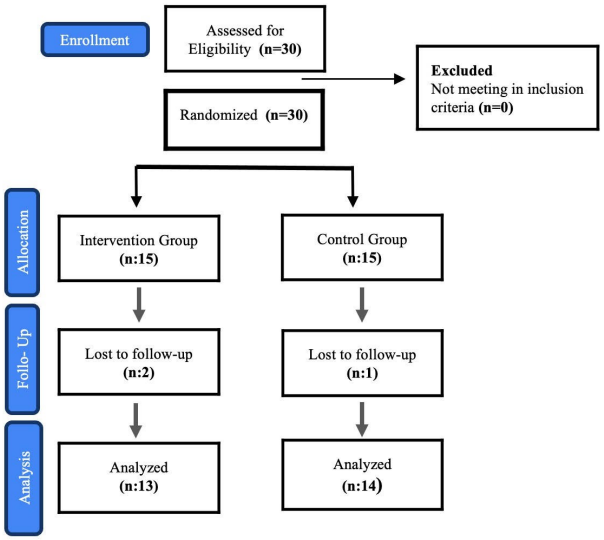

Fig. 1. CONSORT flow chart

2.2. Participants

The study was conducted with a sample of 27 individuals aged between 18 and 65 years, all of whom had a diagnosis of cervical disc herniation. In order to be eligible for inclusion in the study, participants had to be aged between 18 and 65 years, have been diagnosed with cervical disc herniation, and have been diagnosed with cervical disc herniation at the level of bulging or protrusion. Additionally, participants had to have volunteered to participate in the study. Individuals with a history of cervical spine surgery, primary or spinal metastatic malignancy, vascular problems, psychiatric drug use, or upper extremity pathologies (e.g., fracture, ganglion cyst) were excluded from the study.

2.3. Intervention and Procedure

While both groups received conservative treatment, the intervention group also received both IASTM and cupping therapy once a week on non-consecutive days (Fig. 2). The Fremantle Neck Awareness Questionnaire was employed to assess neck awareness, while the Visual Analogue Scale (VAS) was utilized to evaluate pain. Grip strength was quantified with a hand dynamometer, sleep quality was assessed with the Jenkins Sleep Scale, and balance was evaluated with the Single Leg Standing Test (SLST). All assessments were conducted at the outset of the study and its conclusion.

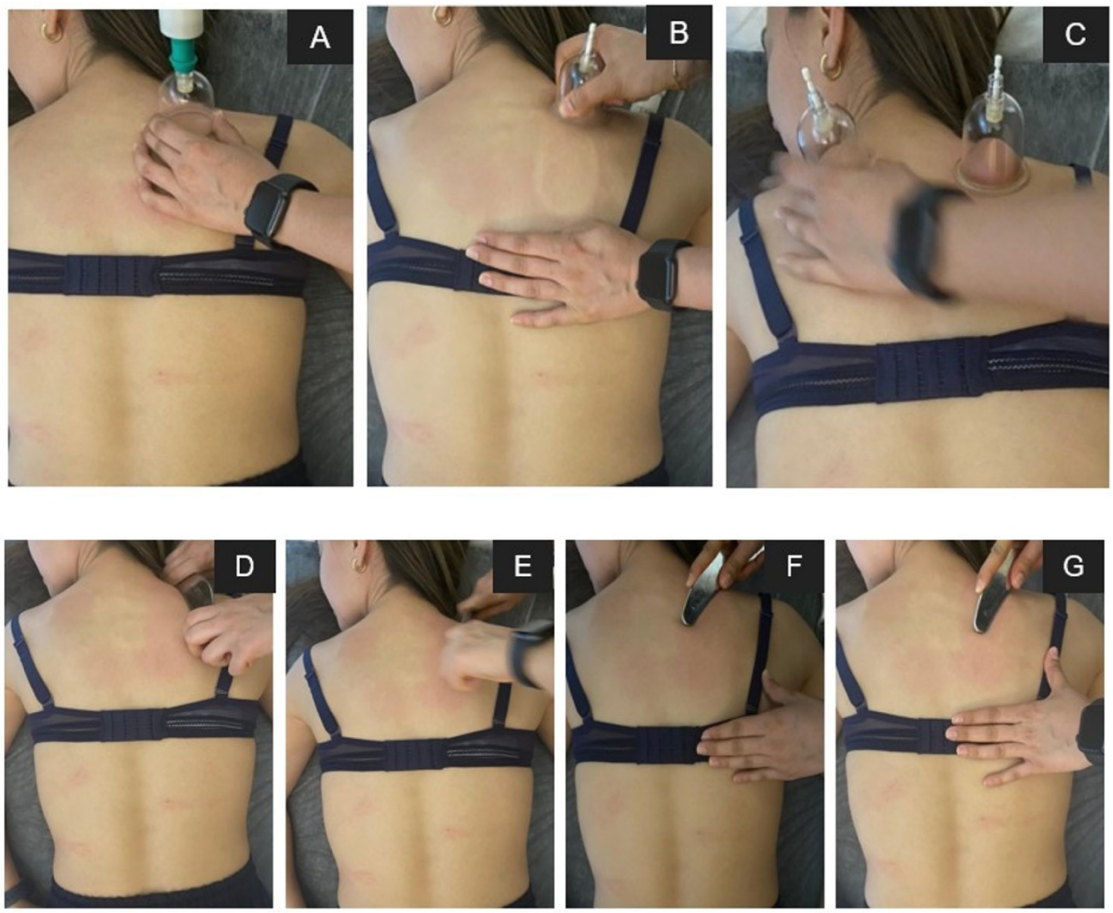

Fig. 2. A, B, C: Cupping therapy, **D, E, F, G**: IASTM technique

2.4. Treatment Method

A conventional physical therapy program was implemented for both groups, comprising ultrasound (Chattanooga Intelect Ultrasound - 1MHz, 1.0 W/cm2, 5 min), NMES (Compex rehab 400 - 20 min), Hotpack (20 min), and Transcutaneous Electric Nerve Stimulation (TENS) (Compex rehab 400 - 20 min), administered five days a week for three weeks.

In addition to the standard physiotherapy programme, participants in the intervention group received Graston and cupping applications in the prone position three days a week, with one day between each application (25). The Graston technique was applied using a sweeping motion for five minutes to observe the presence of hyperemia without forming a hematoma (26). The instrument was used at a 30° to 45°angle with moderate pressure using sweeping strokes across the upper trapezius area in all directions. During the dry cupping technique, a thin layer of liquid paraffin was applied to the skin. This was followed by a single, full traction with a manual pump, which created negative pressure and ensured cup penetration into the skin. Subsequently, the cup was slid in a longitudinal direction for five minutes, during which time the trapezius muscle was massaged. The cup was then left in situ for five minutes. It was ensured that the negative pressure was maintained at a level that would not increase pain (Figure 2). All subjects received IASTM and cupping therapy treatments, with one intervention administered per side of the body, but the order of treatment and side was randomized in advance. After each treatment, the measurements were repeated in the same order. The treatment was administered by a qualified physiotherapist.

2.5. Outcome measurements

A sociodemographic information form was employed to ascertain the demographic characteristics of the study group. Additionally, the Visual Analogue Scale (VAS), Jenkins Sleep Scale, Fremantle Neck Awareness Questionnaire, Hand Dynamometer and Single Leg Standing Test (SLST) were utilized at the commencement and conclusion of the study.

2.6. Socio-demographic Information Form

Participants' age, gender, and dominant hand were questioned.

2.7. Visual Analog Scale (VAS)

It is a methodology employed for the assessment of variables that cannot be quantified numerically. The scale necessitates the inscription of the definitions of the endpoints of the parameter to be evaluated on a 100 mm line, accompanied by the marking of the patient's condition on this line. In the case of pain assessment, the term 'no pain' was inscribed at one end of the line, while 'very severe pain' was inscribed at the other. The patient then marked the point representing their current condition, and the distance from the starting point to this mark indicated the degree of pain. Individuals were questioned about their pain at rest and during activity (27, 28).

2.8. Jenkins Sleep Scale (JSS)

JSS is employed in clinical trials for the assessment of patients'

sleep-related issues. The patients were presented with four questions regarding their sleep difficulties over the previous month and were invited to provide their responses. The options ranged from 0 (almost none) to 5 (23-31 days per month), with 1 (1-3 days per month), 2 (4-7 days per month), 3 (8-14 days per month), and 4 (15-21 days per month) representing intermediate levels of frequency. As the score increases, it is understood that the quality of the individual's sleep declines. This scale is a research tool that is used to assess sleep disorders associated with a range of medical conditions (29). The reliability and validity of this scale in our country was evaluated by Duruöz et al (30).

2.9. Fremantle Neck Awareness Questionnaire (FNAQ)

The questionnaire comprises nine questions and is designed to assess how individuals perceive the communication between the neck and the body, as well as their own body position. The questionnaire evaluates the perception that differs according to the individual. It is a Likert-type questionnaire, with responses ranging from 0 (indicating that the individual never feels this way) to 4 (indicating that the individual always or most of the time feels this way) (31). Turkish validity and reliability have been conducted (32).

2.10. Hand Dynamometer

The patient was positioned in an upright sitting position on a flat surface, with the use of a fixed chair equipped with a backrest. The knee and hip were positioned in 90° flexion, the forearm in a neutral position, the wrist in 0° to 30° extension, and 0° to 5° ulnar deviation. During the measurement, the patient was instructed to squeeze the handles of the dynamometer with maximal force. Three measurements were taken, and the mean values were recorded. Subsequently, the force was recorded in kilograms (33).

2.11. Single Leg Standing Test (SLST)

In this test, one foot was elevated to a position that did not make contact with the supporting leg, and the eyes were initially open. The patient's eyes were then directed towards the head, and he was instructed to close his eyes. The time taken to maintain this position was recorded with a stopwatch, and the test was conducted with the lifted leg in contact with the supporting leg. If the foot made contact with the floor, if there was a bounce or jump, or if any external object was touched for support, this was considered to indicate a balance disorder (34).

2.12. Data Analysis

The data obtained in this study were subjected to analysis using the Statistical Package for the Social Sciences (SPSS) 27 package program. The results of the frequency analyses of the demographic findings are presented. The frequency analyses expressed as n and % values, were calculated for each group. A frequency analysis was conducted on the scale levels, mean, standard deviation (SD), minimum (min), and maximum (max) values of the groups. The Shapiro-Wilk test was employed to ascertain whether the variables were distributed normally. In instances where the scale scores were not deemed to be normally distributed, the Mann-Whitney U test, one of two independent group comparison tests, and the Wilcoxon signed-rank test were employed to examine the differences between dependent continuous variables. The level of statistical significance was set at 0.05.

3. RESULTS

Table 1 presents a comparison of the demographic characteristics of the intervention and control groups. No significant differences were observed between the intervention and control groups concerning gender, dominant hand, and age variables (p> 0.05) (Table 1).

Table 1. Socio-demographic information of the participants

Variable		Intervention Group (n=13)		Control Group (n=14)		p^a
		n	%	n	%	
Gender	*Woman*	7	53.85	8	57.14	0.585
	Man	6	46.15	6	42.86	
Dominant hand	*Right*	11	84.62	13	92.86	0.471
	Left	2	15.38	1	7.14	
		X ± SD	**Min-Max.**	**X ± SD**	**Min-Max.**	p^b
Age		39.85 ± 10.09	25-56	38.64 ± 8.47	28-53	0.923

*p<0.05; a=Chi-Square Test, b=Mann Whitney U Test, n: Number of participants, X: Mean, SD: Standard Deviation, Min: Minimum, Max: Maximum

Table 2 shows the distribution of measurement scores within and between groups. No significant differences were observed between the intervention and control groups in terms of pain at rest and during activity, sleep quality neck awareness, grip strength, and balance scores (p>0.05). However, a significant difference was noted in all measurement scores of the intervention group between the first and last test comparisons (p <0.05). Significant changes were observed in the VAS scores for the control group during rest and activity in the first and last test comparisons (p <0.05). No significant differences were observed in sleep quality, neck awareness, grip strength, and balance scores (p> 0.05) (Table 2).

Table 2. Intragroup and intergroup comparison of measurement scores

Variable		Intervention Group (n=13)		Control Group(n=14)		
		X	SD	X	SD	p[a]
VAS (Rest)	BI	5.12	3.33	6.07	3.10	0.463
	AI	0.85	1.21	1.93	1.98	0.152
	p[b]	**0.002***			**0.002***	
VAS (Activity)	BI	7.58	1.61	5.86	3.46	0.268
	AI	1.92	1.19	2.86	1.83	0.126
	p[b]	**0.001***			**0.005***	
JSS	BI	7.31	4.96	7.07	4.75	0.942
	AI	4.54	4.70	5.43	4.03	0.522
	p[b]	**0.019***			0.054	
FNAQ	BI	10.69	7.67	8.86	5.95	0.627
	AI	6.08	5.28	6.79	5.09	0.575
	p[b]	**0.009***			0.056	
Grip Strength (Right)	BI	34.13	14.30	33.17	13.72	0.923
	AI	38.97	14.67	36.05	13.92	0.611
	p[b]	**0.001***			0.249	
Grip Strength (Left)	BI	30.45	14.10	31.79	15.14	0.884
	AI	36.32	14.72	34.91	15.49	0.645
	p[b]	**0.001***			0.683	
SLST (Right)	BI	6.71	3.50	12.63	14.91	0.497
	AI	10.67	4.48	13.04	13.34	0.577
		0.001*			0.470	
SLST (Left)	BI	5.44	3.29	13.87	16.46	0.073
	AI	9.48	8.36	15.19	15.93	0.191
	p[b]	**0.013***			0.064	

*p<0.05; a: Mann Whitney U Test; b: Wilcoxon Sign Test, n: Number of participants, X: Mean, SD: Standard Deviation, BI: Before Intervention, AI: After Intervention, VAS: Visual Analogue Scale, JSS: Jenkins Sleep Scale, FNAQ: Fremantle Neck Awareness Questionnaire, SLST: Single Leg Standing Test

Table 3 presents a comparison of the discrepancies in measurement scores between the groups. A statistically significant difference was observed between the intervention and control groups in VAS (activity), right-left grip strength, and right SLST scores (p <0.05) (Table 3).

Table 3. Comparison of the differences in measurement scores between groups

Variable	Intervention Group (n=13)		Control Group (n=14)		
	AI-BI		AI-BI		
	X	SD	X	SD	p*
VAS (Rest)	4.27	3.02	4.14	2.82	0.864
VAS (Activity)	5.65	2.17	3.00	2.63	**0.016***
JSS	2.77	3.77	1.64	2.73	0.365
FNAQ	4.62	5.88	2.07	4.27	0.223
Grip Strength (Right)	-4.84	2.41	-2.88	7.83	**0.008***
Grip Strength (Left)	-5.87	4.34	-3.13	10.72	**0.003***
SLST (Right)	-3.96	3.42	-0.41	2.22	**0.012***
SLST (Left)	-4.04	6.35	-1.32	2.48	0.145

*p<0.05; a=Mann Whitney U Test n: number of people, X: Mean, SD: Standard Deviation, BI: Before Intervention, AI: After Intervention, VAS: Visual Analogue Scale, JSS: Jenkins Sleep Scale, FNAQ: Fremantle Neck Awareness Questionnaire, SLST: Single Leg Standing Test

4. Dicussion

The objective of this study was to examine the impact of IASTM and cupping therapy on neck awareness, grip strength, pain, balance, and sleep quality in patients with cervical disc herniation. The present study concluded that IASTM and cupping applications increased neck awareness, grip strength, balance, and sleep quality, while simultaneously decreasing pain in patients with cervical disc herniation.

The existing literature investigating the effects of IASTM remains limited (35). Unuvar et al. (2024) investigated the immediate effects of Kinesio Tape and Instrument-Assisted Soft Tissue Mobilization on pain and proprioception in individuals with chronic neck pain, providing valuable insight into the therapeutic potential of IASTM compared to other manual techniques (36). Gercek et al. (2023) conducted a double-blind randomized controlled trial examining the acute effects of IASTM on pain and joint position error, highlighting

its influence on proprioceptive accuracy in chronic neck pain (37). In one study, IASTM using an M2T blade applied for 4 weeks in individuals with upper trapezius spasm was reported to be a useful tool in reducing pain.(11) Other studies have indicated that IASTM (Graston Technique) is an efficacious intervention for the alleviation of pain and enhancement of function (38, 39). The findings of our study align with those of previous research in this field. The present study indicates that the intervention group exhibited a greater post-treatment improvement in pain intensity compared to the control group. This may be attributed to the induction of tissue microtrauma by IASTM, which resulted in a regional inflammatory process and an increase in fibroblast release. The migration of fibroblasts accelerates the healing process by increasing the synthesis of collagen and the regeneration of tissue (40). Furthermore, the elevation of tissue temperature and blood flow resulting from friction between the instrument and the tissue can enhance tissue oxygenation and facilitate the removal of local waste metabolites (41, 42). The generation of heat results in a reduction in tissue constraints and viscosity, thereby increasing extensibility and imparting a softer texture to the tissue (12). The results of the study indicated a positive correlation between improved sleep quality and reduced pain severity (43). Similarly, there was an observed increase in sleep quality concurrent with a reduction in pain levels in our study cohort.

The application of force to soft tissue can result in the temporary reduction or elimination of pain, a phenomenon known as the analgesic effect. This effect can be achieved through soft tissue manipulation (44). Reduction of pain may lead to muscle relaxation and reduction of muscle protection may provide significant benefits in movement restoration (45, 46). In our study, the application of pressure with IASTM may have resulted in the inhibition of superficial muscles, thereby reducing pain and increasing awareness of the neck muscles.

A number of studies have demonstrated that IASTM can result in enhanced muscle performance (47, 48). In a single study, participants who received IASTM in conjunction with exercise therapy exhibited a more pronounced enhancement in muscle strength than those who underwent exercise therapy alone (48). The results of our study indicated an increase in both right- and left-hand grip strength in the intervention group, which had undergone treatment involving IASTM and cupping techniques. A further study compared the immediate effect of IASTM and kinesiotaping on maximal force output during a handgrip test. It was determined that both IASTM and kinesiotape resulted in significant enhancements in maximal grip strength, whereas the control group did not exhibit any improvement (49). A review of the literature suggests that neuromuscular facilitation (50), an increase in intracellular calcium in muscle tissue(51), and an increase in blood flow (52), may be potential mechanisms by which IASTM may enhance muscle performance.

In a single study, the effects of the IASTM technique and cupping therapy were investigated, and determined that both treatments were effective in improving both short- and long-term range of motion (ROM).(25) In particular, cupping therapy was found to significantly reduce pain (25). A systematic review and meta-analysis indicated that cupping may be a more efficacious treatment for chronic neck or lower back pain than medication (23). A single study employed cupping therapy on the lower trapezius muscle for a period of 10 to 15 minutes on individuals presenting with non-specific neck pain. The results demonstrated a reduction in pain levels on the visual analog scale (VAS) (0-10) at rest and during movement, with a mean decrease of 1.79 and 1.97, respectively, following cupping (53).

Cupping therapy has been demonstrated to be an effective method for manipulating physical structures, including fascia, skin, and musculocutaneous tissues. The underlying theory posits that cupping therapy exerts its beneficial effects by facilitating the elimination of toxins and harmful elements within the treated area. The application of negative pressure suction during cupping therapy facilitates the removal of toxins, stimulates the formation of granulation tissue, and supports the process of wound healing. A substantial body of evidence from numerous studies consistently supports the notion that cupping is beneficial for the early stages of healing in a range of conditions. Moreover, augmented muscle activity and enhanced muscle flexibility have been documented after cupping therapy, which may be attributed to the interplay between muscle length and tension (25). A study was conducted to compare the speed of IASTM results between cupping therapy and the Graston technique. The findings indicated that cupping provided faster pain relief and improved function due to the negative pressure mechanism. The application of cupping to the affected region results in the stimulation of blood flow, ion activity, and neuromuscular junction function. This process has been observed to reduce discomfort within three sessions and enhance flexibility throughout ongoing treatment (25). The application of cupping therapy has been demonstrated to result in vasodilatation and the stimulation of blood circulation, which in turn increases metabolic activity and facilitates the excretion of waste and toxins from the body. This effect has been demonstrated to enhance physical functionality (54). The study's limitations include the lack of clarity regarding the specific mechanisms through which the intervention group's outcomes were achieved. The study only includes short-term post-treatment outcomes. Additionally, the persistence of these outcomes beyond the treatment period remains uncertain. Another limitation is that the therapist is not blinded due to limited facilities. Since there were individuals who dropped out of the study, the study was completed with 27 participants slightly above the minimum threshold. This may compromise the power of the study. Therefore, it would be beneficial to conduct longer follow-up studies with a larger sample size in the future.

Despite the paucity of clinical evidence supporting the use of IASTM and cupping therapy, the findings of the present study indicate that these techniques may be beneficial for individuals with cervical disc herniation. Further studies are needed to recommend that therapists incorporate IASTM and cupping therapy into their treatment plans in addition to traditional physiotherapy. The findings of this study show that neck pain decreased, neck awareness, grip strength, and sleep quality increased, and balance improved in the intervention group after IASTM and cupping therapy without any adverse effects. Alternative methods such as IASTM and cupping therapy have the potential to eliminate the need for analgesics and reduce healthcare costs.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: B.İ., Ö.Ş., Design: B.İ., Ö.Ş., Data Collection or Processing: B.İ., Analysis or Interpretation: B.İ., B.D.H., Literature Search: B.İ., Ö.Ş., B.D.H., Writing: B.İ., B.D.H.

Ethical Statement

Ethical approval was obtained from the Üsküdar University Non-Interventional Research Ethics Committee (61351342/January 2023-28). The protocol is registered with http://clinicaltrials.gov/ (15/August /2023, Clinical Trial, NCT06003907). This study was conducted following the ethical rules specified in the World Medical Association (WMA) Declaration of Helsinki.

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Comparison of ChatGPT-3.5 and Google Bard Performance on Turkish Orthopaedics and Traumatology National Board Examination

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Abstract

This study ia a cross-sectional study to evaluate and compare the responses of two chatbots to compare the performance of ChatGPT-3.5 and Google Bard on the Turkish Orthopaedics and Traumatology National Board Examination. The questions of the Turkish Orthopaedics and Traumatology National Board Examination were asked to the chatbots one by one to have them indicate what the correct answer was and determine the difficulty level of the questions. The examination consists of 100 questions; 92 were included in the study. It was found that ChatGPT-3.5 answered 54.3% of the questions correctly, while Google Bard answered 45.7% of the questions correctly. When the correlation of difficulty and accuracy between the two AI models was evaluated, it was found that both were poorly correlated between the two different AI models (r=0.290 and p=0.005 for difficulty; r=0.314 and p=0.002 for accuracy). Both language models showed about 50% success on the Turkish Orthopaedics and Traumatology National Board Examination. Both found similar levels of difficulty in the questions.

Keywords: accuracy, Bard, ChatGPT-3.5, difficulty, orthopedics

1. Introduction

The scope of artificial intelligence (AI) models in medicine is gradually increasing (1-3). These models, such as ChatGPT and Google Bard, are supported by studies that show success in many areas, such as clinical decision-making, disease diagnosis, imaging of complex conditions, and medical planning (4). In the field of orthopaedics, AI models have several functions, such as suggesting medical treatment, analysing surgical cases, and assisting in teaching (5). For example, predicting early mortality in patients with critical fractures (6), analysing treatment effects in disc herniations using CT images based on AI algorithms (7), and using AI as a learning aid in orthopaedic education for residents (8) are some of the uses of these technologies. Studies have investigated the ability to answer questions correctly in assistant-level board examinations in various fields of medicine (1, 9). Assessing the performance of these AI models in specialised board-style examination questions is very important for understanding and evaluating their clinical utility (1).

This study analysed the answers and comments of ChatGPT 3.5 and Google Bard, both AI models, to the questions of the 1st phase of the Turkish National Board Examination of 2024, which measures national competence in the field of orthopaedics and traumatology, and aimed to compare their performance.

2. Materials and Methods

This study is a comparative, cross-sectional study that evaluates and compares the performance of ChatGPT 3.5 and Google Bard, two AI speech models, on the 2024 Turkish National Board Examination in Orthopedics and Traumatology. The exam consists of 100 questions assessing general orthopaedic knowledge. Eight questions were excluded from the study because they contained photographs. The exam questions were presented individually to both models by two different people. The following introductory sentence was added before each question: "The following question is a national board-level exam question in the field of orthopaedics and traumatology. You are expected to read the question and rate its difficulty as "easy, medium, difficult" and give the correct answer." The performance of each AI model was evaluated by comparing the proportion of correct answers they gave to the questions, their accuracy rates and the level of difficulty they recognised.

2.1. Statistical analysis

Analyses were performed using SPSS v.26 (IBM Corp.,

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Armonk, NY, USA). The chi-square and Fisher's exact tests were used for categorical variables. Independent samples t-test was used for analysis between groups. Pearson's correlation coefficient was used for correlation analysis. The degree of correlation was evaluated according to the coefficient values: r=0.81-1.0 means 'excellent', r=0.61-0.80 means 'very good', r=0.41-0.60 means 'good', r=0.21-0.40 means 'moderate', and r=0.0-0.20 means 'poor' (10, 11). Statistical significance was accepted as $p \leq 0.05$ in all tests.

3. Results

The responses of two different AI models to the Turkish Orthopedics and Traumatology National Board Examination were evaluated. It was found that ChatGPT 3.5 answered 54.3% of the questions correctly, while Bard answered 45.7% of the questions correctly. There was no significant difference between the two groups in the accuracy of the AI models' answers to the questions (p=0.241). When assessing the difficulty of the questions posed by the AI models, ChatGPT 3.5 reported that 3.3% of the questions were easy (n=3), 88% were medium, and 8.7% were difficult. Bard, on the other hand, reported that 3.3% of the questions were easy (n=3), 90.2% were medium, and 6.5% were hard. No significant difference was found between the two AI models in determining the level of difficulty (p=0.654). When the relationship between the accuracy of the answers given and the difficulty of the questions was evaluated within the group, no statistically significant difference was observed between the results of ChatGPT and Bard (p=0.541 and 0.611, respectively) (Table 1). When the correlation between difficulty level and accuracy rate was evaluated between the two AI models, it was found that both were correlated at a low level between two different AI models (r=0.290 and p=0.005 for difficulty level; r=0.314 and p=0.002 for accuracy rate) (Table 2).

Table 1. Assessment of initial artificial intelligence responses by difficulty level as determined by the authors

	Chat GPT 3.5			**Gemini**			***pa***
	Incorrect n(%)	*Correct n(%)*	p^c	*Incorrect n(%)*	*Correct n(%)*	p^c	
Easy	2 (66.7%)	1 (33.3%)		2 (66.7%)	1 (33.3%)		
Medium	36 (44.4%)	45 (55.6%)	0.541	45 (54.2%)	38 (45.8%)	0.611	0.654
Hard	4 (50.0%)	4 (50.0%)		3 (50.0%)	3 (50.0%)		
***p**b*	0.241						

p^a: independent samples t-test for difficulty levels of the questions between two groups, p^b: independent samples t-test for accuracy of the questions between two groups, p^c: chi-square test for analyzing correct answer rate by difficulty category

Table 2. Correlations of difficulty levels and accuracy rates between ChatGPT and Gemini answers

	Difficulty levels (Gemini)	Accuracy rates (Gemini)
Difficulty levels (ChatGPT) *r* *p*	0.290 0.005*	0.146 0.165
Accuracy rates (ChatGPT) *r* *p*	0.185 0.078	0.314 0.002*

4. Discussion

To the best of our knowledge, this is the first study to compare the performance of ChatGPT-3.5 and Google Bard on national board-level questions in Orthopedics and Traumatology. Both models performed similarly, but chatGPT-3.5 led with a success rate of 54.3%. Both AI language models also found similar levels of difficulty in the questions. Moderate correlations were found between the accuracy rates of the two AI models, as well as between their difficulty levels.

