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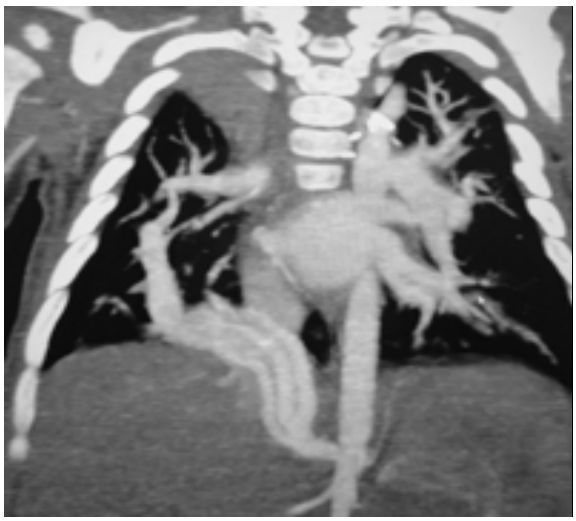

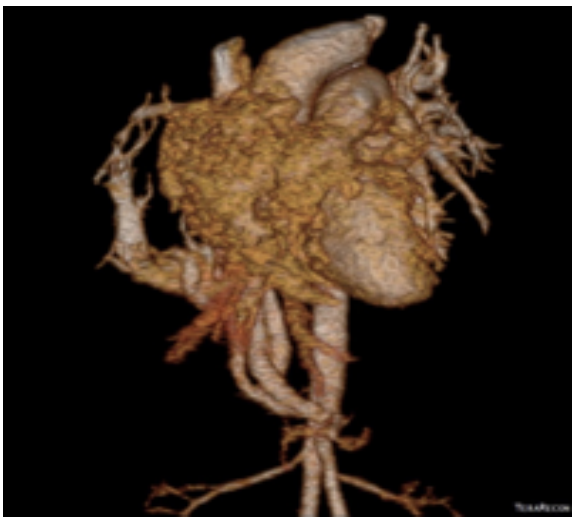

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The effects of treatment on nutrition in children with cancer

Müberra Tanrıverdi[a*], Melikenur Vural[b], Fatma Betül Çakır[c]

[a] *Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Bezmialem Vakıf University, İstanbul, Turkey*
[b] *Faculty of Medicine, Bezmialem Vakıf University, İstanbul, Turkey*
[c] *Department of Pediatric Hematology-Oncology, Faculty of Medicine, Bezmialem Vakıf University, İstanbul, Turkey*

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*** Correspondence to:**
Müberra Tanrıverdi
Department of Physiotherapy and Rehabilitation,
Faculty of Health Sciences,
Bezmialem Vakıf University,
İstanbul, Turkey
e-mail: tanriverdi88@hotmail.com

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ABSTRACT

Chemotherapy affects the diet and feeding habits of children with cancer. They experience side effects such as vomiting, diarrhea and constipation. Feeding disorders occur during treatment period of chronic illnesses such as during the chemotherapy and radiotherapy. The purpose of this study was to determine their feeding habits of the children with cancer. We applied a questionnaire concerning the feeding habits of 21 patients with cancer who received chemotherapy. The questionnaire included where, how, how often and how much children were fed. Nine (42.9%) female patients and 12 (57.1%) male patients participated in the survey. Six (28.6%) lymphomas, 5 (23.8%) sarcomas, 3 (14.3%) kidney tumors, 3 (14.3%) neuroblastomas, 2 (9.5%) brain tumors, 1 (4.8%) acute leukemia and 1 (4.8%) bone tumor were diagnosed. There was a positive statistically significant relationship between regular vegetable consumption and the measurement of body mass indexes ($r=-0.601$; $p<0.05$). Fast food consumption at least once a week was inversely correlated with the measurements of weight and height of the patients ($r=0.683$; $p<0.05$). Body mass index was found to decrease during the acute gastroenteritis periods ($r=-0.470$; $p<0.05$). There was a positive statistically significant relationship between constipation periods and the skinfold thickness measurements ($r=0.714$; $p<0.05$). Healthy feeding is especially important for every child in this patient group. Fast food consumption is one of the factors that negatively affects the health, which adversely affects the patients in the treatment process even more adversely. In the literature, there is no protocol established for the feeding of children with cancer and no guideline has been developed as a result of these studies. Our study is planned to determine this need and it might shed light on the literature with the results.

1. Introduction

Chemotherapy has effects on diet of the children diagnosed with cancer after they were treated with chemotherapy. Children live through various changes on their diet caused by chemotherapy. Because of early feeling of fullness, nausea, vomiting, diarrhea, changes in tastes in mouth, skipping meal, and reasons similar to these, they fall behind of their peers in case of nutrition. Children see various effects on their gastrointestinal system during chemotherapy treatment. During chemotherapy treatment, children do not consume some foods because of change of their taste, skipping meals, nausea-vomiting and diarrhea-constipation problems because of early felling of fullness. Whether main and snack meals provide qualified and sufficient nutrition determines the effectiveness of the treatment (Sala et al., 2004; Moyer-Mileur et al., 2009).

Mechanisms of intense chemotherapy which cause bone loss and effective treatments are unclear. This is an important topic for childhood cancers, because after chemotherapy treatment the healing rate is over 75%, thus the number of people who live with chronic bone deficit is increasing. Researches show that these defects are result of bone loss caused by increased bone marrow generation and bone resorption after chemotherapy. These changes probably were caused by regulatory molecules or changed expression and/or activation of pathways responsible for skeletal cell generation and activity. Recently, there are some preclinical studies that show mechanism of actions and potential health benefits, in some cases decreasing the bone defects caused by chemotherapy, of nutraceuticals including: Resveratrol, genistein, icariin and inflamed fatty acids (Su et al., 2017). Survivors of childhood cancer are at higher risk in terms of developmental conditions such as osteoporosis and cardiovascular diseases. Health promoting behaviors such as healthy diet may decrease this chronic problems. Targeted nutrition interventions may prevent or decrease these chronic diseases (Cohen et al., 2016). Pediatric nutrition and cancer discussions are generally focused on sufficient nutrition intake (enteral and parenteral) on early stages of treatment and after the treatment. However, there are some information that show the vitamins may have additive roles in the care of the children with cancer. In the last decade, researchers showed that maternal bias and perinatal vitamin intake alters the cancer risk of the baby and the children. It's been reported that there is a correlation between prevalence and severity of the side effects of vitamin and antioxidant on children treated with chemotherapy. Vitamin D has a potential anti-cancer activity and many children in North America have low vitamin D values (Stallings, 2008).

In our study, we assessed whether children with various cancers who treated with chemotherapy, received qualified and sufficient nutrition and if they suffered any side effects on their gastrointestinal system. The purpose of our study is to examine children's diet and dietary habits, provide qualified and sufficient nutrition intake and determine the side effects, such as vomiting, diarrhea, during chemotherapy treatment.

2. Material and methods

Study design

Our study is a lifestyle habits examining assessment questionnaire. Our study conducted in our university, Pediatrics Department, Pediatric Hematology and Oncology Division.

Participants

Children and their parent are informed thoroughly and signed informed consent form.

Inclusion criteria

1. Diagnosed with cancer
2. Voluntary attendance
3. Not having any musculoskeletal problems that may prevent assessment

Exclusion criteria

1. Coexistence of a neurologic or orthopedic disorder
2. Lack of cognitive function that prevents communication
3. Lack of mental and physical ability

Outcome measure

Hospital records that contains children's and their parents' personal and clinical information has been scanned and the questionnaire was conducted with those who accepted voluntary attendance. The answers for the questions regarding what kind of snacks they consume, how often they go to fast food restaurants and other similiar subjects were received from the childen diagnosed with cancer. With these results we compared the results from children who was being treated with chemotherapy.

Assessment form

Diagnosis, diagnosis date, treatment, which cure, anthropometric measurements, personal and family history of the person who completed the questionnaire, are recorded. Birthday, gender, place of residence, height, weight, body mass index, upper arm circumference, triceps skin thickness, cancer type, cancer stage, diagnosis date, treatment, which cure of chemotherapy, family history, siblings count, parent education status, school success, personal and family history are the information included in this form.

Diet form

This form has the information about main and snack meal consumptions of children who was undergoing treatment, what they prefer for their meals, the side effects they encountered on their gastrointestinal system during treatment. We found the answers for the following questions; do they consume fruits every day, do they eat fish twice a week, consume vegetable twice a week, do they go fast-food restaurant more than once, do they eat legume more than once a week, do they consume two glass of milk every day, do they consume dessert and sugar several times every day, do they drink energy drink, do they eat egg more than four times a week. How often they consume fruit, vegetable, milk, yoghurt, cheese, cereal, rice and pasta questions are answered. We get information concerning if they experience any gastrointestinal problems during the treatment and if so, whether constipation, diarrhea, early feeling of fullness, nausea or vomiting are present. The questions regarding how many main and

snack meal are consumed and whether they do skip the meals if they skip what they consume instead, what do they drink between meals, why do they skip meals, how often they eat outside are also answered.

Statistical method

Statistical Package for Social Sciences (SPSS) Version 20.0 (SPSS Inc. Chicago. IL. USA) statistics software has been used. Demographic and clinical features of patients analyzed by calculating descriptive statics with minimum, maximum values, average and standard deviation. In all analysis $p<0.05$ (two-sided) values accepted statistically significant. To select the advance statistical analysis which was suitable for the data analysis of this study, it's been checked with "Shapiro-Wilks" test whether data groups have normal distribution. Demographic features of children (gender, age, etc.), diet regimen result's correlation observed with "Spearman Correlation Analysis".

3. Results

Results of the demographic and clinical features of children and diet, has been shown in Table 1. Inter-correlation of results has been shown in Table 2. Results show that nine of them (42.9%) eat fruit everyday regularly, six of them (28.6%) eat fish at least twice a week regularly, 12 of them (57.1%) eat legume more than once a week, nine of them (42.9%) eat vegetables or salads twice a week regularly, 10 of them (47.6%) consume more than 2-3 portions of meat every week 12 of them (57.1%) consume 2 glass of milk every day, 4 of them (19%) consume carbonated drinks, none of them consumes energy drinks, 12 of them (57.1%) eat more than four eggs in a week, three of them (14.3%) consume sugar and dessert several times a day, one of them (4.8%) go to fast-food restaurant more than once a week has been found. When daily dietary habit has been questioned, according most given answers; it has been reported that bread, cereal, rice and pasta consumption as sometimes (n=10, 47.6%), fruit consumption as sometimes (n=12, 57.1%), vegetable consumption as sometimes (n=10, 47.6%), milk, yoghurt or cheese consumption as sometimes (n=7, 33.3%), meat, chicken, fish, legume, egg, appetizers like foods consumption as sometimes (n=10, 47.6%). It has been learnt that children very rarely eat outside (n=15, 71.4%). It's been detected that children have breakfast regularly (n=14, 66.7%), be full very fast frequently (n=8, 38.1%), sometimes have nausea (n=9, 42.9%), sometimes have constipation problems (n=10, 47.6%), most of them have no diarrhea (n=14, 66.4%). It has been found that 12 of children (57.1%) skips meals, most skipped meal is breakfast (n=7, 33.3%), skipping reasons are lack of time (n=7, 33.3%), lack of appetite (n=5, 23.8%), uneasiness about health problems (n=14, 66.7%). It has been determined that fruit is the most preferred snack food (n=14, 66.7%), ayran is the most preferred drink (n=7, 33.3%). It has been found that there is a statistically significant correlation between children's age and eating more than 2-3 portion meat in a week (r=-0.625; p=0.002), between Z-score normal/abnormal status and going fast-food restaurant more than once a week (r=0.683; p=0.004), between going fast-food restaurant more than

Table 1. Demographical and clinical characteristics of children with cancer.

	N	%
Gender (Girl/Boy)	9/12	42.9/57.1
Z-Score (Normal/Abnormal)	3/18	14.28/85.72
Tumor Type		
Leukemia	1	4.8
Lymphoma	6	28.6
Brain tumor	3	14.3
Wilms tumor	3	14.3
Neuroblastoma	3	14.3
Sarcoma	5	23.8
Treatment type		
Chemotherapy	11	52.4
Chemotherapy + Radiotherapy	3	14.3
Chemotherapy + Surgery	2	9.5
Chemotherapy + Radiotherapy + Surgery	5	23.8

	Mean	SD	Min.	Max.
Age (years)	**9.52**	**5.212**	**3**	**18**
Age at diagnosis (years)	7.52	4.844	1	16
BMI (kg/m2)	17.47	4.240	12.2	28.3
Z-Score	48.47	30.824	4	96
Arm circumference	19.58	5.189	14	31
Fat tissue	6.55	1.739	5	9
Chemotherapy cure number	5.16	2.292	1	10
Academic achievement	8.60	1.897	5	10
Number of main meal	2.55	1.099	0	4
Number of snacks	2.47	1.646	0	7

Table 2. The relationship between demographical/physical and related with cancer and treatment characteristics.

		Related with cancer and treatment characteristics					
		Tumor type		Treatment		Chemotherapy cure number	
		z	p	z	p	z	p
Demographical and physical characteristics	Age	-.029	.902	-.005	.982	.160	.512
	Age at diagnosis	-.081	.727	-.137	.553	-.114	.642
	Gender	-.089	.700	-.017	.941	-.335	.161
	Z-Score	.403	.109	.097	.710	.676	.006
	Arm circuference	-.088	.719	.115	.641	.266	.286
	BMI	.128	.613	.203	.419	.461	.072
	Fat tissue	-.062	.866	.047	.898	.056	.886

once a week and consumption of carbonated drinks (r=0.481; p=0.035), between going fast-food restaurant and eating outside (r=0.465; p=0.049), between eating 6-11 meals of bread, cereal, rice and pasta, and BMI (r=-0.470; p=0.049), between eating 6-11 meals of bread, cereal, rice and pasta, and Z-score category (r=0.633; p=0.008), between constipation and skinfold (r=0.714; p=0.020), between early feeling of fullness while eating and treatment (r=-0.568; p=0.007).

4. Discussion

According to our study results, it has been shown that chemotherapy has an effect on nutrition, it has to be assessed in detail during treatment process (diagnosis, treatment and after treatment) and that there is a need for multidiciplinary consultations. Survivors of childhood cancers generally come up against excessive weight gain during early stages of treatment. To prevent obesity and cardiovascular diseases in early stages, it is important to start lifestyle changes early. Studies which focus on lifestyle changes are quite low in number. According to results of studies, the lifestyle changes related cancer are both safe and applicable (Zhang et al., 2017).

In a review which includes 616 participants in total; 275 participants have ALL, all studies have different intervention methods and weak results. There is no clear evidence about bone health related to calcium intake (mean difference (MD) 111.60, 95% confidence interval (CI) -258.97 to 482.17;p=0.56, low quality evidence). Milk consumption (MD 0.43, 95% CI 0.07 to 0.79; p = 0.02, low quality evidence), is meaningful when it's used with calcium supplement day count (MD 11.42, 95% CI 7.11 to 15.73; p<0.00001, low quality evidence), and any calcium supplement (risk ratio (RR) 3.35, 95% CI 1.86 to 6.04; p<0.0001, low quality evidence). It has been reported that there is no significant difference between bone density z-scores in X-Ray DEXA, calcium and vitamin D supplement, and diet and dietary education; at the beginning, during treatment at 12^{th}, 24^{th}, 36^{th} month assessments there was no difference with control group. Multifactorial health behavior had changes even with one behavior change on patients who focused on healthy dietary principals and follow-up phone interviews showed differences when compared with control grouop (MD -0.05, 95% CI -0.24 to 0.14; p=0.60, low quality evidence). There is no clear evidence whether there is a difference between the group who developed healthy dietary behavior, and other. Even though health behavior changes had minor improvement on health behaviors, there is no clear evidence whether it also improves diet. There is no clear evidence about dietary regimen possessing positive or negative effects on cardiovascular and metabolic disorders (Cohen et al., 2016).

Cancer diagnosis and treatment may affect diet Methods which is used to assess dietary intake has not been validated. There is not any specific scale for this topic, and general scales used to collect data are considered not valid (EI-TEE)/TEE × 100%, was 22% for FFQ and 1% for repeated 24HRs.) (Zhang et al., 2015). In the studies which assessed patients diagnosed with cancer and their parents, it was shown that there has to be a support team which is especially important while dealing with side effects of cancer treatment. Adequate management of side effects faced by children treated for cancer, significantly affects their life quality (Bryant, 2003).

