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European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December. The journal publishes content in English.

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Short Communication	1500	200	20	5	1 or total of 5 images
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Letter to the Editor	500	No abstract	5	No tables	No media

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Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

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ORIGINAL RESEARCH ARTICLES

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Original Research

Displaced Femoral Neck Fractures: Anatomic Reduction or Early Surgery?

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ABSTRACT

Objective: Femoral neck fractures are treated with arthroplasty options in elderly patients. In young patients, the aim of the treatment is to protect the hip joint and its functions. In this study, the effect of the timing of surgery and reduction quality on the development of avascular necrosis in patients aged 15-60 years with Garden types 3 and 4 femoral neck fractures were retrospectively analyzed.

Methods: Patients who underwent treatment in our clinic between 2009 and 2016 were retrospectively evaluated. The patients were classified into two groups, including those who underwent surgery within the first 8 hours after injury (mean time, 301 min) and those who underwent surgery 8 or more hours after injury (mean time, 1750 min). The patients were classified according to the Garden classification based on their preoperative radiographs. Reduction quality was evaluated through the Garden Alignment Index using postoperative radiographs. Postoperative radiographs were evaluated on the 1st, 2nd, 3rd, 6th, 12th, 18th, and 24th month after surgery, and the osteonecrosis classification was made using the Ficat-Arlet method.

Results: No significant differences were found among these four groups in terms of operation time, fracture reduction quality, and the development of avascular necrosis ($p>0.05$).

Conclusion: According to the this study the timing of surgery and anatomical reduction have no effect on the development of avascular necrosis in the treatment of displaced femoral neck fractures fixed with three cannulated screws.

Keywords: Femoral neck fractures, trauma, avascular necrosis, internal fixation, anatomic reduction

INTRODUCTION

Femoral neck fractures are usually treated with arthroplasty options in elderly patients. The aim of femoral neck fracture treatments in younger patients is to protect the hip joint and its functions. Internal fixation of femoral neck fractures has high complication rates, with avascular necrosis and nonunion of the femoral head being the most common complications (1-4). In the literature, it has been reported that anatomic reduction and the timing of surgery are effective in preventing the development of avascular necrosis (5, 6).

In this study, we investigated the relationship of avascular necrosis in patients with unstable (Garden grade 3 and 4) femoral neck fractures treated by internal fixation with the timing of surgery and reduction quality. We retrospectively compared avascular necrosis rates between patients who underwent early (<8 hours) and late surgery (>8 hours) with or without anatomic reduction.

METHODS

The study protocol was approved by the Scientific Research Ethics Committee of Gaziantep University Medical Faculty on November 13, 2019 (2019/443). In this study, patients who underwent treatment in our clinic between 2009 and 2016 were retrospectively evaluated. Written consent was obtained from the patients for the surgery. Patients aged 15–60 years, treated with the closed reduction and internal fixation method, using three pieces of 7.3-mm partially grooved cannulated screws, who were followed up for at least two years, developed union, and had femoral neck fractures classified as Garden grade 3 and grade 4 were included in the study (7). Patients with multiple traumas, Garden grades 1 and 2 fractures or pathologic fractures, who underwent arthroplasty and developed nonunion, were excluded from the study. A total of 42 patients met the inclusion criteria. The mean age was 37.16 (15–60) years. Of the patients, 32 were males and 10 were females. Of the femoral neck fractures, 23 occurred in the right hip and 19 in the left hip. The mean follow-up period was 3.2 years.

Patients underwent surgery on a fracture table in the supine position. The reduction was controlled by fluoroscopy, and fixation was provided. The patients received postoperative rehabilitation, and were not load-bearing for six weeks.

The patients were classified into two groups: those who underwent surgery within the first 8 hours after injury (mean time, 301 min) and those who underwent surgery 8 or more hours

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after injury (mean time, 1750 min). The patients were classified according to the Garden classification based on their preoperative radiographs (7). Reduction quality was evaluated with the Garden Alignment Index using postoperative radiographs (8). Postoperative radiographs were evaluated on the 1st, 2nd, 3rd, 6th, 12th, 18th, and 24th month after surgery, and the osteonecrosis classification was made using the Ficat-Arlet method (9). Radiological follow-up was continued for patients who developed avascular necrosis. During this evaluation, the patients were divided into four groups. The first group included patients who underwent surgery in the first 8 hours after injury and whose fractures were anatomically reduced; the second group included patients who underwent surgery in the first 8 hours and whose fractures could not be anatomically reduced; the third group included patients who underwent surgery 8 or more hours after the injury and whose fractures were anatomically reduced; and the fourth group included patients who underwent surgery 8 or more hours after the injury and whose fractures could not be anatomically reduced. The development of osteonecrosis was statistically analyzed among the groups. Pearson chi-square tests were used as the statistical method.