ChatGPT-3.5 and ChatGPT-4 are both known to be successful in important tests. Studies have shown that they successfully pass MBBS and the United States Medical Licencing Exam (USMLE) Steps 1 and 2 (12, 13).

Lum compared the performance of the chatbot and orthopaedic residents on the American Board of Orthopedic Surgery exam and found that ChatGPT answered 47% of the questions correctly. When the response rate was compared according to the duration of orthopaedic specialisation, it was found to be similar for the first-year residents. As the difficulty of the questions increased, the ability to give correct answers decreased (14). Sparks et al. assessed the orthopaedic knowledge of ChatGPT-3.5 on orthopaedic board-style questions using the Orthobullets dataset and found a pass rate of 55.9%. It was reported that the performance of ChatGPT-3.5 on this exam was between the average performance of an intern and a second-year resident (15). Similarly, our study found that ChatGPT answered about half of the national exam questions correctly. This shows that it was not very successful in answering the questions. Studies have reported that chatgpt's low level of judgment and limited logical reasoning ability are the reasons for its inability to choose the appropriate response in clinical scenarios. (1, 16)

Traoré et al. (17) evaluated ChatGPT-3.5's answers to the European Board of Hand Surgery (EBHS) diploma exam, while Thibaut et al. (18) evaluated Google Bard's answers to

the same questions; both studies found that neither ChatGPT nor Bard could pass the first part of the EBHS diploma exam. In our study, the success rate of both AI models was similar, although Bard gave a lower rate of correct answers. The low performance of chatbots in board exams can be explained by the lack of modelling by engineers to train bots in orthopaedics and even medicine, and the lack of resources.

The study has some limitations. One of them is the use of ChatGPT-3.5 instead of ChatGPT-4, which is a more recent version. However, ChatGPT-4 is limited in use because it is paid, and ChatGPT-3.5 is more easily accessible to everyone. Additionally, this study can be considered as a preliminary study to show the differences between GPT-3.5 and GPT-4 by evaluating the performance of GPT-4 in future studies. Secondly, these chatbots cannot analyse videos or images; therefore, the questions with images were not included in this study.

ChatGPT-3.5 and Google Bard had similar performances in answering the Turkish Orthopaedics and Traumatology National Board Examination, but chatGPT-3.5 led with a success rate of 54.3%. The two AI language models also found similar levels of difficulty in the questions. Moderate correlations were found between the accuracy rates of the two AI models, as well as between their difficulty levels.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the manuscript.

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Authors' contributions

Concept: M.K., A.K., Design: M.K., A.K., Data Collection or Processing: M.K., A.K., Analysis or Interpretation: M.K., Literature Search: M.K., A.K., Writing: M.K., A.K.

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The polymorphism of catalase gene C-262T: Impact on ulcerative colitis

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Abstract

Ulcerative Colitis is a chronic inflammatory disease of the digestive tract. Reactive oxygen species (ROS) causes inflammation and are thought to play a role in the pathophysiology of Inflammatory Bowel Disease (IBD). Catalase, one of the antioxidant enzymes, decomposes hydrogen peroxide into oxygen and water and protects the cells from oxidative damage of reactive oxygen species. It has been reported that *CAT* gene promoter polymorphism has a protective effect on oxidative stress-related diseases. The aim of this study was to investigate the association of catalase *C-262T* polymorphism with ulcerative colitis and to determine whether *CATC-262T* polymorphism is a risk factor for the disease. We investigated 80 patients with ulcerative colitis and 90 controls in the Black Sea region in Turkey. After blood sampling, DNA was extracted from the peripheral blood, then we design 2 sets of primers for C and T alleles. The polymorphism was determined by using PCR technique and the statistical analysis was performed by Kolmogorov-Smirnow test. There were no significant association between frequencies of the *CATC-262T*s of the case and the control groups and genotype frequencies were very similar (P=0.996). This is the first report in regard to the association of *CATC-262T* polymorphism with ulcerative colitis in the Black Sea region in the Turkish population. According to the results of this study there were no significant differences in the genotype distribution and allelic frequency between patients and control groups.

Keywords: ulcerative colitis, catalase, ROS, allele frequency, gene polymorphism

1. Introduction

Ulcerative Colitis (UC) is a chronic inflammatory digestive system disease that occurs in the superficial part of the colon mucosa and sub mucosa, spreads from the rectum to the proximal and reaches deeper parts (1). UC can affect any age group, but the peak of the disease is between the ages of 15-25 and between the ages of 55-65, and UC is slightly more common in women than in men (2). Although its etiology still remains unclear, it is stated that environmental factors, genetic factors, immune and some infectious causes contribute to the process (3). Some clinical studies show that genetic factors increase the risk of developing (IBD) (4). The most important risk factor for IBD is positive family history. First degree relatives of 15% of IBD patients are also affected and the risk of developing IBD is 4-20 times higher in first degree relatives (5). Studies of twins have shown that the concordance rates in monozygotic twins are estimated at 16% and 4% for dizygotic twins (6).

It has been reported that the rate of compliance of this disease in monozygotic twins and dizygotic twins are 10% and 3% respectively (7). It is thought that the immune disorder and enteritis developed as a result of genetic predisposition initiates the pathogenesis of UC. Although genetic factors are primarily important for the development of IBD, genes alone are not sufficient for the progressionof the disease. In addition, complex environmental factors are important in disease formation (8). Reactive Oxygen Species (ROS) causing inflammation raise suspicion that it may play a role in the pathophysiology of IBD (9). Catalase, which enables the decomposition of hydrogen peroxide into oxygen and water in living cells, protects the cell from oxidative damages of reactive oxygen species (10). Catalase is present intensely in erythrocyte, liver, kidney and bone marrow cells, especially in peroxisome organelles of living organism . Catalase enzyme genepromoter polymorphism has a protective effect on oxidative stress related diseases (11, 12). For this purpose, the aim of this study was investigation of the role of *CATC-262T* polymorphism in the patients diagnosed with UC in the central Black Sea region of Turkiye.

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2. Materials and Methods

2.1. Study population

Blood samples were collected from 170 subjects consisting of 80 diagnosed ulcerative colitis patients (29 Females, 51 Males) and 90 healthy individuals (45 Females, 45 Males) The allele frequencies are assumed as PT = 88% and PC = 70% respectivly, therefore according to power analyse results were calculated (a power at least of 90 % and the use of 95% confidence interval) and the sample size are obtained as n1 = 80, n2 = 90, respectively.

All individuals in the patient and control groups were selected from individuals living on the Black Sea region in Turkey during 2013-2014 and gave informed consent for this study. Individuals in study groups have similar demographic characteristics. Genotype distributions of ulcerative colitis patients according to age groups were classified in three age groups as 17-29, 30-49, 50-75.

2.2. DNA Extraction

Blood samples were collected from participants into EDTA-containing (ethylenediaminetetraacetic acid) tubes and stored at -20 °C until DNA extraction. The whole genomic DNA was extracted from the blood samples by Vivantis GF-1 Kit (Vivantis, Malaysia) following the manufacturer's instructions. The quantity and quality of the extracted DNA were determined by spectrophotometer (Thermo Scientific-NanoDropOne) at a wavelength of 260/280 nm and agarose gel electrophoreses, respectively. Extracted DNA was kept at -20°C for further processing.

2.3. Polymorphism Genotyping

Determination of *CATC-262T* gene was performed according to method specified in the study of Khodayari et al. (13). The primers used in the study were shown in Table 1. Briefly, each PCR microtube was filled with the following materials and reached a volume of 50 µl: 25 µL of 2X PCR Master Mix (DNA amplification mixture containing Taq DNA Polymerase, reaction buffer, dNTPs, and MgCl2; 0.3 µM of each primer and 4 µL DNA template). The PCR program included the initial denaturation at 95 °C for 5 min, followed by 35 cycles of the denaturation step at 95 °C for 45 s, the annealing step at 56 °C for 45 s, and the extension step at 72 °C for 45 seconds, and the final extension step at 72 °C for 5 min. PCR products were separated on 2% agarose gel with an electrophoresis machine (Nanoboz-Turkey) (Vivantis, Malaysia) using a 130 V power supply for 20-30 min and then visualized under UV-transilluminator (Uvidoc HD6-England).

Table 1. Primers used to determine the genotype of catalase 262C/T polymorphism

Gene	primer	Sequence	Annealing Tm	Product size
C	F	GCCCTGGGTT CGGCTATC	56	400
	R	GGTTTGCTGTGCAGAACACT		
T	F	GCCCTGGGTTCGGCTATT	56	400
	R	GGTTTGCTGTGCAGAACACT		

2.4. Statistical Analysis

Evaluation of genotype and frequencies in controls and UC patients was done with a non-parametric Kolmogorov-Smirnow test and values of P <0.05 were considered statistically significant (14).

3. Results

The gender distribution was 51 (63.75%) males, 29 (36.25%) females in the patients, 50 (50%) males, and 50 (50%) females in the control groups. Genotype distributions of ulcerative colitis patients according to age groups were classified according to the age range of 17-29 (25%), 30-49 (33.75%), and 50-75 years (41.25%). Distribution of 17-29 and 30-49 age groups (P= 0.996ns) and distribution of 30-49 and 50-75 age groups (P= 0.518ns) is shown in Table 2 and 3. The highest and lowest frequency in patients related to age was observed in 50-75 (41.25%) and 17-29 years (25%) and for CC genotype respectively (p= 0.996). For CC genotype, the highest and the lowest frequencies in controls related to age was observed in 30-49 (38.8%) and 50-75 years (24.4%), respectively. Furthermore, the genotype and gender distribution of age for the patient and the control groups is shown in Table 4 and 5.

Table 2. Genotype age distribution of ulcerative colitis patients by groups

Age	Frequency	CAT -262C/T		
		CC	CT	TT
17-29	20 (25%)	15 (75%)	5(25%)	0
30-49	27 (33.75%)	18(66.66%)	6(22.22%)	3(11.11%)
50-75	33 (41.25%)	26 (78.78%)	5 (15.15%)	2(6.06%)

CATC-262T genotype distribution showed similarity in the patient and the control groups by gender (P= 0.996ns). CC-CT-TT genotype distribution of ulcerative colitis patients by gender was given in Table 2 and genotype distribution of the control group by gender is shown in Table 3. When *CATC-262T* polymorphism results of the ulcerative colitis and the

control groups were compared, out of 80 patients 58 (72.5%) were CC homozygous, 17 (21.25%) were CT heterozygous and 5 (6.25%) were TT homozygous. From out of 90 individuals (control group), 56 (62.22%) were CC homozygous, 30 (33.33%) were CT heterozygous and 4 (4.44%) were TT homozygous. According to the PCR results obtained, CAT gene polymorphism was detected in 60 of 78 patients. PCR products for two alleles were 340 bp in length (Fig. 1.).

Table 3. Genotype age distribution of control groups

Age	Frequency	CAT -262C/T		
		CC	CT	TT
17-29	33 (36.6%)	21 (63.63%)	10 (30.30%)	2 (6.06%)
30-49	35 (38.8%)	22 (62.85%)	13 (37.14%)	0
50-75	22 (24.4%)	8 (36.36%)	11 (50%)	3 (13.63%)

Table 4. CC-CT-TT genotype gender distribution of the ulcerative colitis patients

Gender	Frequency	CAT -262C/T		
		CC	CT	TT
Male	51	39	9	3
Female	29	19	8	2
Total	80	58 (72.5%)	17 (21.25%)	5 (6.25%)

Table 5. CC-CT-TT genotype gender distribution of the control group

Gender	Frequency	CAT -262C/T		
		CC	CT	TT
Male	45	25	18	2
Female	45	31	12	2
Total	90	56 (62.22%)	30 (33.3%)	4 (4.44%)

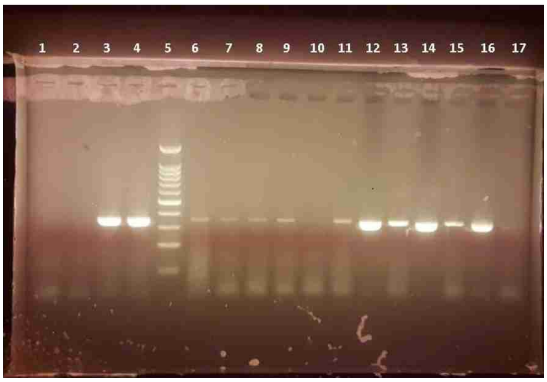

Fig. 1. Gel electrophoresis image of CAT -262C/T under UV-transilluminator 1-2: Negative control, 3: Positive control for C allele, 4: Positive control for T allele, 5: Marker (100bp) 6: DNA sample 1 amplified with the C allele primer, 7: DNA sample 1 amplified with the T allele primer, 8: DNA sample 2 amplified with the C allele primer, 9: DNA sample 2 amplified with the T allele primer, 10: DNA sample 3 amplified with the C allele primer, 11: DNA sample 3 amplified with the T allele primer, 12: DNA sample 4 amplified with the C allele primer, 13: DNA sample 4 amplified with the T allele primer, 14: DNA sample 5 amplified with the C allele primer, 15: DNA sample 5 amplified with the T allele primer, 16: DNA sample 6 amplified with the C allele primer, 17: DNA sample 6 amplified with the T allele primer, (Sample 1: CT, Sample 2: CT, Sample 3: TT, Sample 4: CT, Sample 5: CT, Sample 6: CC)

Statistical analysis showed that genotypes, ages and genders frequencies were not significantly different between the patient and the control groups (P= 0,996 ns). The SNP distribution of the control group was found compatible with Hardy-Weinberg Equation (HWE) (P= 0.007) (Table 6).

Table 6. C and T distribution in the ulcerative colitis and the control groups

	Ulcerative colitis (n=80)	Control (n= 90)	T test
C	133 (83.12%)	142 (78.88%)	$\chi^2 = 7.23$ p= 0.007
T	27 (16.87%)	38 (21.11%)	

4. Discussion

The results of our study indicated that the C allele frequency was found as 83% and 78% in the patient and the control groups, while the T allele frequency was 16% and 21%in the patient and the control groups. When the control and the patient groups were compared, the difference between TT and CC genotype distribution and T and C frequencies was found insignificant. These results reveal that there is no causal relationship between *CATC-262T* polymorphism and UC. The results obtained from this study are compatible with the results of other studies conducted in Turkey (15).

Many research studies show, it is informed that T is less common in the Asian population than C (15,17). On the other hand, "Khodayari et al. (13)" reported that the CC genotype had a protective role. Relation between catalase gene polymorphism and the risk of UC formation is not fully

understood (13). However, catalase activity seems to decrease in many diseases. This situation is associated with increased reactive oxygen species that inactivate the enzyme and cause DNA damage (18,19). In additional to many factors, oxidative stress is thought to be important in the development of ulcerative colitis. The catalase enzyme, which is among the antioxidant defense systems, shares this task with glutathione peroxidase enzyme (GPx) While GPx is effective at low H_2O_2 concentrations, catalase becomes more effective at higher concentrations. However, when the oxygen radicals increase excessively and the proxy/antioxidant balance shifts in favor of the proxydanes, oxidative stress damages the organism by various mechanisms (20, 21). Although the mechanism underlying the change in promoter activity under the influence of the catalase gene *CATC-262T* polymorphism is not fully understood, database searches are not shown that various hypothetical binding sites exist near the polymorphic site for transcription factors such as AP-2 and Sp-1 (20).

There are also conflicting reports in several studies about the role of the *CATC-262T* polymorphism C/C genotype on disease progression. "Ahn et al. (11)" reported that the C/C genotype reduced the risk of breast cancer by 17% compared to the C/T and T/T genotypes (22). Also, the role of the catalase *CATC-262T* gene polymorphism on the risk of prostate cancer (PCa) was investigated in some studies with contradictious results.

In a meta-analysis conducted by "Hu et al. (26)", researchers examined five studies with a total of 3865 cases and 28224 controls (12, 23, 24, 25) and stated that there is a positive correlation between *CATC-262T* polymorphism and the development of prostate cancer (26). In other study, "Jamhiri et al. (27)", investigated CAT mRNA levels in the blood with mutations in the CAT promoter region and reported that CAT mRNA levels in the TC/TC and TT/TT diploids were 2 to 4 times higher than AC/AC diploids. While "Forsberg et al. (20)" reported that T transcription was higher in their study (20), "Ahn et al. (11)" reported that the C was associated with higher enzyme activity in red blood cells (22). Also, "Chistiakov et al. (28)" and "Zotova et al. (29)" reported that T reduced the risk of diabetic nephropathy compared to the C (28, 29). "Mak et al. (17)" reported that the T reduced the risk of asthma in non-smokers. Contradictory results were obtained in the studies in which gene expression was followed by transferring cloned genes into cells (17). It is thought that these contradictory results between studies may be due to usingdifferent techniques to track enzyme activity or mRNA levels, genetics and living conditions between patient and control populations (nutritional, contact with infectious agents, vital stress, etc.). For example, in a study conducted in France, it is reported that thecatalase enzyme activity decreased with age, while an increase by aging to lesser extent was observed in Turkey (15). Another reason for the difference in results is that the relationship between *CATC-262T* polymorphism and UC stages is not evaluated. Considering oxidative stress and oxidative damage caused by free radicals lead to the development of disease in some people, while in others, not causing diseases on other people highlights the genetic difference between individuals (15, 30).

In this study, we investigated the relationship between *CATC-262T* polymorphism and UC. Statistically no significant relationship was observed. However, it is recommended to conduct more comprehensive studies in wider experimental groups including factors such as expression of the catalase enzyme gene and different UC stages.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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None to declare.

Authors' contributions

Concept: N.N.M., H.B., Design: N.N.M., H.B., Data Collection or Processing: B.Y., İ.B., T.A., Analysis or Interpretation: N.N.M., H.B., Literature Search: N.N.M., H.B., Writing: N.N.M., H.B.

Ethical statement

Ethical committee permission was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee (2013/311).

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Frequency, distribution, and impact on prognosis of *BCR-ABL1* kinase domain mutations in tyrosine kinase inhibitor resistant chronic myelogenous leukemia patients

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Abstract

Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by the *BCR::ABL1* fusion gene. Despite the improved outcomes with the tyrosine kinase inhibitors (TKIs) treatment, primary and acquired resistance has become a big challenge. Through the various mechanisms, *ABL1* tyrosine kinase domain (TKD) mutations play a major role in the resistance. Of the 287 patients in our study, 261 patients' resistance status were available, and 110 (42.2%) were resistant to imatinib (IM). Ninety of those 110 patients' *BCR-ABL1* TKD mutation analyses were available, and 13 of them (14.4%) had mutations. 8 of them had the T315I mutation, 2 had the Y253H mutation, and the remaining patients had one E255K, V299L, and F317L mutation each. In the IM-resistant patients, the mean size of the spleen was larger, peripheral white blood cell count and plasma β2-microglobulin levels were higher, and hemoglobin and hematocrit levels were lower ($p<0.05$). Also, it could not be detected any significant correlation between fusion signal patterns and the rate of IM resistance. In conclusion, *ABL1* TKD mutations are essential causes of TKI resistance in CML patients and must be used to choose the appropriate subsequent TKI.

Keywords: chronic myeloid leukemia, IM resistance, ABL1 TKD mutations

1. Introduction

Chronic myeloid leukemia (CML) is a clonal hematopoietic disorder characterized by the fusion of the BCR and ABL1 genes. BCR::ABL1 fusion gene encodes a chimeric oncoprotein with abnormally high and constitutive tyrosine kinase activity (1). Aberrantly activated kinase causes enhanced proliferation and differentiation arrest (2). Clinical signs and symptoms are seen due to differentiation arrest and accumulation, including fatigue, and a loss of appetite, granulocytosis, granulocytic immaturity, basophilia, thrombocytosis, and splenomegaly (1).

Chronic myeloid leukemia has three distinct clinical stages: chronic phase, accelerated phase, and blast crisis. Without treatment, the disease progresses from chronic phase to accelerated and blastic phase, resulting in death within an average of 4 years (2). Targeted therapies using tyrosine kinase inhibitors (TKIs) have drastically improved life expectancy (3). Imatinib was the first TKI introduced, and it increased the 5-year survival rate from 22% to 70% compared to pre-imatinib. Treatment fails in some patients, and several TKIs, including dasatinib, bosutinib, nilotinib, ponatinib, and asciminib, have been introduced to overcome this resistance (4). TKD mutations are well-known causes of TKI resistance and have the potential to direct treatment (5). Different methods with advantages and disadvantages have been used to detect TKD mutations, and different mutation frequencies and distributions have been determined so far among studies (6, 7).

The present study aims to assess the frequency and distribution of BCR::ABL1 TKD mutations in TKI-resistant CML patients.

2. Materials and Methods

2.1. Patients

We examined a group of BCR::ABL1 TKD mutations in 93 imatinib-resistant patients. Among the 287 consecutive CML patients followed by Ondokuz Mayıs University Faculty of Medicine between January 2008 and March 2017, TKI-resistant patients were enrolled in this study. The age, gender, blood cell count, plasma LDH and β2-microglobulin levels, TKIs used, survival rates, BCR::ABL1 fusion patterns detected with FISH and BCR::ABL1 TKD mutation results were collected.

2.2. BCR-ABL Fusion Detection with FISH and RT-qPCR Methods

FISH (fluorescence in situ hybridization) analyses were conducted with Dual Color, Dual Fusion Translocation Probes provided by either Vysis or Cytocell companies (Vysis,

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Downers Grove, IL; Cytocell, Cambridge, UK).

BCR::ABL1 fusion transcript quantification was performed with RT-qPCR (Reverse transcription-quantitative polymerase chain reaction) analysis by Roche LightCycler BCR-ABL1 or Ipsogen BCR-ABL1 Mbcr IS-MMR kits. Total RNA was extracted from peripheral blood using the QIAamp RNA Blood Mini Kit. Then, cDNA was obtained using an ipsogen RT Kit, and the quality of synthesized cDNA was checked by cDNA-PCR amplification of wild-type ABL1 housekeeping gene. For the quantification of BCR-ABL1 Mbcr fusion gene transcripts, Ipsogen® BCR-ABL1 Mbcr kit was used according to the manufacturer's recommendations.

2.3. Sequencing Analysis of BCR::ABL1 TKD Mutations

Pyrosequencing of the eleven targeted mutations provided below was performed on Qiagen Q24 Pyromark. The PCR products obtained for the RT-qPCR mentioned above study were used as input material and pyrosequencing was performed with four different primer pairs to detect specific mutations: Primer Pair-1: Y253H, Y253F, E255K, E255V; Primer Pair-2: V299L; Primer Pair-3: T315A, T315I, F317V, F317L; Primer Pair-4: F359C, F359V. Sequencing results were analyzed with Pyromark Q24 software.

2.4. Statistical Analysis

Statistical analyses were performed using IBM SPSS Version 22.0 (SPSS, Chicago, USA). The Mann-Whitney U test and Student's t-test were used to compare the differences between the groups. Kaplan-Meier and log-rank tests were used for survival analysis. P-value <0.05 was considered as significant.

3. Results

3.1. Patient characteristics

A total of 287 consecutive CML patients, 143 females and 144 males, were enrolled in this study, and patients' characteristics were summarized in Table 1. Two hundred sixty-one patients' follow-up information was available. One hundred ten (42.2%) of the 261 patients were resistant to TKI.

The mean overall survival of the cohort was 177.5 months, and IM-resistant patients had shorter overall survival (139.5 months vs 206.6 months; Log-rank p<0,05). Analysis of baseline clinical findings is presented in Table 2. Imatinib-resistant patients' mean spleen size (181.8 vs. 153.0 mm), white blood cell count (153.2 vs. 104.3 x10^9/L), and ß2-microglobulin level (3,040 vs. 2,581 ng/mL) were significantly higher, and hemoglobin levels (10.7 vs. 11.5 g/dl) were lower than the imatinib-sensitive patients' (p<0.05) (Table 2).

Table 1. Characteristics of patients cohort

Sex	
Female/Male	143/144
Age of onset (mean+SD)	52.5±17.3
FISH fusion patterns	
2F1G1R (dual fusion)	178
1F1G1R (deletion of BCR and ABL1)	21
1F2G1R (deletion of ABL1)	10
1F2G2R (complex translocation)	8
3F1G1R (*+Ph*)	3
1F1G2R (deletion of BCR)	2
2F2G2R	2
N/A	63
Imatinib response status	
Sensitive	151 (%52.6) (57.8%)
Resistant	110 (%38.3) (42.2%)
No follow-up	26 (9.0%)
Phase in Resistant group	
Accelerated phase	7
Blastic phase	16
Chronic phase	87
***BCR::ABL1* TKD mutation**	
Yes	13
No	77
N/A	20

Table 2. Comparison of the baseline clinial features of IM-resistant and IM-responsive patients

	Resistant	Responsive
Onset Age	50±18,5 yrs	53±16.2 yrs
Liver size (mm)	181.2±25.9	178.7±25
Spleen size (mm)	181.8 ±61.1	153±41.2*
WBC (x10^9/L)	153.2±136	104.3±85.9*
Hemoglobin (g/dL)	10.7±2.1	11.5±2.1*
Hematocrit (%)	32.2±6.2	35.4±6.2*
Thrombocyte (/dL)	430,153±322,002	497,983±353,318
ESR (mm/h)	28.8±27,1	20.2±21.9
LDH (U/L)	987.6±586.6	903.8±647.7
B2M (ng/mL)	3,040.1±1,513.7	2,581.5±914*

WBC: White Blood Cell, ESR: Erythrocyte Sedimentation Rate, LDH: Lactate DeHydrogenase, B2M: β2 microglobulin, yrs=years * p < .05

3.2. FISH Analysis Patterns at Baseline

FISH analysis patterns for BCR::ABL1 with dual fusion probes at diagnosis were available for 224 patients. Results are depicted in Table 1. 79.5% (178/224) of the patients had classical dual fusion (2F1G1R), and the remaining 20.5% (46/224) had various fusion patterns. Of the 46 patients, 21 patients (45.7%) had 1F1G1R (loss of derivative 9), ten patients (21.7%) had 1F2G1R (deletion ABL1), eight patients (17.4%) had 1F2G2R (complex variant translocation), three patients (6.5%) had 3F1G1R (additional Ph), two patients (4.3%) had 1F1G2R (deletion BCR), two patients (4.3%) had 2F2G2R. The mean age of diagnosis of the patients with FISH fusion patterns other than dual fusion was earlier than the patients with classical dual fusion FISH patterns (54.3±17.7 vs 48.2±15.2 years; p<0.05), but no statistical difference was detected between the classical fusion pattern and the other group in terms of imatinib resistance rate (44.7% vs 54.5%; p>0,05) and overall survival (154.3 months vs. 117.4 months, log-rank p=0.8).

3.3. BCR::ABL1 TKD Mutation Analysis

Among the 110 imatinib-resistant patients, the mutation status of 90 imatinib-resistant patients could have been assessed, and 13 (14.4%) patients had mutations in the BCR::ABL1 TKD (Table 3). T315I was our study's most common mutation, and the detected mutations are depicted in Fig. 1. T315I was detected in 8 patients, Y253H in 2 patients, and E255K, V299L, and F317L mutations were detected in one patient each. Of the 13 patients with the mutation, 7 (36,8%) were in the accelerated/blastic phase. The BCR::ABL1 TKD mutations were clustered statistically significantly higher in patients with accelerated/blastic phase (36.8% vs 8.4%; $p<0.05$). Patients with Y253H and E255K were also resistant to subsequent nilotinib treatment, V299L and F317L were also resistant to subsequent dasatinib treatment, and patients with T315I mutation were resistant to both dasatinib and nilotinib treatments. We also investigated whether there was a relationship between BCR::ABL1 TKD mutation carriers and FISH fusion pattern types among IM-resistant patients and found no significant differences.