Recently, survival rate of childhood cancers has been increased. Increasing survival rates also increased the focus on supportive care for these patients. The regulation of the diet is a supportive method which helps to increase tolerance to chemotherapy included in the anticancer treatment, to increase survival rates, to increase quality of life and to decrease infection risk. Guides and evaluation criteria for care management that includes diet, pharmacology and psychosocial hardships considered in diet regimen of children with cancer, has been suggested, but no applicable guides with high quality evidence is available (Ladas et al., 2005).

Anthropometric measurements are being used while assessing diet status (Sacks et al., 2014). There is a correlation between dietary and infection rate on leukemia patients, thus supplementary diet is suggested during chemotherapy to reduce infection rates (Taj et al., 1993). Children with ALL have a higher risk because of unhealthy weight gain. Weight measurements are regularly repeated for weight management. Early weight management is important (Folta et al., 2017).

Acute and chronic hunger are mostly correlated with chilhood cancer. Despite gastrointestinal complications that interrupts chemotherapy and radiotherapy treatments, supplamentary diet should be maintained (Filler et al., 1977). Children treated with chemotherapy and/or radiotherapy are under the risk of malnutrition because of nausea, vomiting, lack of appetite and mouth ulcer-like side effects. Malnutrition during treatment increases the infection risk, decrease the tolerance to the treatment and even effects general survival rates. Personal risk factors should also be examined (Robinson et al., 2012).

Having more survivors of chilhood cancer, revealed that central nervous system treatment may have serious long term consequences on cognitive and endocrine functions. Most common endocrinopathies related to radiation are hypothyroidism and lack of growth hormone. The effects of the treatment on the rate of growth are multifactorial and includes lack of growth hormone, spinal shrinking, early puberty, undetermined

and malnutrition. It is essential to maximize long term impact assessment until non-neurotoxic treatment completed (Duffner, 2004). Chemotherapy often causes intestinal damage (mucositis). There is no clinical evidence whether mucositis caused by chemotherapy endangers absorption. After chemotherapy, mucositis and/or diarrhea toxicity scores increase (De Koning et al., 2007). After chemotherapy, tibia and femur fracture risk increases because of the bending stiffness decreases. These are caused by malnutrition and accompanied by weight loss and direct effects of chemotherapeutic agents on skeletal system. Signs and symptoms suggestive of these complications should be looked out during the follow-up of children treated with chemotherapy (Van Leeuwen et al., 2003). Increasing the survival rates of chilhood cancer, long-term effects become crucial. Oral health during cancer treatment process is important for general health, nutrition level, quality of life and holistic care. Oral health is especially important for neuroblastoma patients treated with chemotherapy (Hutton et al., 2010).

Limitations of our study are the small number of participants included in our study, the lack of the ability to divide into subgroups according to their appropriate demographic and clinical characteristics, the disease progression and survival rates of different cancer types, and the presence of different eating culture habits, even if experienced in the same society.

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Evaluation of external carotid artery flow and thyroid gland functions after carotid endarterectomy

Zeki Talas[a*], Hasan Tüzün[b]

[a] *Department of Cardiovascular Surgery, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey*
[b] *Department of Cardiovascular Surgery, Cerrahpasa Faculty of Medicine, İstanbul University Cerrahpasa, İstanbul, Turkey*

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*** Correspondence to:**
Zeki Talas
Department of Cardiovascular Surgery,
Faculty of Medicine,
Kocaeli University, Kocaeli, Turkey
e-mail: zekitalas@gmail.com

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ABSTRACT

In developed countries, atherosclerosis is the major reason for morbidity and mortality. The external carotid artery is an important collateral in atherosclerotic carotid artery disease. Blind endarterectomy is performed to the external carotid artery during carotid endarterectomy surgery. After the surgery performed by this method, a 5-16% occlusion rate in the external carotid artery was reported in the short and long term. The aim is to show the early pathology on the external carotid artery and its clinical importance after standard carotid endarterectomy. In our study, 30 patients who underwent carotid endarterectomy for carotid artery stenosis were recruited in Istanbul University Cerrahpaşa, Department of Cardiovascular Surgery. Twenty-one patients were male and nine females. The mean age was 63.6 (±9.0). Preoperative duplex ultrasonography was performed in all patients. After discharge, during outpatient control FT3, FT4, and TSH were evaluated with blood tests and flow rates with color duplex. Rate evaluation: <50% stenosis if flow rate is <150 cm/s, 50%-75% stenosis if flow rate is between 150-250 cm/s, and >75% stenosis if flow rate is >250 cm/s. In the duplex examination, we did not detect any significant change in flow rates, external carotid artery flow rates in the preoperative and early postoperative period. However, as expected, a significant reduction in the internal carotid artery flow rates after the operation was recorded. In the analysis of thyroid functions, no significant difference was detected between preoperative and postoperative values. Stenosis, occlusion and dissection can be seen on the external carotid artery after carotid endarterectomy. There are no signs or symptoms due to these lesions. Carotid endarterectomy is a safe procedure for external carotid artery and thyroid superior artery and thyroid gland.

1. Introduction

The most important cause of mortality and morbidity in developed countries is atherosclerosis (Ros, 1993). Atherosclerosis leads to various diseases by creating lesions in blood vessels in different parts of the body. One of these clinical conditions is stroke due to carotid artery involvement. Stroke ranks third among all causes of mortality in the United States, followed by cardiovascular mortality. Today, the incidence of stroke cases is 120-180/100.000 every year (Car et al., 1996). This creates a negative socio-economic effect on society, patient and families. In all treatment protocols, the aim is to prevent cerebral infarction. A transient ischemic attack (TIA) is an alarm that warns us to prevent subsequent stroke or death. Research has mostly focused on this subject for symptomatic or

non-symptomatic carotid artery patients and the cases where surgery is required have been determined as a result of multidisciplinary meetings.

Carotid endarterectomy surgery for stroke prevention gained acceptance rapidly and became the most performed operation. The external carotid artery is an important collateral artery in atherosclerotic carotid artery disease (Aleksi et al., 2009). During carotid endarterectomy, many surgeons perform blinding endarterectomy to the external carotid artery. External carotid artery occlusion has been reported at a rate between 5-16% in the early or late postoperative period with this method (Joseph, 1998). Studies have shown > 50% stenosis in the external carotid artery after carotid endarterectomy in 18% of cases and > 75% stenosis in 10% of cases (Asce et al., 1996). From this point of view, we evaluated the effects of blinding endarterectomy on both the external carotid artery and the superior thyroid artery during carotid endarterectomy and their feeding regions, by evaluating external carotid artery flow parameters (by duplex ultrasonography) and thyroid function tests. Thus, we aimed to investigate the pathology created by standard carotid endarterectomy in the external carotid artery -which is a good collateral artery in the early period- and its clinical significance.

2. Material and methods

This study was conducted on 30 patients who underwent carotid endarterectomy for carotid artery stenosis in Istanbul University Cerrahpaşa Medicine Faculty Department of Cardiovascular Surgery. Ethics committee approval was obtained from the Ethics Commission of Istanbul University Faculty of Medicine (Capa Medicine Faculty) before the study.

Thirty patients were included in the study. Twenty-one patients were male and nine were female. The mean age was 63.6 (± 9.0) years. Twenty five (83.3%) patients were neurologically symptomatic and five (16.6%) were asymptomatic. Of the symptomatic patients 12% had a stroke, one (4%) had amaurosis fugax, one (4%) had syncope and 80% had transient ischemic attacks. When the risk factors were evaluated, 19 (63.3%) patients had hypertension, 11 (33.3%) patients had diabetes mellitus, 20 (66.6%) patients had hyperlipidemia, and 19 (63.3%) patients had a history of tobacco use.

Duplex ultrasonography (100%) was performed in all patients during the preoperative preparation period. Carotid system angiography and carotid system MR angiography were also used for the evaluation of surgical indications. Patients underwent routine carotid endarterectomy (shunts were routinely used and the main carotid artery, the internal carotid artery were

Table 1. Demographic data of patients.

Patient Number	Age	Gender	Symptom	Smoking	DM	HT	History of thyroidal disease	LDL-C mg/dl	HDL-C mg/dl	Total C mg/dl
1	70	M	+	+	-	+	-	98	28	155
2	73	M	+	+	-	+	-	109	34	174
3	55	F	+	+	+	+	-	112	35	209
4	72	F	-	-	+	-	-	164	57	242
5	65	M	+	+	-	+	-	90	33	138
6	70	F	+	-	-	+	-	138	37	203
7	65	M	+	-	+	+	-	143	39	213
8	55	M	+	+	+	+	-	84	33	138
9	73	M	+	-	-	+	-	89	27	139
10	63	F	-	+	+	-	-	186	40	245
11	40	F	-	-	+	-	-	118	23	192
12	59	M	-	+	-	-	+	143	53	211
13	60	M	-	+	-	-	-	205	50	288
14	67	M	+	+	-	+	-	132	29	200
15	64	M	+	-	+	+	-	125	32	180
16	83	M	-	-	-	-	-	85	48	160
17	70	M	-	+	-	-	-	138	34	186
18	59	M	-	+	-	-	-	153	30	199
19	56	F	+	-	-	+	-	122	38	201
20	58	M	-	+	-	-	-	161	41	220
21	58	M	+	-	+	+	-	84	31	156
22	67	F	+	-	+	-	-	82	34	165
23	54	F	+	-	+	+	-	135	47	201
24	57	M	-	+	-	+	-	200	36	268
25	56	M	+	+	+	+	-	98	36	164
26	60	M	+	+	-	+	-	107	50	201
27	62	M	+	+	-	+	-	80	45	147
28	80	M	+	+	-	+	-	159	44	227
29	78	M	+	+	-	+	-	84	39	142
30	60	F	-	+	-	-	-	179	44	245

clamped with the vascular clamp, the external carotid artery and the superior thyroid artery were clamped with the vascular loop) and followed up in the appropriate ward after the procedure. The patients were discharged on average five days postoperatively. The patients were recalled to our polyclinic between 15th and 30th postoperative days for follow-up. Routine blood tests and sT3, sT4, TSH blood tests were performed. Carotid system flow velocities were evaluated by color duplex ultrasonography (USG).

In duplex USG evaluation, peak systolic flow velocities in the main, internal and external carotid arteries were calculated and velocity increases in these vessels were evaluated. Similar to Ascer et al., a flow velocity of <150 cm/sec was evaluated as <50% stenosis, a flow velocity of 150-250 cm/sec was evaluated as 50-75% stenosis, and a flow velocity of > 250 cm/sec was evaluated as > 75% stenosis (Ascer et al., 1996; Table 1, 2).

Statistical analysis

The obtained values were expressed as mean and ± standard deviation values. All data were performed using the SPSS 15.0 statistical software package. The groups were compared by using the Student-t test. p-value <0.05 was considered statistically significant.

3. Results

Duplex USG results revealed that there was no significant change in the external carotid artery flow rates between the preoperative and early postoperative periods in patients who underwent carotid endarterectomy (p=0.184). However, as expected, flow rates of both the main carotid artery and the internal carotid artery were significantly decreased in the postoperative period (p <0.001; Table 3).

Table 3. Comparison of mean peak systolic flow velocities in carotid arteries before and after carotid endarterectomy.

	Preoperative	Postoperative	p value
Main Carotid Artery	105.2 (±30.0) cm/sec (n=30)	82.9 (±18.7) cm/sec (n=30)	< 0.001[x]
Internal Carotid Artery	228.2 (±70.9) cm/sec (n=30)	110.8 (±30.7) cm/sec (n=30)	< 0.001[x]
External Carotid Artery	117.9 (±47.4) cm/sec (n=30)	96.7 (±63.4) cm/sec (n=30)	> 0.984

Blood fT3, fT4 and TSH levels were measured before and after the operation for thyroid gland control. Based on the statistical analysis performed, there was no significant difference between preoperative and postoperative values.

Table 2. Dependent variable parameters.

Patient Number	Preoperative MCA velocity cm/s	Preoperative ECA velocity cm/s	Preoperative ICA velocity cm/s	Preoperative TSH mU/ml	Preoperative fT3 pg/ml	Preoperative fT4 ng/dl
1	103.5	140.7	289	2.57	3.04	1.01
2	132.2	119	275.7	1.2136	2.6	0.92
3	126.2	204	312.1	0.8802	0.56	1.38
4	99.4	106.7	275.8	0.4468	2.44	1.42
5	107.3	92	219.2	1.3815	2.37	1.28
6	132	95	294.7	1.211	2.32	1.19
7	154.3	188	289.4	0.6522	2.7	1.15
8	105.2	112.7	259.2	1.1762	2.75	1.11
9	98.6	123	248.2	1.214	1.72	1.03
10	104.7	134.7	246.4	3.811	2.8	1.01
11	84.3	104	374.2	1.563	2.21	1.34
12	99.4	116	176.5	0.019	2.75	1.41
13	137.2	125.2	199.4	1.401	3.67	1.32
14	104.6	145	154.9	1.1425	2.63	1.19
15	67	166	128.5	0.4182	2.62	1.11
16	117.4	88.5	247.3	2.19	3.5	1.11
17	64.3	109	183.2	0.65	1.7	1.07
18	87.9	60.1	213.7	0.43	3.17	1.2
19	104.5	96.7	154	4.459	3.25	1.27
20	83	167.5	221.4	1.989	2.52	1.06
21	214	0	275.7	1.6342	2.57	1.08
22	78.3	114	215.6	0.981	2.28	1.34
23	85.7	98.9	178.6	2.71	2.71	1.3
24	127	250	402.5	1.49	3.83	1.52
25	105	90.8	208	1.375	3.1	1.2
26	86	154.7	89.4	0.9819	3.12	1.18
27	74.6	104.8	123.9	1.06	2.9	1.26
28	103	70	220	0.9	3	1.09
29	67.5	102.7	148	0.96	2.65	1.56
30	103	58	220	0.74	3.13	1.02

When external carotid artery flow velocities were compared according to the presence of disease risk factors (i.e. hypertension, hyperlipidemia, gender, smoking history and the presence of diabetes), no significant increase was found in flow velocities (p=0.567). Similarly, there was no significant change in thyroid function tests according to risk factors (Table 4, 5). Postoperatively, there was no significant change in thyroid function tests between diabetic and non-diabetic patients (p=0.784; Table 6).

Table 4. Evaluation of thyroid function tests results before and after carotid endarterectomy.

	Preoperative value	Postoperative value	p value
fT3 (pg/ml)	2.7 (±0.6) (n=30)	3.0 (±0.8) (n=30)	> 0.072
fT4 (ng/dl)	1.2 (±0.2) (n=30)	1.2 (±0.2) (n=30)	> 1.00
TSH (mU/ml)	1.4 (±1.0) (n=30)	1.4 (±0.8) (n=30)	> 1.00

Table 5. Evaluation of the effect of diabetes on mean peak systolic flow velocity in the external cartois artery after carotid endarterectomy.

	DM + (11 patients)	DM – (19 patients)
Preop ECA peak systolic flow velocity (cm/sec)	120.0 ± 55.0	116.7 ± 44.0
Postop ECA peao systolic flow velocity (cm/sec)	128.4 ± 91.2	78.4 ± 29.8
P value	> 0.784	> 0.996

Table 6. Evaluation of the effect of diabetes on mean free T3, free T4 and TSH values after carotid endarterectomy.

	DM + (11 patients)		DM – (19 patients)		p value
	preop	postop	preop	postop	
TSH (mU/ml)	1.4 ± 1.0	1.5 ± 1.0	1.4 ± 1.0	1.3 ± 0.7	> 1.02
fT3 (pg/ml)	2.4 ± 0.7	2.8 ± 0.9	2.8 ± 0.6	3.1 ± 0.7	> 1.08
fT4 (ng/dl)	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	> 1.00

One (3.3%) of the patients, flow velocity in the external carotid artery could not be detected by Doppler ultrasonography on the 15th postoperative day (occlusion). Patient had no significant complaints and there were no physical examination findings. Therefore, it was decided to continue the patient's treatment without any changes and the patient is being followed clinically.

In one patient (3.3%), although the external carotid artery flow velocity was 80.7 cm/sec, Doppler ultrasonography showed dissected vessel wall and flap. 65-year-old female patient had no risk factors other than hypertension. Dissection was detected during routine follow-up and the patient was taken to the outpatient clinic follow-up due to the lack of complaints or clinical symptoms (Fig. 1).