Statistical Analysis

Continuous variables are presented as mean and standard deviation, and categorical variables are presented as absolute numbers and percentages. The Shapiro-Wilk test was used for testing the normality of numerical data. Mann Whitney U test was used to compare numerical variables that were not normally distributed in two groups. The relationship between categorical variables was analyzed by the Chi-square test. The SPSS 22.0 (IBM SPSS Corp.; Armonk, NY, USA) software package was used for the analyses. For all analyses, a p value of <0.05 was considered statistically significant.

RESULTS

Forty-two patients (32 males, 10 females) who met the inclusion criteria and had at least two years of follow-up data were included in the study, and their radiographies were evaluated. Among the postoperative controls, the rate of avascular necrosis in the femoral head was determined to be 52.38%. The duration of avascular necrosis development in the patients was 1.67 years (621.22 days). Avascular necrosis was seen in 45.75% of the patients who underwent surgery before 8 hours and whose fracture was reduced anatomically (Figure 1–8). Avascular necrosis was seen in 63.63% of patients who underwent surgery before 8 hours and whose fracture could not be reduced anatomically (Table 1a, 1b). Avascular necrosis was seen in 58.33% of patients who underwent surgery after 8 hours and whose fracture was reduced anatomically. Avascular necrosis was seen in 8.50% of pa-

Main Points:

- As we have shown in our study, in 52% of the patients, avascu- lar necrosis can develop after an average of 1.67 years, although postoperative union is provided for displaced femoral neck fractures.
- According to the this study the timing of surgery and anatomical reduction have no effect on the development of avascular necrosis in the treatment of displaced femoral neck fractures fixed with three cannulated screws.
- In the treatment of these patients, we believe that changing the treatment method or turning to new treatment methods may increase the success rate.