Table 3. Type and frequency of *BCR::ABL1* TKD mutations among Imatinib-resistant patients with CML

Mutations	Frequency	Percent
Presence of mutations		
Yes	13	14.4
No	77	85.6
Type of TKD mutations		
P-loop mutations		
Y253H	*2*	2.2
E255K	1	1.1
Non-P-loop mutations		
V299L	1	1.1
T315I	8	8.9
F317L	1	1.1

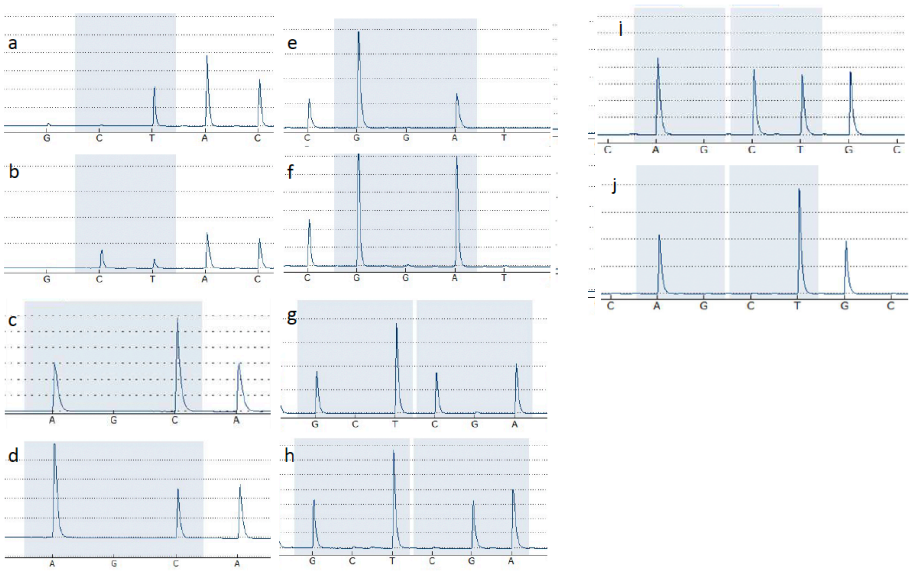

Fig. 1. Pyrosequence images of the patients with certain BCR-ABL1 TKD mutations and wild type control. a. Y253H wild type b. Y253H (TAC>CAC) mutant c. V299L wild type d. V299L (ACC>AAC) mutant e. E255K wild type f. E255K (GGA>GAA) mutant g. F317L wild type h. F317L (TTC>TTG) mutant i. T315I wild type j. T315I (ACT>ATT) mutant

4. Discussion

Tyrosine kinase inhibitors have caused significant advances in the treatment of CML and have improved the overall survival of patients considerably. However, some patients do not respond to the treatment initially or develop TKI resistance over time. For patients with CP-CML, imatinib achieves a major molecular response (MMR) in 50%-60% of cases. Following the literature, our study found the MMR rate to be 57.8% (8). Two of the most extensive studies, IRIS and DASISION, appear to have very different results on the MMR rate. However, when the IRIS study, in which the rates were low, was carefully examined, it could be recognized that approximately 20% of the patients needed follow-up. Therefore, the rates might be lower (9, 10). IM resistance also affects the overall survival of CML patients. In a recently published comprehensive study, IM-resistant patients had a shorter 3-year OS than IM-responsive patients, which is consistent with our results (11).

Although many causes of imatinib resistance have been identified, the most important is BCR::ABL1 gene TKD mutations. Mutation rates vary widely across studies, between 10.3% and 63%, depending on the selection criteria for mutation analysis and the method used. Mutation rates were 11.6% and 10.3% regardless of resistance status in two studies conducted with Sanger sequencing. In studies conducted with Sanger sequencing analysis on resistant patients, quite different results were obtained, ranging from 22.4% to 63% (12, 13). Although highly variable mutation rates, 10.5-45%, have been reported in the literature, most studies fell in 10-20%. In this study, we analyzed a group of targeted mutations among the IM-resistant patient group and found the rate to be 14.4%, consistent with the literature. The most common mutation was

T315I, and the mutation distribution was similar to that in the literature (8). BCR::ABL1 TKD mutation type is essential for choosing subsequent treatments. T315I mutation has been associated with both nilotinib and dasatinib treatments, Y253H and E255K mutations have been associated with nilotinib treatments, and V299L and F317L mutations have been associated with dasatinib treatments in the literature. Our patients' data were also consistent with the literature.

Predicting the prognosis of the disease at diagnosis is essential for the management and follow-up of the treatment. The age at diagnosis, spleen size, blast count, eosinophil, basophil percentages, platelet count, and chromosomal abnormalities are well-defined parameters for prediction. This study examined these parameters among the imatinib-resistant and -sensitive patients. Compared to imatinib-sensitive patients, the imatinib-resistant patients showed higher leukocyte counts and β2 microglobulin levels, larger splenic sizes, and lower hemoglobin levels at diagnosis. These findings were also following the literature data (14, 15).

It has also been suggested that the type of FISH fusion patterns could be used to predict IM resistance. These patterns could be the signifier of dual fusion patterns, deletion of chromosome 9q or chromosome 22q on the fused chromosome, loss of whole fused chromosome 9, additional Philadelphia chromosome, and complex translocations related to BCR and ABL1. In the literature, the percentage of fusion patterns other than dual fusion ranged from 9.2% to 28.5% (16-18). Our study found that the rate within this range and the distribution of the patterns were also consistent with the literature. Few studies have been carried out on the prognostic significance of this derivative chromosome structure, and contradictory results have been obtained. Early reports in the IM era revealed that in the deletion group, complete hematological response rate, progression-free survival, and MMR were lower (19, 20). However, subsequent studies (21-23) found no difference in survival and remission rates between the deletion and the non-deletion groups. Our study was consistent with these literature data.

One of the most striking FISH groups is variant translocations. El-Zimaity (24) found that patients with variant translocations showed similar response rates and survival times. Also, no significant differences were found regarding white blood cell and platelet counts and hemoglobin levels. Many other studies have shown that patients with variant translocations do not differ prognostically. However, variant translocation has been associated with poor prognosis in some studies. In our study, the number of patients with complex translocation is inadequate for a good comparison, but there was no statistically significant difference among groups.

This study had some limitations related to the incomplete data for initial prognosis assessment and clinical failure. Clinical prediction scores like Sokal, ELTS, and EUTOS could not be calculated due to incomplete data like blast percentage at the diagnosis. Also, our study had various advantages and significant disadvantages due to the mutation analysis method. We were able to analyze only a limited number of mutations.

In conclusion, the most critical challenge in treating CML is the development of TKI resistance, which is seen in many patients. Predicting the prognosis of patients at the time of diagnosis and identifying the BCR::ABL1 mutation type in resistant patients could guide the subsequent treatment and improve patient care. New techniques like high-throughput sequencing can identify more mutations, and different mechanisms that lead to imatinib resistance will be identified. This could pave the way for treatments that can be used to care for patients with CML and overcome resistance.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: O.S.A., G.O., M.T., Design: O.S.A. G.O., Data Collection or Processing: O.S.A, M.T., D.O., Analysis or Interpretation: O.S.A, G.O. E.A, Literature Search: O.S.A., E.K., U.A., Writing: O.S.A., U.A.

Ethical Statement

The study was approved by the ethics committee of the Faculty of Medicine of Ondokuz Mayıs University (approval date 05.05.2017 and file number B.30.2ODM.0.20.08/916). Written informed consent was obtained from all patients.

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Research Article

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Histopathological and radiological correlation of lung mass lesions with transthoracic biopsy and endobronchial biopsy

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Abstract

The aim of this study is to correlate radiological CT imaging approaches to the lesion and pathology results in lung lesions. In this study, lung biopsy pathology results of 54 cases sent to the pathology department between 2020-2023 were compared with existing radiological CT images. The cases were grouped pathologically as benign and malignant, and the malignant cases were classified according to their tumor types. Radiologically, the cases were divided into groups as benign or malignant, peripheral or centrally located. In this study, core biopsies of a total of 54 cases, 15 female (27.7%) and 39 (72.3%) male, were evaluated. The average age of the patients was found to be 61.1 (21-95). Of a total of 54 lung solid mass lesions, 38 (70.4%) were peripheral and 16 (29.6%) were centrally located. In the radiological double-blind evaluation, 29 (53.7%) of the cases were diagnosed as malignant, while 24 (44.4%) were pathologically diagnosed as malignant. While squamous cell carcinoma (SCC), the most common pathologically malignant tumor, was seen in 9 cases, the second most common small cell carcinoma (SCLC) was detected in 7 cases. In conclusion, pathology and radiological imaging in lung lesions are important in early diagnosis, effective treatment and prolonged survival, and correctly performed radiological imaging and histopathological correlation with biopsy with today's interventional techniques in early suspicious cases have an important place in reducing morbidity and mortality.

Keywords: Lung cancer, transthoracic biopsy, histopathology, malignant, benign

1. Introduction

Lung cancers have a significant impact on the public health system as they are the most common disease with 2.1 million cases worldwide and are the leading cause of cancer deaths. According to epidemiological studies, it is twice as common in men as in women, and the rate is gradually increasing in middle-aged men (1, 2).

Solitary lesions detected in the lung are very common and early diagnosis and treatment of these lesions are very important because of the possibility of mortality. Therefore, the diagnosis should be made with a careful and accurate pathological and radiological approach.

The first method used in lung parenchyma imaging is direct chest radiography. Although its cost, radiation dose, availability and ease of performance are quite successful compared to its diagnostic approach; It has been observed that it can miss 10-15% of symptomatic infiltrative diseases, 30% of bronchiectasis and 60% of emphysematous diseases. For this reason, multi-slice Computed Tomography (CT) methods are needed (3, 4). The most reliable imaging method for lung parenchymal lesions is contrast-enhanced CT, and in these imaging, the next step is taken in the preliminary diagnosis according to the morphological or density difference according to the contour edge features of the lesion.

The decision for lung biopsy based on CT findings is discussed by the radiologists and clinicians with a joint decision on the necessity of biopsy. CT imaging plays an important role in evaluating the characteristics of the lesion and determining the best approach for biopsy. Some of the criteria for lung biopsy based on CT findings are as follows (5):

1. Location of the Lesion:

Peripheral Lesions: Transthoracic biopsy is usually the first choice for lesions located close to the outer edge of the lung. These lesions can be more easily accessed with a needle inserted through the chest wall.

Central Lesions: Lesions close to the central airways or the hilum are usually attempted to be histopathologically established using endobronchial biopsy or bronchoscopy

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because these masses are closer to the airways.

Subpleural Lesions: Lesions close to the pleura or chest wall are usually easier to sample percutaneously with transthoracic biopsy. Inaccessible or deep lesions: Deep lesions located in difficult-to-reach areas and close to vascular structures are preferred for endobronchial ultrasound (EBUS) guided biopsy.

2. Lesion Size: Lesions 1 cm and larger are generally easier to target and sample during biopsy, but smaller lesions are more difficult to obtain adequate biopsy samples and require greater precision and experience in imaging-guided procedures.

3. Shape and Border Features: For example, spiculated borders are a sign of malignancy, especially lung cancer. However, more regular-circumscribed lesions are more likely to be benign. However, they do not exclude malignancy, especially if they are rapidly growing.

4. Density and Contrast Enhancement: Heterogeneous Density may indicate malignancy. If there is an air-filled space in a cavitary lesion, it may indicate certain types of malignancy, such as infection, tuberculosis, or squamous cell carcinoma. Biopsy allows differentiation between benign and malignant lesions. Contrast Enhancement can also be a sign of malignancy, as cancerous tissues usually show increased blood supply and therefore increased contrast.

5. Lymphadenopathy: Enlarged lymph nodes seen with a lung mass on CT increase the suspicion of metastasis or primary lung cancer.

6. Growth Over Time or Rapid Growth is suspicious for malignancy, especially small cell lung cancer or metastasis.

Stable or Slow Growth, on the other hand, tends to be benign lesions, but malignant lesions can present with rapid changes.

7. Location Relative to Other Structures: If the lesion is near large blood vessels such as the aorta or pulmonary artery, the risk of bleeding during biopsy may be increased. In such cases, advanced imaging techniques or other approaches may be preferred. Lesions that appear to invade adjacent structures are highly suspicious for malignancy.

8. Calcification Pattern: A lesion with central or popcorn-like peripheral calcification is usually benign.

9. Presence of Associated Features such as Atelectasis or Pneumonitis may indicate malignancy or an infectious process. Presence of Pleural Effusion The presence of pleural effusion, especially if exudative, may be associated with malignancy. Considering all these findings, biopsy and histopathological diagnosis should be performed on suspicious lesions (6).

Transthoracicor bronchial biopsy is recommended for benign and/or malignant cases in CT scans detected incidentally in the lung or in the presence of symptoms.

The pathology results are guiding in terms of the preoperative medical treatments of the case, the type of operation, molecular studies and targeted treatments.

In addition to diagnostic H&E stains in transthoracic or bronchoscopic biopsies sent from lung lesions, immunohistochemical stains are often used and molecular and next-generation sequencing are used to determine further targeted treatments.

Although pathological examination is the gold standard in the diagnosis of lung cancer, the importance of early diagnosis reveals the importance of accurate and reliable radiology.

2. Materials and Methods

Cases whose CT findings were suggestive of malignancy or were considered benign but malignancy could not be excluded, and whose medical treatment was considered by the clinician to be planned and whose biopsy was indicated were included in the study.

In this study, 59 cases with a pathological diagnosis were retrospectively evaluated between June 2020 and 2023, at Van Training and Research Hospital. 5 cases were not included in the study because they were reported as inadequate in pathology. Retrospective CT scans of patients with sufficient pathology specimens and a pathological diagnosis were examined, and a total of 54 cases were evaluated and included in the study.

Statistical data of the study; Variables are presented as mean ± standard deviation. SPSS Windows version 21.0 package program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

2.1. Radiological evaluation:

The study was conducted retrospectively by examining the cases in which the radiological examination images of the patients were positive from the Picture Archiving and Communication Systems (PACS), which is radiological data archiving system. Images of the patients were obtained using 16-slice Computed Tomography (Siemens, medical solution); CT images obtained with a slice thickness of 3 mm, a dose of 120 kV, and without contrast during the biopsy were evaluated. For the study, axial image data taken from the PACS (karpacs wiever v.1.0) system was examined.

In this study, after radiologists and clinicians made a decision for biopsy together, CT images of the cases were evaluated by a second radiologist for biopsy decision. In case of discrepancy between the radiologists, a third radiologist was consulted. Lung lesions were defined as benign or malignant according to their location, central or peripheral, and morphological features, and the probability of benign or malignant was evaluated. Lung biopsy procedures:

Under CT guidance, an opaque marker was placed on the skin level and the entry point was determined at the lesion level. Then, following local anesthesia (Prilocaine2%)

injection under the skin.

Using the coaxial system, the lesion level was reached with the coaxial needle and sheath, then the needle part of the coaxial needle was removed and the tru-cut biopsy needle was advanced to the lesion over the needle sheath, and a tissue piece was removed in at least 2 pieces with a thick needle. It was taken and sent to pathology in formalin. To reduce the possibility of pneumothorax, if air was seen in the pleural space, it was tried to be minimized by negative aspiration with a 10cc syringe. The last control image was taken and complications such as pneumothorax, hemothorax or parenchymal hemorrhage were tried to be seen and the treatment plan was made accordingly.

2.2. Pathological evaluation:

Transthoracic and bronchoscopic biopsies sent to the pathology. H&E slides prepared from sections obtained from 10% buffered formalin-fixed, paraffin-embedded tissues, and immunohistochemical studies were taken from the archive and re-evaluated. The cases were divided two categories: benign lesions and malignant lesions. Non-diagnostic biopsies were not included in the study. Malignant lesions were grouped as small cell lung carcinoma (SCLC), adenocarcinoma, squamous cell carcinoma (SCC), mucoepidermoid carcinoma, large cell neuroendocrine carcinoma, lymphoma and metastases. The results of the pathology specimens were compared with Computed Tomography images.

3. Results:

There were 54 patients diagnosed with core biopsy. The gender rate of these cases was %27.8 female (15 cases) and %72.2 male (39 cases) The average age of the patients was 61.1 years, and the age range was 21–95 years. Localization of the lung lesions were 70.4% peripheral (38 cases) and 29.6% (16 cases) were centrally located. Transthoracic biopsy was performed in 36 patients and EBUS was performed in 18 patients. In the double-blind radiological evaluation, 46.3% (25) of the cases were interpreted as benign and 53.7% (29) as malignant. Pathologically, 30 (55.6%) cases were diagnosed as benign and 24 (44.4%) as malignant. Pathologically malignant cases are as follows; 9 patients were reported as SCC, 7 patients as SCLC, 3 patients as adenocarcinoma, 3 patients as renal cell carcinoma metastasis, 1 patient as lymphoma and 1 patient as large cell neuroendocrine carcinoma.

Of the pathologically confirmed malignant cases, 20 (83.3%) were men and 4 (16.7%) were women. The average age among malignant cases was 60.3 years; the average age was 56.7 years for women and 61.0 years for men. In the radiological and pathological comparison, the correct diagnosis was made radiologically with a sensitivity of 66.67% (95% confidence interval) and a specificity of 60%.

4. Discussion

Since lung cancers are among the most common causes of cancer-related death, the distinction between malignant and benign is of critical importance. Studies show that although survival rates for lung cancer are increasing, as in all cancers, survival rates are among the cancers with the lowest rates due to late diagnosis.

CT has an important place in the diagnosis of lung lesions, especially small lung nodules and lung cancers. Treatments for lung lesions require a multidisciplinary approach, radiologically, pathologically and clinically. For this reason, the earliest pathological diagnosis of patients and early treatment of these cases are very important. Targeted therapies, which have become increasingly important in lung cancer in particular, increase the need for molecular studies and the importance of pathological correlation.

High-resolution computed tomography has increased success rates in detecting small lung lesions and indistinct ground-glass opacity lesions (7).

Solid can be benign or malignant. Solitary pulmonary nodules are defined as nodules smaller than 3 cm surrounded by lung parenchyma. While 10-70% of radiologically described solitary pulmonary nodules are lung cancers, 80% of the remaining are granulomas from benign lesions. If the lesion density is <20 HU, it usually suggests cystic lesions such as simple parenchymal cysts, abscess or hydatid cysts. Lesions >20 HU are considered as solid density lesions and re-evaluated. Morphological features of the lesion; It is evaluated according to the contours of the lesion, whether it contains calcification, the central-peripheral location of the calcification, and whether it is solid or semi-solid. Histopathological verification should be performed for every patient considered malignant. For this purpose, the appropriate biopsy method, accompanied by radiological findings, should be preferred with a low complication rate and a high probability of diagnosis.

While transthoracic lung biopsies (Fig. 1) are an important procedure for peripherally located lesions, fiberoptic bronchoscopes and endobronchial ultrasonographies are more often important for histopathological sampling in centrally located lesions, as they are technically easier and have fewer complications.

CT-guided transthoracic biopsies (TTBx) have a sensitivity of 90% in diagnosis. Although invasive methods have very successful results in diagnosis. TTBx has complications, especially in peripherally located lesions. Pneumothorax is the most common complication and may occur at rates of 17% and 42%, and pulmonary bleeding may occur secondary to invasive procedures at a rate of 27% (7). In our study, there was only one patient who underwent tube thoracostomy with significant and clinical implications. Alveolar hemorrhage was detected as a procedure complication in 6 patients, which was not clinically significant but was reflected in imaging findings.

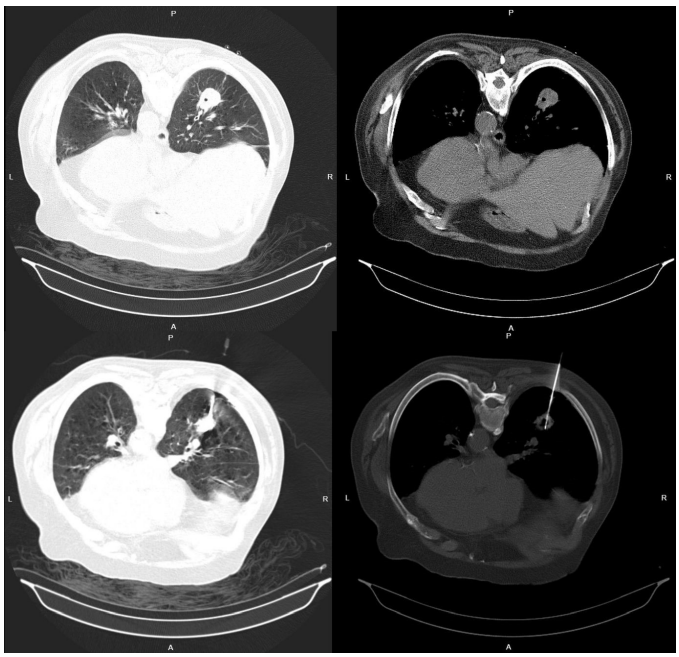

Fig. 1. An 80-year-old male patient with a central necrotic thick-walled lesion in the lower lobe of the right lung received the pathological diagnosis of malignant neoplasia as a result of transthoracic biopsy performed in the prone position. During and after the procedure, a mild pneumothorax, which is not clinically significant, is observed in the posterior due to minimal pleural separation.

Although lung cancer has recently increased in the female population due to the increase in smoking among women, it is seen on average 2 times more frequently in men than in women (1-9). In our study, 20 of 24 malignant cases were detected in male patients, in line with the literature. The average age of occurrence of lung cancer is over 60 years of age in men and women worldwide, and the average age of occurrence is 70, but non-small cell carcinomas, especially adenocarcinomas, can also be seen under the age of 55. In our study, the average age of malignant cases was evaluated as 60.3 years, and since these cases also included metastatic lesions, it was thought that they were proportionally different from the literature. The fact that the average age of women (56.75) is lower than that of men supports existing studies (9)

Pathologically, lung cancers are divided into two main categories: small cell lung cancers and non-small cell lung cancers. Non-small cell cancers are as follows; adenocarcinoma, squamous cell carcinoma, neuroendocrine carcinomas (large cell neuroendocrine carcinomas) and carcinoids (9). Although the most common cancers change over time, the most common non-small cell carcinomas in recent data are adenocarcinomas, accounting for more than 40% of all cancers. The rate of SCCs has decreased in recent years with changes in smoking habits (10-11) (Fig. 2).

We thought that the difference observed in our study proportionally with the literature would be due to the small number of cases and factors such as smoking and air pollution.

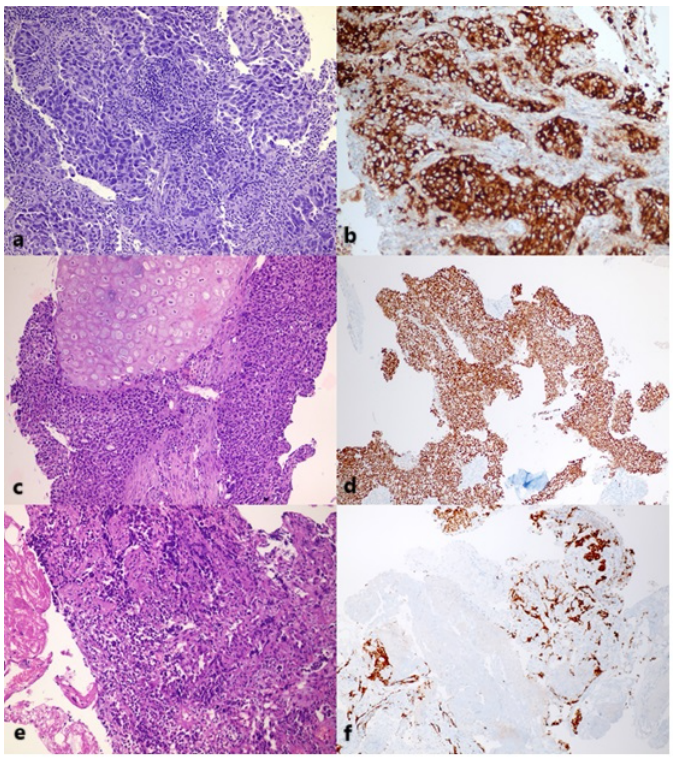

Fig. 2. a: Solid poorly differentiated adenocarcinoma with large hyperchromatic nuclei and pleomorphic gland formations. 200 X H&E; **b:** Adenocarcinoma, strong cytoplasmic immunoexpression in tumor area. 200 X Napsin A; **c:** Squamous cell carcinoma, cartilage tissue invasion. 200X H&E; **d:** Squamous cell carcinoma, strong nuclear immune expression 100 X P63; **e:** Small cell lung carcinoma with widespread crush artifacts, small hyperchromatic nuclei, and no cytoplasm visible. 200 X H&E; **f:** Strong nuclear immunoexpression in area of small cell lung carcinoma. 100X TTF-1

Lung cancers are classified as central or peripheral, depending on their radiological location. While SCLC and SCC are often centrally located, adenocarcinomas are more peripherally located. Of our 3 adenocarcinoma cases, 2 were found to be peripherally located, 2 of the SCCs were centrally located, 7 were peripherally located, and 5 of the SCLCs were centrally located and 1 was peripherally located. Although adenocarcinomas and SCLCs were proportionally compatible with the studies in our cases, the incompatibility observed in SCCs was associated with the numerical limitation of our case series (8, 11, 12).

Pathology and radiological imaging of lung lesions are important for early diagnosis, effective treatment and prolonged survival. Since lung cancers are in the first place in cancer deaths, the distinction between malignant lesions and benign lesions is of critical importance. Careful and accurate radiological imaging is the first step in approaching the patient. Although the radiological and pathological correlation in our study was lower than previous studies, this rate is higher in large case series.

Ethical Statement

Approval was obtained from Van Training and Research Hospital Ethics Committee of Science Health University on

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Conflict of interest

Authors declare that there is no conflict of interest for this article.

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Authors' contributions

Concept: S.T., Design: T.O., Data Collection or Processing: S.T., Analysis or Interpretation: S.T., Literature Search: T.O., S.T., Writing: S.T., T.O.

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Research Article

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Intravenous imunoglobulin treatment and evaluation of autoinflammatory and ımmunodeficiency-associated recurrent parotitis cases in children

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Abstract

Recurrent juvenile parotitis is a rare inflammatory condition characterized by recurrent, non-obstructive, and non-suppurative inflammation of the parotid gland during childhood. Diagnosis of the disease is based on clinical symptoms and laboratory tests. Although cases of recurrent parotitis are rare, various diseases can contribute to its etiology. Case series in the literature have reported diagnoses of immunodeficiency and autoimmune diseases. This study presents five cases diagnosed with autoimmune disease and immunodeficiency, successfully treated with intravenous immunoglobulin.

Keywords: recurrent parotitis, immunodeficiency, autoinflammatory, IVIG

1. Introduction

Recurrent parotitis is an infrequent inflammatory disease characterized by unilateral or bilateral swelling of the parotid gland, presenting with recurrent attacks. It is the second most common disease of the salivary gland after mumps (1). Diagnosis in patients experiencing two or more attacks is generally based on history, physical examination, ultrasound, and elevated serum amylase levels. During physical examination, the opening of the parotid duct is usually dilated, surrounded by white-yellow plaques. Ultrasound is used to confirm the diagnosis by detecting sialectasis, hypoechoic areas, and punctate calcification during the examination (2,3). Although the exact cause of the disease is not fully understood, various etiological factors have been reported in children, including congenital ductal malformations, genetic factors, autoimmune diseases such as Sjögren's syndrome, allergies, sarcoidosis, and immunodeficiencies such as selective IgA deficiency. In adults, it is often associated with HIV (4).

The aim of this study is to focus on the rare recurrent parotitis disease in children and emphasize the potential role of immunodeficiency and autoimmune diseases in its etiology. Additionally, the study will discuss the potential of intravenous immunoglobulin (IVIG) treatment in reducing the frequency of attacks.