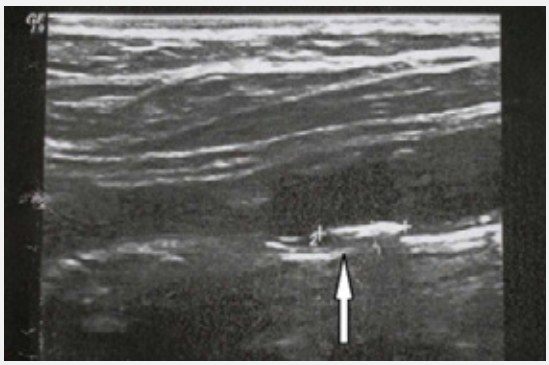

Fig. 1. Flap in the external carotid artery after the operation (arrow).

In addition, flow velocity above 250 cm/sec was detected in two (6.6%) patients. This rate is compatible with >75% stenosis. Less than 50% stenosis was detected in the rest of the patients (86.6%) according to the peak systolic velocity of the external carotid artery. The mean systolic velocity of the external carotid artery after carotid endarterectomy was 96.7 ± 63.4 cm/sec.

4. Discussion

In carotid endarterectomy, endarterectomy for the external carotid and superior thyroid arteries is performed blindly. Pathologies such as restenosis, occlusion and dissection may develop in these arteries due to blinding endarterectomy for the external carotid artery performed as a routine surgical method. Cerebrovascular disease caused by embolism in the retrograde internal carotid artery due to thrombosis of the external carotid artery may also be seen (Ascer et al., 1996). In our series, two (6.6%) patients had >75% stenosis, one patient (3.3%) had occlusion, and one patient (3.3%) had dissection after carotid endarterectomy according to peak systolic flow velocities and color Doppler ultrasonography. In total, three (10%) patients developed >50% stenosis. The rate of non-severe external carotid artery stenosis was 86.6% with 26 patients.

In a study by Archie et al. 10% (±2) of 313 patients undergoing carotid endarterectomy operation had severe stenosis or >75% stenosis in the external carotid artery postoperatively. The rate of patients with <50% external carotid artery stenosis in the early period was 74% (±6.9) (Joseph, 1998). Ascer et al. also detected <50% early postoperative stenosis in 93 (82%) out of 116 patients who underwent blinding external carotid artery endarterectomy. 50-74% stenosis in the external carotid artery was detected in 11 (10%) patients, whereas >75% stenosis was detected in nine (8%) patients. Full occlusion was not observed in any patient after the operation (Ascer et al., 1996). In their 2009 study, Aleksic et al. measured flow volume in carotid arteries by Doppler ultrasonography. According to the

results of this study, the volume in the external carotid artery has decreased by 4% in the early postoperative period (Aleksic et al., 2009).

Although no complications related to the superior thyroid artery have been reported in the literature, we wanted to evaluate the thyroid gland using thyroid function tests. Based on the results of our study, it was seen that there was no significant change in thyroid function tests. When the patients were examined according to risk factors, no significant results were found in terms of thyroid function tests. There are studies in which the superior thyroid artery is used as a graft for the patch. In a study by Jenkins et al., an ipsilateral superior thyroid artery was used as the patch. The authors reported that using the superior thyroid artery as a patch did not create any complications on thyroid gland function (Jenkins et al., 1997).

The optimal method of protecting the external carotid artery during carotid endarterectomy is unclear. In the study comparing the blinding and eversion methods performed by Archie et al., external carotid artery restenosis rates were similar (Joseph, 1998). The importance of the external carotid artery during carotid endarterectomy does not only apply to the area perfused by the external carotid artery. At the same time, the external carotid artery may be a source of embolism that may affect the internal carotid artery during and after the operation (Ascer et al., 1996). Therefore, the external carotid artery should be carefully protected during and after the operation. Moore reported three cerebrovascular embolism cases that developed in an antegrade route from the internal carotid artery due to the flap forming in the external carotid artery following carotid endarterectomy, and progressed in a retrograde route (Moore et al., 1990). Therefore, complications and disease may occur if the external carotid artery is not maintained during the operation and insufficient endarterectomy is performed.

In the study of Ascer et al., the rate of patients with >75% stenosis in both external and internal carotid arteries after carotid endarterectomy was found to be 8%. Approximately 80% of the patients had stenosis in the external carotid artery, though less than 50%. The point to be taken here is that in order for a postoperative ischemic event related to the internal carotid artery to develop, the external carotid artery may need to be occluded. Severe stenosis or occlusion of the external carotid artery does not cause significant ischemic clinical problems and is usually a benign disease (Ascer et al., 1996). Ascer et al. performed endarterectomy by transecting the atherothrombosis in the external carotid artery orifice in order to avoid blinding endarterectomy to the external carotid artery, and argued that this technique did not cause postoperative or perioperative neurological complications and severe stenosis of the external carotid artery (Ascer et al., 1996). In this study, the rate of severe (> 75%) stenosis in the external carotid artery was found to be 8% in the early postoperative period. In the study of Archie et al., blinding endarterectomy and eversion methods were compared. Here, longitudinal arteriotomy was performed after transecting the external carotid artery and complete plaque removal was achieved. Postoperative severe external carotid artery stenosis rate was found to be 10% (Joseph, 1998). These two studies reported highly similar results.

As Aleksic emphasized in his study, for the better results, performing the controls of the arteries with caution and as less traumatic as possible, considering the traumatic effect of the clamps and even the vascular loops used, exploration of the external carotid artery with its branches if necessary, and extending the arteriotomy to the appropriate site are acceptable options (Aleksic et al., 2009). In other studies, external carotid artery thrombosis, either symptomatic or asymptomatic, is reported in the literature in varying rates between 5% and 16% (Ascer et al., 1996). Stenosis or occlusion of the external carotid artery following carotid endarterectomy may not cause significant clinical problems and many of these may not require intervention. However, the external carotid artery should still be preserved during surgery because of its collateral importance, especially in patients with lesions in the contralateral carotid artery. The superiority of the methods performed for this purpose such as eversion endarterectomy and shunt application to the external carotid artery over standard carotid endarterectomy could not be demonstrated.

In a study in which shunt application was performed to the external carotid artery during carotid endarterectomy, it was stated that shunt application to the external carotid artery did not create a significant change in surgical technique and did not change the clamp time. In this study, shunt was applied to the external carotid artery at the time of a neurological event in patients operated under locoregional anesthesia and routine shunt application was not performed. The importance of the external carotid artery as a collateral vessel was investigated (Belardi et al., 2001). However, as a result, if a shunt was to be applied to the external carotid artery, proximal branches were considered and it was decided to apply the shunt up to the first 3 cm of the artery and it was concluded that shunt application to the external carotid artery did not have significant effects in terms of the operation (Belardi et al., 2001). This study has a number of limitations worth noting. First, we conducted a retrospective study. Second, the number of patients, which were included in our study, may seem relatively small compared to other studies. Third, it's a single-center design. Further prospective randomized trials with large volumes are needed to compare evaluation of external carotid artery flow and

thyroid gland functions after carotid endarterectomy.

Postoperative external carotid artery stenosis or occlusion is roughly attributed to incomplete endarterectomy or the flap remaining in the lumen. During the operation, endarterectomy on the external carotid artery with an open technique (non-blinding endarterectomy) should be performed by visually observing the most distal part of the thrombotic plaque and not leaving any residual flap as in the internal carotid artery. However, in this way, factors such as prolonged operation time, greater dissection and increased anastomosis length will come into play. In order to make a healthy and more definitive evaluation, we need to perform further external carotid endarterectomy operations and evaluate their results. Carotid endarterectomy is a safe procedure for external carotid artery and thyroid superior artery and thyroid gland.

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The role of CT angiography in preoperative evaluation of aortic coarctation

Tülay Demircan[a*], Onur Işık[b], Muhammet Akyüz[b], Ahmet Ergin Çapar[c], Kayı Eliaçık[d]

[a] *Department of Pediatric Cardiology, Tepecik Training and Research Hospital, Izmir, Turkey*
[b] *Department of Pediatric Cardiovascular Surgery, Tepecik Training and Research Hospital, Izmir, Turkey*
[c] *Department of Radiology, Tepecik Training and Research Hospital, Izmir, Turkey*
[d] *Department of Pediatrics, Tepecik Training and Research Hospital, Izmir, Turkey*

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*** Correspondence to:**
Tülay Demircan
Department of Pediatric Cardiology,
Tepecik Training and Research Hospital,
Izmir, Turkey
e-mail: tulay.sirin@hotmail.com

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ABSTRACT

Aortic coarctation (CoA) constitutes 5-8% of all congenital heart diseases. Physical examination findings and imaging methods are helpful in diagnosis. Computed tomographic (CT) angiography and transthoracic echocardiography are the common diagnostic tools for aortic coarctation. In this study, we aimed to evaluate the intracardiac and extracardiac anomalies that we detected in our cases. We also evaluated the contribution of preoperative diagnosis of extracardiac anomalies in preventing surgical complications. From January 2016 and May 2018, we enrolled 37 infants with clinically suspected CoA who underwent CT angiography and transthoracic echocardiography and operated in our hospital. The extracardiac and intracardiac anomalies associated with CoA were evaluated. Extracardiac anomalies that were not seen in transthoracic echocardiography but diagnosed by CT angiography were evaluated. The contribution of CT angiography in surgical planning was determined. The patients (24 males and 13 females) were aged from one day to 240 months. Of this sample, 54% of thirty-seven patients were in the neonatal period. When we examined the accompanying intracardiac pathologies, ventricular septal defect was present in three cases, atrial septal defect in seven cases, subaortic membrane and Shone complex in two cases. Extracardiac anomalies such as, tracheal duplication, Scimitar syndrome, pulmonary vein course anomaly and left bronchial pressure were detected by CT angiography in 2.7% of the patients. Abnormal right subclavian artery was present in 10.8% of the cases and the surgical team was more sensitive to paraplegia measures. In 19% of the longsegment coarctation; if the surgical team consider it too far for end-to-end anastomosis, they chose synthetic tube graft for the patient prior to the operation. The results indicated that the CT angiography is a beneficial non-invasive method for confirming CoA and detecting the accompanying extracardiac anomalies in children with CoA. Preoperative morphological features and extracardiac anomalies which were detected by CT angiography were found to be reliable for surgical planning.

1. Introduction

Coarctation of the aorta (CoA) is a common congenital heart disease that appears approximately three in every 10000 births (Ringel et al., 2012; Beekman, 2013). Generally, the diagnosis is made during childhood, but there are frequent cases that are not diagnosed

until adulthood (Brickner et al., 2000). It often occurs in a discrete or tubular form in the distal of the left subclavian artery. CoA could be an isolated disorder as well as it could be seen with aortic arch hypoplasia, left heart lesions (aortic stenosis, mitral stenosis, hypoplastic left heart syndrome), atrial septal defect (ASD), ventricular septal defect (VSD), bicuspid aorta, Turner syndrome and aneurysm of circle of Willis (Attenhofer et al., 2002).

In addition to the physical examination, imaging methods are helpful in the diagnosis (Mellander et al., 2006; Gómez-Montes et al., 2013). Today transthoracic echocardiography, CT and MR angiography are the most preferred diagnostic methods. Computerized tomography angiography is one of the most frequently used methods due to its short duration of examination, sedation time, and its applicability in patients with poor general condition (Lee et al., 2004; Tsai et al., 2008). The location of coarctation, collateral circulation, relation with the left subclavian artery, arcus hypoplasia, and other extravascular anomalies that may accompany can be detected by CT angiography (Frush, 2005; Taylor, 2008). However, the role of detecting these extracardiac anomalies for the success of surgery has not been studied enough yet. Catheter angiography is used today for treatment rather than diagnosis. Compared to catheter angiography, CT angiography is exposed to a lower dose of radiation.

In the current study, we investigated the role of CT angiography in the preoperative evaluation of patients and its effectiveness on mortality during surgery.

2. Materials and methods

Patient population

We reviewed the charts of 37 consecutive infants who were diagnosed as CoA clinically and admitted to our hospital between January 2016 and May 2018. The research procedure was approved by the local ethical committee (No:2018/7-13). Demographics of the patients were also analyzed: Symptoms, age, gender distribution, weight, height, imaging data and operation procedure. At the same time, we evaluated the frequency of congenital heart defects associated with aortic coarctation. We included patients with mild associated cardiac anomalies that were not addressed at the time of the coarctation repair (ie, atrial septal defect or ventricular septal defect bicuspid aorta, aortic stenosis and Shone complex) but excluded complex congenital heart defects (ie, single ventricular physiology double outlet right ventricle -DORV and hypoplastic left heart syndrome- HLHS etc.). We evaluated the surgical benefits of knowledge of extravascular anomalies not detected in echocardiography and detected in CT angiograhy. We investigated the role of CT angiography in the evaluation of preoperative patients and its effectiveness on mortality.

Transthoracic echocardiography

All transthoracic echocardiography was performed by the pediatric cardiologist using the Philips Ultrasound System and the S8-3, S3-1 probe. The transthoracic echocardiography protocol recommended by the European Guidelines was applied to all patients (Evangelista et al., 2008). Intracardiac defects were evaluated by the apical four-chamber view, left ventricular long axis, suprasternal examination, parasternal short-long axis and subcostal examinations. During the suprasternal examination, the location and length of the CoA, diameter of the coarctation zone and the transverse aorta were evaluated. Color Doppler imaging was used to evaluate the blood flow and measure the differential pressure and maximum speed in the location of the CoA.

CT image acquisition

Common indications for CT angiography included transverse arch hypoplasia, poor echo windows, complex geometry, or uncertain arch branching pattern; surgical preference for three-dimensional (3-D) imaging was also a considerable factor. Computerized tomography angiography scan was performed with a Siemens Definition AS 128 section device and required cases were treated with chloral hydrate in a sedative manner with a dose of 50 mg/kg rectally or orally. Patients were examined in supine position, and we took images extending from the base of the neck to the diaphragm. A low radiation and contrast agent protocol was applied in the procedure according to age and weight. The non-ionic contrast agent was administered with a dosage of 0.8 cc/kg using antecubital vein. The lowest radiation dosage was applied to the tissue using 'care dose' technology according to the body area (collimation 16x1.25 mm, slice thickness 0.75 mm, slice interval 0.625 mm, pitch set automatically, reconstruction interval 0.625 mm).

The following analyses were performed in each patient for diagnostic evaluation.

i) Axial single-phase low dose multidetector CT scans and two-dimensional sagittal and coronal reconstructions.

ii) Maximum intensity projection.

iii) 3D image reconstruction included multiplanar reformatting (MPR), curved planar reformatting, maximum intensity projection (MIP), and volume rendering (VR).

All data were evaluated by a pediatric cardiologist and cardiac surgeon on the basis of preoperative chest x-ray and echocardiography.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA) statistical package program. Descriptive statistics are given as

number of units (n), percentage (%), mean ± standard deviation. The sensitivity and specificity were used to delineate the diagnostic accuracy of CT angiography and TTE for the extracardiac and intracardiac malformations in each group.

3. Results

During the study period the data of thirty-seven patients who underwent surgery for CoA were evaluated. The patients were aged from one days to 240 months and the mean body weight ranged from 2 to 80 kg. Of this sample, 54% of 37 patients were in the neonatal period. According to the examination by gender, 35% were female and 65% were male. A murmur of the heart was found in 44% of admitted patients, followed by respiratory distress with 19% and antenatal diagnosis with 12%. Short segment coarctation was present in 81% of the patient and in 19% long segment coarctation was seen.

When we examine the accompanying intracardiac pathologies, VSD was present in three cases, ASD in seven cases, subaortic membrane and Shone complex in two cases. Pulmonary artery banding operation was performed in two of our patients with large muscular VSD. The prevalence of bicuspid aortic valve was 30%. The (patent ductus arteriosus) PDA was observed in 11 cases in the newborn period. There was one case who had Turner syndrome, one had VACTERL (vertebral defects, anal atresia, cardiac malformations, tracheoesophageal fistula, renal anomalies and extremity anomalies), one with reactive airway disease, one case with a single kidney and one had epilepsy. Aberrant right subclavian artery was seen in 10.8% of the cases (Fig.1). Also with the help of CT angiography, one patient was diagnosed with tracheal duplication, one patient with Scimitar syndrome, (Fig. 2), one patient with a pulmonary vein course anomaly, and one patient with left bronchial pressure.