2. Materials and Methods

The study included pediatric patients who had experienced at least two clinically confirmed episodes of parotitis, with diagnoses supported by laboratory and imaging findings, and who were evaluated for underlying immunodeficiency or autoimmune conditions.In this retrospective case review, the files of patients who presented to the Department of Pediatric Immunology and Allergy at - Faculty of Medicine were examined, and necessary permissions were obtained from the patients' families and the hospital ethics committee. The evaluation of patients was conducted using a previously established method (5,6). The files of five children diagnosed with recurrent parotitis were thoroughly examined. The diagnosis of recurrent parotitis was confirmed by clinical and ultrasonographic findings in children who had at least two acute non-suppurative parotitis attacks. The age at the onset of each attack, duration, affected gland, and frequency of attacks were recorded for each patient. Additionally, family history was assessed for signs of autoimmune disease (skin rash, dry mouth, and eyes, joint swelling), frequent illnesses, growth retardation, persistent wounds, and vaccine unresponsiveness. Physical examination findings, acute phase reactants (erythrocyte sedimentation rate and serum C-reactive protein levels), serum amylase levels, and immunological function tests (absolute lymphocyte and neutrophil counts, immunoglobulin G, A, M levels) were analyzed. Serum rheumatoid factor, autoantibody profile (antinuclear antibody (ANA), anti-double-stranded DNA (anti-dsDNA) antibodies, anti-RNP antibodies, anticardiolipin, anti-smooth muscle antibody (anti-ASMA), antiphospholipid antibodies, anti-Ro (SS-A), anti-La (SS-B) antibodies), serum complement levels (C3, C4), and infectious markers (anti-mumps IgM, anti-HAV

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IgM, HbsAg, anti-HCV, anti-HIV, anti-CMV IgM, anti-EBV IgM, anti-toxoplasma IgM) were also evaluated.

2.1. Case 1

A 12-year-old male patient experienced recurrent parotitis attacks starting at the age of 6, totaling 14 episodes. The swelling was usually observed in the patient's right salivary gland, and the attacks lasted for 7-10 days. The patient also exhibited symptoms such as swelling, redness, and pain in the hands and feet. There was no consanguinity between the parents. The patient's mother also had similar arthritis and dermatitis complaints. Although the patient did not have familial Mediterranean fever (FMF) mutations, a nod2 mutation (c.2798+158C > T) was detected. Despite repeated empirical antibiotic treatment during parotitis attacks, the attacks persisted. The patient responded well to intravenous immunoglobulin (IVIG) treatment (400 mg/kg/day, every three weeks) and colchicine therapy.

2.2. Case 2

An 11-year-old female patient, whose parents were cousins, experienced her first attack at the age of 9 and had a total of 6 recurrent parotitis attacks.The attacks were concentrated on the right side of the patient. Additionally, elevated liver enzymes, hypothyroidism, and lymphopenia were present. Although serum immunoglobulin values were within normal limits for her age, high levels of antinuclear antibodies (ANA+++) were detected in the tests. Mutations M696V and R202Q were identified in investigations for recurrent fever and joint pain. The patient responded positively to colchicine and IVIG treatment, and her symptoms and liver enzyme levels returned to normal.

2.3. Case 3

A 13-year-old male patient experienced his first attack at the age of two and had a total of 6 recurrent parotitis attacks. The patient's parents were consanguineous. The patient had a history of frequent infections, and his mother had psoriasis. Serum IgG and IgM levels were found to be low for his age. With a diagnosis of common variable immunodeficiency, the patient was started on IVIG treatment. Before the treatment, the patient experienced recurring attacks every 3-4 months, but after the treatment, the attacks significantly decreased, and no attacks were observed in the last three years. The patient was found to have a TNFRSF13B C104R heterozygous mutation through genetic testing.

2.4. Case 4

An 11-year-old male patient, who first presented at the age of 3 with recurrent fever, neck swelling, frequent infections, joint pain, and mouth sores, was evaluated, with no consanguinity between the parent. Streptococcus pyogenes growth was detected in the patient's throat culture. Attention deficit hyperactivity disorder symptoms were observed in follow-ups, and the patient was considered to have pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). Monthly long-acting penicillin treatment was initiated, and psychiatric follow-up was conducted. However, the swelling in the patient's neck continued, and attacks occurred almost every month. During this process, no growth was detected in throat cultures. Familial Mediterranean fever (FMF) and PFAPA syndrome were excluded through tests and steroid treatment. Although the patient's immunological function tests tests were normal, high levels of thyroid autoantibodies were found. Considering autoimmunity and PANDAS, the patient responded positively to IVIG treatment.

2.5. Case 5

A 6-year-old male patient experienced his first attack at the age of 3. The patient, who used medication for epilepsy, had a history of frequent infections, prolonged moniliasis in the neonatal period, and prolonged febrile periods after vaccinations. The patient's sibling had recurrent fever and joint pain, and there was a history of FMF in the family, with the parents being consanguineous.Despite normal immunodeficiency tests, high levels of thyroid autoantibodies were found. Considering autoimmunity and PANDAS, the patient was started on IVIG treatment and showed no attacks during the IVIG treatment period.

Based on the patients' medical history, physical examination findings, and laboratory results, differential diagnoses of allergy, sarcoidosis, and Sjögren's syndrome were considered, but no consistent findings were identified. When evaluating the diagnosis and treatment of our patients, the second case received IVIG treatment due to suspicion of autoimmunity. Similarly, the fourth case underwent IVIG treatment under comparable circumstances. The third case was associated with TACI deficiency, while the fifth case presented suspicion of common variable immunodeficiency. The first case was diagnosed with Blau syndrome associated with a nod2 mutation. Each patient had received various antibiotic treatments (such as penicillin, ampicillin-sulbactam, cefuroxime, etc.) prior to their presentation to our clinic. Following evaluations conducted at our clinic, the patients were administered regular IVIG treatment for approximately 6 months. In cases 1 and 2, colchicine treatment was initiated in addition to IVIG therapy. In case 1, a parotitis attack recurred once after discontinuation of the medication. Due to their diagnosis of CVID, cases 3 and 5 continue IVIG treatment to mitigate the risk of additional infections.

Table 1. Clinical and laboratory characteristics of children diagnosed with recurrent parotitis

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	male	female	male	male	male
Age of Patient	12 years	11 years	13 years	10 years	6 years
Age of First Attack	6 years	9 years	2 years	3 years	3 years
Total Number of Attacks	14	6	6	numerous	4
Attacks Time	7-10 day	7 day	10 day	7-10 day	10 day
Side of Attacks	right	right	bilateral	right /bilateral	right
Tracking Time	1 years	3 years	6 years	7 years	1 years
Leukocyte	11490	15780	9120	8000	16960
ESH (mm/s)	56	21	12	28	70
CRP (mg/dl)	37	48	34	12	144
Amylase (U/L)	266	1530	388	269	386
Autoantibodies	negative	ANA+++	negative	Thyroid autoantibodies positive	negative
Infection Viral Markers	negative	negative	negative	negative	negative
Concomitant Disease	NOD2 mutation (Blau syndrome)	Hypothyroidism, FMF (M694V mutation)	CVID (TNFRSF13B C104R Heterozygous Mutation)	autoimmune thyroiditis	CVID
Treatments	IVIG colchicine	IVIG colchicine	IVIG	IVIG	IVIG
IgG (mg/dl)	1040 (N:907-1958)	895 (N:835-2094)	590 (N:907-1958)	916 (N:835-2094)	610 (N:764-2134
IgA (mg/dl)	94 (N:96-465)	210 (N:67-433)	90 (N:96-465)	74 (N:67-433)	83 (N:70-303)
IgM (mg/dl)	133 (N:83-232)	160 (N:47-484)	54 (N:83-232)	68 (N:47-484)	185 (N:69-387)
IgG Subgroups	normal	normal	normal	normal	normal
Lymphocyte %	%27	%17	%25	%13	%26
T helper (CD3+CD4+ %)	%31 (N:27-57)	%34 (N:27-57)	%36 (N:27-57)	%42 (N:27-57)	%34 (N:26-48)
Cytotoxic T cells (CD3+CD8 +%)	%43 (N:19-38)	%33 (N:19-38)	%17 (N:19-38)	%27 (N:19-38)	%34 (N:20-42)
B cells (CD19+ %)	%13 (N:10-30)	%13 (N:10-30)	%14 (N:10-30)	%10 (N:10-30)	%15 (N:10-27)
Natural killer cells (CD3- 16+56+ %)	%11 (N:8-30)	%10 (N:8-30)	%27 (N:8-30)	%13 (N:8-30)	%9 (N:8-27

(ESH: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, N: Neutrophil count, ANA: Antinuclear Antibody, FMF: Familial Mediterranean Fever, IVIG: Intravenous Immunoglobulin, Ig: Immunoglobulin)

3. Discussion

Recurrent parotitis refers to intermittent inflammation of one or both parotid glands. Although its incidence is not precisely known, small case series have been reported in the literature. This condition, more commonly observed in males, typically has an onset age between 3 and 6 years. It usually manifests in episodes occurring every 3-4 months, lasting 4-7 days. Three of our patients were observed to exhibit recurrent parotitis symptoms within this age range (1). However, two of our patients had different onset ages; one experienced initial attacks at 1.5 years, while the other at 9 years. Late-onset cases were also encountered in a series of 20 cases by Ericson et al. (4). Similar to the literature, the ratio of male patients was higher in our series.

Patients with recurrent parotitis experience attacks at least twice. Symptoms observed during these attacks include swelling, pain, fever, redness, and increased warmth in the parotid gland (2). All our patients had signs of swelling and fever in the parotid gland. Diagnoses were confirmed through ultrasound and laboratory tests. In all patients, mumps vaccination had been administered before, and mumps IgM was negative, while IgG was positive. Since mumps-associated parotitis cases can occur in adults, anti-HIV tests were also negative to exclude this condition. Tests for other viral diseases yielded negative results.

Recurrent parotitis cases associated with immunodeficiencies such as selective IgA deficiency, IgG3 deficiency, X-linked hypogammaglobulinemia, common variable immunodeficiency, and autoinflammatory diseases like Sjögren's syndrome have been reported in the literature. However, the exact cause remains uncertain (2, 7). Due to the unclear etiology of recurrent parotitis, there is no consensus on treatment. While many experts do not use antibiotics during acute attacks, the benefit of prophylactic antibiotic use has not been demonstrated.

A prospective case-control study conducted by Wu et al. in patients with juvenile recurrent parotitis (JRP) showed that as age increases, CD4+ levels decrease, while CD8+ T levels

relatively increase. CD4+ T cells are essential components of the immune system, particularly crucial for signaling CD8+ cytotoxic cells that destroy infected cells. Therefore, the disturbance in the CD4/CD8 T cell ratio can lead to infectious or autoimmune diseases (8,9). In the same study, significant differences were found in IgG, IgE, IgA, and C3 levels. The authors suggested that immune function in JRP patients differed from the general population, indicating decreased cellular immunity and insufficient antibody production. They proposed that immunotherapy aiming to improve immune responses systemically and in the parotid gland should be prioritized (8,9).

Intravenous immunoglobulin (IVIG) treatment is among the mechanisms affecting the immune system. Specifically, IVIG has been observed to promote the expansion of CD4+CD25+ regulatory T cells (Tregs), playing a significant role in the control of autoimmune diseases and inflammatory conditions. IVIG also exhibits immunomodulatory effects by suppressing T and B cell activation. It has been demonstrated that IVIG preparations work by directly influencing T cell proliferation and reducing B cell receptor (BCR)-mediated activation of B cells (1,10).

The efficacy of IVIG therapy in autoimmune and inflammatory diseases has been demonstrated in various studies. A long-term study on rheumatic diseases reported that IVIG treatment led to remission in the majority of patients; however, a decrease in treatment response was observed in some cases after seven years (11). Similarly, research on treatment-resistant uveitis has shown that IVIG is an effective and well-tolerated option for controlling inflammation (12). Nonetheless, evaluations in pediatric rheumatology patients have revealed that approximately half of the cases experienced mild to moderate adverse effects such as headache, nausea, and aseptic meningitis following IVIG administration (13). Additionally, rapid infusion rates and inadequate hydration have been associated with serious complications such as renal failure and thromboembolic events. Therefore, during long-term use of IVIG therapy, it is recommended that both clinical efficacy and potential adverse effects be closely monitored (14).Cases 2 and 4 from our study were treated with IVIG due to suspicions of autoimmunity, case 1 with Blau syndrome associated with nod2 mutation, case 3 with TACI deficiency, and case 5 with suspected common variable immunodeficiency. After treatments, only case 1 experienced two more attacks at longer intervals, while the others had no further attacks. In cases with underlying autoimmunity or immunodeficiency, as indicated in our study, IVIG treatment resulted in a reduction in the number and frequency of attacks. These findings highlight the importance of immunological and genetic evaluation in cases of recurrent parotitis and suggest that IVIG and colchicine treatments can be effective in preventing attacks.

Recurrent parotitis cases in children may be associated with autoinflammatory diseases and immunodeficiency conditions. Understanding the underlying causes of this disease is critically important for determining effective treatment strategies. IVIG treatment can be considered as a treatment option in recurrent parotitis cases associated with autoinflammatory diseases and immunodeficiency in children. This treatment can modulate the immune system, reduce inflammation, and balance immune responses. However, further research is needed to better understand the role of IVIG in the treatment of recurrent juvenile parotitis (JRP) in children. Future studies should focus on better understanding the reasons for recurrent parotitis in children and developing more effective treatment methods. Continued research in this field will contribute to a better understanding of the disease and the development of treatment approaches.

Our study suggests that in children with recurrent parotitis and underlying immunodeficiency or autoimmunity, IVIG may be an effective alternative in cases unresponsive to first-line treatments. Therefore, early immunological evaluation and consideration of IVIG in resistant cases are clinically important.

This study has several limitations. First, as it is a retrospective and observational study, it is not possible to establish a causal relationship. The small sample size limits the generalizability of the findings, particularly regarding the efficacy and safety profile of IVIG therapy. Additionally, the lack of a standardized clinical scoring system for evaluating treatment response may introduce subjectivity into the assessments. The duration of long-term follow-up varied between cases, and long-term outcomes could not be fully evaluated in some patients due to limited observation periods. Moreover, concomitant treatments such as colchicine or supportive therapies may have influenced the treatment outcomes, representing a potential confounding factor. Therefore, these findings should be supported by larger, prospective and controlled studies to validate their significance.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 27/02/2020 and the number of ethical committee decisions is 2020/93.

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Statistical evaluation of the effects of stroke risk factors on NIHHS AND MRS

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Abstract

Stroke is the second leading cause of death and the third leading cause of disability worldwide (1,2), with a global prevalence estimated at 0.2% (3). It also significantly contributes to healthcare costs (4). Ischemic strokes, which result from disrupted cerebral blood flow, make up approximately 80–85% of all stroke cases. Key risk factors for stroke include hypertension, diabetes mellitus, heart disease, hyperlipidemia, smoking, and lifestyle factors. This study investigated the impact of stroke risk factors and lesion localization on stroke severity, as measured by the NIHSS scale, and patient independence at discharge, as evaluated by the Modified Rankin Scale (MRS). The analysis included 159 patients, comprising 82 women (51.6%) and 77 men (48.4%), with a mean age of 68.1±14.2 years, 93.1% of whom were over 45 years old. Stroke types were distributed as follows: 41.5% anterior circulation, 37.1% posterior circulation, and 21.4% lacunar strokes. The factors affecting the difference between NIHSS values (Delta NIHSS) at admission and discharge were analyzed by univariate and multivariate analysis methods. According to the results of univariate analysis, the presence of hypertension, myocardial infarction, coronary artery and carotid disease, previous insulin use, intravenous-tPA (iv-tPA), intra-arterial-tPA (ia-tPA) and thrombectomy treatments, age, NLR, mean blood pressure and exit MRS values were significantly associated with Delta NIHSS ($p<0.05$). Multivariate analysis revealed a positive correlation between Delta (Δ) NIHSS value and CAD and iv-tPA values and a negative correlation with exit MRS value ($p=0.021$; $p=0.012$ and $p<0.001$ respectively). Our model provides consistent results for the relationship between NIHSS and MRS scores and positive and negative functional outcomes.

Keywords: stroke, NIHSS score, Shapiro-Wilk test, Mann-Whitney U test, Spearman Rho test, regression analysis

1. Introduction

Globally, stroke ranks as the third most common cause of morbidity, and the second most common cause of death (1,2) The prevalence of stroke is 0.2% globally (3) Stroke also significantly affects expenses for healthcare in addition. (4) About 80% to 85% of all strokes are ischemic strokes, which are characterized by the disturbance of cerebral blood flow. (5) Stroke is a preventable neurological condition, and physicians have been working on modifiable risk factors for years. The main risk factors for stroke are hypertension, diabetes mellitus, heart disease, hyperlipidemia, smoking, and lifestyle. In addition to these factors that determine the prognosis, the treatment methods used, the presence, and degree of carotid stenosis, the localization of the stroke in the brain, and the neutrophil/lymphocyte ratio also contribute to the process. The National Institutes of Health Stroke Scale (NIHSS) is often used to measure stroke severity in emergency or neurology departments. This scale was first designed in 2001 by Neurologist Dr. Lyden et al. to assess the results of Recombinant Tissue Plasminogen Activator (rt-PA) administration in acute stroke. (6). NIHSS is a widely used tool for assessing stroke severity, comprising 11 categories with a total score range from 0 to 42. It evaluates various aspects of brain function, including vision, sensation, motor abilities, consciousness, speech, and language. A score of 42 represents the most severe and catastrophic stroke. The scoring system categorizes strokes as follows: 0 indicates no stroke, 1–4 corresponds to a minor, 5–15 to a moderate, 15–20 to a moderate-to-severe, and 21–42 to a severe stroke (6). Various outcomes scales assess stroke outcomes. One of the most commonly used outcome scales is the Modified Rankin Scale (MRS), which was developed to assess independence specifically. The MRS also evaluates whether the patient can perform all of the activities that he/she could do before the stroke (7). The levels of the scale are defined as follows: 0 - No

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symptoms; 1 - Symptoms are present but do not cause significant impairment; the individual can carry out regular tasks and activities. 2: Mild impairment; cannot perform all previous activities, but can do his/her work without assistance; 3: Moderate impairment; needs some assistance, and can perform activities; 2: Mild disability; cannot fulfill all previous activities, Able to perform their tasks independently without requiring help. Level 3: Moderate disability, where some assistance might be needed, but the individual is still capable of walking unassisted. Level 4: Moderately severe disability, where walking without assistance is not possible, and the individual cannot manage their own needs without help. Level 5: Severe disability, characterized by being bedridden, incontinent, and dependent on continuous care. (8). In this study, the relationship between stroke risk factors and stroke lesion localization detected in patients admitted to neurology services with a stroke clinic, and the NIHSS scale, and MRS at discharge were examined.

2. Method

Adana City Training and Research Hospital Scientific Ethics Committee (312/9) permission was obtained for the cross-sectional study on stroke. This study comprised 159 individuals who were monitored in the neurology clinic after being diagnosed with ischemic stroke. Patients were classified as having posterior-anterior or lacunar infarcts based on their clinical findings, following the categorization outlined by Blanford et al. in 1991 (9). For each case, data on the NIHSS, MRS, carotid Doppler ultrasonography, laboratory results, comorbid conditions, and demographic details were collected. The MRS, which ranges from 0 to 6, is used to assess the level of dependency and disability in stroke patients during their daily activities.

A negative functional outcome was linked to MRS≥ 3, while a favorable functional outcome was linked to MRS <3. The study assessed some variables, such as demographics, the presence of comorbid conditions like diabetes mellitus (DM) and hypertension (HT), atrial fibrillation (AF), heart valve disease, history of stroke, heart failure, serum lipid and cholesterol levels and coronary artery disease (CAD). The study also evaluated the impact of medications, including antihypertensives, acetylsalicylic acid, clopidogrel, antilipidemic agents, and insulin, alongside treatments administered during hospitalization, such as intravenous (IV) and intra-arterial (IA) thrombolytics (tissue plasminogen activator, TPA) and thrombectomy. Furthermore, it analyzed parameters such as the neutrophil-to-lymphocyte ratio (NLR), admission glucose, fibrinogen, and creatinine levels, as well as neurological NIHSS scores at admission and discharge (or mortality) and the MRS (Table 1).

2.1. Statistical Evaluation

The data in the study were analyzed using SPSS version 27.0 (Statistical Package for the Social Sciences). Categorical variables were presented as frequencies and percentages, whereas continuous variables were summarized using the mean and standard deviation, along with the median, minimum, and maximum values when applicable. To ascertain whether the parameters were normally distributed, the Shapiro-Wilk test was employed. For non-normally distributed parameters, the Shapiro-Wilk Test determines whether a collection of data fits a normal distribution. It is particularly useful for small samples. The assumption of normal distribution is rejected and if the p-value is less than 0.05, it is accepted that the data are not normally distributed (10).

The Mann-Whitney U test was employed for pairwise group analysis. This nonparametric test is designed to compare two independent groups that do not follow a normal distribution. It evaluates whether there is a significant difference in the ranks between the two groups. Serving as a nonparametric alternative to the independent t-test, it is particularly suitable for data that does not meet the assumption of normality (11).

Spearman's rho correlation test was used to determine the relationship between continuous measures. The Spearman's Rho Correlation Test quantifies the degree and direction of a monotonic relationship—one that is rigorously non-linear—between two variables but if the data are ordinal or not normally distributed. A significant positive or negative association is indicated by a number around +1 or -1, whilst no relationship is indicated by a value near 0 (12).

Multivariate linear regression analysis was employed to investigate the factors influencing changes (Δ) in NIHSS scores. For all statistical tests, a significance level of $p<0.05$ was used. This analytical technique extends the principles of linear regression by modeling the relationships between multiple dependent variables and one or more independent variables. It is especially beneficial in situations where the response variables are correlated, providing a more detailed understanding of the data structure compared to univariate regression, which is limited to analyzing a single response variable (13).

The link between a dependent variable (outcome) and several independent variables (predictors) is modeled using multivariate linear regression. In this instance, the variables influencing the shift in the NIHSS score are investigated. Understanding how the predictor variables—such as age, therapy, etc.—affect the result while accounting for the impact of other variables is the goal.

Scientific Significance (p-value < 0.05). In these tests, a p-value below 0.05 is accepted as statistically significant. In other words, the observed outcome is unlikely to have happened by accident, and the null hypothesis—that is, the idea that there is no difference between the two groups is firmly rejected.

Table 1. Clinical and Demographic Characteristics on Admission to Hospital

	Numbers (n)	Percent (%)
Posterior infarct	59	37,1
Anterior infarct	66	41,5
Laküner infarct	34	21,4
Age		
≥ 45	11	6,9
<45	148	93,1
Gender		
Female	82	51,6
Male	77	48,4
Hypertension	121	76,1
Diabetes Mellitus type 2	59	37,1
Insulin Use	25	15,7
Atrial fibrilation	28	17,6
Valve disease	36	22,6
Heart failure	20	12,6
Myocardial infarction	18	11,3
Bypass	14	8,8
Cigarette	53	33,3
Alcohol	11	6,9
Coronary artery disease	44	27,7
Previous CVD	26	16,4
iv-tPA	11	6,9
ia-tPA	7	4,4
Thrombectomy	6	3,8
Hemorrhagic infarct	10	6,3
Substance use	2	1,3
Carotid artery stenosis		
<%50	98	61,6
>%50	61	38,4
Vertebral artery stenosis	19	11,9
Use of antilipidemic drugs	10	6,3
Acetyl salicylic acid-clopidogrel use	49	30,8
Use of antihypertensive drugs	83	52,2
NOACs use	6	3,8
Coumadin use	12	7,5
	Avarage±Sd	**Med**
Age	68,1±14,2	70
EF	53,9±8,9	55
HDL	37,2±11,8	36
LDL	118,8±38,5	117,2
Triglyceride	163,7±89,4	142
Fibrinogen	382,2±96,7	374
N/L	5,52±4,5	4,24
N/Platelet	0,034±0,02	0,03
Glucose level	177±104,5	138
Average blood pressure	108,1±22,9	103,4
INR	1,14±0,21	1,10
Creatine Level	1,03±0,7	0,89
NIHSS at hospital admission NIHSS	10,4±4,7	10
NIHSS on discharge from hospital	8,06±6,3	8
Delta(Δ) NIHSS	2,35±3,7	2
Output mRS	3,21±1,9	3

N:neutrophil L: lymphocyte NOAC: New Oral Anti Coagulant INR: International Normalized Ratio EF: ejection fraction HDL: high-density lipoprotein LDL: low-density lipoprotein

3. Results

The study included 77 (48.4%) male and 82 (51.6%) female patients. The patients' average age was 68.1±14.2 years, and 93.1% of them were older than 45. Of the patients, 34 (21.4%) experienced a lacunar stroke, 59% (37.1%) experienced a posterior circulation stroke, and 66 (41.5%) experienced an anterior circulation stroke. Previous studies suggest that higher NLR and NPR levels recorded at initial admission in acute ischemic stroke cases are closely linked to worse functional outcomes and higher mortality rates, highlighting their significance as prognostic biomarkers. This research utilized both univariate and multivariate analyses to determine the factors influencing changes in patients' NIHSS scores between admission and discharge (Δ NIHSS).

When factors affecting (Δ) NIHSS are examined in univariate analysis; The presence of hypertension, myocardial infarction, coronary artery, and carotid disease, previous insulin use, iv-tPA, ia-tPA, and thrombectomy treatments, age, NLR, mean blood pressure and output MRS were associated ($p<0.05$) (Table 2).

Table 2. Factors affecting delta(Δ)NIHSS; univariate and multivariate analyses

	Univariate	Multivariate			
			%95 CI		
	p	β	Lowest	Highest	p
Posterior infarct	0,195				
Anterior infarct	0,471				
Laküner infarct	0,447				
Gender					
Female	0,058				
Male					
Hypertension	**0,022***	-0,681	-1,817	0,456	0,238
Diabetes Mellitus type 2	0,598				
Insulin use	**0,032***	-0,287	-1,539	0,966	0,652
AF	0,103				
Valve disease	0,308				
Heart failure	0,851				
MI	**0,009****	-1,053	-2,745	0,639	0,221
Bypass	0,656				
Cigarette	0,961				
Alcohol	0,072				
CAD	**0,049***	1,429	0,221	2,636	**0,021***
Previous CVD	0,395				
iv-tPA	**<0,001****	2,925	0,644	5,205	**0,012***
iatPA	**0,004****	1,768	-4,003	7,538	0,546
Thrombectomy	**0,016***	-0,273	-6,035	5,489	0,925
Hemorrhagic infarct	0,201				
Substance use	0,859				
Carotid artery stenosis					
<%50	**0,040***	-0,160	-1,150	0,829	0,749
>%50					
Vertebral artery stenosis	0,059				
Use of antilipidemic drug	0,109				
Use of Asa -clopidogrel	0,930				
Use of antihypertensive drugs	0,293				
NOACs usage	0,409				
Warfarine usage	0,159				
Age	**0,003****	-0,026	-0,061	0,010	0,153
EF	0,287				
HDL	0,157				
LDL	0,269				
Triglyceride	0,731				
Fibrinogen	0,486				
N/L	**0,006****	-0,070	-0,171	0,031	0,174
N/Platelet	0,063				
Glucose level	0,886				
Average Blood Pressure	**0,009****	-0,010	-0,030	0,011	0,354
INR	0,919				
Creatinine level	0,349				
Output mRS	**<0,001****	-1,091	-1,344	-0,838	**<0,001****

*p<0,05, *p<0,01, Delta(Δ)NIHSS: Difference between Input NIHSS and Output NIHSS
AF: atrial fibrillation MI: myocardial infarction CAD: coronary artery disease CVD:cerebrovascular disease NOACs: New oral anticoagulants asa: acetyl salicylic acid INR: International Normalized Ratio EF: ejection fraction HDL: high-density lipoprotein LDL: low-density lipoprotein N:neutrophil L: lymphocyte

A negative Delta (Δ)NIHSS value indicates that the NIHSS value increased at the end of the treatment, whereas a positive (Δ) NIHSS value indicates that the NIHSS value decreased at the end of the treatment.

When the factors affecting (Δ) NIHSS were analyzed in the univariate analysis, it was found that there was a significant relationship between HT, Insulin use, MI, CAD, iv-tPA, iatPA, Thrombectomy, Core disease, Age, N/L, Mean BP and Outcome MRS values ($p<0.05$). The model outlined in Table 2 incorporates the parameters identified as significant during the univariate analysis into the subsequent multivariate analysis. Multivariate analysis revealed a positive correlation between (Δ) NIHSS value and CAD and iv-tPA values and a negative correlation with Out MRS value ($p=0.021$; $p=0.012$ and $p<0.001$ respectively).

A multivariate analysis model was developed by incorporating the parameters identified as significant through the univariate analysis. As a result of multivariate analysis, it was determined that (Δ) NIHSS value had a positive relationship with coronary artery disease and the use of iv-tPA in treatment, and a negative relationship with the output MRS value ($p=0.021$; $p=0.012$; $p<0.001$, respectively), (Table 2). Accordingly, while a positive functional result (high (Δ) NIHSS) was detected in those with a history of concomitant coronary artery disease and those receiving IV-TPA treatment, a negative functional result (negative) was detected in those with high MRS or low (Δ) NIHSS. Our model provides consistent results for the relationship between NIHSS and MRS scores and positive and negative functional outcomes.

4. Discussion

According to data from the Global Burden of Disease, Injuries, and Risk Factors Study (GBD) spanning 1990 to 2019, stroke ranks as the second leading cause of death worldwide (14). Similarly, World Health Organization statistics for 2021 identify stroke as the leading cause of death after ischemic heart disease and COVID-19 (13). The organization's database reports that approximately 15 million people globally suffer a stroke each year, with one-third dying from it and another third experiencing long-term disabilities. This imposes significant social, psychological, and economic challenges on societies (15). GBD stroke project data predicts that between 2020 and 2050, stroke-related mortality and disability, as well as the economic burden on societies, will approximately double (16,17). Many risk factors cause stroke and most of these are modifiable causes such as hypertension, hyperlipidemia, and high blood sugar (18,19). Therefore, preventive medicine remains the most important pillar of treatment. Governments need to know the common stroke risk factors in their communities and why these risk factors are not being rehabilitated, inform physicians and citizens, and put forward feasible health programs. Another important issue is that physicians should know the main factor or factors that cause stroke in their patients as well as the other players that contribute to the disease. Because main and secondary factors will affect the prognosis. The incidence of stroke tends to increase especially in individuals younger than 55 years of age, which we will define as young stroke (16,17). In addition, the prevalence of metabolic syndrome and its components such as high blood pressure, overweight, and diabetes are increasing. Many different components such as processed food consumption, environmental factors, increased stress factors in living conditions, and epigenetic causes play a role in this. In our study, hypertension, diabetes, and cardiac pathologies were identified as the most important risk factors. In addition, it was determined that the neutrophil/lymphocyte ratio, which was calculated by considering the hemogram data of the patient during hospitalization, could be used as a prognostic indicator in our study as in many studies (20). When we looked at the treatment modalities applied after stroke, it was noteworthy that intravenous thrombolytic therapy and thrombectomy positively affected the prognosis and thus MRS. This once again highlights the importance of time of onset, patient assessment, and access to neuroimaging modalities in ischemic stroke. The data from our study are important to inform adequate healthcare planning, resource allocation, and prioritization for stroke, and to assess the success or failure of measures to reduce the burden of stroke. In medicine, many disease-specific scales have been developed to assess the severity of the disease and the current condition of the patient. NIHSS and MRS scales are only two of them (21,22). In our study, we statistically evaluated the effect of stroke risk factors and lesion localizations caused by stroke on the NIHSS scale and tried to reveal the effectiveness of medical treatments applied to the patient at the discharge stage with the MRS scale in addition to vascular risk factors. The information obtained guided the toolbar, which is being developed by us to determine the prognosis in stroke cases. In addition, it has contributed to the creation of mathematical models by setting targets that will reveal the strengths and weaknesses of the treatments applied in comorbid conditions accompanying stroke. In conclusion, the importance of early diagnosis and personalized treatment of stroke is undeniable. In solving this problem, health and engineering sciences should produce more joint projects and offer different perspectives on the subject.

Conflict of interest

The authors declare that there are no conflicts of interest related to this manuscript. No financial relationships, personal connections, or any other affiliations that could be perceived as influencing the objectivity or integrity of this research exist. All authors have contributed to the study's design, data collection, analysis, and writing, and the results presented are solely based on the scientific data without any external bias. The authors further confirm that there are no competing interests that could have influenced the results or interpretation of the findings.

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Ethical statement

Adana City Training and Research Hospital Scientific Ethics Committee (312/9) permission was obtained for the cross-sectional study on stroke.

Authors' contributions

Concept: G.G.K., M.Z.B., P.B.B., Design: G.G.K., M.Z.B., P.B.B., Data Collection or Processing: P.B.B., Analysis or Interpretation: G.G.K., M.Z.B., Literature Search: G.G.K., M.Z.B., P.B.B., Writing: G.G.K., M.Z.B., P.B.B.

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Review

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The oncogenic role of Holliday junction recognition protein in hepatocellular carcinoma

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Abstract

The relationship between centromere dysfunction leading to numerical chromosomal changes and cancer is well-known. Holliday Junction Recognition Protein (HJURP) is a chaperone of the centromere-specific protein, Centromere Protein A (CENP-A), and is considered a critical factor in determining centromere identity. There are researches showing that CENP-A is overexpressed in many types of human cancer. However, the role and dynamics of HJURP in tumorigenesis have only recently been clarified. In this review, the connection between HJURP and liver cancer, as well as the role of HJURP in cancer development, has been summarized.

Keywords: CENP-A, Chaperone, HJURP, hepatocellular carcinoma, liver cancer, prognosis

1. Introduction

Today, liver cancer remains a significant global health concern due to its high incidence and mortality rates. Cirrhosis, chronic liver disease, viral hepatitis and excessive alcohol consumption are the main risk factors for liver cancer (1-3). Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer, representing around 75-90% of all cases. Smoking, type 2 diabetes, viruses, aflatoxin-contaminated food products, excessive alcohol consumption, and obesity are among the primary risk factors for HCC (Figure 1) (3).

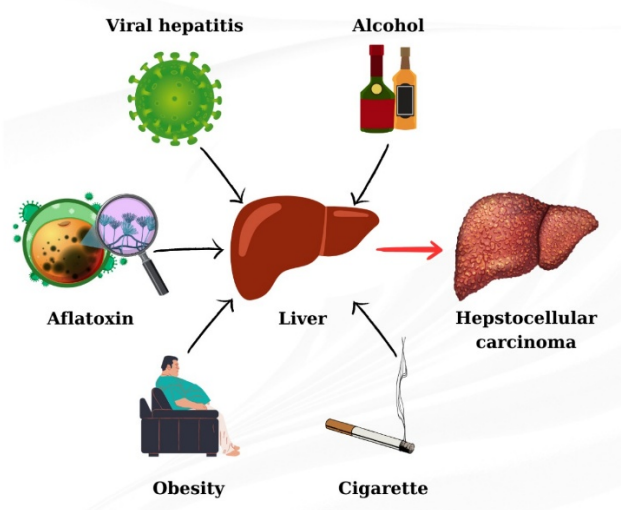

Fig.1. Various risk factors contributing to hepatocellular carcinoma development

Despite various treatment approaches, the general 5-year survival rate observed in patients with liver cancer, due to delayed diagnosis and advanced stages, does not exceed 20%. Surgery is the primary treatment option for early-stage HCC. Liver transplantation can also be performed in patients who are not suitable for surgical resection and have liver dysfunction. The ablation therapy is also considered for the patients with early-diagnosed HCC (4). Due to its anti-proliferative and anti-angiogenic effects, sorafenib, recognized for its role as a potent tyrosine kinase inhibitor (TKI), is used as a long-term first-line treatment in patients with advanced HCC or cases that have relapsed and show poor progression after regional therapy. A distinct kinase inhibitor, lenvatinib, is noted for its reduced incidence of hand-foot skin reactions when compared to sorafenib, while it has an increased incidence of hypertension, proteinuria, and anorexia (5). Ramucirumab, a vascular endothelial growth factor receptor 2 (VEGFR-2) antagonist, represents the first application of biomarker-based therapy for advanced patients and serves as a highly successful second-line treatment option demonstrating favorable responses. Likewise nivolumab, a humanized anti- programmed cell death protein 1 (PD-1) monoclonal antibody and an immune checkpoint inhibitor, demonstrates promising potential for treating patients with advanced HCC. Durvalumab, another inhibitor developed in recent years, is a programmed death-ligand 1 (PD-L1) monoclonal antibody that works by enabling the

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recognition of cancer cells by the immune system and stands out with its clinical phase study results. Positive results of the clinical phase I/II study of durvalumab and tremelimumab combined treatment have been reported (5, 6).

Chromosomal instability (CIN) is the most frequently encountered pathology in HCC cells. Additionally, various mechanisms, including errors in chromosomal segregation, defects in DNA repair processes, and the inhibition of the tumor suppressor gene *p53* are correlated with HCC (7). The segregation errors occurring during mitotic division result in cells characterized by an abnormal chromosome count, known as aneuploidy, which is largely associated with cancer (8). The errors that occur during cell division result in the activation of p53. Activation of p53 occurs following inappropriate chromosome segregation and leads to cell cycle arrest, senescence or apoptosis. Therefore, p53 depletion often contributes to aneuploidy in cancers (8, 9). P53, which is crucial for preserving genomic stability, serves as the primary checkpoint during cell division, inducing cell cycle arrest upon detecting DNA damage and promoting apoptosis in the event that the issue is unresolved. In addition, it is known that *p53*, one of the most frequently mutated tumor suppressor genes in cancers, has a strong relationship with HCC pathogenesis and is one of the most frequently mutated genes (10-12).

One of the critical points in ensuring chromosomal stability is the centromere. The centromere functions for kinetochore construction, kinetochore-microtubule connection, and the spindle assembly checkpoint (SAC). As detailed in the following sections, Constitutive Centromere Associated Network (CCAN) proteins, such as CENP-C and CENP-N, and the CENP-A nucleosome form a complex to bring together the kinetochore and kinetochore-associated proteins to ensure normal segregation of chromosomes (13). The histone H3 variant specific to the centromere, known as CENP-A, is found to be overexpressed in aggressive cancer cells (14, 15). Additionally, the relationship between the levels associated with the centromeric chaperone Holliday Junction Recognition Protein (HJURP) and CENP-A has shown that HJURP is essential for the localization of CENP-A at centromeres and serves a crucial function in the completion of CENP-A nucleosome formation (16, 17). The high expression of CENP-A and its chaperone HJURP correlates with one another and results in cancer prognosis in cells lacking p53. The accumulation of CENP-A causes mitotic errors, loss of centromere function, and CIN, characteristic features of cancer (18).

Despite the availability of various alternative therapeutics, the expected outcome and survival rates in patients with HCC are not very high. Therefore, identifying the biomarkers to facilitate early diagnosis and investigation of the new target molecules remains important for achieving better outcomes in HCC patients. Since genomic instability is a significant parameter driving the formation and advancement of HCC, elucidating the mechanisms that trigger genomic instability is considered crucial for identifying new biomarkers and target molecule s. Consequently, there is a significant need to identify new proteins with low or absent expression in normal liver cells but elevated expression in HCC as diagnostic markers. Furthermore, developing new treatment strategies by targeting these proteins is possible.

In this review, we concentrated on the potential for CENP-A and its chaperone HJURP, that play a key role during mitotic segregation, as diagnostic, prognostic, and therapeutic cancer markers in liver cancer.

2. Nucleosome Formation and Histone Chaperones

Eukaryotes maintain their genomes through a densely packed nucleoprotein complex called chromatin within the cell nucleus (19, 20). Nucleosomes, the fundamental units of tightly organized chromatin, are formed by wrapping a ~147 base pair DNA sequence in the vicinity ofa histone octamer, which includes an H3-H4 histone heterotetramer surrounded by H2A-H2B histone heterodimers on both sides. This formation is completed by incorporating linker DNA and a linker histone (21, 22). The formation of the nucleosome complex is a extremely complex process that involves the coordinated action of many proteins within the cell. Central to nucleosome formation are ATP-dependent chromatin remodeling complexes and ATP-independent histone chaperones (23). The remodeling of chromatin structure is necessary to ensure the continuity of eukaryotic cells. Nucleosome formations occur repeatedly in various processes, such as DNA replication. Following DNA replication, ancestral histones together with newly synthesized histones ensure the formation of replication-dependent nucleosomes, while replication-independent nucleosome formation occurs during gene transcription (24, 25).

Histone proteins have a positive charge, making them prone to easily bind to negatively charged DNA. Nevertheless, they also possess the ability to interact undesirably with all nucleic acids and various cellular components (26). It is recognized that histones precipitate when mixed with DNA in solution at physiological ionic strength, provided that proteins known as chaperones are absent. First described by Laskey et al. as nuclear proteins that inhibit improper interactions between histones and DNA in frog oocyte extracts (27), chaperones ensure the proper folding of histones and prevent their positive charges from engaging in nonspecific interactions (28, 29). Histone chaperones are a family of histone-binding proteins that maintain non-nucleosomal histone-DNA interactions. They separate core histones from DNA until a proper nucleosomal arrangement is achieved and, together with ATP-dependent chromatin remodelers, reshape nucleosomes to resolve chromatin structure and provide accessible DNA templates for cellular processes (19). Generally, histone chaperones are a highly conserved family of proteins participating in chromatin-linked cellular processes, including

histone and nucleosome biosynthesis/biodegradation, remodeling, central dogma mechanism, and DNA repair. Unlike ATP-dependent chromatin remodeling complexes that interact with DNA, chaperones function as histone-binding proteins. Depending on their selectivity for targeted histones, they can have broad functions in many biological processes central to chromatin structure, including the eukaryotic FACT (facilitates chromatin transcription) complex, or very specific, limited functions, such as Scm3 in yeast and HJURP in humans, which facilitate the formation or maintenance of centromeric chromatin (26). Histone chaperones, classified according to the histone substrates they bind to, are often categorized as H3–H4 or H2A–H2B chaperones based on their binding to H3-H4 or H2A-H2B oligomers. Some, such as FACT, are known to bind to both hetero-oligomers with dissimilar domains. A few histone chaperones can bind to specific histones (canonical or variant) alone, and this binding pattern often contributes to the chaperone's localization and/or functions (30, 31). They perform different functions at various stages of nucleosome formation. Initially, histone proteins are produced in the cytosol and subsequently transferred to the nucleus for nucleosome assembly. Certain histone chaperones, like Nap1, facilitate this transport by partially regulating the importin-histone interaction. Second, during stress conditions, a soluble histone pool must be maintained continuously, and some histone chaperones, like nuclear autoantigenic sperm protein (NASP), act as a histone reservoir and respond to histone demand. Histone chaperones and histone-binding proteins including RbAp46 and Asf1 ensure the continuity of interactions between histones and histone-modifying enzymes by directly regulating the enzymatic activity of these enzymes. Finally, histone chaperones are immediately involved in the deposition of histones onto DNA for nucleosome formation (31). They also act as necessary regulators of chromatin structure and function, often being misregulated in cancer, with significant effects on tumor growth and survival rates (32).

Both genetic and epigenetic changes contribute to cancer pathogenesis. Research indicates that histone associated proteins, effector proteins, and chromatin remodelers play a role in the initiation and advancement of cancer (33). The centromeric nucleosomes possess a kinetochore, the region where chromosomes attach to spindle microtubules during mitotic division. The seamless transfer of genetic material to daughter cells during cell division is achieved by the specific binding of chromosomes to spindle microtubules (34). The centromeres and kinetochores play a critical role in the separation of chromatids that make up the chromosomes during division. Consequently, the errors in centromere and/or kinetochore formation lead to various chromosomal aberrations in the form of chromosomal gains and losses (aneuploidy) and are the primary course of chromosomal instability observed in cancer cells (35-37). The centromeres and kinetochores, together with centromeric chromatin, consist of inner and outer kinetochore structures. The structural core component for centromeric chromatin and kinetochore formation is the histone H3 variant CENP-A. The assembly of CENP-A depends on the HJURP chaperone brought to the centromere by the MIS18 complex. This assembly also requires several CCAN components, such as CENP-C, CENP-H/-I/-K, and CENP-N/-L/-M complexes. Errors associated with CENP-A result in chromosome segregation defects and aneuploidy. Notably, high expression of CENP-A, HJURP, and certain centromeric proteins has been linked with poor prognosis in some cancers, such as liver cancer (38-40).

3. CENP-A Nucleosome and Hepatocellular Carcinoma

During cell division, the centromere, which is responsible in the accurate transmission of the chromosome set to daughter cells, functions with a complex called the kinetochore. This complex assembles centromeric DNA and consists of over 90 proteins, ensuring proper attachment of spindle fibers to the chromosome (41, 42). Loss of centromere structure and/or function leads to chromosome segregation errors, which often result in the formation of micronuclei and aneuploidies linked with the presence of abnormal chromosomes in the cell. Consequently, the accumulation of these errors causes chromosomal instability in cells, leading to cancer (18, 43).

The centromere consists of two regions: the core centromeric chromatin and the pericentric heterochromatin. In the course of the cell cycle, the centromere is formed by a protein complex known as CCAN, which includes 16 centromere proteins (e.g., CENP-C, CENP-H, CENP-I, CENP-K, CENP-U, CENP-W, and CENP-X) (44). The arrangements of the kinetochore structure during mitosis is thought to involve the CCAN proteins (44). The formation of kinetochore at the centromere during cell division and the attachment of spindle fibers to the kinetochore are important for a healthy cell cycle. The formation of the kinetochore complex at the centromere, which has a special importance for the occurrence of a normal mitotic phase, is determined by the localization of the histone H3 variant CENP-A, and CENP-A stands out as an important protein that confers epigenetic identity to the centromere (39, 45). In addition, CENP-A nucleosomes are centromere-specific, distinguishing them from other H3 nucleosomes found in chromatins (44).

CENP-A and other proteins interact with spindle microtubules to form a network with chromatin, bridging the centromeric chromatin and the mitotic kinetochore (46, 47). The position of centromeres on chromosomes should be maintained through cell generations, making the retention of CENP-A in centromeric chromatin essential. Indeed, CENP-A levels at centromeres are stable across numerous cell divisions (39). Loss of CENP-A, which is necessary for the localization of all kinetochore components, leads to disruptions in kinetochore function, improper chromosome segregation, and subsequent impairments in cell viability and function. Therefore, the continuity of centromere characteristics and function is proportional to the presence of CENP-A

nucleosomes on each chromosome (42). Additionally, overexpression of CENP-A, leading to mislocalization to non-centromeric regions, has the potential to form ectopic kinetochores or weaken normal kinetochores, causing chromosomal segregation errors and genomic instability (45).

Cancer requires a process involving the accumulation of genetic mutations, such as chromosomal translocations or aneuploidy, which lead to structural rearrangements in genes or imbalances in gene dosage (48). Cancer cells derived from solid tumors exhibit chromosomal instability and aneuploidy linked with aggressive tumor behavior and adverse prognosis (48, 49). The situation is similar in liver cancer, where HCC cells carry abnormal chromosomes with various genetic rearrangements such as translocations, deletions, and gene amplifications (50). Increased chromosomal instability induced by irregularities in mitotic control mechanisms leads to aneuploidy, which is much more common compared to other oncogenic or tumor suppressor mutations in cancer (51). It has been reported that the changes in chromosomes are observed in about 90% of solid tumors (52), and the chromosome loss or gain can lead to the development of treatment resistance in cancer cells (https://www.cancer.gov/). The formation of aneuploidy can result in the simultaneous occurrence of multiple genetic changes necessary for both tumor initiation and progression (53, 54). The mutations in the mitotic control genes and overexpression of these gene products are frequently observed in CIN-related cancers. The irregular activity, particularly high expression of mitotic control genes such as *CENP-A/E*, is known to lead to chromosomal aberrations, aneuploidy, and rearrangements in HCC cells (50).

CENP-A, one of the first identified components of the kinetochore in humans, which has an important role in mitotic regulation, is a centromere-specific protein of 17 kDa encoded by the *CENPA* gene. It regulates the kinetochore formation and establishes centromere identity through epigenetic mechanisms during mitosis and meiosis. It is also necessary for the localization of all other centromere and kinetochore components (53). The mutations in or knockouts of CENP-A, which have a primary role in mitotic division and normal chromosome segregation, cause chromosome missegregation (53).

The evidence so far indicates that CENP-A and other centromeric proteins are commonly overexpressed in cancers, and that this protein excess is linked with the formation of aneuploidy, a characteristic of tumour cells (15). High levels of CENP-A have been noted in various cancers, including colorectal (53), hepatocellular (15), lung (55, 56), prostate (57), ovarian (58), and breast cancers (59). In human hepatocellular carcinoma, CENP-A mRNA expression is substantially higher in immortalized HepG2 cell lines compared to SMMC-7721 cells, and overexpression of CENP-A is also observed in primary tumor tissues. CENP-A levels correlate with histological grade progression in patients, and a significant relationship has been observed between CENP-A and P53 protein levels. Furthermore, siRNA-mediated inhibition of overexpressed CENP-A in HepG2 cells has reversed cancerous properties (15, 60). Data obtained from bioinformatic analyses revealed that CENP-A overexpression is associated with poor prognostic features such as poor survival, late-stage tumor and tumor size, and vascular invasion in HCC (61). It has been suggested that CENP-A functions as a transcriptional regulator with Yin Yang 1 (YY1) in the pathogenesis of HCC and stimulates HCC. YY1, which is composed of a transcriptional activation domain, transcriptional repression domain, spacer domain and DNA-binding domain, binds to CENP-A via a zinc finger region. The fact that YY1 is a part of the GL-Kruppel family of zinc finger DNA binding proteins that can differentially regulate gene expression as a transcriptional activator and repressor, and the demonstration of its interaction with CENP-A, reveals the function of CENP-A as a transcriptional regulator in the pathogenesis of HCC. It has also been suggested that lactylation of CENP-A ubiquitylation on lysine 124 (K124) facilitates HCC tumor progression by stimulating the transcriptional activation of CENP-A (62). Data have been provided that CENP-A may suppress cell ferroptosis and enhance tumor progression in HCC by inducing the transcription of stathmin1 (STMN1), a cytoplasmic phosphorylated protein that regulates the cell cytoskeleton in HCC pathogenesis. (63). It has also been reported that the COOH-terminal deletion of hepatitis B virus X protein (HBx), which is linked with hepatocellular carcinoma, is positively correlated with CENP-A expression, may indirectly increase CENP-A expression and may be effective on tumour progression in HCC (64).

4. CENP-A Chaperone Holliday Junction Recognition Protein (HJURP) and Hepatocellular Carcinoma

In mammalian cells, the dynamics of CENP-A are closely linked with cell cycle progression. HJURP is recognized as the chaperone for CENP-A, based on structural differences in H3 variants that particularly recognize CENP-A (65). HJURP is crucial for the accumulation of CENP-A at human centromeres during the late mitosis/early G1 phase of the cell cycle in a CDK-dependent manner (32). It facilitates the incorporation of CENP-C at the centromere, aiding in the assembly of functional kinetochores, which mediate cell division and chromosome segregation (66).

While HJURP is known to regulate the cell cycle, its regulatory mechanism is considered more complex than merely managing cell cycle progression. Various proteins that affect HJURP function, as well as downstream proteins regulated by HJURP, have been reported to interact with HJURP. The most prominent molecule regulated by HJURP is the histone H3 variant CENP-A. The collaboration between CENP-A and its chaperone HJURP is crucial for normal cell cycle progression, whereas ectopic activation of HJURP is associated with chromosomal instability and immortality in

cancer cells (66).

In *Saccharomyces cerevisiae*, overexpressions of Scm3p and HJURP have been linked to chromosomal loss phenotypes. The studies involving GFP-tagged HJURP in transfected human HeLa cancer cell lines have observed increased HJURP expression leading to nuclei with micronuclei or delayed chromosomes compared to controls. These findings indicate that improper regulation of HJURP results in defects in kinetochore function and chromosomal instability in human cells (67).

Cancer cells exhibit high levels of CIN, characterized by frequent chromosomal segregation errors leading to aneuploidy (48). This relationship has been validated in studies on solid tumors, confirming the high expression of HJURP in cancer cells. Research by Tatsuya Kato and colleagues identified HJURP as a newly overexpressed gene in non-small cell lung cancer (NSCLC) in comparison with normal lung tissues through cDNA microarray analysis (68). HJURP is similarly overexpressed in various cancers, as seen with other histone chaperones. A 2020 study on colorectal cancer found that HJURP acts as an oncogene and may serve as a potential prognostic biomarker and therapeutic target. Inhibition of HJURP with siRNA suppressed cancer cell proliferation, migration, invasion, and tumor formation (69). In liver cancer, HJURP expression is significantly increased compared to healthy tissues and could be a biomarker of poor prognosis (66). Overexpression and mislocalization of HJURP have also been noted in lung cancer cell lines (68). It has been proposed that HJURP expression levels are linked with radiation therapy response, making it a prognostic factor for disease-free and general survival and a predictive biomarker for radiation sensitivity (40). The effect of HJURP on cancer mechanisms has started to emerge in recent years, though studies are still limited. The expression levels of HJURP protein in various cancer cells and their association with tumor behavior are summarized in **Table 1**. For all cancers examined, high levels of HJURP have shown a strong association with poor prognosis. Additionally, HJURP may contribute to restoring DNA double-strand breaks, potentially increasing resistance to genotoxic agents (70).

Table 1. Expression levels of HJURP protein in various cancer and tumor behavior

Cancer Type	Expression Level	Association with Tumor Behavior	Related Pathway	Reference
Liver	High Expression	High HJURP expression is associated with poor prognosis.	p21 ubiquitination via MAPK/ERK1/2 and AKT/GSK3β pathways	(66, 75)
Breast	High Expression	HJURP mRNA level is a prognostic factor for disease-free and overall survival in breast cancer patients and a predictive biomarker for radiosensitivity.	-	(33, 40)
Pancreas	High Expression	Patients with high HJURP levels have significantly worse survival rates compared to those with low HJURP levels.	Regulation of MDM2 expression via H3K4me2 dimethylation.	(32)
Colorectal	High and Low Expression	High HJURP expression significantly reduces cancer-specific survival rates compared to low HJURP expression.	-	(69)
Bladder	High Expression	The prognostic relationship is not specified.	Regulation of ROS metabolism and cell cycle via PPARγ-SIRT1 feedback loop	(77)
Lung	High Expression	HJURP expression is associated with the progression and metastasis of NSCLC.	Activation of the Wnt/β-catenin pathway	(78)
Glioma	High Expression	HJURP levels are related to patient prognosis.	-	(79, 80)
Ovarian	High and Low Expression	High HJURP expression levels are significantly associated with lymph node metastases and lower overall survival.	-	(81)
Prostate	High Expression	HJURP levels may be associated with patient prognosis.	-	(82)
Renal Cell Carcinoma	Low Expression	HJURP expression may be associated with poor prognosis in RCC patients.	Regulation of cell apoptosis via PPARγ/SIRT1	(83)

Determining HJURP expression levels is important not only for classifying high-risk patients but also for selecting suitable candidates for radiotherapy. To date, little is known about the function of HJURP expression in human cancer, and there is still no specific HJURP inhibitor or treatment that can block its role in cancer cells. The high correlation of HJURP with cancer highlights the potential of HJURP inhibitors for future therapeutic applications (81).