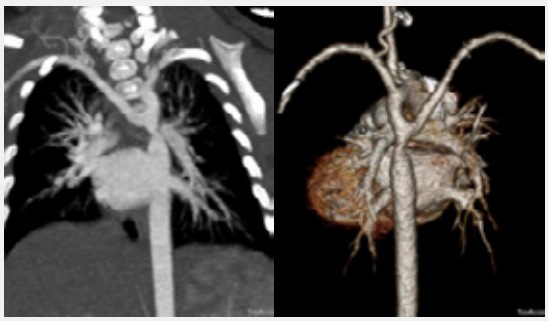

Fig. 1. CT angiography of 3 years old girl with aortic coarctation and aberrant right subclavian artery association in coronal image. The same patient 3D reconstruction image also seen on the left part of image.

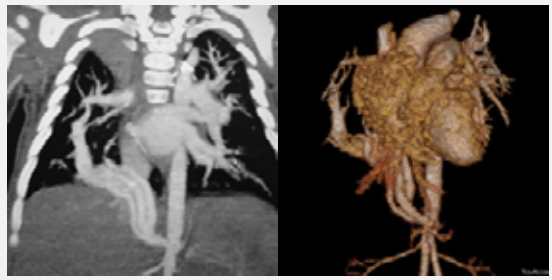

Fig. 2. Coronal CT angiography of a 6-months old case with Scimitar syndrome + recoarctation. Aorto-pulmonary collateral artery, Scimitar vein and the clips in the recoarctation region. The same patient 3D reconstruction image also seen on the left part of the image.

Enlarged end-to-end anostomosis was performed on 29 (78%) of our patients after resection, four patients were treated with tube graft interposition (10.8%) and aortoplasty with xenograft pericardial patch and glutaraldehyde treatment was administered in three cases (8.1%). If it seems too far for end-to-end anastomosis with measurements by CT angiography, a synthetic tube graft was chosen for the patient prior to the operation. In one case, an arcus hypoplasia was detected and arcus reconstruction surgery was performed (Fig. 3). Isthmus hypoplasia was detected in five cases with CT angiography and the operation plan was not altered because it was not severe. Abnormal right subclavian artery was present in 10.8% of the cases and the surgical team was more sensitive to paraplegia measures. In a15 days old case re-coarctation was encountered due to PDA clips or fibrotic stenosis at the site of surgery (Fig. 4). In one case, left bronchial compression was observed due to the abnormal course of the aortic arch, and after end-to-end anastomosis, posterior aortoplasty procedure was performed on the descendant aorta. Aortic patch plasty was preferred only in patients with re-coarctation. Tube graft interposition was favored in patients with long segment stenosis and isthmus hypoplasia in adult type aortic coarctations. In all other patients, extended end-to-end anastomosis operation was performed after resection. All patients' operations were performed using a simple cross-clamping technique. There was no complication in our patients and there was no death. Detailed information of the patients is given in Table 1.

4. Discussion

In our study detection rate of transthoracic echocardiography for intracardiac anomalies and CoA were 100% while the rate for extracardiac anomalies such as an aberrant right subclavian artery (ARSA) and Scimitar syndrome were 86.5% (using surgery as the gold standard). With the advances

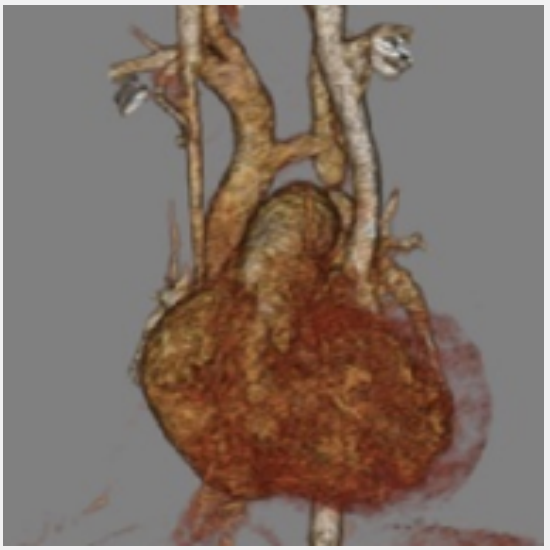

Fig. 3. CT Angiography 3D reconstruction image. four-month old; aortic coarctation + arcus hypoplasia.

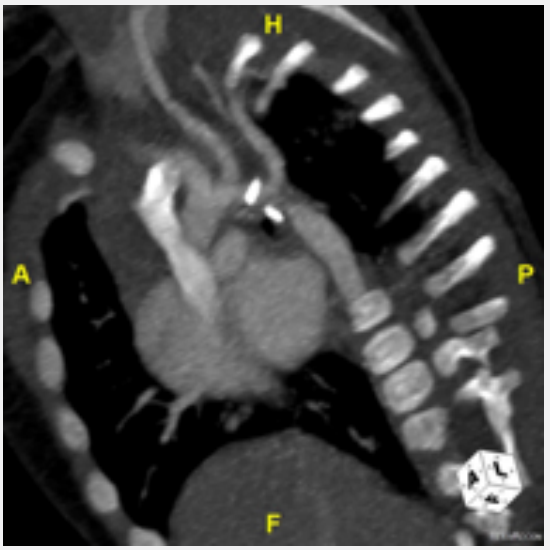

Fig. 4. Sagittal multiplanar examination showed re-coarctation due to PDA clips or fibrotic stenosis at the site of surgery in the 15-days-old case operated for aortic coarctation.

in transthoracic echocardiography devices and the increased experience of the clinicians performing it, it is now one of the best noninvasive and easily applied methods in the diagnosis of congenital heart diseases in infants and young children. Other commonly used methods are catheter, CT and MR angiography. There are some studies comparing CT angiography with TTE in diagnosis of congenital CoA and accompanying malformations (Al-Azzazy et al., 2014). In the diagnosis of CoA; sensitivity of CT angiography was found to be 100%, while the sensitivity of transthoracic echocardiography was 91%, and for the diagnosis of VSD, the specificity of CT angiography was found as 91.7% (Turkvatan et al., 2009; Rose-Felker et al., 2017). Didier et al., observed MR, MR angiography and transthoracic echocardiography results were evaluated preoperatively and postoperatively and it was observed that especially in postoperative patients transthoracic echocardiography was less effective in showing pseudo-aneurysms, isthmus hypoplasia, and re-coarctation. (Didier et al., 2006). Lee et al., reported 14 newborn cases referred for catheter angiography were correctly diagnosed with CT angiography and catheter angiography was no longer required. (Lee et al., 2006).

Preoperative knowledge of the extracardiac anomalies and arcus hypoplasias by the surgeon provide significant benefit in terms of operation selection (Stone et al.,1990; Adaletli et al., 2011). In our cases of long and hypoplastic segment coarctation the surgical team prepared for patch enlargement of arcus aorta and patchplasty procedure can be done safely when the PDA was open. In addition if the surgical team consider the case too far for end to end anastomosis they may choose a syntetic tube graft for the patient. It may be difficult to decide tube grafts size intraoperative moment but if the team was prepared on images for the case this is not an issue for procedure.

Our cases who were diagnosed with ARSA were between the ages of 0-3 years and all of them were localized behind esophagus and caused esophagus compression. In these patients, surgical division performed on ARSA to release the aorta in order to anastomose the tips and decompress the pressure on the esophagus. When aortic coarctation is accompanied by ARSA the rate of morbidity and surgical strategy change. If the patient have an aberrant artery associated with coarctation then the surgeons awareness is very important because we know that these patient groups have higher paraplegia ratio (Kieffer et al., 1994). At this situation the surgical team may be more sensitive for paraplegia precautions. Complications did not develop in our patients.

Computerized tomography angiography was helpful in the diagnosis of some patients. One of these patients (case 31) who was referred to our unit from another center with re-coarctation diagnosis. The echocardiographic evaluation revealed significant enlargement on the right side structures and pulmonary hypertension. The patient had obvious respiratory distress, CT angiography was planned hence the general condition of the patient was not appropriate for catheter angiography. Our case with right lung hypoplasia was found to have Scimitar vein and large aorta pulmonary collateral arteries. In another patient (case 19), partial tracheal duplication was detected by CT angiography. This anomaly was an uncommon and

Table 1. Demographic characteristics, symptoms and results of various imaging methods in 37 patients with CoA.

No	Age	G	Symptoms	Type of CoA	BAV	Accompanying intracardiac pathology	The defect detected in CT-angiography	Comorbidity	Type of operation
1.	1 M	M	Murmur	Short		PFO			End-to-end
2.	20 Y	F	Murmur	Long				Turner Syndrome	Tube Graft
3.	1 M	M	Tachypnea	Long		PFO, PDA, hypoplasia of arcus aorta	Hypoplasia of İsthmus, PDA		End-to-end
4.	1 M	M	Antenatal diagnosis	Short		PFO, large muscular VSD	VSD		End-to-end, pulmonary artery banding
5.	5 M	M	Murmur	Short					End-to-end
6.	17 Y	M	Control	Long	+	Shone complex, subaortic membrane			Tube Graft
7.	8 M	M	Murmur	Short	+	Shone complex			End-to-end
8.	2 Y	F	Tachycardia	Short					End-to-end
9.	1 M	F	Murmur	Short			Moderate hypoplasia of arcus	Epilepsy	End-to-end
10.	5 D	M	Dyspnea	Short		PDA,PFO, Arcus hypoplasia	Hypoplasia of isthmus, PDA	Meningo-myelocele	End-to-end
11.	12 Y	M	Growth retardation	Long	+	PDA	PDA	Left renal agenesis	Tube Graft
12.	2 D	M	Dyspnea	Short		PDA, ASD	PDA		End-to-end
13.	10 D	M	Murmur	Short	+	PFO			End-to-end
14.	2 D	F	Dyspnea	Short		PDA	PDA		End-to-end
15.	1D	F	Antenatal diagnosis	Short		PDA	PDA		End-to-end
16.	5 D	M	Dyspnea	Short	+	PDA	Hypoplasia of isthmus, PDA		End-to-end
17.	1 D	F	Newborn congenital anomaly	Short		PDA, ASD	Hypoplasia of isthmus, PDA	VACTERL	End-to-end
18.	1 D	M	Murmur	Short	+	PDA, ASD	PDA		End-to-end
19.	10 D	F	Murmur	Short		Moderate hypoplasia of arcus aorta		Tracheal duplication	End-to-end
20.	1 M	F	Murmur	Short	+	LPSVC	ARSA, LPSVC		End-to-end, division of ARSA
21.	3 M	M	Murmur	Short	+		ARSA		End-to-end, division of ARSA
22.	4 M	F	Murmur	Long		Large muscular VSD, hypoplasia of arcus aorta	Hypoplasia of arcus aorta, VSD		Reconstruction of arcus aorta, pulmonary artery banding
23.	3 D	M	Dyspnea	Short		PDA	PDA		End-to-end
24.	5 D	F	Dyspnea	Short	+	ASD,TAPVD?	pulmonary vein course anomaly		End-to-end
25.	15 Y	M	Hypertension	Short	+				Tube Graft
26.	3 Y	F	Murmur	Short		VSD,subaortic membrane	ARSA		End-to-end, division of ARSA
27.	1 D	M	Antenatal diagnosis	Short		Right pulmonary agenesis? ASD	Hypoplasia of right PA, ARSA		End-to-end
28.	1 D	F	Antenatal diagnosis	Short		PFO,PDA	PDA		End-to-end
29.	2 M	F	Murmur	Short		ASD			End-to-end
30.	1 M	M	Murmur	Short		ASD			End-to-end
31.	6 M	M	Dyspnea	Short		Recoarctation,Pulmonary hypertension	Scimitar syndrome, APKA	Scimitar syndrome, APKA	End-to-end, Scimitar vein anastomosis, ligation of APKA
32.	3 Y	M	Murmur	Long				Reactive airway disease	Patch aortoplasty
33.	12 Y	M	Control	Long		Recoarctation			Pericardial patch
34.	8 Y	F	Control	Short		Recoarctation			Pericardial patch
35.	19 D	M	Dyspnea	Short		PDA, PFO			End-to-end
36.	4 M	M	Murmur	Short	+				End-to-end
37.	3,5 M	F	Murmur	Short			Compression of arcus to left main bronchi		End-to-end

G: Gender, M:Male, F:Female, PFO: Patent foramen ovale, ASD: Atrial septal defect, VSD:Ventricular septal defect, CoA: Coarctation of the aorta, TAPVD:Total anomalous pulmonary venous, LPSVC: Left-sided persistent superior vena cava, PDA: Patent ductus arteriosus, ARSA:Aberrant retro esophageal subclavian artery, CT: Computed tomography, MRI: Magnetic resonance imaging, TTE: Transthoracic echocardiography.

asymptomatic situation that is diagnosed incidentally. As long as they do not cause respiratory problems, they do not require treatment (Karcaaltincaba et al., 2004) and we did not perform any additional intervention on our patient. But sometimes fistulas can accompany and knowing the existence of a fistula pre-operatively is beneficial in planning the anesthesia management of the patient. In another patient with re-coarctation (case 28), we found that metallic clips used for PDA closure caused re-coarctation or fibrotic stenosis at the site of surgery. In this patient, we preferred surgery instead of angiography. CT angiography is excellent at finding associated extracardiac anomalies but it is not useful for visualizing the aortic gradient or other cardiac malformations (Huang et al., 2017). In the case of recoarctation it may be vital the examining the CT images before operation. Because the if the surgeon examines CT images; dissection may be more easy and safe. At such cases the surgical team may find a reason for coarctation (large titanium clips applied to ductus, intraaortic thrombosis, pseodoaneurism etc.). We believe that the planning the operation and discussion from obtained images may help to improve safety and quality of the procedure. Maybe the clinicians use CT for the diagnose but on the other side the surgical team uses images for planning, avoiding complications. Our study has some limitations that to be considered. First, relatively small sample size is foremost limitation of our study. Secondly, this study was limited by its retrospective design and clinically obtained data.

As a result, we conclude that transthoracic echocardiography is the first choice for the coarctation diagnosis depending on the experiment of the clinician and CT angiography is useful for patients with poor acoustic window for TEE and extracardiac malformation suspicion. The location of coarctation, collateral circulation, relation with the left subclavian artery, arcus hypoplasia, and other extravascular anomalies that may accompany can be detected by CT angiography. The main purpose is planning a better management with the preoperative data and imaging and preventing possible complications. Knowing preoperative vascular anomalies may change the type of surgery. It can also prevent re-surgeries.

Conflict of interest

The authors declared no conflicts of interest.

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Effects of preoperative planning on the treatment of the tibial plateau fractures using 3-column classification on radiological results

Emre Gültaç*, Hıdır Tanyıldızı, Cem Yalın Kılınç, Hüseyin Aydoğmuş, Rabia Mihriban Kılınç, Nevres Hürriyet Aydoğan

Department of Orthopaedics and Traumatology, Faculty of Medicine, Sıtkı Koçman University, Muğla, Turkey

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*** Correspondence to:**
Emre Gültaç
Department of Orthopaedics and Traumatology,
Faculty of Medicine, Sıtkı Koçman University,
Muğla, Turkey
e-mail: emregultac@yahoo.com

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ABSTRACT

Tibial plateau fractures are common and difficult to manage. The purpose of the treatment of tibia plateau fractures is to obtain knee joint which is stable, painless and has functional range of motion. Schatzker's classification and AO classification are most commonly used for the categorisation and to guide the treatment of these fractures. The 3-column classification is a useful tool in the preoperative surgical planning of tibial plateau fractures, especially in fractures extending to the posterolateral corner. The purpose of this study is to evaluate the effect of planning the treatment of the tibial plateau fractures using 3-column classification prior to surgical treatment on radiological results. A retrospective examination was made of 43 patients (32 males, 11 females, mean age 48.91) patients with operatively treated plateau fractures at Muğla Sıtkı Koçman University Medical Faculty was obtained. Preoperative and postoperative 6th month and 1st year radiological images were collected. Radiological evaluation of 43 patients included in the study revealed that 22 had a posterior column fracture. 11 of these posterior column fractures were fixated by posterior plate except medial and-or lateral plate or screw fixation. In addition, only interfragmanter screws were used in fixation of four patients and only plate oriented posterior screws were used in three patients with posterior column fracture. On the other hand, in four patients with posterior column fracture, no fixation was found except medial and-or lateral fixation for the posterior fractured components. The average preoperative modified rasmussen score of 22 patients with posterior column fracture was 3.5. Postoperative Modified Rasmussen Scores were calculated as 7.54 and 6.09 in the 6th months postoperative and 1st year, respectively. At the postoperative 1st year time nine patients in Kellgren-Lawrence grade 1, 18 patients in grade 2-3 and 16 the patients had grade 4 osteoarthritis. Grade 4 OA patients were all patients without any extra fixation for posterior column fracture. Modified Rasmussen Scores of these patients were also lower than other patients. Eleven patients who had fixation with posterior plate had higher radiological scores and less osteoarthritis findings. It was observed that there was no significant difference in terms of radiological scoring and osteoarthritic changes between patients who received an interragmenter screw and those who had a posterior oriented screw over the plate. As it is well known that postoperative negative changes and degeneration in the joint result from non-anatomic fixation. We think that in patients with proximal tibia fracture, diagnosis of fractures and recognition of fracture patterns in the posterior column are important and the most accurate diagnosis will be possible with CT imaging. We believe that efforts should be made for the anatomical detection of the fracture diagnosed.