Although the relationship between HJURP and cancer mechanism has been revealed, there are a limited number of research investigating its potential oncogenic role in the emergence of HCC (summarized in **Table 2**). In hepatocellular carcinoma tissues from 164 liver cancer patients, higher HJURP expression was observed, particularly in tumor tissues larger than 5 cm. This underscores HJURP's role in supporting HCC cell proliferation and also shows that patients with elevated HJURP expression have poorer survival rates contrasted to those with low expression (66). In HBV-related HCC patients, the non-synonymous SNP in exon 8 of the HJURP gene (rs3771333) was significantly linked with the

onset of HCC. Individuals carrying the rs3771333 C allele (A/C or C/C genotypes) have a higher risk of HCC development contrasted to those with the A/A genotype (73). Genome integrity is regulated through the collaboration of cell cycle checkpoints and DNA repair systems. Disruptions in the genes responsible for regulating genome integrity contribute to genomic instability, premature aging, and cancer susceptibility (84). The hepatocarcinogenesis process, like other carcinogenesis processes, is quite complex and includes heterogeneous mechanisms involving abnormalities in multiple signaling pathways. However, similar to many other carcinogenic processes, deregulation of the cell cycle is commonly observed in liver cancers (85, 86). The cell cycle is closely controlled by cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors (CKIs), and the retinoblastoma protein family (pRb) (87). Key regulators of the cell cycle include cyclin-dependent kinase inhibitors (CDKIs) such as p21, p27, and p16, which are known as tumor suppressors (88). p21 is known to suppress tumors by inducing cell cycle arrest in response to various stimuli. It has also been shown to act as a major effector in many tumor suppressor pathways, causing anti-proliferative effects independently of the p53 tumor suppressor mechanism. However, recent research suggests that under certain conditions, p21 can induce cellular proliferation and exhibit oncogenic effects. These data suggest that p21 is dysregulated in human cancers but can act as either a tumor suppressor or an oncogene depending on cellular conditions (89). HJURP inhibition has been described to induce cell cycle arrest in the G0/G1 phase in HCC cells without inducing apoptosis, indicating that HJURP could be an important regulator in the cell cycle. Chen et al. are showing that HJURP suppresses p21 expression in HCC cells and alters p21 stability through MAPK/ERK1/2 and AKT/GSK3β signaling pathways. It is known that SKP2, CDT2, LRR1, and CDC20 E3 ligases are responsible for p21 degradation. The research has shown that HJURP is significantly associated with SKP2, but not with LRR, CDT2, or CDC20, and that HJURP supports ubiquitination-mediated p21 degradation (75).

Table 2. The role of HJURP protein in hepatocellular carcinoma and its relationship with prognosis

Cancer Type	Expression Level	Association with Tumor Behavior	Related Pathway	Reference
Hepatocellular carcinoma	High Expression	Hypomethylation-induced overexpression of the HJURP promoter is inversely associated with survival; a potential prognostic biomarker and therapeutic target	DNA hypomethylation.	(71)
Hepatocellular carcinoma	High Expression	HJURP is significantly associated with the immunosuppressive tumor microenvironment, T cells, dendritic cells and B cells in hepatocellular carcinoma and has potential in determining prognosis.	Immune system related pathway	(72)
Hepatocellular carcinoma	Single nucleotide polymorphism	A non-synonymous SNP rs3771333 in exon 8 of the HJURP gene is significantly associated with the onset of hepatocellular carcinoma.	Single nucleotide polymorphism	(73)
Hepatocellular carcinoma	High Expression	HJURP and ASF1A histone chaperones are more effective in determining the prognosis of HCC patients with the two-gene model, rather than alone.	-	(74)
Hepatocellular carcinoma	High Expression	HJURP deregulated p21 via MAPK/ERK1/2 and AKT/GSK3β signaling pathways and induced ubiquitin-mediated degradation of p21; Induced HCC cancer cell proliferation and associated with poor prognosis.	p21 ubiquitination via MAPK/ERK1/2 and AKT/GSK3β pathways	(75)
Hepatocellular carcinoma	High Expression	HJURP expression is a prognostic marker for HCC, and its high expression stimulates the proliferation of HCC cancer cells.	-	(66)
Hepatocellular carcinoma	High Expression	High expression of HJURP upregulates Sphingosine kinase 1, stimulates epithelial-mesenchymal transition, increases migration and invasion of cancer cells, and reduces survival.	Sphingosine kinase1 (SPHK1) upregulation	(76)

Epithelial-to-mesenchymal transition (EMT), which plays a essential role in cancer cell invasion, metastasis, or therapy resistance, is characterized by the loss of epithelial cell junctions and apical-basal polarity, rearrangement of the cytoskeleton, changes in cell shape, and activation of genes associated with a mesenchymal phenotype, along with downregulation of epithelial gene expression profiles. As a result, cell mobility increases, and a more invasive phenotype is exhibited (90-92). EMT is generally controlled by the transcription factors SNAIL, zinc-finger E-box-binding (ZEB), and basic helix–loop–helix (bHLH), which suppress epithelial-specific genes such as E-cadherin and cytokeratin and upregulate genes that lead to a mesenchymal phenotype. Expression changes at the gene level prevent the formation of epithelial cell-cell junctions and result in loss of epithelial function. In addition, the degradation of epithelial cell junctions is also supported by the increased expression of mesenchymal proteins such as fibroblast-specific protein (FSP-1) as well as EMT-transcription factors such as SNA1, SNA2 (92, 93). HJURP's role in the epithelial-mesenchymal

transition in hepatocellular carcinoma has been revealed. It has been shown that HJURP facilitates EMT, supporting HCC migration and invasion and that HJURP degradation inhibits HCC cell migration and invasion by upregulating E-cadherin and downregulating N-cadherin and Vimentin. In addition, HJURP overexpression in Huh7 cells has been linked with reduced E-cadherin and increased Vimentin expression. Microarray analysis in Huh7 cells identified 20 EMT-related genes among 164 differentially expressed genes, with sphingosine kinase 1 (*SPHK1*) changing in association with HJURP regulation. KEGG pathway analysis supported that HJURP regulates sphingosine metabolite processes. HJURP degradation led to increased SPHK1 expression and reversed EMT marker expression in HCC cells, reducing invasion capabilities (76). SPHK1, as a regulator of sphingolipid metabolism, contributes to HCC development. SPHK1 converts sphingosine, which induces tumor suppression via apoptosis, into sphingosine-1-phosphate (S1P), which promotes cell proliferation and survival. Increased protein and mRNA levels of SPHK1 in HCC tissues induce S1P expression and support metastasis in HCC cells. Inhibition of SPHK1 with an inhibitor or siRNA suppresses cell migration and invasion in human liver cancer cells (94, 95). Liu et al. reported that SPHK1 supports EMT by inducing autophagy and stimulating the lysosomal degradation of the epithelial marker CDH1 in hepatocellular carcinoma cell lines. The effect of SPHK1 on the EMT process has also been shown in non-small cell lung cancer and colorectal cancer cells (96, 97).

In recent years, the CENP-A chaperone HJURP has gained increasing importance in cancer mechanisms and its association with prognosis. Several research have shown that high expression of HJURP in hepatocellular cancers is linked with poor prognosis and lower survival rates. However, there is very little research exploring the mechanisms that mediate the significant increase in HJURP expression in hepatocellular carcinoma. The study showed that HJURP promoter region methylation levels are lower in cancer tissues compared to adjacent normal tissues. The study suggested that the overexpression of HJURP in HCC is associated with hypomethylation of HJURP. The same study performed cell cycle and apoptosis analyses to determine the underlying mechanism of HJURP's negative impact on HCC prognosis and found that HJURP inhibition led to G0/G1 phase arrest in HuH7 and SK-HEP-1 cells. In addition, the apoptotic cell rates were significantly increased in hepatocyte-derived carcinoma cell line (HuH7) and human liver adenocarcinoma cell line (SK-HEP-1) lacking HJURP. In HepG2 cells with induced ectopic expression of HJURP, G0/G1 phase arrest and apoptotic cell rates were significantly reduced. Based on these data, it is thought that HJURP supports cancer cell proliferation by inhibiting G0/G1 arrest and apoptosis in HCC cells (71).

Hepatocellular carcinoma is commonly linked with the inactivation of the tumor suppressor p53, significant chromosomal instability, and factors causing chronic hepatocyte death (98). Chromosomal abnormalities and genomic instability are particularly common in HBV and HCV-related HCCs (99). In the molecular pathogenesis of HCC, different genetic mechanisms such as somatic mutations in the p53 tumor suppressor gene and activation of the WNT signaling pathway are known to be significant (100-103).

High levels of CENP-A and HJURP are linked with poor prognosis in human cancers, making both factors prominent as prognostic and predictive biomarkers in recent years. The tumor suppressor p53 is known to induce an antiproliferative response in the cell when various cellular stress factors that stimulate oncogenic signaling are involved. In addition, its importance in chromatin organization is now known. Gain-of-function p53 mutations can upregulate key chromatin regulators such as MLL1 and MLL2 through epigenetic mechanisms in cells. Additionally, p53 can induce cell cycle arrest in response to nucleosome depletion, leading to extended S phase and eventual cell death in p53-deficient cancer cells. Therefore, p53 is a significant sensor of altered chromatin environments, and loss-of-function or gain-of-function mutations in p53 often cause chromatin changes that affect tumor development. Research has shown that CENP-A and HJURP gene expression is specifically upregulated in p53-deficient human cancers. Researchers have proposed that HJURP and CENP-A genes may be suppressed by intact p53 in normally proliferating cells. Mutant or deficient p53 is therefore thought to play a key role in promoting HJURP and CENP-A expression and regulating chromatin changes in cancer cells (104).

Centromeric factors have emerged as significant elements in cancer biology, serving as both prognostic markers and potential therapeutic targets. Studies focusing on pharmacological targeting of histone chaperone complexes have reported that targeting FACT, a histone chaperone that promotes chromatin reclamation during transcription, with drug-like small molecules may yield significant results in cancer treatment. These data demonstrate the importance of identifying histone chaperones as important target proteins involved in cancer mechanisms and targeting them for treatment in cancers such as HCC, where successful survival rates are still not achieved (105).

5. Conclusion

This summarized information suggests that HJURP is linked to tumor development and metastasis in many solid malignancies, including hepatocellular carcinoma. Determining its expression could significantly contribute to molecular diagnosis in clinic, and developing anti-cancer drugs targeting this protein may offer a novel therapeutic approach.

Conflict of interest

All authors declared no conflict of interest.

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Authors' contributions

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Hyperprolactinemia and infertility female and male

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Abstract

Hyperprolactinemia is characterized by elevated, often pathologic serum prolactin concentrations affecting the reproductive health of both men and women. Prolactin is a hormone the anterior pituitary gland produces that serves numerous reproductive functions. In elevated amounts, prolactin interferes with the hypothalamic-pituitary-gonadal (HPG) axis, causing reproductive dysfunction. In women, hyperprolactinemia manifests with irregular menstruation, anovulatory cycles, or galactorrhea, while in men, low libido, erectile dysfunction, or oligospermia may ensue. Such effects are often linked to infertility and impaired conception. Diagnosis begins with measuring prolactin levels in the serum to confirm hyperprolactinemia and MRI imaging for suspicion of pituitary adenomas, a common cause. Most cases could be managed by dopaminergic treatment, such as bromocriptine and cabergoline, to help restore gonadal function, while surgical intervention would be resorted to in cases of large or resistant prolactinomas. To effectively frame treatment approaches, one has to understand the pathology behind hyperprolactinemia and infertility. This review discusses the etiology, pathophysiology, clinical presentation, and management of hyperprolactinemia, focusing on its reproductive health and fertility consequences.

Keywords: hyperprolactinemia, prolactin, hypogonadism, prolactinoma, gonadal dysfunction, dopamine agonists

1. Introduction

1.1. Defining Hyperprolactinemia and Infertility

Hyperprolactinemia refers to a condition characterized by excessive release of the hormone prolactin, which also facilitates lactation and other reproductive functions. The prolactin levels are normally controlled by dopamine, which inhibits its release from the lactotrophs of the anterior pituitary gland. Any alteration in the regulation of prolactin secretion would alter the production of gonadal hormones, thus affecting fertility in both males and females. The clinical definition of infertility is 12 months of unprotected sexual intercourse without a conception event.. This rate is pegged around 15% of couples worldwide, hyperprolactinemia being an essential contributory cause behind it. High prolactin levels inhibit gonadotropin-releasing hormone (GnRH) secretion, which drops down follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, which are critical for ovulation and spermatogenesis. Thus, hyperprolactinemia is now considered a primary endocrine disorder affecting fertility outcomes.

1.2. Etiology of Hyperprolactinemia

Hyperprolactinemia has both physiological, pathological, and pharmacological causes.

The physiological causes include pregnancy, lactation, stress, and sleep, which are natural elevations of prolactin levels (7). Pathological hyperprolactinemia results mostly from prolactin- secreting pituitary adenomas (prolactinomas), hypothyroidism, chronic kidney disease, or liver cirrhosis (8). Prolactinomas are the most common cause, with microadenomas (<10 mm) or 3 macroadenomas (>10 mm) creating sustained prolactin elevations (9). Others include any pituitary and hypothalamic disorder that might induce prolactin secretion, for example, stalk compression from non-functioning adenomas (10). Hyperprolactinemia could be caused by medications such as antipsychotics (including risperidone and haloperidol), antidepressants (mainly selective serotonin reuptake inhibitors), and antihypertensives (like verapamil) (11). These drugs act by interfering with dopamine signaling, taking off the inhibitory control prolactin release had, thus predisposing to hyperprolactinemia.

1.3. Impact of Hyperprolactinemia on Fertility in Males and Females

1.3.1. Effects on Female Fertility

High prolactin levels disturb ovarian function in females by suppressing GnRH secretion, which results in decreased levels of FSH and LH. This inhibition results in anovulation, menstrual irregularities (e.g., oligomenorrhea or amenorrhea), and reduced estrogen production (12). Galactorrhea, the inappropriate secretion of breast milk, may also be another prominent symptom in hyperprolactinemic women, but it does not always arise (3). Extended hyperprolactinemia hampers

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endometrial receptivity, possibly reducing implantation success (4). Primary tenets of reproductive endocrinology are that hyperprolactinemia is seen in 10-20% of women who are infertile (5). Long-standing hyperprolactinemia can lead to suppression of estrogen, which can affect bone mineral density and postulate the risk of osteoporosis (6).

1.3.2. Effects on Male Fertility

By suppressing the secretion of GnRH in men, hyperprolactinemia causes hypogonadotropic hypogonadism, reducing LH and FSH levels and thus decreasing testosterone production and interfering with spermatogenesis (3). Just like in females, reduced libido, erectile dysfunction, and gynecomastia (breast enlargement) are common symptoms in males as well (6).

High prolactin levels are found in 3-11% of men with oligozoospermia (low sperm count), which leads to high oxidative stress in spermatozoa, causing sperm DNA fragmentation and decreased fertilization potential (12). Although many men may not see hyperprolactinemia manifest as overt symptoms so frequently, it exerts profound impacts on sperm morphology, motility, and fertility. Evidence suggests that untreated hyperprolactinemia will lead to irreversible gonadal dysfunction, even after normalization of prolactin levels

2. The Mechanisms of Hyperprolactinemia and Its Impacts on Reproductive Physiology

2.1. Disruption of the Hypothalamic-Pituitary-Gonadal Axis

Hyperprolactinemia mainly disrupts the hypothalamic-pituitary-gonadal (HPG) axis and affects reproductive physiology. The hypothalamus controls reproductive function through the secretion of gonadotropin-releasing hormone (GnRH), acting upon the anterior pituitary for the subsequent release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (1).

These hormones are indispensable for ovarian follicle development, ovulation, corpus luteum function in females, and testosterone production and corpus luteum function in females; the primary results are decreased FSH and LH levels and spermatogenesis in males. Excess levels of the hormone prolactin inhibit GnRH secretion, causing low levels of FSH and LH and ultimately thwarting regular reproductive action (2).

The hormonal imbalance in females appears to be responsible for several clinical manifestations relating to reproductive physiology, such as anovulation, irregular menstrual cycles, and decreased estrogen levels, rendering females infertile. On the other hand, hyperprolactinemia inhibits the secretion of estradiol, the hormone responsible for maintaining endometrium thickness necessary for implantation of the fertilized egg. So even if ovulation occurs, the endometrium may not be ideal for implantation, reducing the chances of conception. In males, prolactin excess suppresses testosterone secretion by inhibiting Leydig cell function in the testes, resulting in hypogonadotropic hypogonadism (4). Manifestations include diminished sexual drive, erectile dysfunction, and deficient sperm production. The significance of testosterone to spermatogenesis means that reduced levels are often paired with the manifestation of oligospermia (low sperm count) or azoospermia (absence of sperm in semen), both contributing to male infertility.

2.2. Direct Effects on Reproductive Tissues

Hyperprolactinemia has direct effects as well as direct results beyond endocrine suppression on reproductive tissues. In women, prolonged exposure to high prolactin levels inhibits ovarian follicle maturation. These inhibitory effects primarily affect the granulosa, a cell type essential for follicular maturation and estrogen synthesis (6). High levels of blood prolactin impair oocyte-creating fertilization rates and embryo developmental competence. In addition, it negatively affects the receptivity of the endometrium, affecting implantation failure and increasing the risk of early pregnancy loss. Likewise, hyperprolactinemia disrupts the testicular microenvironment in men. Studies have shown that prolactin interacts with Sertoli cells, supporting sperm development and causing abnormal spermatogenesis (8). Besides producing increased prolactin levels, it has heightened oxidative stress in spermatozoa, resulting in sperm DNA fragmentation, decreased motility, and low fertilizing potential (9). These factors hinder sperm function to such an extent that it becomes difficult to conceive, even when the concentration is otherwise normal. One of the most significant concerns, however, is the long- term impact on reproductive function from hyperprolactinemia. After normalization of serum prolactin levels, long-persisting gonadotropin suppression can succeed in causing irreversible gonadal dysfunction. In females, this condition manifests as premature ovarian insufficiency, while males would have persistent testicular atrophy associated with compromised fertility potential. Early diagnosis and treatment of hyperprolactinemia play a vital role in preventing irreversible reproductive damage.

2.3. Psychological and Systemic Manifestations

Hyperprolactinemia has effects on the system and psyche, as well as the reproductive function. High prolactin levels indicate mood disorders like depression and anxiety that may further reduce sexual desire and fertility (11). The continual estrogen deficiency in these women poses an even higher risk for the development of osteoporosis and fractures, whereas chronic suppression of testosterone in men results in loss of body mass and increased fatigue (12). This makes a case for holistic management to address issues of fertility and general well-being due to hyperprolactinemia.

3. Clinical Manifestations and Diagnostic Approaches

Hyperprolactinemia is an endocrinological condition involving an increased serum level of blood prolactin and is mainly mediated through the inhibition of hypothalamic-pituitary-

gonadal axis function. Given that multiple aspects of the reproductive and systemic physiology are impacted by GnRH pulsatility, the increase in prolactin levels can lead to severe consequences. The signs and symptoms of hyperprolactinemia are not always obvious, are influenced by sex and age, and may be accompanied by endocrine or metabolic disorders. It is crucial to be mindful of these manifestations to ensure timely diagnosis and management.

3.1. Clinical Presentation in Females

In women, hyperprolactinemia is most commonly manifested by menstrual irregularities. This encompasses the following: oligomenorrhea, which is the infrequent occurrence of enstruation; amenorrhea, which is the complete lack of menstruation; and anovulatory cycles due to the suppression of GnRH and the subsequent suppression of LH and FSH by prolactin. This leads to all their fructose-dependent physiology, including ovulation and follicular development, being severely impacted by the resulting hypoestrogenic state (6). Another intriguing feature that is present, although not in all patients, is galactorrhea, the spontaneous production of breast milk in females who are not pregnant or breastfeeding. They may also demonstrate other features of chronic estrogen deprivation, like vaginal atrophy, painful intercourse, and low sexual desire. Hypoestrogenemia contributes to osteopenia and osteoporosis, particularly in prospective menopausal females, resulting in an enhanced fracture rate and long-term skeletal unreliability (7). These endocrine pathophysiologic changes happen insidiously, and many patients do not seek medical advice until fertility issues arise or other more generalized symptoms manifest.

3.2. Clinical Presentation in Males

Unlike the more severe symptoms seen in females, male symptom patterns are less apparent and sometimes overlooked. The typical clinical manifestations of hyperprolactinemia include decreased libido, erectile dysfunction, gynecomastia, and, rarely, galactorrhea in men. As with hyperprolactinemia, the mechanism is hypogonadotropic hypogonadism due to the suppression of the secretion of gonadotropins by prolactin. It results in lower testosterone levels in the serum, thus impacting sexuality, mood, skeletal muscle mass, and bone density. First of all, it should be mentioned that hyperprolactinemia influences spermatogenesis significantly.

Because the intratesticular microenvironment is especially vulnerable to changes in gonadotropin stimuli, raised prolactin levels are thought to adversely affect sperm concentration, motility, and morphology (9). Thus, conditions such as oligospermia or azoospermia may be present in effected individuals after an appropriate workup to identify contributing factors to subfertility or infertility.

3.3. Neuropsychiatric and Metabolic Consequences

In addition to its effects on reproduction, hyperprolactinemia has been associated with neuropsychiatric and metabolic alterations. Dopamine antagonism in the hypothalamic area due to endogenous or exogenous factors can facilitate mood disorders like depression, anxiety, irritability, and cognitive deterioration. These effects are most apparent in people with prolactinomas or cases of prolonged disease without treatment (19). It is believed that the neuropsychiatric alterations of hyperprolactinemia are due to changes in dopaminergic modulatory tone in mesolimbic and mesocortical circuits. Additionally, other studies conducted in the past years have correlated chronic hyperprolactinemia with metabolic syndrome, which includes central obesity, insulin resistance, and dyslipidemia (11). The prolactin receptors in the adipose tissue indicate that prolactin might regulate adipose tissue formation and lipid homeostasis. This broadens the suggested use of prolactin in fertility, endocrinology, and prospects in cardiovascular diseases.

3.4. Diagnostic Framework

Thus, properly diagnosing hyperprolactinemia implies systematically identifying biochemical, radiological, and clinical findings. The primary diagnostic test is a prolactin-level blood test. Prolactin levels increase during sleep, exercise, and in response to stress, whereas they decrease in response to food intake; therefore, blood samples should be collected before 10 am after denying the patients food and ensuring they have sat for 30 mins (12). PRL reference values differ from the lab, but >25 ng/mL in women and >20 ng/mL in men are considered high. In those patients with mild elevation of PRL, it is recommended to repeat the test for confirmation of hyperprolactinemia. Moderate hyperprolactinemia (widespread >200 ng/mL) indicates a chance for a prolactinoma, specifically prolactin-secreting pituitary macroadenoma. On the other hand, actual moderate elevation can have a secondary cause, such as steroids, thyroid medication, kidney diseases, or stress. Consequently, a comprehensive review of the patient's medication list is imperative. Prolactin can be raised by various medications, including dopamine receptor antagonists like antipsychotics, risperidone, and haloperidol; SSRIs; and several antihypertensives, including verapamil, due to tuberoinfundibular dopamine (18).

3.5. Neuroimaging and Hormonal Assessments

MRI is the imaging study of choice when hyperprolactinemia is present and involves imaging the sella turcica for the detection of pituitary microadenomas (less than 10 mm) and macroadenomas (greater than 10 mm) and evaluation of the extent of the tumor and the possibility of chiasmal compression by tumor. Patients with macroadenomas should be tested for bitemporal hemianopsia and visual fields, and perimetry should be tested (14). Further endocrinological assessment by systematic blood examination, specifically TSH and free T4, to rule out hypothyroid-induced hyperprolactinemia, which results from stimulation by TRH. It also is necessary to perform renal and hepatic function tests to exclude systemic causes. In women, precisely, the serum levels of estradiol, LH, and FSH help determine the extent of reproductive depression. In men, assessing testosterone, LH, and FSH helps describe the

hypothalamic- pituitary-testicular axis. Under some circumstances, macroprolactin level assays may be relevant. Macroprolactin is a biologically inactive, high molecular weight prolactin complex that, when present in circulation, can lead to raised serum prolactin levels without clinical manifestations.

The PEG precipitation test can distinguish macroprolactin from the biologically active monomeric prolactin (15).

4. Treatment Strategies and Fertility Management in Hyperprolactinemia

The management of hyperprolactinemia needs to be rational and individualized to achieve normalization of serum prolactin levels, the amelioration of hypogonadism and fertility if impaired, and the identification and treatment of the causative factor, which may be a functional pituitary tumor, the use of a drug with hyperprolactinemic effects, or a systemic disorder. Therapeutic management depends on the degree of hyperprolactinemia, tumor size, symptomatology, and the patient's childbearing plan.

4.1. Pharmacologic Therapy

4.1.1. Dopamine Agonists as First-Line Treatment

Dopamine agonists are at present considered the first-line treatment option for hyperprolactinemia. They work on D2 receptors in the pituitary to inhibit prolactin release and to cause tumor regression in prolactinomas. Cabergoline and bromocriptine are the two most commonly used ergot derivatives, but cabergoline is recommended because of its higher efficacy, longer duration of action, and better side effect profile (1). Cabergoline reverses hyperprolactinemia in more than 85% and causes tumor shrinkage in many micro- and macroprolactinomas (19). It is taken twice a week, increases compliance, and diminishes the gastrointestinal side effects seen with bromocriptine. Although adverse effects have been reported in studies, bromocriptine still has its uses, especially for pregnant women, because of its efficacy and fewer side effects than other dopamine agonists. Therapeutic targets are aimed at achieving normoprolactinemia, symptom control (such as galactorrhea or amenorrhea), and recovery of regular menses or testosterone levels. The frequency of monitoring hormonal reassessment is usually 1 to 3 months in the first year, and the intervals between subsequent evaluations are longer if biochemical remission is attained.

4.1.2. Management of Drug-Induced Hyperprolactinemia

The treatment of choice for patients with medication-induced hyperprolactinemia is usually changing or ceasing the causative drug. If cessation is not possible (for instance, in psychotic illnesses), the use of a dopamine agonist or switching to a non-prolactinogenic medication like aripiprazole should be considered (4). However, this should be done under close consultation with the prescriber to prevent risks of psychiatric decompensation.

4.1.3. Addressing Underlying Conditions

Other violations include hypothyroidism, which requires levothyroxine as it inhibits TRH-stimulated prolactin secretion. Hyperprolactinemia due to chronic diseases such as kidney diseases or cirrhosis may resolve with treatment of the underlying condition, but dopamine agonists may be necessary if galactorrhea persists after treatment.

4.2. Surgical and Radiation Options

4.2.1. Transsphenoidal Surgery

Surgical intervention is required in patients for whom dopamine agonist treatment is contraindicated or could be discontinued or in patients who experience compressive symptoms from the macroprolactinoma, such as visual field compromise from optic chiasm compression. The best procedure is adenomectomy through the transsphenoidal approach, which involves direct resection of the lesion with minimal damage to other pituitary gland tissues. Surgical success has been reported to depend on tumor size, its stage or extent of growth, and whether the tumor is well-defined or invasive. Microprolactinomas are reported to have more than 70% remission rates, while macroadenomas have lower remission rates and a high potential for recurrence (18). Hence, there is a general preference for surgical intervention in severe conditions or when all the available medical therapies have been exhausted.

4.2.2. Radiation Therapy

Radiation therapy is not used initially but may be indicated in large prolactinomas that are aggressive or resistant to both medical and surgical management. Stereotactic radiosurgery (Gamma Knife) is preferred as it is less invasive and causes less hypopituitarism as compared to conventional radiotherapy/fractionated radiotherapy.