1. Introduction

Tibial plateau fractures account for approximately 1% of all fractures (Cole et al., 2009). Tibial plateau fractures are common and difficult to manage injuries that can be due to high-energy trauma in young adults or low-energy trauma in elderly patients. Tibial plateau fractures occur due to a combination of axial loading and varus/valgus applied forces leading to articular depression, malalignment and an increased risk of posttraumatic osteoarthritis and should be considered as complex injuries representing a wide fracture spectrum, soft-tissue compromise, neurovascular damage, compartment syndrome, and ligament and meniscus tears (Lansinger et al., 1986; Honkonen et al., 1995).

Conservative treatment is limited only to simple non-displaced fractures and elderly patients with co-morbidity. With the development of surgical techniques and imaging methods, tibial plateau fractures are treated with surgical methods more frequently. Anatomical reduction of the joint surface, absolute stability and minimal soft tissue dissection is extremely important in surgical treatment (Mahadeva et al., 2008).

There are various surgical techniques used in the surgical treatment of tibia plateau fractures. Open reduction of the joint and fixation with screws and/or plates, arthroscopy or scopy assisted percutaneous screw fixation, external fixation, plaster or traction application are the treatment options. Currently, open reduction internal fixation using locked plate systems is accepted as the gold standard treatment method (Biggi et al., 2010; Wang et al., 2013).

The purpose of the treatment of tibia plateau fractures is to obtain a knee joint which is stable, painless and has functional range of motion. Fragmented fracture and poor soft tissue make it difficult to treat these injuries and may result in high complications and morbidity. The risk of wound complications is very high in the surgical treatment of tibial plateau fractures caused by high-energy traumas. Surgical timing should be set according to the condition of the soft tissues, and if necessary soft tissues should be waited until they become able to allow surgical intervention (Moore et al., 1987; Katsenis et al., 2005).

Understanding the local anatomy, fracture patern and mechanism and preoperative planning are important in order to achieve good results. The surgeon should individualize operative treatment with respect to a variety of factors, such as the patient's age, pre-existing levels of activity, medical morbidity and expectations. Injury considerations should include the extent of fracture comminution and joint impaction, associated injuries, and most important, the condition of the soft tissues (Rasmussen et al., 1973; Lansinger et al., 1986).

Schatzker's classification and AO classification are the most commonly used for the categorisation and to guide the treatment of these fractures (Schatzker et al., 1979). Schatzker classification and AO Classification evaluate fractures according to the anterior posterior graphs. However, only anterior posterior radiographs and fractures in the coronal plane can be overlooked. Currently, with the widespread use of computed tomography, the detection of fractures in the coronal plan is made easier, and recent studies emphasize the importance of evaluations made with computed tomography in preop planning in the preoperative period (Hoekstra et al., 2017). Posterior shear fractures, which can be evaluated on side radiographs and computed tomography sections, are defined in 3-column classifications unlike other classifications (Piątkowski et al., 2015). The 3-column classification is a useful tool in the preoperative surgical planning of tibial plateau fractures, especially in fractures extending to the posterolateral corner. According to the Schatzker classification, the reliability among the observers is quite high (Hoekstra et al., 2017).

The purpose of this study is to evaluate the effects of planning the treatment of the tibial plateau fractures using 3-column classification prior to surgical treatment on radiological results.

2. Material and methods

Approval for the study was granted by the Local Ethics Committee. A retrospective examination was made of patients aged 18-80 years who were operated because of tibia plateau fracture at Muğla Sıtkı Koçman University Medical Faculty between January 2014 and November 2018. All data were abstracted from the electronic medical record, histories and physicals, operative notes, clinic visit notes, and radiographs. Variables such as fracture type, fixation method, timing of surgery were recorded.

An initial list of 55 patients with operatively treated plateau fractures was obtained. Twelve patients were excluded for lost to follow-up. Exclusion criteria included non-acute fractures (>4 weeks after the incident), open fractures and pediatric fractures (aged <18 years). 43 patients (32 males, 11 females, mean age 48.91 [range 20-76 years]) were included in the study. Preoperative and postoperative 6th month and 1st year radiological images were collected. Besides radiographs, all patients had computed tomography scans (CT) for accurate fracture evaluation and classification according to Schatzker et al., and 3-column classification (Schatzker et al., 1979; Luo et al., 2010).

All of the patients (43) were treated surgically. All patients were treated using the standard techniques of exposure, open reduction, and stable internal fixation.

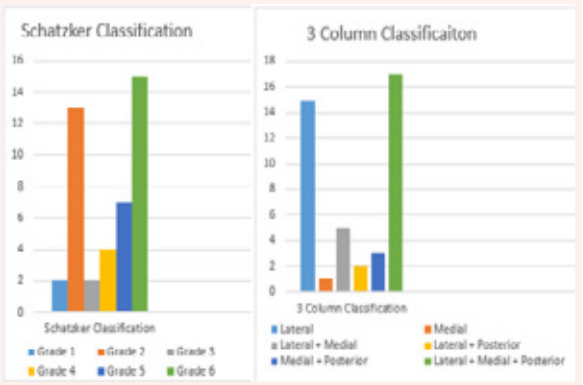
In general, fractures were stabilised by open reduction and internal plate fixation. The mean time to operation was 48 hours (range three hours to seven days). A midline incision or lateral and medial incision was used for open reduction and internal fixation. Joint surface depressions were elevated and stabilised by autograft or allogreft and a buttress plates were used in fixation. In fractures involving posterior column, a posterior butress plate was used for fixation in addition to other butress plates. If needed, additional cannulated screws were used to support the joint surface in order to avoid depression. The postoperative management protocol included early mobilization using a hinged knee brace. All patients were instructed to be non-weight bearing for the first six to ten weeks, followed by partial weight bearing for a further two to six weeks depending on the operating surgeon's intraoperative assessment of fracture comminution and stability.

Radiological assessment were performed regularly for all patients. Preoperative fracture classification according to Schatzker and 3-column classification, degree of joint depression, reduction quality, the presence of osteoarthritis, union time were reviewed by the senior author. Preoperative, immediate postoperative and postoperative one year modified Rasmussen score were calculated by the senior author.

Radiological features of osteoarthritis included the presence of joint space narrowing, articular margin osteophytes, as well as subchondral cysts and sclerosis. Union was defined as evidence of bone healing by direct or indirect means in at least two radiographic planes.

Descriptive statistics were calculated to examine the characteristics of the sample. T-tests were conducted for comparisons between the two groups on continuous variables and x2 tests for comparisons between the two groups on unranked categorical variables, and Fisher exact test where appropriate. For all analyses, significance was set at the $p<0.05$ level.

Table 1. Schatzker and 3 column classifications made by direct x-ray and computed tomography images taken before the operation are summarized.



3. Results

43 patients (32 men, 11 women) were included in the study. The mean age was 48.91 [20-76 years]. Schatzker and 3-column classifications made by direct x-ray and computed tomography images taken before the operation are summarized in Table 1. All patients could be grouped according to Schatzker and 3-column classifications. The degrees of articular depression, condylar widening, varus/valgus angulation, and osteoarthritis were measured and recorded from the preoperative and postoperative radiological images. In addition to this, all patients had Modified Rasmussen Scores preoperatively and postoperatively.

Radiological evaluation of 43 patients included in the study revealed that 22 had a posterior column (17 had 3-column fractures, five had two column fractures). In the postoperative radiological evaluation of 22 patients with posterior column fracture; 11 of these posterior column fractures were fixated by posterior plate except medial and-or lateral plate or screw fixation (Fig.1). In four patients with posterior column fracture;

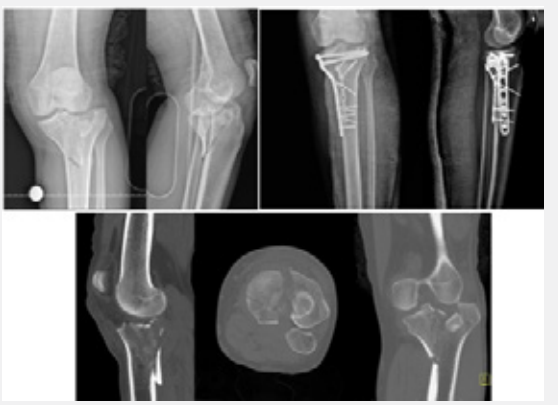


Fig. 1. Preoperative and postoperative images of a 42-year-old patient with a tibial plateau fracture after a traffic accident.

interfragmanter screw were used in fixation and plate oriented posterior screws were used in three patients with posterior column fracture. On the other hand, in four patients with posterior column fracture, no fixation was found except medial and-or lateral fixation for the posterior fractured components.

The average preoperative modified rasmussen score of 22 patients with posterior column fracture was 3.5. Postoperative Modified Rasmussen Scores were calculated as 7.54 and 6.09 in the 6th months postoperative and 1st year, respectively. Postoperative Modified Rasmussen Scores were worse and osteoarthritis findings were higher in four patients without any fixation to the posterior column. When these four patients were not statistically analyzed, it was seen that the average Modified Rasmussen Score

was calculated as 7.77 and 6.66 in the 6th month and 1st year, and the radiological score was improved. In particular, 11 patients who had fixation with posterior plate had higher radiological scores and less osteoarthritis findings. It was observed that there was no significant difference in terms of radiological scoring and osteoarthritic changes between patients who received an interfragmenter screw and those who had a posterior oriented screw over the plate.

4. Discussion

The aim of our study is to evaluate the effect of preoperative planning of surgical treatment of the tibial plateau fractures using 3-column classification on radiological results and to examine the postoperative radiological results of patients with posterior column fracture in the tibial plateau. We believe that the selected fixation method and the achieved reduction success are the most important factors in preventing the development of osteoarthritis. In the literature, it has been shown that a joint line displaced more than 2 mm may be associated with poor clinical outcomes, and traumatic osteoarthritis develops in approximately 20-44% of patients after a proximal tibial fracture (Rasmussen et al., 1973; Volpin et al., 1990; Giannoudis et al., 2010).

X-ray imaging is still the gold standard in controlling the progression of post-traumatic osteoarthritis (Lizaur-Utrilla et al., 2015). In our study, we used X-ray imaging in radiological follow-up. Based on x-ray imaging, traditional classification systems (Hohl and Moore, Schatzker, OTA / AO 2007) play an important role in guiding surgical treatment (Schatzker et al., 1979). With the advent of computed tomography, more different classifications have emerged and facilitated the understanding of fracture morphology. In 2016, Wang et al. made a prospective cohort study involving 287 patients and proposed the concept of three columns (medial, lateral and posterior columns) (Wang et al., 2016).

3-column classification approach is a valuable addition to the Schatzker classification in the current form. Although the 3-column classification approach of tibial plateau fractures has already shown to be very helpful to understand the fracture pattern and determine the surgical strategy preoperatively, 3-column classification approach allows both lateral buttressing and posterolateral support of lateral tibial plateau fractures extending into the posterolateral complex. According to Zhu et al. the inter-observer reliability of the 3-column classifications is higher than the Schatzker classification (Zhu et al., 2012). On the other hand, several studies have compared reliability and reproducibility among commonly used classification systems, but no consensus has been reached on the best choice (Gicquel et al., 2013).

The medial part of the knee is concave to the tibial side and the femoral condyle is highly mobile in the tibial space. During the axial loading of the femur, it creates an explosive effect on the tibia. Since the tibial plateau charge is directed posteriorly, the resulting force vector is directed to the posterior, thereby leading to a posterior division. Therefore, it is important to keep in mind the possibility of developing a posteromedial fracture (Eggli et al., 2008). We suggest that the posterior fracture detection is very important in treatment success. In this regard, we think that conventional X-ray imaging may not be sufficient and CT imaging should be routinely performed in every patient with a proximal tibia fracture.

In our department, we routinely use CT scans in tibia plateau fractures to determine the fracture pattern preoperatively and X-ray imaging in follow-up period postoperatively. Preoperative CT images were collected from our patients' records. By classifying 3-columns on CT records, we detected patients with posterior colon fracture. In our study, 22 of 43 patients had posterior column fracture. Modified Rasmussen Scores were calculated on X-ray imaging in the preoperative, postoperative 6th months and 1st year time. Radiological evaluation was also performed for posttraumatic arthritis examination. At the postoperative 1st year time nine patients in Kellgren-Lawrence grade 1, 18 patients in grade 2-3 and 16 the patients had grade 4 osteoarthritis. Grade 4 OA patients were all patients without any extra fixation for posterior column fracture. Modified Rasmussen Scores of these patients were also lower than other patients. On the other hand, in our study, 79% of the patients that operated because of tibia plateau fracture had mild or severe osteoarthritis, osteoarthritis findings may also develop regardless of the type of fixation like other intraarticular fractures. Articular incongruities in the tibial plateau are well tolerated, with little evidence that anatomical reduction of the more common fracture patterns improves patient outcome or prevents arthritis. Factors other than articular congruity, such as joint stability, retention of menisci, and coronal alignment, play a more important role in determining outcome. Despite anatomical joint reconstruction, development of osteoarthritis may still occur secondary to the initial articular cartilage and meniscal injury (Honkonen et al., 1995; Marsh et al., 2002). Limitations of this study; the number of patients was low and the follow-up was short. Meniscus repair was not performed in any patient. This may have affected the results.

As it is well known that postoperative negative changes and degeneration in the joint result from non-anatomic fixation. Articular step-off and widening of the lateral condyle correlate strongly with the clinical outcome of tibial plateau fracture treatment. We think that in patients with proximal tibia fracture, diagnosis

of fractures and recogniation of fracture patterns in the posterior column is important and the most accurate diagnosis will be possible with CT imaging. We believe that efforts should be made for the anatomical detection of the fracture diagnosed.

Declaration of conflicting interests

The author declares that there is no conflict of interest.

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Association of anteroseptal hypokinesia after myocardial infarction with LDLR variation: A cross-sectional case-control study

Pegah Pourzargham[a], Mahboobeh Azadi[a], Monireh Ataollahi[a], Hamed Fouladseresht[a], Shahdad Khosropanah[b], Mehrnoosh Doroudchi[a*]

[a] *Department of Immunology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[b] *Department of Cardiology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

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*** Correspondence to:**
Mehrnoosh Doroudchi
Department of Immunology,
Faculty of Medicine,
Shiraz University of Medical Sciences,
Shiraz, Iran
e-mail: mdoroud@sums.ac.ir

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ABSTRACT

Cholesterol-rich LDL (LDL-C) is a major atherogenic lipoprotein. Elevated content of LDL-C in serum is associated with the higher risk of atherosclerosis. The plasma LDL-C level is regulated by LDL receptor. The T allele of rs2228671 in LDLR may be associated with decreased LDL-C levels. We investigated the association of rs2228671 of LDLR with myocardial infarction (MI) in people from Fars province of Iran. In this study 248 cases with MI and 256 healthy blood donors were tested for their rs2228671 LDLR polymorphism by PCR-RFLP method. The CC genotype of the rs2228671 single nucleotide polymorphism tended to be more common in patients while TT showed a higher frequency in the control group. Patients with anteroseptal hypokinesia had a significantly higher frequency of the CC genotype compared with other patients (p=0.04, OR 8.217 and 95% CI 0.4755 to 142). Also frequency of C allele was increased as compared with that of the T allele in patients with anetroseptal hypokinesia (p=0.05, OR 7.637 and 95% CI 0.4367 to 124.6). There was also a significant increase of CT genotype in patients with abnormal heart rate (p=0.014). A significantly higher frequency of the CC genotype in patients with anetroseptal hypokinesia and its decrease in patients with abnormal heart rate suggest a complex relationship between LDLR variants and complications of MI.