4.3. Fertility Restoration Strategies

4.4.1. Ovulation Induction in Women

Ovulation is an important biological phenomenon, and hyperprolactinemia poses a massive threat to women's fertility through anovulation. The restoration of ovulatory function is usually accomplished with dopamine agonist therapy only. It has also been found that once the prolactin levels are brought back to normal, as many as eighty percent of women can get back their periods and can conceive again (8). In case it does not return, ovulation can be induced through the use of drugs like clomiphene citrate or letrozole. However, since many couples do not obtain pregnancy even after prolactin levels return to normal, ART, such as in vitro fertilization (IVF), may be used when other infertility factors are present (9).

4.4.2. Male Fertility Management

In men, dopamine agonist therapy raises testosterone concentration and helps to increase the chances of conception by improving sperm production and sexual function. Some studies show that the elevation of prolactin levels is associated

with poor sperm concentration, movement, and morphology (10). In the case of chronic idiopathic oligozoospermia, additional treatments like gonadotropin therapy, including human chorionic gonadotropin (hCG) or follitropin injections to promote spermatogenesis, might be used. It is therefore advocated that a complete workup to rule out other causes of infertility, such as varicocele, testicular failure, and genetic issues, should be done (20). Fertility preservation strategies can also be used in patients with an increased testosterone level that is >20 ng/mL or in those patients receiving pharmacological management for prolactinomas or undergoing surgery or radiotherapy.

4.4. Monitoring and Long-Term Follow-Up

Patients with hyperprolactinemia, especially those with prolactinomas, are usually followed up endocrinologically to monitor executive functioning and the return of tumor activity. It is recommended that prolactin measurements be taken serially and pituitary imaging be performed every 1-2 years in macroadenomas or recurrent microadenomas (17). The selective endocrine evaluation comprises pituitary hormone axes, especially in patients who undergo surgery or radiotherapy for pituitary tumors, as hypopituitarism may be experienced. Screening bone density is recommended if the initiation of treatment with gonadotropin is delayed or if chronic hypogonadism is present.

5. Emerging Research and Future Directions

Concerning hyperprolactinemia, the diagnostic tools and approaches to treatment have significantly changed during the last decades, but new information in the field still comes to light regularly. In this chapter, the current topics of hyperprolactinemia molecular pathophysiology, precision medicine, new drugs, and pharmacology, as well as future perspectives on fertility and systemic effects, have been discussed.

5.1. Advancements in Molecular and Genetic Understanding

Some molecular evidence, such as gene and protein expression maps, has revealed that prolactinomas are heterogeneously characterized tumors. MEN1, the MEN type 1 gene, has been linked to familial and sporadic pituitary tumors, which points to hereditary causes in some patients (18). Such pathways may enable risk assessment of individuals and early diagnosis in high-risk populations. New studies on gene variations of dopaminergic receptors also reveal why certain patients do not respond to dopamine agonists. Further, research has pointed to carriers of the D2 receptor gene (DRD2) with treatment-resistant prolactinomas, leading to receptor- selective drugs that bypass such resistance mechanisms (20). Moreover, other molecular changes, including histone methylation and DNA methylation of particular genes, have also been identified in prolactinoma tissues with concerning indications for breast cancer aggressiveness and recurrence.

5.2. Innovations in Pharmacologic Treatment

Even though this medication is deemed the most effective, certain drawbacks have been observed. Chemotherapy-induced toxicities, drug resistance mechanisms, and incomplete eradication of tumors, in some instances, require tweaking of the approach. One of these is the extended-release dopamine agonists, which increase receptor occupancy and reduce dosing frequency, thus enhancing patient compliance and decreasing side effects (4). There are new classes of agents under study. SERMs and SPRMs are under consideration for their ability to intervene in prolactin secretion indirectly, based on hormonal regulation (5). In addition, other early-phase trials with non-selective and selective GnRH kisspeptin agonists, presumed to enhance secretion of GnRH, appear to have the potential to eradicate hyperprolactinemia- induced anovulation without necessarily suppressing prolactin.

5.3. Precision Medicine and Targeted Therapies

The advancement of the concept of precision medicine is also bringing significant changes in the field of endocrine oncology. Practical significance: Using biomarkers such as genetic, hormonal, and imaging, new and existing treatments can be adjusted to be the most effective in treating clients while avoiding potential harm. For instance, when patients presented with tumors with high Ki-67 scores, he could recommend surgery earlier or additional treatment (7). It may also help clinicians select a specific DA or predict patients likely to need a higher dose or a combination therapy. With these approaches becoming more widespread, they are expected to increase long-term remission rates significantly.

5.4. Emerging Technologies in Fertility Preservation

In the field of reproductive medicine, hyperprolactinemia continues to be an essential, although fully reversible, factor behind infertility. Still, distinct possibilities are emerging for new techniques for patients with potential infertility due to long-term gonadal failure. Oocyte vitrification, egg freezing, and testicular sperm extraction, or TESE, are being used more commonly in patients who are to be subjected to invasive cancer treatments or are to receive long -term medical therapies that may affect their vision (8). Stem cell-based ovarian/testicular regeneration is still in the experimental stage but has the potential to restore fertility in hypogonadal patients with secondary hypogonadism attributed to hyperprolactinemia (9). Moreover, machine learning algorithms use multiple parameter data sets to predict ovulatory response and IVF success in hyperprolactinemia (10).

5.5. Future Directions and Research Gaps

There are still some gaps in knowledge despite these accomplishments. One of the reasons is that the long-term effects of untreated or chronic hyperprolactinemia on cardiovascular and neurocognitive health have not been explored adequately. Thus, considering the role of prolactin in endothelial function and dopaminergic signaling, longer-term prospective studies must evaluate the cardiovascular risks of

atherosclerosis and hypertension, as well as cognitive impairment and mood disorders, among patient populations (11). Second, more research is needed to find biomarkers to help distinguish between diseases such as FH and prolactinomas, specifically when prolactin levels are low. Circulating microRNA and prolactin isoform ratios are currently being explored (18). In addition, limited research on hyperprolactinemia among transgender, intersex, and non-binary patients indicates that hormone therapy may have more complex effects in these populations.

6. Conclusion and Recommendations

6.1. Conclusion

The current paper focuses on the clinical and endocrine consequences of hyperprolactinemia, a multifaceted affliction with impacts on the reproductive system, psychological functions, and overall body functioning. It results in hypothalamic-pituitary- gonadal (HPG) axis dysfunction due to the suppression of GnRH by prolactin with hypogonadotropic hypogonadism in both sexes. The outcome is not uncomplex: it may run from menstrual disorders, galactorrhea, and infertility in women to erectile dysfunction, decreased libido, and oligospermia in men. Moreover, hyperprolactinemia is no longer only associated with neuropsychiatric disorders but also metabolic syndrome and future cardiovascular disease. The diagnosis depends on biochemical evaluation, imaging of the sellar region, and exclusion of other pathophysiologic conditions that can cause a similar presentation, like hypothyroidism, renal failure, or drug side effects. Including pituitary MRI and hormone assays, which help differentiate prolactinomas and manage patients.

Currently, dopaminergic agents, especially cabergoline, are therapeutically most effective and represent the gold standard in prolactinoma treatment, tumor shrinkage, and recovery of reproductive function. Surgery and radiation therapy are reserved for patients with persistent or progressive tumors that are also rapidly growing; hence, risk stratification is crucial. Although there have been significant achievements in the treatment of cancer, it remains an intervening issue in non-responsive cases, drug allergies, and maintenance of complicated diseases. Luckily, information regarding molecular genetics, receptor pharmacology, and the molecular basis for personalized medicine is gradually improving. Incremental improvements are anticipated to not only advance the outcomes of reproduction but also ameliorate the permanent threats arising from excessive prolactin concentrations.

6.2. Recommendations

6.2.1. Enhance Early Detection and Screening

In women, reproductive endocrinologists and primary care or internal medicine physicians should have a heightened awareness of hyperprolactinemia in women with irregular or oligo menstrual cycles, infertility, galactorrhea, or sexual dysfunction. By including prolactin in the tests associated with infertility and amenorrhea, doctors could make the diagnosis and start treatment on time. Promote interdisciplinary management. Due to the interaction of hyperprolactinemia with various systems, patients should be managed by several specialists, such as endocrinologists, reproductive specialists, neurologists, and psychiatrists, as required. This way is integrated to systematically take care of hormonal and psychological consequences.

6.2.2. Personalize Pharmacologic Therapy

Clinicians should, therefore, adjust the dopamine agonist treatment based on the patient's tolerance level, pharmacogenomic testing, and tumor activity. Monitoring the side effects and prolactin levels regularly is vital to effectively address the symptoms and noise while preventing negative consequences.

6.2.3. Integrate Fertility Preservation Strategies

As previously stated, ALL adult patients and women of childbearing age, including those requiring surgery or taking long-term medications, should discuss fertility preservation with their clinician. Methods like oocyte vitrification, sperm cryopreservation, or embryo banking help to protect future reproductive self-determination.

6.2.4. Continuity, Long-Term Commitment, and Follow-Up

Patients should be followed up longitudinally for tumor recurrence, hypopituitarism, and potential complications like osteoporosis or newly developed metabolic syndrome. Correspondingly, bone density scans, cardiovascular risk, and psychosocial assessment should be integrated into the post-treatment follow-up regimens. Expand research into understudied populations. More studies should be conducted regarding hyperprolactinemia risk and its effects on various population groups like adolescents, the elderly population, transgender people, and those with other endocrine disorders. Awareness of differences in risk-protective factors and treatment intervention in these groups will improve the clinician's effectiveness and sensitivity to cultural differences.

6.2.5. Advanced Public and Provider Education

Informative public health campaigns targeting healthcare professionals and the general populace may help spread knowledge concerning hyperprolactinemia's signs, consequences, and management. It is a powerful strategy to educate patients and engage them in their care to better follow through with therapy regimens.

Conflict of interest
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Ethical Statement

This study is not required ethics approval.

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Case Report

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Osteopetrosis of temporal bone causing bilateral peripheral facial nerve paralysis: MRI and CT findings

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Abstract

Osteopetrosis is a rare, heterogeneous group of genetic disorders characterized by osteoclast dysfunction, resulting in excessive bone sclerosis. When it affects the skull base and temporal bone, it can lead to Eustachian tube dysfunction, recurrent otitis media, hearing impairment, and cranial nerve deficits. This report highlights the computed tomography (CT) and magnetic resonance imaging (MRI) findings of temporal bone osteopetrosis in an 18-year-old male presenting with acute-onset bilateral facial nerve paralysis. Radiological evaluation revealed significant narrowing of the bilateral internal acoustic canals, a hallmark feature of the disease. The patient received a short course of corticosteroid therapy, resulting in complete resolution of symptoms. This case underscores the critical role of imaging in diagnosing and managing cranial nerve complications in temporal bone osteopetrosis.

Keywords: computed tomography; magnetic resonance imaging; temporal bone; osteopetrosis; facial paralysis

1. Introduction

Osteopetrosis is a rare genetic bone disorder characterized by sclerosis of the bones resulting from defective osteoclast function. This dysfunction impairs the resorption of bone and mineralized cartilage, leading to dense but fragile bones that are prone to fractures and other complications (1). The involvement of the skull base and temporal bone in osteopetrosis can result in a variety of clinical manifestations, including Eustachian tube dysfunction, recurrent otitis media, hearing loss, and cranial nerve deficits (2,3). Such cases require thorough radiological assessment to guide diagnosis and management. Herein, we report a case of osteopetrosis in an 18-year-old male presenting with acute-onset bilateral facial nerve paralysis, highlighting the computed tomography (CT) and magnetic resonance imaging (MRI) findings associated with this rare presentation.

2. Case presentation

An 18-year-old male patient with a known history of osteopetrosis presented to hospital with complaints of acute-onset bilateral facial nerve paralysis. Laboratory investigations, including hemoglobin levels and white blood cell counts, were within normal limits. On physical examination, there was notable weakness of the facial muscles on both sides, affecting the forehead and lower face symmetrically. Otoscopic examination revealed intact tympanic membranes and normal external auditory canals, with no evidence of hearing loss. High resolution CT of the temporal bones showed diffuse sclerosis involving the temporal bones and skull base (Fig. 1). Notably, there was significant narrowing of the bilateral internal acoustic canals. MRI of the same region demonstrated decreased signal intensity of the affected bones on both T1-weighted (T1W) and T2-weighted (T2W) sequences, consistent with sclerotic changes (Fig. 2). The patient was admitted to the hospital and initiated on a three-day course of corticosteroid therapy. His symptoms resolved entirely during hospitalization, and he was discharged in stable condition with no residual facial nerve deficits.

3. Discussion

Osteopetrosis encompasses a heterogeneous group of heritable bone disorders characterized by impaired bone resorption due to osteoclast dysfunction leading to bone sclerosis (1). This dysfunction may result from defective differentiation of hematopoietic stem cells into osteoclasts or from a failure in the function of already differentiated osteoclasts. Although the affected bones appear radiologically dense, they are structurely fragile and prone to fractures, highlighting the paradoxical nature of this disorder (4).

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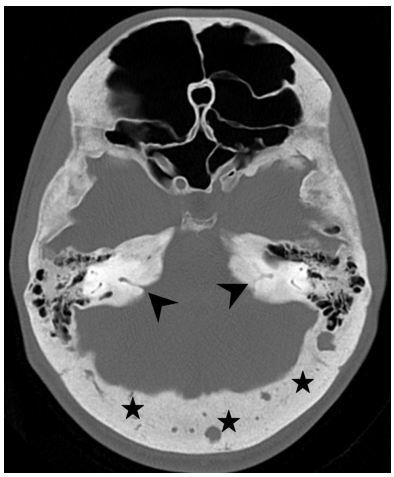

Fig. 1. Axial CT image of the temporal bone showing diffuse sclerosis involving the temporal bones and entire skull base (asterix). Note the significant narrowing of both internal acoustic canals (arrowheads).

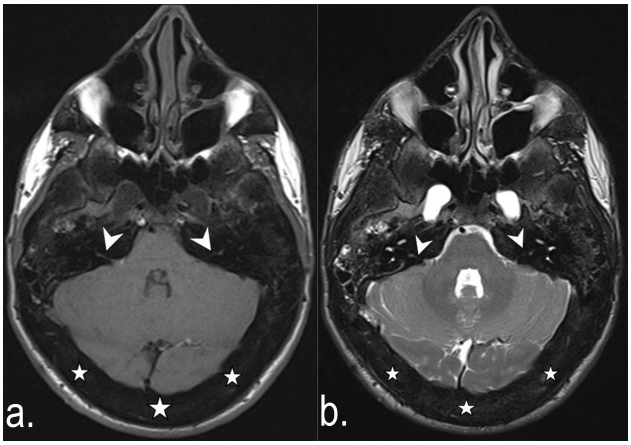

Fig. 2. T1W and T2W images of the temporal region reveal pronounced signal loss in the medullary bone (asterisk), indicative of extensive sclerosis. Severe narrowing of the bilateral internal acoustic canals is also evident (arrowheads), correlating with the radiological findings on CT.

The clinical presentation of osteopetrosis varies based on its genetic subtype. Autosomal-dominant osteopetrosis, commonly diagnosed in young adults, generally has a more favorable prognosis and is divided into type I and type II. Type I typically spares the skull base and temporal bones, while type II frequently involves these regions (3). By contrast, the autosomal-recessive form, also known as malignant osteopetrosis, has a more severe course and manifests in infancy with higher morbidity and mortality.

Temporal bone involvement in osteopetrosis can lead to significant clinical complications, including stenosis of both external and internal auditory canals, ossicular ankylosis, vascular narrowing, Eustachian tube dysfunction, otitis media, sensorineural hearing loss, and cranial nerve deficits (2,3). Facial nerve compression, as seen in this case, may manifest as peripheral facial nerve paralysis, clinically resembling Bell's palsy.

Imaging plays a pivotal role in diagnosing temporal bone osteopetrosis. High-resolution CT typically reveals diffuse sclerosis, obliteration of medullary cavities, reduced diploic spaces, and narrowing of auditory canals (3). The stapes may also appear thickened in some cases. On MRI, sclerotic bone is characterized by decreased signal intensity on both T1W and T2W sequences.

Currently, there is no definitive preventive treatment for osteopetrosis. Management is primarily symptomatic and supportive. Cranial nerve dysfunction, including facial nerve paralysis, may be addressed with decompression surgery, although evidence supporting its efficacy remains limited (4,5). In cases of severe sensorineural hearing loss, cochlear implantation can provide functional improvement (6).

In conclusion, temporal bone osteopetrosis is a rare but clinically significant manifestation of this complex disorder. The disease frequently presents with increased bone density and extensive sclerosis, leading to complications such as hearing loss and facial paralysis. This case highlights the characteristic CT and MRI findings in an 18-year-old male with osteopetrosis.

Conflict of interest

The authors declare that they have no competing interests.

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Case Report

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Endovascular management of a persistent primitive trigeminal artery aneurysm initially misdiagnosed as pituitary adenoma: A Case Report

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Abstract

Persistence of primitive intracranial embryonic anastomoses such as the permanent primitive trigeminal artery (PPTA) is rare, with the PPTA representing 80-85% of these cases. Aneurysms associated with PPTA are rare. This presents diagnostic and therapeutic challenges. We report a 64-year-old woman, initially evaluated for headache and dizziness, where cranial computed tomography (CT) incidentally suggested a hypodense sellar lesion. Subsequently, on CT angiography, a 9 mm saccular aneurysm in the PPTA and an 18 mm aneurysm in the right internal carotid artery (ICA) cavernous segment were identified. Endovascular treatment with flow diverter stent implantation was successfully performed, followed by post-operative ticagrelor and acetylsalicylic acid therapy. The patient was discharged neurologically intact after three days of care in the hospital and there was no deterioration in neurological examination at 1-year follow-up. This case underscores the necessity of careful angiographic evaluation in patients with suspected pituitary masses to avoid potentially catastrophic misdiagnoses. It also highlights the critical role of imaging techniques in identifying rare vascular anomalies.

Keywords: Aneurysm, carotid-vertebrobasilar anastomoses, case report, endovascular technique, persistent primitive trigeminal artery

1. Introduction

PPTA is one of the four fetal anastomoses that spontaneously closes with the development of the posterior communicating (PCOM) arteries and vertebral arteries, which provide posterior blood flow between the carotid and vertebrobasilar circulations during the fetal period. In rare cases where closure fails, these anastomoses persist into adulthood and are called permanent carotid-vertebrobasilar anastomoses (1, 2). PPTA is the most common type of permanent carotid-basilar anastomosis (1-3).

It is usually found incidentally in cases of aneurysms accompanying PPTAs or patients with hormonal disorders due to pituitary compression (4,5). Various vascular abnormalities such as trigeminal artery-cavernous fistulas, Moyamoya disease may be associated with PPTA (6).

The literature shows that cases accompanied by aneurysms are encountered in patients with PPTA, but aneurysms originating from PPTA are rare. Treatment can be performed with surgical and endovascular methods (7, 8).

In this article, a case of PPTA incidentally detected in a patient with suspected pituitary mass due to headache and dizziness during cranial imaging was presented.

2. Case presentation

A 64-year-old woman without a previous medical history presented with a two-month history of headaches and dizziness. Her neurological examination had no significant findings. In the cranial computed tomography (CT) imaging, a 13 mm hypodense nodular lesion was observed in the right half of the sella turcica (Fig. 1). Further evaluation with cranial CT angiography revealed the presence of two saccular aneurysms: an approximately 9 mm aneurysm in the proximal region of the PPTA at the cavernous segment of left ICA and an 18 mm aneurysm at the cavernous segment of the right ICA (Fig. 1 and 2). The location of the aneurysms and their presence in two vessels were evaluated by the neurovascular council and the endovascular method was recommended.

After premedication with 300 mg of acetylsalicylic acid and 180 mg of ticagrelor 12 hours before treatment. While the patient was under general anesthesia, a 6F 088 long sheath (Neuron MAX, Penumbra, Alameda, CA, USA) and a Sofia distal access catheter (Microvention, Tustin, California, USA) were placed through the right femoral arterial access to provide access.

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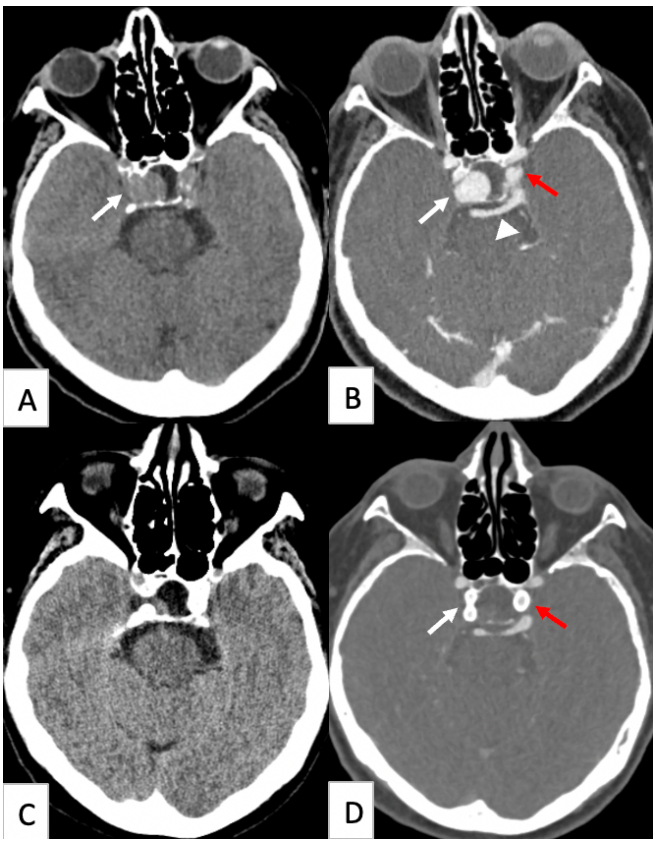

Fig. 1. Preoperative (A-B) and first year (C-D) cranial CT and CT angiography axial images. A: White arrow shows the lesion in the right half of the sella confused with the pituitary mass. B: White arrow shows cavernous segment aneurysm of the right internal carotid artery, triangular head shows PPTA and red arrow shows PPTA aneurysm. C: Postop first year control cranial CT, D: Postop first year control cranial CT angiography

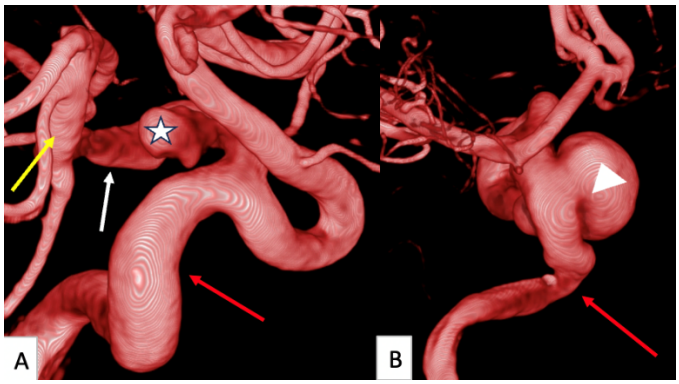

Fig. 2. Images of the patient's aneurysm. A: Red arrow indicates internal carotid artery (ICA), white arrow indicates PPTA, yellow arrow indicates basilar artery, asterisk indicates PPTA aneurysm. B: Arrowhead indicates cavernous segment aneurysm of the right internal carotid artery

Due to the position, size and symptomatology, it was decided that coils would not be suitable for treatment and flow-directing stent placement would be more suitable. While irrigating with heparin (total 12500 IU), a guiding catheter was introduced to the right ICA and 5.5x40 mm and 5x25 mm Pipeline Embolisation Device (PED-Shield, Medtronic Neurovascular, Irvine, California, USA) flow-directing stent were placed in the aneurysm neck in the cavernous segment under roadmap guidance. In the same session, a guiding catheter was placed in the ICA on the left side and a 6x16 mm Pipeline Embolization Device (PED-Shield, Medtronic Neurovascular, Irvine, California, USA) flow-directing stent was placed in the neck of the aneurysm in the PPTA localization under the road map. On control angiography images, it was observed that the aneurysm necks were closed by the stents and flow in the aneurysm lumens was reduced (Fig. 3). The intervention was concluded without complications.

The patient was started on ticagrelor 90 mg ticagrelor daily and acetylsalicylic acid 300 mg acetylsalicylic acid daily. The patient was discharged after 3 days of follow-up without any change in neurological examination and no residual aneurysm was found in the control first-year cranial CT angiography images (Fig. 3).

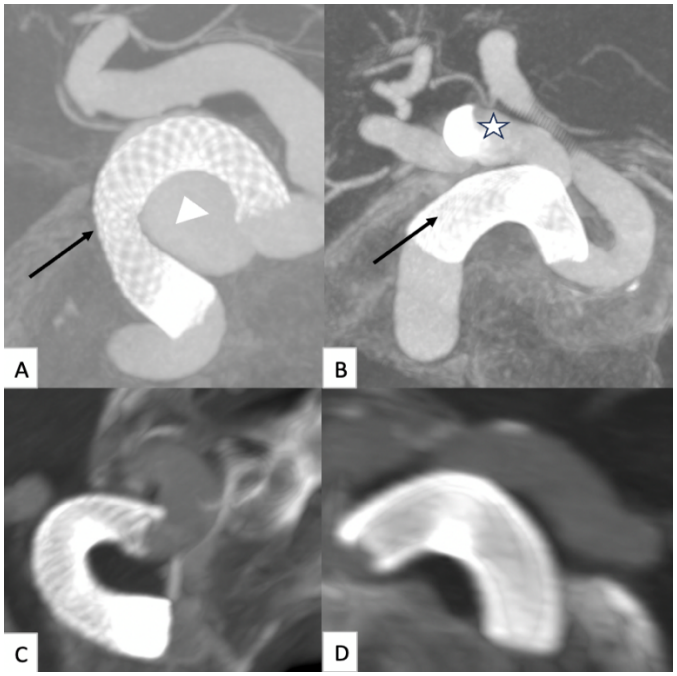

Fig. 3. Postoperative (A-B) and first-year (C-D) control CT angiography images of the patient. No residual aneurysm was seen in the control first year cranial CT angiography images. Black arrow: flow diverter stent, arrowhead: right ICA cavernous segment aneurysm, asterisk: PPTA aneurysm

3. Discussion

It is very significant to investigate the presence of PPTA when planning surgical approaches to the skull base. The presence of an unrecognized PPTA may lead to injuries following approaches to the sellar or para-sellar regions and the cavernous sinus (2-4, 9). There are different results regarding the frequency of the association of PPTA with intracranial aneurysms and other vascular pathologies. Aneurysms in PPTA can occur at the PPTA-ICA junction, PPTA-basilar artery junction (7, 10). Aneurysms can be saccular, fusiform, small, large, single or multiple. It is rare for multiple aneurysms to accompany PPTA patients (11). Because of the rarity of PPTA aneurysms and the paucity of relevant studies,

the bleeding risk of PPTA aneurysms is unknown (10). Since PPTA aneurysms are usually located deep in the vicinity of cranial nerves and perforating vessels, surgical treatment is difficult and endovascular treatment is often preferred (12, 13). We planned endovascular treatment of the aneurysm instead of follow-up for our patient.

In PPTA classifications, Salas and modified Saltzman classifications are used (2,3,14). Salas et al. (14) classified PPTAs as sphenoidal and petrosal types according to their course on angiography. In the sphenoidal subtype, PPTA closely surrounds the pituitary gland with an intrasellar, trans-pituitary course and can cause hormonal disorders such as hyperprolactinemia and hypopituitarism by compressing the stalk (5). Similarly, due to PTA's close anatomical relationship with nerves, it can cause ophthalmoparesis, trigeminal neuralgia, and oculomotor and abducens nerve palsies (15).