1. Introduction

Cardiovascular diseases (CVDs) have been categorized as a main health problem in Middle East and Iran in recent years (Hadaegh et al., 2013; Jahangiri-Noudeh et al., 2014; Kheirandish et al., 2014; Moghaddam et al., 2014; Hadaegh et al., 2015; Lotfaliany et al., 2015; Hadaegh et al., 2017; Mohseni et al., 2017). Coronary Artery Diseases (CADs) are the most common CVDs with a greater frequency in men compared to women which appear less frequently in younger people (Nicolini et al., 2017). On average, a first heart attack for men happens at the age of 65 years but the average age of a first heart attack in women is 72 years (Harvard).

Acute myocardial infarction (AMI), also known as heart attack, represents a leading cause of hospital admissions and mortality around the world (Moran et al., 2014). The prevalence of AMI in Iran is high and is increasing (Mohseni et al., 2017). AMI occurs

in case of a mismatch between myocardial oxygen supply and demand. Therefore, damage happens as heart encounters the inability of oxygenation (Boyette and Manna, 2019). AMI is a complex multifactor condition of familial and environmental nature (Rose, 1964). Generally, atherosclerotic plaque rupture and subsequent thrombosis of advanced atherosclerotic lesion cause partial or complete blockade in the artery (Libby, 2006), which in case of coronary arteries it can cause AMI (Boyette and Manna, 2019). It is widely accepted that the elevation of low density lipoprotein (LDL) content, as a result of hereditary or life style risk factors, causes embolism (Gofman and Lindgren, 1950; Krenz and Korthuis, 2012).

Moreover, one of the major atherogenic lipoproteins is the cholesterol-rich LDL (LDL-C). The content of cholesterol in serum is carried by LDL-C, where high levels of LDL-C accumulate and accelerate atherosclerosis (Soutar, 2001; Rafieian-Kopaei et al., 2014). The plasma LDL-C level is regulated by LDL receptor (LDLR). Therefore, variations in LDLR may lead to abnormal levels of LDL-C (van Zyl et al., 2014). LDLR is a type I transmembrane protein that is encoded by a gene located in the 19p13.2 region and is 45 kb in length, including 18 exons and 17 introns. Thirteen of the 18 exons encode sequences of protein which are similar to sequences in other proteins: five of these exons encode sequences similar to the sequences in the C9 component of complement; three exons encode sequences similar to those in the precursor of epidermal growth factor (EGF) and in three proteins of the blood clotting system (factor IX, factor X, and protein C); and five other exons encode sequences that are shared with the EGF precursor and are not repeated (Sudhof et al., 1985).

The LDLR pathway is mostly limited to tissues in which there are high interactions for cholesterol such as liver, adrenal gland and reproductive organs (Soutar, 2001). An in vivo study in mice showed that LDLR expression is differently regulated in liver and intestine by dietary cholesterol and dietary saturated fat. Dietary cholesterol regulates LDLR at the transcriptional level, while dietary fatty acids do not (Srivastava et al., 1995). Changes caused by mutation in the LDLR may decrease expression or function of LDL receptors and consequently defected LDL-C uptake (Leigh et al., 2017). Therefore, the perturbed catabolism of LDL-C may lead to an increase in its content (Motulsky, 1989). The more intensely a homozygote is affected, the less they have competent receptors and consequently they show higher levels of cholesterol (Punzalan et al., 2005). The association of elevated LDL cholesterol levels with the higher risk of atherosclerosis is already shown but there seems to be inter-population differences (Ference et al., 2017). A recent genome-wide association study (GWAS) represented various single nucleotide polymorphisms (SNPs) at the LDLR locus that provide inter-individual variations in serum lipid concentrations (Sandhu et al., 2008). Among these variations, the minor variant of rs688 (Asn591 ACC_ ACT), which is located within exon 12, is reported to be associated with a 4–10% elevation in plasma cholesterol levels (Jha et al., 2018). Backing this, another study on rs688 reported its association with LDL-C and total cholesterol in a gender-dependent manner (Zhu et al., 2007). The rs1122608 SNP is reported to be associated with lower serum levels of LDL-C and the lower risk of coronary artery disease (Martinelli et al., 2010). However, a meta-analysis established that rs1122608 of LDLR was not associated with the risk of Coronary Heart Disease (CHD) (Zhang et al., 2013). It is shown that in German and British populations, the T allele at SNP site of rs2228671, which is located in exon 2, is associated with decreased LDL-C levels and CHD risk (Linsel-Nitschke et al., 2008). By contrast, no association between this SNP and cardiovascular diseases in Chinese population is seen (Ye et al., 2014). It is also suggested that the T allele of rs2228671 is associated with LDL-C levels but no association has been found with the risk of CAD (Martinelli et al., 2010).

Given the importance of LDLR in CAD, and the inter-population differences of association of rs2228671 with CAD, the objective of this study was to investigate the frequency of this SNP in a sample of patients in southwestern Iran and controls from the same region, to determine the importance of this LDLR variant in our population.

2. Material and methods

Study population

All approved MI cases (n=248, mean age=59.64 ± 12.44 y) included in this study, were referred to the affiliated hospitals of Shiraz University of Medical Sciences in southwest of Iran, between 2014 and 2017. The sample size was calculated based on the minor allele frequency of 8% and CI of 95% and the DEFF of 1.8 using EPI info statistical analysis software (https://www.openepi.com/SampleSize/SSPropor.htm). MI diagnosis was confirmed by the collaborating cardiologist, on the basis of typical ECG variations and changes in cardiac markers. All individuals had evidences in favor of acute MI and sampling and data recording were done in less than 24 hours as of progression of symptoms. Confirmation of MI was based on more than 50% stenosis in one or more of the coronary arteries in coronary angiography. The clinical data of patients who underwent echocardiography was also recorded.

The patients' clinical criteria are shown in Table 1. Based on the criteria defined by American Heart

Association, systolic blood pressure more than 140 mmHg and diastolic blood pressure more than 90 mmHg were considered as high systolic and high diastolic blood pressure, respectively (https://www.heart.org/en/health-topics/arrhythmia/about-arrhythmia). BMI was calculated by division of weight in kilograms to the square height in meters for each patient.

Table 1. Demographical criteria of patients with AMI.

Characteristics	
Number of subjects	248
Age(mean ± SD)	59.64 ± 12.44
Sex (male/female)	183/65
BMI (129)	no. (%)
<25	68 (27.42)
25-30	48 (19.35)
30-35	12 (4.84)
>35	1 (0.40)
Missing	119 (47.98)
Familial history (157)	
Yes	45 (18.15)
No	112 (45.16)
Missing	91 (36.69)
Hypertension (157)	
Yes	48 (19.35)
No	109 (43.96)
Missing	91 (36.69)
Diabetes mellitus (156)	
Yes	38 (15.32)
No	118 (47.58)
Missing	92 (37.1)
Smoking (157)	
Yes	76 (30.65)
No	81 (32.66)
Missing	91 (36.69)

Control individuals (n=256, mean age=45.45 ± 9.26 y) were recruited from among healthy blood donors between 2014 and 2017 who referred to Fars Blood Transfusion Center and resided in the same geographic region as of patients. The blood donors were also examined by a physician and were assessed for systemic diseases, including: hypertension, dyslipidemia, stroke, coronary artery disease and also they were approved of not being cured with drugs for related disorders. Also the individuals in control group were all non-smokers, -alcoholics or -drug addicts. The potential sources of bias between patients and controls was the mean age differences between the two groups, as the patients had a significantly greater age ($p<0.001$).

Blood samples and DNA extraction

Ten ml venous blood was collected from all individuals in tubes containing ethylene diamine tetra acetic acid (EDTA) 5% w/v (weight/volume) anticoagulant. DNA extraction was further performed by salting out method. DNA quality and quantity was evaluated using Eppendorf Biophotometer. Also DNA concentration and protein contamination were determined by means of spectrophotometer (Agilent) in 260 and 280 nm wavelength. Samples were kept in -40°C until used. Samples with 1.6 to 1.9 optical densities (O.D.) were used for polymerase chain reaction (PCR).

Genotyping

PCR and RFLP methods were used for genotyping of coding rs2228671 polymorphism in LDLR gene. PCR procedure was performed in a 25 µL total reaction volume containing 0.75 µL of dNTP, 2 µL genomic DNA (50 ng/ml), 0.75 µL of $MgCl^2$, 2.5 µL10X PCR Buffer, 2 µL Taq DNA polymerase, 16 µL distilled water, and 1.0 µL of each of the forward and reverse primers. Mentioned components were mixed in each single tube and 40 µL mineral oil was added to each one. After 10 seconds of centrifugation, tubes were placed in the thermocycler (Techne Flexigene).

For RFLP, the required restriction enzyme was added to PCR products and incubated at 37°C for 6-10 hours in a dry block. The enzymatically cleaved products were electrophoresed on a 3.5% agarose gel containing 2.5 µL safe stain and visualized by UV light at 254 nm (Fig. 1). The rs2228671-T allele was identified as a 194 bp band and the rs2228671-C allele as a 174 bp band in the gel. Due to their small fragment size, the 20 bp bands were not clearly defined on the gel. The primer sequences, required restriction enzyme, recognition site of the restriction enzyme, and the length of cleaved products are represented in Table 2. All the experiments were performed in our laboratory in the Department of Immunology, Faculty of Medicine, Shiraz University of Medical Sciences.

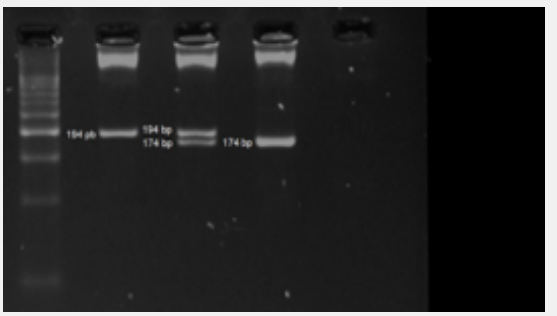

Fig. 1. PCR-RFLP products corresponding to the LDLR rs2228671 polymorphism.

Table 2. Primer sequences, restriction endonucleases, PCR product lengths, and restriction patterns.

SNP	**Primer sequences**	**Restriction enzyme**	**Product length (bp)**	**Length of final fragments (bp)**
LDLR rs2228671	Forward-5'CTCTCAGTGGGCGACAGACG-3' Reverse-5'-CAACATGGCGAGACCCTGTC-3'	BstUI 5' CG↓CA 3' 5' GC↑GC 3'	194	CC: 20, 174 CT: 20, 174, 194 TT: 194

Statistical analyses

SPSS software (Version 19) was used to perform statistical analysis. $\chi 2$ test and Fisher's exact test were performed to investigate the differences in the frequencies of genotype and allele between patients and controls as well as the correlation of clinical manifestations with genotypes and alleles. Also binary and multinomial logistic regression analyses were performed to confirm the primary $\chi 2$ results, where applicable. Odds ratios and relative risks were calculated using online EPI info statistical analysis software (www.openepi.com/TwobyTwo/TwobyTwo.htm). The significance level was set at and below 0.05. We used the STREGA reporting guidelines for reporting this study (Little et al., 2009).

3. Results

Genotype and allelic distribution

SNP analysis was performed in a population of 248 MI cases (65 females and 183 males) and 256 controls (18 females and 238 males). Table 3 illustrates the distribution of genotypes and allelic frequencies of the LDLR (rs2228671 C/T) polymorphism in MI patients and healthy control group. As shown in Table 3, rs2228671 alleles ($\chi 2$ p=0.07, OR 1.4) and genotypes ($\chi 2$ p= 0.06, OR: CC VS TT= 0.098, CT VS TT= 0.117, TT= 1) had no significant difference between MI patients and healthy controls. Hardy-Weinberg Equilibrium was tested for each group and genotypes in both patients (p=0.38) and controls (p=0.47) were in equilibrium. The proportion of genotypes among patients increased progressively from rare homozygous genotype, TT (0%) to heterozygotes, CT (16.13%) to common homozygous genotype, CC (83.87%). Compared with the control group, CC was more common in patients while TT showed a higher frequency in the control group. Accordingly, the decrease in frequency of the rs2228671-T allele in patients was notable (Table 3). The relative risk for rs2228671-C and -T alleles showed that the C allele has a greater non-significant risk for myocardial infarction (p=0.07, RR=1.219; Table 3). The CC genotype of the rs2228671 SNP also showed a higher non-significant relative risk in comparison with other genotypes (p=0.06, RR=1.162; Table 3).

Association of LDLR polymorphism with clinical manifestations of the disease

Cross-tabulation was performed to determine the association of genotypes/alleles with clinical manifestations in patients. Genotype was significantly correlated with anteroseptal hypokinesia. Of 131 (100%) cases with known echocardiography data, 18 (13.74%) individuals were positive for anteroseptal hypokinesia who all (100%) had CC genotype, however, of 113 (86.26%) negative cases of anteroseptal hypokinesia, 92 (81.42%) had CC and 21 (18.58%) had CT genotype. Individuals with anteroseptal hypokinesia had a significantly higher frequency of the CC genotype in comparison with other genotypes (p=0.046, OR 8.217 and 95% CI 0.4755-142). Also frequency of C allele was increased as compared with that of the T allele in patients with anteroseptal hypokinesia (p=0.057, OR 7.637 and 95% CI 0.4367-124.6).

We also investigated the association of high systolic or diastolic blood pressure with genotypes and alleles of LDLR (rs2228671 C/T) polymorphism. There were no significant association between genotype and systolic blood pressure in $\chi 2$ test (p=0.19; Table 4), or regression analysis (p=0.23; Table 4). However, there was a significant increase ($\chi 2$ p=0.075, regression p=0.014, OR 2.242; Table 4) in the CT genotype in patients with abnormal heart rate (i.e., the heart rate below 60 BPM or over 100 BPM). By inclusion of age in the regression analysis and after correction for age the difference in the genotype frequencies based on rate, stayed significant (p=0.009). Similarly, a significant increase in T allele was observed in patients with abnormal heart rate (p=0.02; Table 5). No association was observed for BMI with genotypes (p= 0.10) and alleles (p=0.12). The genotypes and alleles of LDLR (rs2228671 C/T) polymorphism did not show any significant differences in other wall motion abnormalities (Table 6, Table 7).

Table 3. The distribution of rs2228671 of LDLR in MI patients and control group.

Genotype and Allele	Patients N (%)	Controls N (%)	p value	OR (95%CI)	Relative Risk
Genotypes			0.06		
CC	208 (83.87)	204 (79.69)		CC VS TT: 0.098 (0.005-1.806)	1.162
CT	40 (16.13)	47 (18.36)		CT VS TT: 0.117 (0.006-2.217)	0.918
TT	0 (0)	5 (1.95)		TT: 1 (Reference)	0.183
Alleles			0.07	1.4000 (0.9143-2.1436)	
C	456 (91.94)	456 (89.06)			1.219
T	40 (8.06)	56 (10.94)			0.82

Table 4. The association of clinical features with LDLR rs2228671 genotypes.

Clinical manifestations	LDLR rs2228671 N (%)		P Chi²	P Regression	OR (95%CI)
	CC	CT			
Arrhythmia			0.44	0.15	2.621 (0.702-9.784)
Positive	70 (81.4)	16 (18.6)			
Negative	138 (85.19)	24 (14.81)			
Rate			0.075	0.014	2.971 (1.245-7.088)

Normal	110 (88)	15 (12)			
Abnormal	98 (79.67)	25 (20.33)			
SBPA *			0.19	0.23	2.242 (0.599-8.392)
Positive	14 (73.68)	5 (26.32)			
Negative	129 (85.43)	22 (14.57)			
DBPA **			0.36	0.89	1.102 (0.268-4.533)
Positive	13 (76.47)	4 (23.53)			
Negative	130 (84.97)	23 (15.03)			
QT			0.41	0.74	1.39 (0.566-3.414)
Normal	71 (86.59)	11 (13.41)			
Abnormal	137 (82.53)	29 (17.47)			

*Systolic blood pressure on admission
**Diastolic blood pressure on admission

Table 5.The association of clinical features with LDLR rs2228671 alleles.

Clinical manifestations	**LDLR rs2228671 N (%)**		**P Chi²**	**P Regression**	**OR (95%CI)**
	C	T			
Arrhythmia			0.46	0.17	2.267 (0.699-7.359)
Positive	156 (90.7)	16 (9.3)			
Negative	300 (92.59)	24 (7.41)			
Rate			0.09	0.02	2.626 (1.163-5.927)
Normal	235 (94)	15 (6)			
Abnormal	221 (89.84)	25 (10.16)			
SBPA *			0.21	0.25	2.029 (0.600-6.863)
Positive	33 (86.84)	5 (13.16)			
Negative	280 (92.72)	22 (7.28)			
DBPA **			0.38	0.98	1.095 (0.296-4.053)
Positive	30 (88.24)	4 (11.76)			
Negative	283 (92.48)	23 (7.52)			
QT			0.43	0.50	1.341 (0.575-3.130)
Normal	153 (93.29)	11 (6.71)			
Abnormal	303 (91.26)	29 (8.73)			

*Systolic blood pressure on admission
**Diastolic blood pressure on admission

Table 6. The association of Wall Motion Abnormalities with LDLR rs2228671 genotypes.

Wall Motion Abnormalities	**LDLR rs2228671 N (%)**		**P Chi²**
	CC	CT	
Apicoseptal Akinesia			
Positive	12 (80)	3 (20)	
Negative	98 (77.78)	18 (22.22)	0.66
Missing	117		
Apicoseptal Hypokinesia			
Positive	6 (100)	0 (0)	
Negative	100 (82.65)	21 (17.35)	0.24
Missing	121		
Apicolateral Akinesia			
Positive	16 (88.89)	2 (11.11)	
Negative	94 (83.2)	19 (16.8)	0.54
Missing	117		
Apicolateral Hypokinesia			
Positive	11 (84.62)	2 (15.38)	
Negative	96 (76.80)	19 (23.20)	0.92
Missing	120		
Anterior Hypokinesia			
Positive	4 (100)	0 (0)	
Negative	103 (83.06)	21 (16.94)	0.37
Missing	117		
Anterolateral Akinesia			
Positive	3 (75)	1 (25)	
Negative	107 (84.25)	20 (15.75)	0.62
Missing	117		
Anterolateral Hypokinesia			
Positive	4 (100)	0 (0)	
Negative	106 (83.46)	21 (16.54)	0.37
Missing	117		
Anteroseptal Akinesia			
Positive	8 (100)	0 (0)	
Negative	102 (82.93)	21 (17.07)	0.20
Missing	117		
Anteroseptal Hypokinesia			
Positive	18 (100)	0 (0)	
Negative	92 (81.42)	21 (18.58)	0.046
Missing	117		
Inferior Akinesia			

Positive	4 (66.67)	2 (33.33)	
Negative	103 (84.43)	19 (15.57)	0.25
Missing	120		
Inferior Hypokinesia			
Positive	6 (66.67)	3 (33.33)	
Negative	101 (84.87)	18 (15.13)	0.155
Missing	120		
Inferoseptal Akinesia			
Positive	3 (75)	1 (25)	
Negative	103 (83.74)	20 (16.26)	0.64
Missing	121		
Inferoseptal Hypokinesia			
Positive	6 (75)	2 (25)	
Negative	101 (81.17)	19 (15.83)	0.50
Missing	120		
Dyskinesia			
Positive	4 (80)	1 (20)	
Negative	106 (84.13)	20 (15.87)	0.80
Missing	117		

Table 7. The association of Wall Motion Abnormalities with LDLR rs2228671 alleles.

Wall Motion Abnormalities	**LDLR rs2228671 N (%)**		**P Chi²**
	C	T	
Apicoseptal Akinesia			
Positive	27 (90)	3 (10)	
Negative	214 (92.24)	18 (7.76)	0.67
Missing	234		
Apicoseptal Hypokinesia			
Positive	12 (100)	0 (0)	
Negative	221 (91.32)	21 (8.68)	0.29
Missing	121		
Apicolateral Akinesia			
Positive	34 (94.44)	2 (5.56)	
Negative	207 (91.59)	19 (8.41)	0.56
Missing	117		
Apicolateral Hypokinesia			
Positive	24 (92.31)	2 (7.69)	
Negative	211 (91.74)	19 (8.26)	0.92
Missing	240		
Anterior Hypokinesia			
Positive	8 (100)	0 (0)	
Negative	227 (91.53)	21 (8.47)	0.39
Missing	234		
Anterolateral Akinesia			
Positive	7 (87.5)	1 (12.5)	
Negative	234 (92.13)	20 (7.87)	0.63
Missing	234		
Anterolateral Hypokinesia			
Positive	8 (100)	0 (0)	
Negative	233 (91.73)	21 (8.27)	0.40
Missing	234		
Anteroseptal Akinesia			
Positive	16 (100)	0 (0)	
Negative	225 (91.46)	21 (8.54)	0.22
Missing	234		
Anteroseptal Hypokinesia			
Positive	36 (100)	0 (0)	
Negative	205 (90.71)	21 (9.29)	0.057
Missing	234		
Inferior Akinesia			
Positive	10 (83.33)	2 (16.67)	
Negative	225 (92.21)	19 (7.79)	0.27
Missing	240		
Inferior Hypokinesia			
Positive	15 (83.33)	3 (16.67)	
Negative	220 (92.44)	18 (7.56)	0.17
Missing	240		
Inferoseptal Akinesia			
Positive	7 (87.5)	1 (12.5)	
Negative	226 (91.87)	20 (8.13)	0.66
Missing	242		
Inferoseptal Hypokinesia			
Positive	14 (87.5)	2 (12.5)	
Negative	221 (92.08)	19 (7.92)	0.52
Missing	240		
Dyskinesia			
Positive	9 (90)	1 (10)	
Negative	232 (92.06)	20 (7.94)	0.81
Missing	234		

4. Discussion

In the current study we found that the frequency of rs2228671 SNP at LDLR locus was non-significantly different between patients with AMI and healthy blood donors in southwest of Iran. Our results are in line with the finding of a study in northern Italy (Martinelli et al., 2010), however, due to the lower number of cases in our study, the difference did not reach a significant level. A potential source of bias between patients and controls was the mean age differences between the two

groups, as the patients had a significantly higher age ($p<0.001$). However, this difference was inevitable due to the higher age of MI incidence in which age finding healthy volunteers is less likely.

Our most important finding was an increase in the frequency of the CC genotype in patients with anteroseptal hypokinesia, which may suggest the association of CC genotype with this type of wall motion abnormality (WMA). The relation of segmental WMA to cardiovascular events is shown in acute myocardial infarction (Peels et al., 1996; Fleischmann et al., 1997; Stein et al., 1998; Carluccio et al., 2000). WMAs have been shown to increase the re-polarization time of the heart and also cause inhomogencity of re-polarization, which results in myocardial arrhythmia. Similarly, abnormal heart rate increases the re-polarization time (Han and Moe, 1964; Kuo et al., 1983; Zareba and Moss, 1995; Opthof et al., 2012). In our analysis, however, there was a significant decrease in the CC genotype among patients with abnormal heart rate. Also a significant increase in the T allele was also observed in patients with abnormal heart rate (Table 5). Therefore, individuals with T allele were less likely to show anteroseptal hypokinesia but were more likely to have increased heart rate. All but one patient had heart rates less than 140 beats per minute and it is possible that the increased heart rate had maintained blood pressure and/or contractility, therefore resisting hypokinesis. Whether the increased heart rate in these individuals has a compensatory effect for lower contractility of the heart muscle through "Treppe phenomenon" needs to be investigated (Mulieri et al., 1992). Of note, despite expectations, we did not find any correlation between anteroseptal hypokinesia and arrhythmia in our patients (Opthof et al., 2012).

The limitations of this study were the relatively low number of cases, missing values and the age difference between cases and controls, as well as lack of clinical and paraclinical data of controls, which may have hampered the clear conclusion on the differences of cases ad controls.

Our study showed an association between rs2228671 CC genotype of LDLR and anteroseptal hypokinesia, while this genotype decreased in patients with abnormal heart rate. Further investigation of this SNP along with haplotypic combinations of LDLR polymorphisms may provide information on the part these variants and their products play in mechanical complications of heart after MI.

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Conflict of interest: The authors declare no conflict of interest.

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Can dehydroepiandosterone prevent chemotherapy-related damage? Investigation of protective effects of dehydroepiandosterone against paclitaxel-induced toxicity damage in rat ovaries

Önder Sakin[a], Muhammet Ali Oruç[b], Ali Doğukan Anğin[a], Yasemin Alan[c], Mustafa Gökkaya[a], Hasan Sağdiç[a], Emre Mat[a], Kayhan Başak[d], Murat Alan[e*]

[a] *Department of Obstetrics and Gynecology, Kartal Training and Research Hospital, University of Health Sciences, Istanbul, Turkey*
[b] *Department of Family Medicine, Faculty of Medicine, Ahi Evran University, Kırsehir, Turkey*
[c] *Department of Obstetrics and Gynecology, İzmir Metropolitan Municipality Eşrefpaşa Hospital, İzmir, Turkey*
[d] *Department of Pathology, Kartal Training and Research Hospital, University of Health Sciences, Istanbul, Turkey*
[e] *Department of Obstetrics and Gynecology, Tepecik Education and Research Hospital, Universty of Health Sciences, İzmir, Turkey*

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*** Correspondence to:**
Murat Alan
Department of Obstetrics and Gynecology,
Tepecik Education and Research Hospital,
University of Health Sciences,
İzmir, Turkey
e-mail: gozdealan@hotmail.com

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ABSTRACT

Our aim is to evaluate whether dehydroepiandosterone has a protective effect on paclitaxel-induced ovarian damage. Group 1 (the control group): No treatment was administered. Intact ovarian tissue was removed and blood samples were taken for anti-mullerian hormone (AMH) test. Group 2 (the paclitaxel group): Rats received paclitaxel intraperitoneally at a single dose of 7.5 mg/kg. Group 3 (the paclitaxel + DHEA group): Rats received paclitaxel intraperitoneally at a single dose of 7.5 mg / kg at baseline and DHEA subcutaneously for 10 days at a dose of 60 mg / kg daily. Rats in groups 2 and 3 were sacrificed at the end of 10 days, ovarian tissues were removed and blood samples were taken for AMH test. The edema score was higher in the paclitaxel+DHEA group than in the normal group. Vasculary congestion score was higher in the paclitaxel and paclitaxel+DHEA groups than in the normal group. Cellular degeneration score was higher in paclitaxel group than normal group. Total score was higher in the paclitaxel and paclitaxel+DHEA groups than in the normal group. In the paclitaxel group, the number of tertiary follicles and ovarian volume were lower than in the normal group. Primordial follicles, secondary follicles, tertiary follicles, AMH level and ovarian volume of paclitaxel+DHEA group were lower than normal group. In conclusion DHEA was found to increase damage in paclitaxel-treated rats, leading to a decrease in follicle counts and AMH.

1. Introduction

The incidence of cancer has recently shown a steady increase (Siegel et al., 2018). World Health Organization estimates that 1.4 million women of reproductive age will be diagnosed with cancer annually by 2030 (Lyttle Schumacher et al., 2017). In parallel with these

increasing numbers, new populations have emerged in recent years that include cancer survivors and individuals exposed to harmful side effects of treatments such as young adults, and their numbers are likely to continue to increase (Sonigo and Beau, 2019). Young cancer patients may have to undergo chemotherapy treatment, and most of them may experience fertility loss (Ili et al., 2019).

Paclitaxel is a natural product derived from the bark and needles of Pacific yew tree, Taxus brevifolia (Massey et al., 2019; Zhu and Chen, 2019). This drug is a microtubule inhibitor that promotes polymerization and inhibits the separation of microtubules (Bang and Na, 2019). It has high activity against epithelial ovarian cancer, and exhibits notable penetration capacity in cells up to 80 cell layers (Dwivedi et al., 2019). It is one of the most successful and widely used natural anticancer drugs owing to its unique anticancer mechanism (Wani et al., 1971). It is commonly used in the treatment of solid tumors including ovarian, breast, head, neck and non-small cell lung cancers (Yucebilgin et al., 2004; Bang and Na, 2019).

It is an extremely important drug in terms of side effects. Poor water solubility, severe toxicity and lack of selective antitumor activity are factors limiting its clinical application (Wani et al., 1971; Bernabeu et al., 2016; Holden and Varcoe, 2019; Moku and Layek, 2019; Zajdel and Wilczok, 2019). Several studies have shown that menstrual irregularities and amenorrhea may develop following anthracycline-based chemotherapy (Davis et al., 2005; Fornier et al., 2005; Abusief et al., 2010). Animal studies have revealed that paclitaxel damages healthy mature oocytes by inducing cell death, and impacts the short-term reproductive potential in a dose-dependent manner (Yucebilgin et al., 2004; Tarumi et al., 2009). Further, paclitaxel damages the primordial follicles that constitute a large portion of the ovarian reserve (Nicosia et al., 1985; Meirow et al., 2007).

Dehydroepiandrosterone (DHEA) is an important pro-hormone in the ovarian follicular steroidogenesis (Tartagni et al., 2015). It increases follicular insulin-like growth factor-1 (IGF-1) levels that promote folliculogenesis by increasing the effect of gonadotropin and by reducing follicular arrest (Ubaldi et al., 1996; Bosch et al., 2003; Al-Azemi et al., 2012). It has been shown that, in this way, it can improve the quantitative and qualitative ovarian response (Jayaprakasan et al., 2014).

Several studies have shown the useful effect of DHEA on ovarian reserve. At reproductive centers in 45 countries worldwide, DHEA is used to improve outcomes in approximately one-third of the in vitro fertilization cycles (Zhang et al., 2014). In two separate studies, it was reported that DHEA support can improve oocyte retrieval (Tsui et al., 2015) and an improvement in pregnancy rate (Tsui et al., 2015). A meta-analysis of eight studies reported between 2006 and 2014 concluded that DHEA supplementation increased pregnancy rates without resulting in a significant improvement in oocyte retrieval or quality (Li et al., 2015; Klinge et al., 2018).

In this experimental study, we aimed to investigate whether DHEA has protective effects on paclitaxel -related ovarian damage.

2. Material and methods

This study was conducted at the Animal Testing Laboratory of University in July 2019, after the approval of the ethics committee.

Care of rats

Norvegicus species in our study, Wistar albino genus, 10-12 weeks, and female rats weighing 190 to 216 grams were used. The rats received light at least 12 hours per day in a cage of four animals, at a temperature of 21 to 23 degrees Celsius, at a moisture content of 40-50%, they reached the standard rodent pellet without restriction and drank tap water without restriction.

Study groups

Group 1 (the control group): These rats underwent a laparotomy procedure at baseline and the ovaries were removed. Blood was drawn from the inferior vena cava for AMH testing.

Group 2 (the paclitaxel group): Rats received paclitaxel intraperitoneally at a dose of 7.5 mg/kg at baseline (Yucebilgin et al., 2004) and underwent an oophorectomy procedure at the end of day 10 of the study. After sacrificing the rats, at least 2-3 ml of blood was collected for AMH testing. Then laparotomy was performed and both ovaries were excised for histopathological examination.

Group 3 (the paclitaxel + DHEA group): Rats received paclitaxel intraperitoneally at a dose of 7.5 mg/kg at baseline. In addition, they received DHEA (Cayman Chemical, Michigan, USA, CAS registry no: 53-43-0, item no:15728) subcutaneously for 10 days at a dose of 60 mg/kg daily as dissolved in 0.1 ml of sesame oil (Wang et al., 2014; Hassa et al., 2015). After sacrificing the rats, at least 2-3 ml of blood was collected for AMH testing. Then laparotomy was performed and both ovaries were excised for histopathological examination.

Paclitaxel

Robotic chemotherapy drug preparation device in our hospital prepared the drugs under conditions in accordance with international standards. Double HEPA air cleaning system, waste safety system, dose measurement to ISO 5, class 100 and GMP class A standards.

Surgical procedures

Animals were decapitated before laparotomy. Surgical procedures were performed after iodized solution

cleaning. In order to prevent the drying effect of the air, the treatments were carried out in less than 5 minutes. Ovaries were excised with scissors (Fig. 1).

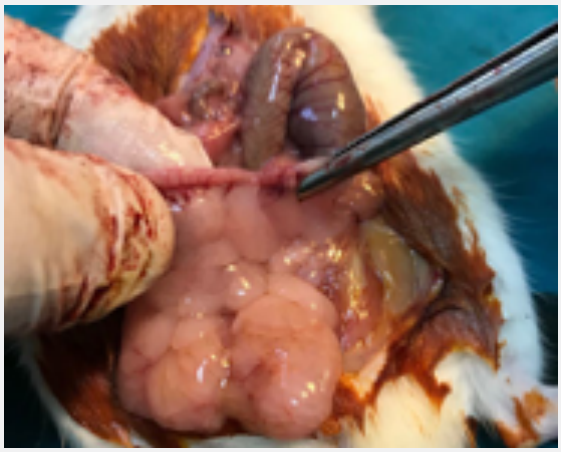

Fig. 1. Excision of the ovary.