The modified Saltzman classification consists of 3 types. In type I, PPTA supplies the basilar artery, superior cerebellar artery, and posterior cerebral arteries. PCOM arteries, vertebral artery, and proximal BA may be absent or hypoplastic. For this reason, PPTA-related pathologies during the interventional procedure may result in infarction in the posterior circulation of the brain. In Saltzman type 2, preservation of PPTA may not be necessary as blood flow to the posterior circulation is predominantly via the PCOM arteries (6). Our case had Saltzman type 2 and PPTA was preserved during the procedure. Detailed examination of the vascular anatomical structure of the PPTA is important in the success of the treatment and studies with new classifications are performed with CT and MRI imaging in current studies. Weon also classified PTA into five subtypes according to haemodynamic characteristics (16, 17). Consequently, aneurysm cases associated with PPTA are rare in the literature, and as case reports increase, we will gain more knowledge about diagnosis and treatment.

Informed consent

Written informed consent was obtained from the patient.

Conflict of interest

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An often-forgotten diagnosis: Midgut malrotation and volvulus in an adult

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Abstract

Intestinal malrotation is a rare congenital abnormality that typically presents in the neonatal period or within the first year of life. However, it is exceedingly rare for this condition to persist without symptoms or go undiagnosed until adulthood. Case Report: A 70-year-old man sought medical assistance due to severe abdominal pain, nausea, vomiting, and bloating. Despite experiencing intermittent abdominal pain for 15 years, he had never undergone any abdominal procedure. Upon examination, no tenderness or distension was observed, but an abdominal radiograph indicated a distended small bowel. Subsequent contrast-enhanced abdominal computed tomography (CT) scan confirmed midgut malrotation with volvulus, showing a whirlpool sign. The patient received symptomatic treatment and was discharged for an elective Ladd's procedure. Conclusion: Intestinal malrotation is often missed in adults with non-specific abdominal symptoms, leading to delayed diagnosis. This emphasizes the importance of considering it in the differential diagnosis of suspected small bowel obstruction.

Keywords: Midgut malrotation, midgut volvulus, intestinal malrotation, adult

1. Introduction

Intestinal malrotation is a rare congenital abnormality resulting from the failure of the usual 270° counterclockwise midgut rotation around the superior mesenteric vessels during embryonic development (1). This condition generally involves bilious vomiting during the neonatal period or infancy, but it can also be asymptomatic and become apparent later in childhood or adulthood (2). This condition is rare, affecting approximately 1 in every 6000 newborns and only 0.2% of adults (3, 4). Despite its rarity, the condition is often overlooked in adults due to its nonspecific clinical presentation, which mimics more common gastrointestinal disorders such as irritable bowel syndrome or peptic ulcer disease (5, 6). This diagnostic ambiguity frequently delays identification until complications such as volvulus or bowel ischemia occur (7). We report a case of a seventy-year-old patient presenting with intermittent abdominal symptoms, diagnosed with midgut malrotation with volvulus, and successfully managed with conservative treatment before undergoing an elective Ladd's procedure.

2. Case Report

A 70-year-old male patient sought medical attention due to abdominal pain accompanied by nausea, vomiting, and bloating, persisting for two days. He reported a 15-year history of intermittent abdominal discomfort, which he attributed to dietary indiscretions or stress. The patient had not undergone any previous abdominal procedure, and his medical history is only remarkable for hypothyroidism. The initial vital signs recorded were as follows: temperature 36.3°C, respiratory rate 16 breaths/min, blood pressure 110/80 mmHg, and pulse 89 beats/min. Upon initial evaluation, the patient was oriented, and his abdomen was soft, with no tenderness or distension or peritoneal signs. Laboratory findings, including complete blood count, electrolytes, and renal/liver function tests, were within normal limits. An abdominal radiograph showed signs of a distended small bowel loops (Fig. 1), prompting further imaging. Subsequently, a contrast-enhanced abdominal CT scan confirmed the diagnosis of midgut malrotation with volvulus. No obstruction was present, but imaging revealed a 360-degree clockwise rotation of the small bowel around the superior mesenteric artery (SMA) and vein (SMV) at the root of the mesentery (whirlpool sign) (Fig. 2). Also diffuse intestinal wall edema was noted in the intestinal loops (Fig. 3). Given the absence of hemodynamic instability, bowel ischemia, or perforation, the patient was managed conservatively with bowel rest, nasogastric decompression, and intravenous hydration along with symptomatic treatment. Immediate surgical intervention was deferred in favor of close clinical monitoring. During the follow-up, there was no deterioration in the clinical condition and laboratory values. The patient was discharged on the third day with a good state of health. He attended for a follow-up appointment and for planning elective Ladd's procedure seven days later when he was found to be asymptomatic.

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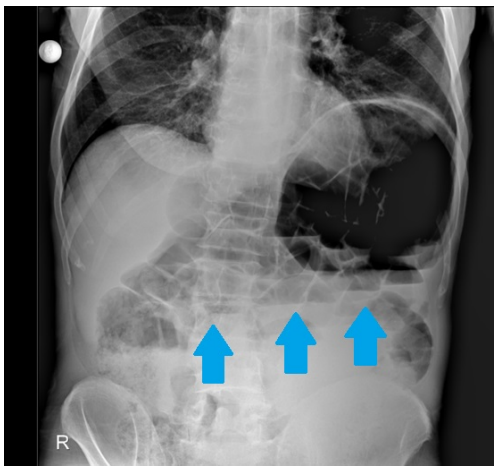

Fig. 1. An abdominal radiograph shows signs of a distended small bowel (the blue arrows)

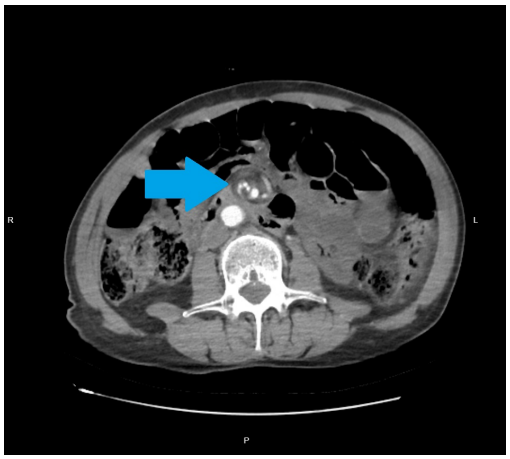

Fig. 2. An axial CT image shows the typical whirlpool sign (the blue arrow) and a distended small bowel

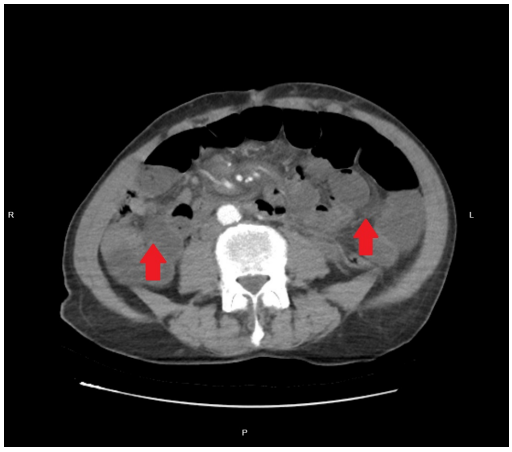

Fig. 3. An axial CT image showing widespread edema in the intestinal loops (the red arrows)

3. Discussion

Adult patients with intestinal malrotation often present with a protracted history of recurrent, nonspecific abdominal pain. This persistent discomfort leads to frequent medical consultations; however, the elusive nature of the symptoms often results in the absence of a definitive diagnosis. In the early stages, patients with malrotation may not show any symptoms. However, some patients may experience long-term abdominal discomfort or, less often, sudden abdominal pain. Due to these non-specific gastrointestinal symptoms, the diagnosis of the condition is usually delayed. Also, patients may be subjected to inappropriate diagnostic conclusions and unnecessary medical interventions. These symptoms may be caused by peritoneal fibrous bands called Ladd's bands, which can cause acute or chronic intestinal obstruction or volvulus (9). Neville J et al. observed that symptoms of midgut volvulus in adults can manifest differently but often involve recurring abdominal pain and intermittent vomiting, mirroring the experiences of our patient (6). The gold standard for diagnosing malrotation is either an upper gastrointestinal series or a CT scan with oral and intravenous contrast. For adults, a CT scan is preferred. Both scans can help detect malrotation by demonstrating the unusual position of the caecum to the left and the out-of-the-ordinary relationship between SMA and SMV (In malrotation, SMA is located to the left of SMV). Midgut volvulus can be detected in an upper gastrointestinal series by the corkscrew sign of the proximal small bowel, or in a contrast CT scan by the whirlpool sign (the mesentery and SMV have a rotating appearance around SMA). Other features that may be seen include obstruction of the duodenum and congestion of the mesenteric vasculature (2, 7). Midgut malrotation can be managed either symptomatically or surgically, depending on clinical presentation (5, 8). Ladd's procedure, which involves division of Ladd's bands, widening of the mesentery, and appendectomy, remains the definitive treatment to prevent recurrent volvulus (9). Immediate surgery is mandatory in cases of acute volvulus with ischemia, obstruction, or hemodynamic instability (4). In stable patients without complications, as seen in this case, bowel rest and decompression may suffice to stabilize the patient before elective surgery (7). This approach minimizes surgical risks in elderly or comorbid patients while addressing acute symptoms. The decision to delay surgery in our patient aligns with evidence supporting elective Ladd's procedure in non-emergent cases (4). However, clinicians must weigh the risks of recurrent volvulus against surgical morbidity, particularly in older adults. Our patient did not require immediate surgical intervention and was successfully managed with symptomatic treatment, followed by an elective Ladd's procedure. Our patient presented with intermittent symptoms, including nausea, vomiting, and abdominal pain, which are typically associated with small bowel obstruction. Unfortunately, he delayed seeking medical attention as he believed the symptoms were not severe enough. Consequently, his diagnosis was delayed.

Our case was particularly intriguing and noteworthy because no signs of ischemia were observed despite the 360-degree rotation of the mesenteric root, especially in an elderly patient. Intestinal malrotation is a comparatively infrequent cause of bowel obstruction in adult patients. As a result, it is usually not considered as a possible diagnosis when patients show non-specific abdominal symptoms until complications like volvulus or bowel ischemia occur. This underscores the critical importance of maintaining a high index of suspicion.

Diagnosing malrotation in adults can be challenging, but including it in the differential diagnosis of small bowel obstruction can make it attainable.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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Ethical Statement

Ethics committee approval is not required for this study.

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A candy catastrophe: case report on methamphetamine poisoning in a child

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Abstract

Methamphetamine, a potent amphetamine derivative, is increasingly implicated in pediatric poisonings and can present with severe neurological, cardiovascular, or behavioural disturbances. Clinicians must remain vigilant when managing undifferentiated status epilepticus, as toxic etiologies may masquerade as primary medical causes. A previously healthy 3-year-old boy was found convulsing at home and received rectal diazepam without improvement. On arrival at the emergency department, he exhibited generalised tonic-clonic seizures, tachycardia, and dilated pupils despite intravenous benzodiazepines and levetiracetam. Suspicion arose when inconsistent caregiver accounts and mention of "candy" ingestion led the team to perform toxicology screening, which revealed methamphetamine as the offending agent. The child required endotracheal intubation and continuous midazolam infusion to control his refractory seizures. Subsequent investigations showed lactic acidosis but no intracranial pathology. Both parents later admitted to methamphetamine use prompting urgent child-protection involvement. The boy was successfully extubated after two days, recovered without neurological deficits, and was discharged on hospital day eight. This case underscores the importance of maintaining a high index of suspicion for toxic etiologies in unexplained pediatric seizures. Early recognition, prompt seizure management, and vigilant social assessment are critical to achieving favourable outcomes and ensuring child safety.

Keywords: methamphetamine poisoning ; methamphetamine toxicity ; methamphetamine induced seizure ;child neglect ; child abuse

1. Introduction

Methamphetamine is the second most widely used illicit drug worldwide after cannabis(1). A synthetic derivative of amphetamine, methamphetamine exerts powerful sympathomimetic effects by enhancing the release of dopamine, norepinephrine, and serotonin. Oral ingestion of methamphetamine typically leads to symptom onset within about 20 minutes, with peak plasma concentrations reached in two to three hours and a half-life of approximately 10 hours(2).

Commonly known on the street as "ice" or "crystal," methamphetamine is popular among recreational users for its capacity to elevate mood and induce euphoria. Typical sympathomimetic effects include tachycardia, hypertension, hyperthermia, agitation, arrhythmias, and, less commonly, seizures. There is no specific antidote for methamphetamine toxicity; therefore, care is primarily supportive.

Globally, there have been increasing reports of pediatric methamphetamine poisoning, with methamphetamine identified as a frequent cause of illicit drug-related pediatric admissions in certain regions like the United States. In Malaysia, childhood poisoning is a significant public health issue, affecting children aged 0 to 5 years in 63% of overall reported incidents; of these, 96.7% are believed to be unintentional(3). However, local data specifically regarding methamphetamine poisoning in children remains scarce.

The effects of methamphetamine on the pediatric population are only partially understood, given the limited number of case reports and studies. Many management strategies were adapted from anecdotal experiences with adult patients, while others relied more on a theoretical understanding of the pathophysiology instead of robust evidence. In this report, we present a rare case of status epilepticus in a 3-year-old child following accidental methamphetamine ingestion, which was successfully managed, leading to a favourable outcome.

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2. Case description

A previously healthy 3-year-old boy was brought to the Emergency Department (ED) after being discovered convulsing on the bathroom floor by his father. Initially, the father sought care at a private clinic, where the child received rectal diazepam. Because the seizure persisted, he was urgently transferred to our hospital via ambulance. On arrival to the emergency department, the child was having generalised tonic-clonic movement of bilateral upper and lower limbs with up rolling of eyes. He was tachycardic with a heart rate of 174 beats/min with a bounding pulse, blood pressure of 79/52mmHg, oxygen saturation of 98% on high flow oxygen and capillary glucose level of 8.2mmol/L upon arrival. The pupils were dilated bilaterally. Initial pharmacotherapy included intravenous (IV) diazepam (total of 4 mg) followed by IV midazolam (0.2 mg/kg once, then 0.1 mg/kg twice). Because the seizures continued, second-line therapy with IV levetiracetam was administered. As the patient was in status epilepticus, the patient was intubated for airway protection. Post-intubation, there were still persistent tonic-clonic movements, for which continuous midazolam infusion finally controlled the seizure.

Subsequent examination revealed hyperreflexia in both upper and lower limbs but no clonus. The child was afebrile and showed no signs of meningismus, as both Kernig's and Brudzinski's signs were negative. Cardiovascular and respiratory examinations were unremarkable, with no bruises, wounds, or bite marks observed. Point-of-care blood gases revealed lactic acidosis (pH 7.08, pCO_2 65 mmHg, HCO_3 19.3 mmol/L, lactate 7.5 mmol/L, base excess –11.3). An electrocardiogram demonstrated sinus tachycardia.

Further history disclosed that the child's older brother had witnessed him climbing up to an unlocked medicine cabinet shortly before the event, where he had allegedly eaten some "candy." This detail raised the suspicion of possible accidental poisoning, prompting a urine toxicology screening that tested positive for methamphetamine. A non-contrast computed tomography (CT) scan of the brain showed no evidence of haemorrhage or ischemic changes.

The child was admitted to the Intensive Care Unit (ICU) and remained intubated for two days. After extubation, he was weaned off oxygen and transferred to the general pediatric ward. He was discharged after an 8-day hospital stay without apparent neurological deficit.

Upon further investigation, both parents admitted to being methamphetamine users, though they claimed the "medicine cabinet" was typically locked. They stated they had forgotten to lock it after their last methamphetamine consumption. Given the high suspicion of child neglect, the hospital's Suspected Child Abuse and Neglect (SCAN) team was activated. The father was subsequently charged with child neglect and incarcerated, and the mother lost custody of both children. The boy and his sibling were placed in the care of their maternal grandmother. At the latest follow-up, the child showed no developmental regression or other complications.

3. Discussion

This case illustrates several important aspects of pediatric methamphetamine toxicity. While most pediatric exposures manifest with sympathomimetic signs, our patient presented with the rare complication of status epilepticus, estimated to occur in only 5% of methamphetamine poisonings(4). The rapid onset and severity of symptoms correlate with methamphetamine's enhanced lipid solubility and accelerated blood-brain barrier penetration(2,5)

The management of methamphetamine-induced status epilepticus presents a therapeutic challenge due to limited pediatric-specific protocols. Current evidence supports benzodiazepines as first-line therapy for both agitation control and seizure management in methamphetamine-poisoned children(4) Our stepwise approach—progressing from benzodiazepines to levetiracetam and finally continuous midazolam infusion—aligned with recent toxicology guidance for refractory drug-induced seizures(6). Notably, we avoided phenytoin based on evidence suggesting limited efficacy in toxin-induced seizures (7,8)

This case emphasises several critical clinical lessons. First, clinicians must maintain a high suspicion for toxicological etiologies in pediatric patients presenting with unexplained neurological, cardiovascular, or behavioural manifestations. Second, thorough history-taking, including environmental assessment and caregiver reliability, is essential for identifying potential exposures. Inconsistent histories, evasive behaviour, and the social conditions of the caregiver can provide valuable insights into possible exposure to toxins

Third, the standard approach to status epilepticus may require modification in toxin-induced cases, with emphasis on benzodiazepines and consideration of alternative second-line agents.

From a public health perspective, this case highlights the intersection of substance use disorders and child safety. Recent studies demonstrate increasing rates of unintentional pediatric exposures to illicit substances in households where drugs are present(9). Emergency physicians serve as critical sentinels in identifying potential child neglect and initiating appropriate protective measures. In Malaysia, the Child Act 2001 provides the legal framework for healthcare professionals to collaborate with welfare and law enforcement agencies to protect vulnerable children.

This rare presentation of methamphetamine poisoning-induced status epilepticus in a toddler highlights the need for early recognition and prompt, aggressive management of illicit drug exposures in pediatric populations. Emergency and pediatric teams must remain vigilant, particularly when presented with nonspecific or unexplained clinical findings. This case also emphasises the critical importance of caregiver

education, secure storage of potentially harmful substances, and the role of social services in preventing recurrent harm. With appropriate treatment and follow-up, the prognosis can be favourable, as illustrated by the child's return to normal developmental milestones.

Conflict of interest

The authors would like to declare the were no conflicts of interest in publishing this case report.

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Authors' contributions

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Postoperative hemodynamic stabilization with terlipressin: A case of liver transplantation

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Abstract

This report presents the successful management of a patient with acute liver failure on the background of chronic liver disease who underwent liver transplantation. Terlipressin, a vasoactive agent, managed hepatorenal syndrome and acute kidney injury by promoting splanchnic vasoconstriction and intrahepatic vasodilation, improving renal blood flow and hemodynamic stability. The case involves a 41-year-old woman with autoimmune hepatitis and primary biliary cirrhosis who required triple vasoactive therapy postoperatively. Bolus doses of terlipressin reduced the need for other vasopressors, stabilized renal function, and facilitated her recovery. This case underscores the potential of terlipressin as a valuable therapeutic option in the postoperative management of liver transplantation patients with refractory hemodynamic instability. Careful dosing and monitoring are essential to optimize outcomes and minimize risks.

Keywords: Hepatorenal syndrome, liver transplantation, terlipressin

1. Introduction

Acute liver injury developing on the background of chronic liver disease is defined as a severe clinical condition resulting in the sudden loss of liver functions (1). These patients may present with severe symptoms of liver failure as well as extrahepatic manifestations (2). Among extrahepatic organ failures, renal failure is the most common and is considered a significant prognostic factor independent of mortality (3). HRS typically develops in patients with decompensated liver cirrhosis. It can also emerge as a complication of fulminant liver failure and acute hepatitis. HRS is a syndrome characterized by severe acute kidney injury (AKI) resulting from renal artery vasoconstriction (4). The recommended pharmacotherapy for treating HRS-AKI includes using albumin combined with a vasoconstrictor agent (5).

Terlipressin, a vasopressin analog, exerts its effects primarily through two mechanisms. First, it binds to V1 receptors on the vascular wall, causing vasoconstriction. Second, it binds to V2 receptors, enhancing water reabsorption from the distal renal tubules and collecting ducts (6). By inducing splanchnic vasoconstriction, terlipressin reduces portal blood flow and lowers portal hypertension, playing a critical role in treating acute kidney injury observed in hepatorenal syndrome. Additionally, it decreases intrahepatic arterial resistance, leading to intrahepatic vasodilation and a subsequent reduction in hepatic venous pressure. This mechanism redistributes blood from the splanchnic region to the central vascular compartment, regulating renal blood flow and increasing mean arterial pressure (7). Compared to other vasoactive agents like norepinephrine, terlipressin has not demonstrated a significant difference in mortality rates but has been reported to have favorable effects in reversing hepatorenal syndrome (8). This article presents the successful management of a patient with acute liver failure on the background of chronic liver disease who underwent liver transplantation following treatment with terlipressin.

2. Case Report

A 41-year-old female patient with a 15-year history of autoimmune hepatitis and primary biliary cirrhosis was enrolled in a liver transplantation program. She presented to the emergency department with complaints of nausea, vomiting, poor oral intake, and fatigue. Due to rapidly rising bilirubin levels and changes in the MELD (Model for End-Stage Liver Disease) score, she was admitted to the internal medicine ward for further monitoring and management.

The patient's medical history included Hashimoto's thyroiditis, celiac disease, and a history of surgeries due to recurrent vertebral fractures. She regularly took ursodeoxycholic acid, furosemide, spironolactone, propranolol, and levothyroxine. On physical examination, her

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general condition was moderate, vital signs were stable, and she was alert, cooperative, and oriented. Systemic examination revealed widespread jaundice, tense ascites in the abdomen, and pretibial edema (+/+), with no other pathological findings noted.

Laboratory tests revealed elevated total bilirubin levels with a predominance of direct bilirubin (19.31–19.77 mg/dL), hyperammonemia (98 µmol/L), and an increased INR (2.06). Abdominal computed tomography (CT) scans showed no new pathology that could explain the patient's current complaints. The MELD (Model for End-Stage Liver Disease) score was calculated as 25, and the patient was included in the organ transplantation waiting list.

The patient underwent orthotopic liver transplantation with a cadaveric donor liver. In the postoperative period, she was admitted to the intensive care unit (ICU) on triple inotropic and vasopressor therapy (norepinephrine, dopamine, and dobutamine). The need for triple vasoactive agents persisted during postoperative hemodynamic monitoring, and reduced urine output was observed. The patient was administered a 1 mg intravenous bolus of terlipressin. Subsequently, the vasoactive agents were gradually tapered and discontinued, and the patient was extubated. Due to the positive effects of terlipressin, it was decided to administer 1 mg every 6 hours with a plan to taper the dose based on the patient's clinical condition. The patient's vasoactive drug requirements, hemodynamic parameters, and fluid management within the first 24 hours are shown in **Fig. 1**.

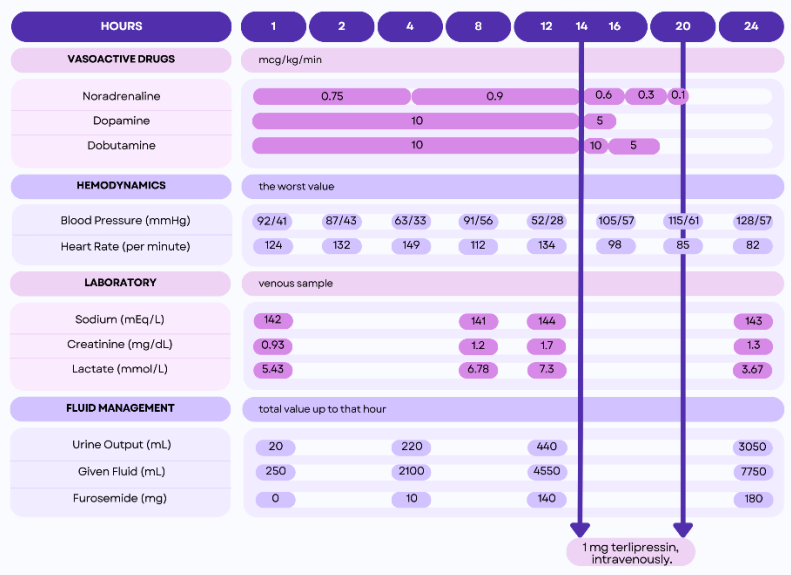

Fig. 1. First 24 hours after liver transplantation

Terlipressin was administered via slow intravenous push following a gradual dose reduction protocol. On the first day, the patient received 1 mg every 6 hours (total 4 mg/day); on the second day, 1 mg every 8 hours (total 3 mg/day); on the third day, 1 mg every 12 hours (total 2 mg/day); and on the fourth day, 1 mg once daily (total 1 mg/day). The medication was discontinued, and treatment was terminated on the fifth day.

The patient's creatinine levels, which were normal upon admission to the intensive care unit, progressively increased in the postoperative period as urine output decreased. Consequently, a furosemide infusion was initiated. The dose of furosemide was titrated to maintain a urine output rate of 0.5 mg/kg/hour. By the third day of follow-up, the patient's urine output and creatinine levels stabilized, and renal replacement therapy was not required.

The patient's concurrent prophylactic antifungal and immunosuppressive therapies were continued without interruption. The patient achieved clinical stability on the fifth intensive care unit follow-up day and was transferred to the general ward.

3. Discussion

Terlipressin has been shown to reduce the incidence of postreperfusion syndrome (PRS), particularly in cases of cadaveric donor liver transplantation, highlighting its potential to mitigate complications such as severe PRS (9). In our case, the patient required multiple vasopressor therapies in the postoperative period, and bolus terlipressin administration successfully restored hemodynamic perfusion, thereby reducing the need for other vasopressor agents.

Although some studies suggest that continuous terlipressin infusion may be more effective than bolus administration, in our case, adequate blood pressure was achieved with intermittent bolus administration of terlipressin (10). Terlipressin is considered a promising agent for managing hemodynamic instability and renal dysfunction during and after transplantation. However, despite its efficacy for the intended use, it carries significant risks of serious side effects, such as cardiac and ischemic complications and electrolyte imbalances (11).

While terlipressin reduces the incidence of PRS and improves renal function, its effects on portal pressure and potential side effects, such as increased pulmonary capillary wedge pressure, require careful evaluation. In our case, no adverse effects associated with terlipressin use were observed. To optimize liver transplantation outcomes, terlipressin administration should be closely monitored and tailored to individual patient needs, with consideration of potential side effects. In our case, a bolus dosing strategy combined with gradual tapering led to successful clinical improvement without developing adverse effects.

The severity of liver disease significantly impacts treatment response; however, it has been reported that a response to terlipressin can be achieved regardless of disease severity, leading to better outcomes in these patients (11). Additionally, terlipressin has been shown to have a more favorable effect on survival than norepinephrine (4). In our case, the preoperative MELD score was calculated as 25, consistent with severe liver disease. Nevertheless, the patient responded well to terlipressin therapy, achieving clinical improvement. Initially requiring triple vasopressor therapy, the patient experienced gradual discontinuation of other vasopressors following the initiation of terlipressin, resulting in hemodynamic stability.

This case report highlights the effectiveness of terlipressin in managing hemodynamic instability postoperatively in a patient requiring liver transplantation due to autoimmune hepatitis and primary biliary cirrhosis. Compared to other vasopressor agents, terlipressin provides a superior hemodynamic profile, particularly in patients with hepatorenal syndrome, and is associated with improved renal blood flow. However, further clinical experience and research are needed to evaluate terlipressin's benefits and potential risks comprehensively.

Informed consent

Informed consent was obtained from the patient's legal guardian, and ethical standards were followed.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

There is nothing to declare.

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Authors' contributions

Concept: M.İ., F.Ü., Design: Ö.Y.Ç., Data Collection or Processing: M.İ., T.S.A., Analysis or Interpretation: Ö.Y.Ç., F.Ü., Literature Review: M.İ., T.S.A., Drafting: M.İ., Ö.Y.Ç.

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