Histopathological examinations

The extracted ovarian tissue was placed in 10% formalin and taken to the laboratory for pathological examination. Paraffin blocks were prepared for approximately 24 hours and tissue sections of five micrometers were taken. Follicular activity was evaluated by taking five random samples from each ovarian tissue. The preparations were first stained with hematoxylin eosin and then examined with a light microscope (Olympus Clinical Microscope, Tokyo, Japan) and a microtome blade was used for the preparation of paraffin blocks (Leica, Nussloch, Germany).

Histopathological damage scores were performed according to the evaluations of Celik et al. (Celik et al., 2004). Tissues that appeared completely normal with no changes were evaluated as grade 0. Grade 1 indicated mild edema, mild vascular congestion, absence of hemorrhage or leukocyte infiltration; Grade 2 indicated moderate edema, moderate vascular congestion, absence of hemorrhage or leukocyte infiltration; Grade 3 indicated severe edema, severe vascular occlusion, minimal hemorrhage and minimal leukocyte infiltration, Grade 4 indicated severe edema, severe vascular occlusion, hemorrhage and leukocyte infiltration (Fig. 2).

All follicles were counted to assess ovarian reserve. Primordial, primary, secondary (pre-antral), tertiary (antral) and atretic follicles were counted (Fig. 3, 4). Follicles were evaluated as described by Parlakgumus et al. (Parlakgumus et al., 2014). Primordial follicle is described as an oocyte surrounded with only one layer of flattened ovarian follicular epithelial cell layer, primer follicle is surrounded with one or more layer of cuboidal granulosa cells. Secondary/ pre-antral follicle is surrounded with more than two cell layers and consists of antrum folliculi and zona pellucida. Tertiary follicle is defined if there are antrum, stratum granulosum and surrounding cumulus oophorus layers. Atretic follicle; the basement that separated the oocyte from granulosa cells often thickens to become the glassy membrane. Fibrous material replaces the granulosa cells and loss of cohesion may also occur in granulosa cells.

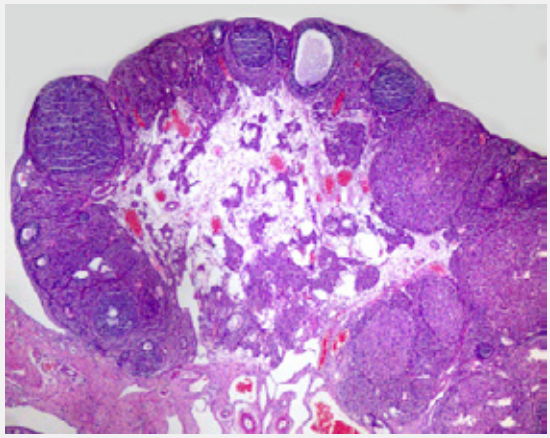

Fig. 3. Edema in medulla x100 hematoxylin-eosin.

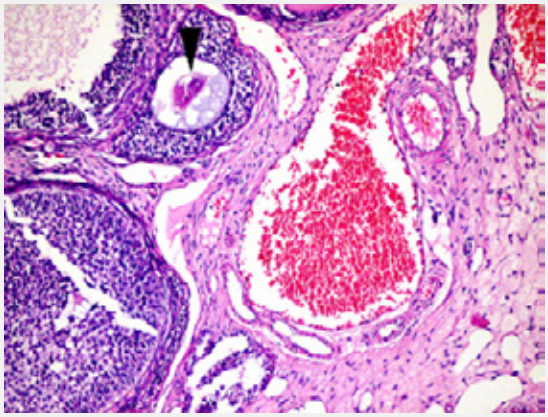

Fig. 2. Distinct dilated vessels x200 hematoxylin-eosin.

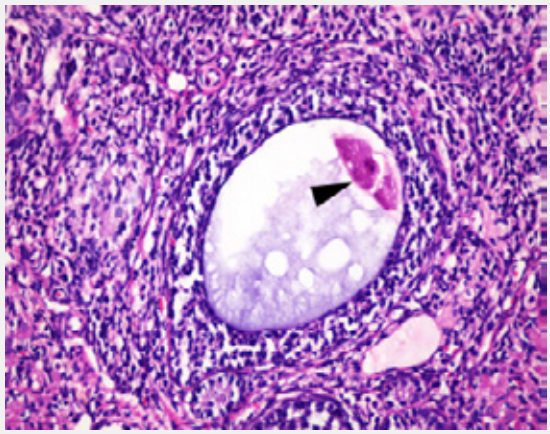

Fig. 4. Defragmented oocyte x400 hematoxylin-eosin.

AMH assays

The laboratory technician was blinded by not knowing which blood belonged to which animal, and all samples were analyzed in the same experiment. Blood samples were taken into lithium heparinized tubes (BD Vacutainer Plasma tubes, Manchester, UK). Blood was centrifuged before 30 minutes (15 minutes at 1000 G). Serum was first removed and the remaining plasma was transferred to an Eppendorf tube and stored at -20°C until the day of analysis. The sensitivity of the AMH kit was 0.10 g/mL, with a detection range of 0.16 to 10 ng / mL (Elabscience®, Rat AMH kit; Houston, Texas, USA).

Statistical analysis

Statistical analysis was performed with the help of SPSS version 17.0 program. The suitability of the variables to normal distribution was examined by histogram graphs and Kolmogorov-Smirnov test. Mean, standard deviation, median and IQR values were used to present descriptive analyzes. Non-parametric variables were evaluated between the two groups and Mann Whitney U Test was used. Spearman Correlation Test was used to analyze the measured data with each other. The cases where the p-value was less than 0.05 were evaluated as statistically significant results.

3. Results

Histopathological damage scores were compared between the groups. The edema score was higher in the paclitaxel+DHEA group than in the normal group. Vascular congestion score was higher in the paclitaxel and paclitaxel+DHEA groups than in the normal group. Cellular degeneration mean score in normal ovary group is 0.00±0.00, in paclitaxel group 0.63±0.52 and in paclitaxel+DHEA group 0.25±0.46. Cellular degeneration score was higher in paclitaxel group than normal group (Table 1).

Total damage score was calculated by summing all the scores of edema, vascular congestion, inflammation, cellular degeneration and hemorrhage. Total score was higher in the paclitaxel and paclitaxel+DHEA groups than in the normal group (Table 1).

Follicle numbers and AMH values were compared according to the groups. In the paclitaxel group, the number of tertiary follicles and ovarian volume were lower than in the normal group. Primordial follicles, secondary follicles, tertiary follicles, AMH level and ovarian volume of paclitaxel+DHEA group were lower than normal group (Table 2).

Correlation between AMH and rat weight, ovarian volume, total damage score, number of atretic follicles,

Table 1. Comparison of histopathological damage scores of normal ovary vs paclitaxel, paclitaxel + DHEA (Mann Whitney U Test).

		Normal ovary	Paclitaxel	p*	Paclitaxel+DHEA	p**
Edema	Mean SD	0.00±0.00	0.38±0.52	0.063	1.50±0.76	0.001
	Median- IQR	0.00(0.00-0.00)	0.00(0.00-1.00)		2.00(1.00-2.00)	
Vascular congestion	Mean SD	0.00±0.00	0.50±0.53	0.025	1.50±1.07	0.003
	Median- IQR	0.00(0.00-0.00)	0.50(0.00-1.00)		2.00(0.50-2.00)	
Inflammation	Mean SD	0.00±0.00	0.13±0.35	0.317	0.00±0.00	1.000
	Median- IQR	0.00(0.00-0.00)	0.00(0.00-0.00)		0.00(0.00-0.00)	
Cellular degeneration	Mean SD	0.00±0.00	0.63±0.52	0.009	0.25±0.46	0.143
	Median- IQR	0.00(0.00-0.00)	1.00(0.00-1.00)		0.00(0.00-0.50)	
Hemorrhage	Mean SD	0.13±0.35	0.00±0.00	0.317	0.00±0.00	0.317
	Median- IQR	0.00(0.00-0.00)	0.00(0.00-0.00)		0.00(0.00-0.00)	
Total damage score	Mean SD	0.13±0.35	1.63±1,19	0.009	3.25±1.83	0.002
	Median- IQR	0.00(0.00-0.00)	2.00(0.50-2.50)		4.00(2.00-4.50)	

Table 2. Comparison of normal ovary vs paclitaxel, paclitaxel + DHEA groups in terms of follicle count and AMH values (Mann Whitney U Test).

		Normal ovary	Paclitaxel	p*	Paclitaxel +DHEA	p**
Primordial follicle	Mean SD	12.75±1.91	8.63±5.10	0.063	3.87±2.30	0.001
	Median- IQR	12.50(11.50-14.00)	10.00(3.50-12.00)		3.00(2.00-5.50)	
Primary follicle	Mean SD	10.50±2.33	14.75±8.84	0.792	8,13±3,98	0.224
	Median- IQR	11.00(8.50-12.00)	12.00(7.00-23.50)		8.50(4.50-11.00)	
Secondary (pre-antral) follicle	Mean SD	12.25±1.83	10.75±3.15	0.365	7.88±3.64	0.017
	Median- IQR	12.50(10.50-13.50)	11.00(9.00-13.50)		8.50(4.50-10.50)	
Tertiary (antral) follicle	Mean SD	21.50±3.21	15.00±3.96	0.010	17.00±5.15	0.050
	Median- IQR	22.00(19.50-23.50)	14.00(12.50-15.50)		18.50(12.00-21.00)	
Athretic follicle	Mean SD	0.25±0.46	0.63±0.52	0.143	0.25±0.46	1.000
	Median- IQR	0.00(0.00-0.50)	1.00(0.00-1.00)		0.00(0.00-0.50)	
AMH (ng/mL)	Mean SD	3.42±0.79	2.95±0.73	0.294	2.07±0.80	0.010
	Median- IQR	3.37(2.64-4.06)	2.95(2.55-3.47)		2.02(1.45-2.70)	
Ovary volume (mm^3)	Mean SD	55.49±9.14	39.14±8.22	0.003	37.99±12.91	0.008
	Median- IQR	54.12(50.19-55.53)	39.05(33.07-42.40)		36.23(28.74-45.35)	
Rat weight (grams)	Mean SD	197.25±13.60	203.38±6.57	0.247	204.00±7.33	0.139
	Median- IQR	193.0(189.0-207.0)	205.50(201.0-207.0)		203.0(198.0-209.0)	

secondary + tertiary follicles were evaluated for each group. Accordingly, there is a strong positive correlation between AMH and ovarian volume in normal group (Table 3).

Table 3. Correlations between rat weights, ovary volume, total damage score, number of atretic follicles, and AMH levels (Spearman Correlation Test *p<0,050 **p<0,010).

	Normal	Normal ovary	Paclitaxel
Rat weight (grams)	AMH	Paclitaxel AMH	Paclitaxel +DHEA
Ovary volume (mm3)	AMH	-0.500	0.476
Total damage score	0.082	-0.309	-0.466
Atretic follicle count	0.000	0.169	-0.630
Pre-antral + antral follicle count	-0.072	-0.024	0.252

4. Discussion

Paclitaxel binds and stabilizes cellular microtubules that cause cell death. It also initiates apoptosis by various mechanisms (Wang et al., 2000). However, the mechanisms that lead to chemotherapy-induced follicular destruction of follicles have not yet been elucidated and require further research (Gucer et al., 2001). Previous studies have shown that paclitaxel application in mice causes depletion of the follicular reserve. This effect of paclitaxel causes premature ovarian failure and infertility in young patients (Gucer et al., 2001). We performed histopathological evaluations to examine the damage of paclitaxel to the ovarian tissue. We determined that there was a significant increase in vascular congestion, cellular degeneration, and total damage scores in paclitaxel-treated tissues compared with those observed in normal ovarian tissues. We determined that there was increase in edema, vascular congestion and total damage scores in the group receiving the paclitaxel+DHEA treatment (Table 1). When ovarian volumes were examined, we identified a significant decrease in ovarian volumes in both groups (Table 2). We observed that paclitaxel caused damage to ovarian tissue in both groups, however this damage did not change with DHEA supplementation.

Moreover, it has also been documented that combined chemotherapy regimens exert a significant cytotoxic effect on the ovaries and that a combination of paclitaxel and cisplatin has a maximum cytotoxic effect on both primordial and mature follicles (Ozcelik et al., 2010). In mature oocytes, paclitaxel induces meiotic maturation delay and spindle defect, which results in aneuploid oocytes. In addition, mature follicles show higher susceptibility to chemotherapy-induced damage (Gucer et al., 2001). In their first study conducted on mice in 2000, Gucer et al. identified the damage caused by the use of paclitaxel on primordial cells in the ovaries. They observed that this damage developed in all doses of 2.5, 5.0, and 7.5 mg/kg of paclitaxel (Gucer et al., 2001).

In the study conducted by Yucebilgin et al., rats that received 7.5 mg/kg paclitaxel and 5 mg/kg cisplatin underwent oophorectomy after seven days, and there was a significant decrease in ovarian follicles in both groups. In our study, we examined the ovarian follicles and observed a significant decrease in the antral follicles in the paclitaxel group compared with the normal ovary group. In the paclitaxel+DHEA group, we found that there was a decrease in primordial, preantral and antral follicles. We determined that the addition of DHEA was associated with an increase in follicular damage (Table 2). We think that DHEA could not prevent primordial follicle damage because it did not prevent the increase in edema, vascular congestion and total damage scores. Primordial follicles are known to be sensitive to chemotherapy. In previous studies, as a result of applying chemotherapy, primordial follicles decreased because of the damage (Ozcelik et al., 2010) The ovarian toxicity of taxane-based chemotherapies remains unclear and the dose dependence of this toxicity is uncertain, partially due to the fact that an adequate evaluation of ovarian toxicity through animal studies is lacking. Paclitaxel is an important agent for breast cancer as well as for gynecological malignancies, and the use of paclitaxel as a neoadjuvant chemotherapy is an important treatment strategy. Therefore, whether the incidence of amenorrhea is dependent on paclitaxel dose is an important issue for the treatment of young women (Tarumi et al., 2009). Taraumi et al. identified a decrease in the primordial and antral follicle counts as a result of the use of paclitaxel. They found that the number of tertiary follicles also increased. In hormonal assessments, estradiol and progesterone hormone levels did not vary depending on paclitaxel. Based on their study results, they reported that paclitaxel damaged antral follicles, but did not affect less mature follicles (Tarumi et al., 2009). In this study, no AMH evaluation was carried out. AMH has been confirmed in many studies as a reliable molecular biomarker of ovarian reserve (Broer et al., 2011; Iliodromiti and Nelson, 2015; Cheng et al., 2019). Reduction of AMH to minimal levels may be correlated with reduced ovarian follicle count (Stracquadanio et al., 2018). Although there are numerous ovarian reserve tests with varying predictive capabilities, antral follicle count and anti-mullerian hormone levels have been determined to provide the best diagnostic accuracy for constantly predicting poor ovarian reserve (Jayaprakasan et al., 2010). We investigated the effect of the use of paclitaxel on AMH. In conclusion, we determined that the AMH levels of rats that were administered paclitaxel alone were similar to those of normal rats, whereas there was a decrease in AMH levels in rats who received paclitaxel+DHEA. We determined that the addition of DHEA was associated with an increase in AMH decline (Table 2).

DHEA, a precursor of estradiol and testosterone, has been recognized as a potential intervention to enhance supplementation, improve the ovarian status, and improve assisted reproductive technique results in women with low ovarian response (Tsui et al., 2014). Although DHEA is more widely used in patients with poor response, many different opinions remain among many clinicians. Most of the published studies are based on retrospective and/or observational data, and their results can be biased (Yeung et al., 2014). However, initial reports on DHEA supplementation in patients with low ovarian response remain controversial due to the lack of large-scale, well-designed confirmatory studies (Barad and Gleicher, 2006; Barad et al., 2009). Based on the result of our study that investigated whether DHEA supplementation in addition to paclitaxel would reduce ovarian damage, it was determined that DHEA supplementation has an overall negative effect because rats treated with paclitaxel+DHEA experienced a decrease in ovarian volume, an increase in histopathological damage scores, a decrease in AMH levels, and a greater decrease in follicle counts compared with the group using paclitaxel alone.

In the paclitaxel+DHEA group, we found that there was a decrease in primordial, preantral and antral follicles. The addition of DHEA was associated with an increase in follicular damage.

It was determined that DHEA does not exert any positive effect in reducing ovarian damage caused by the use of paclitaxel in rats and that on the contrary, it has a negative effect on follicle counts and AMH levels, which are the most important indicators of infertility.

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Atakum, 55200, Samsun, Turkey
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