Figure 1. Preop antreposterior (AP) x-ray

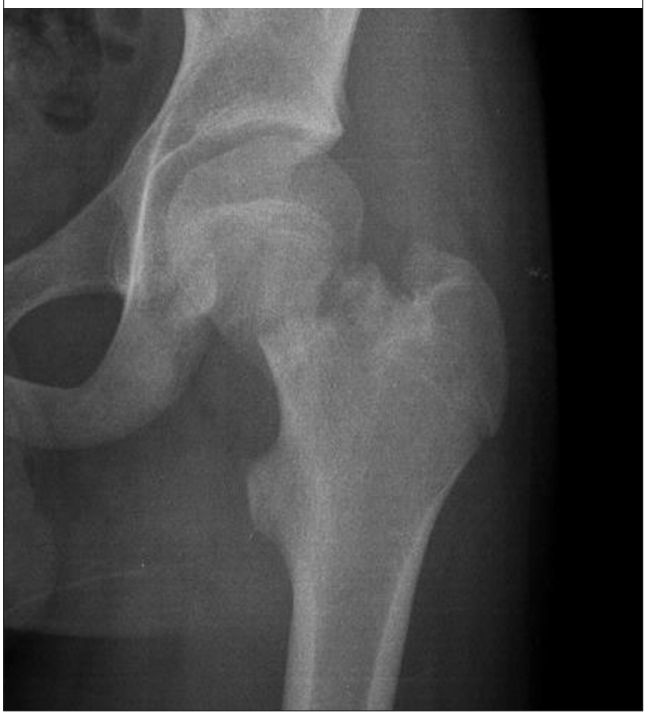


Figure 2. Preop lateral (LAT) x-ray

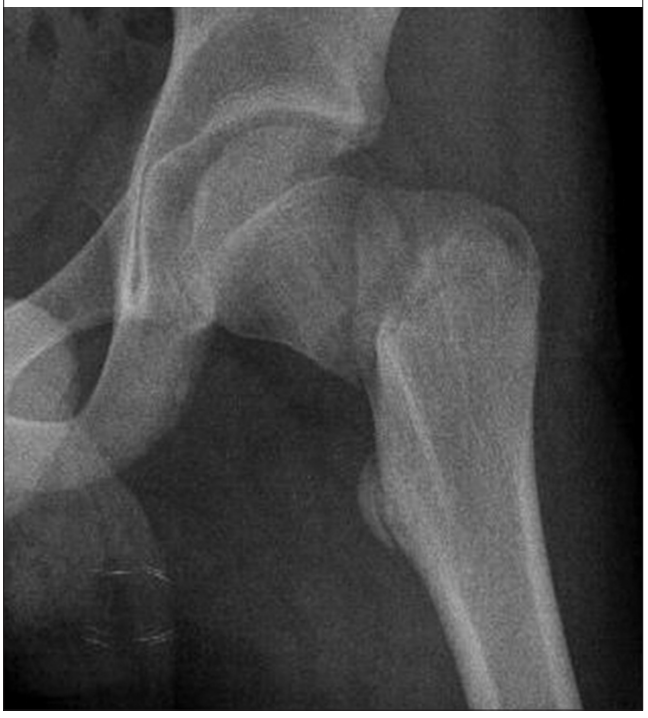


Figure 3. Postop AP x-ray

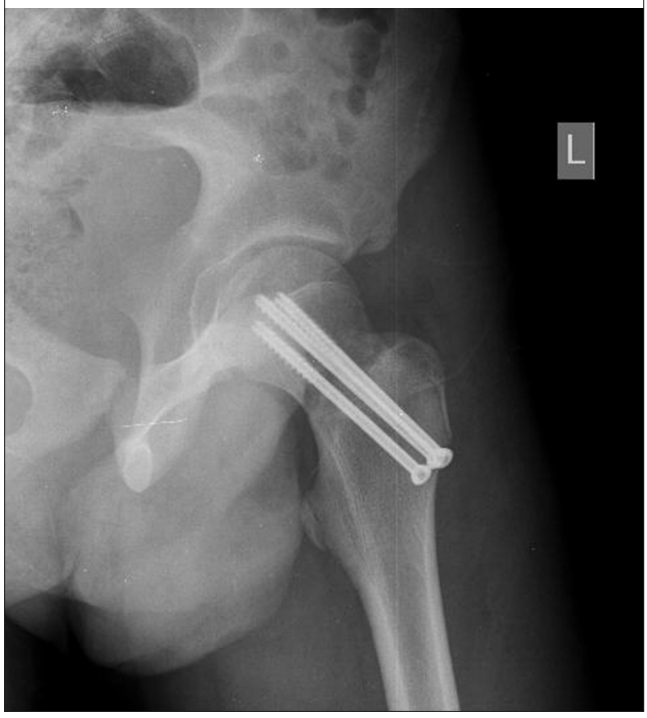


Figure 4. Postop LAT x-ray

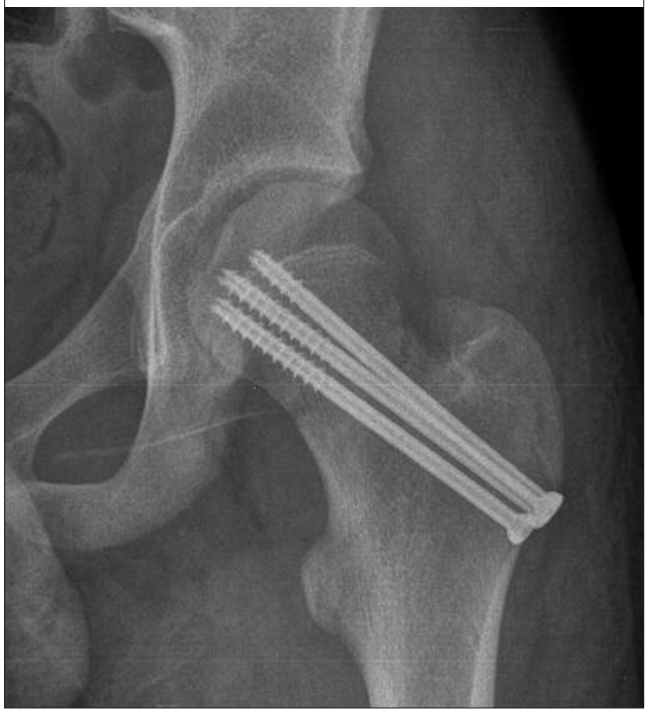


Figure 5. Postop 4th months AP x-ray

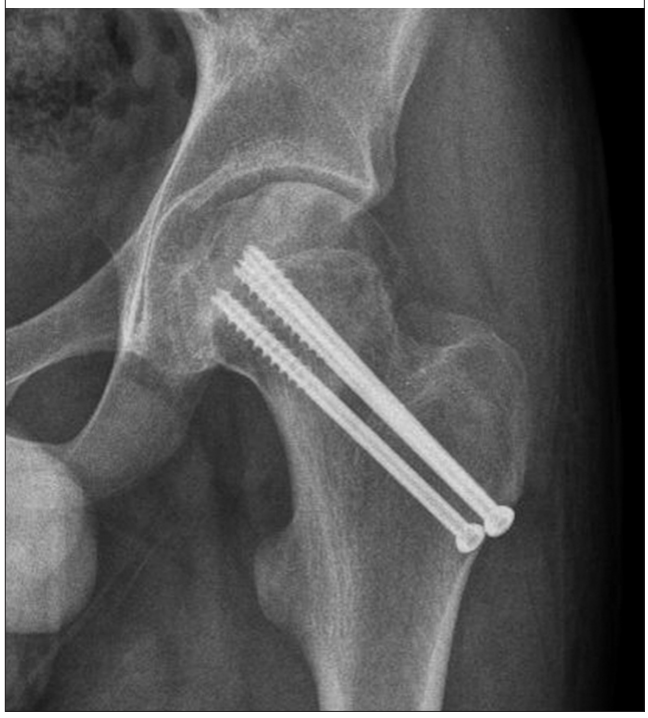
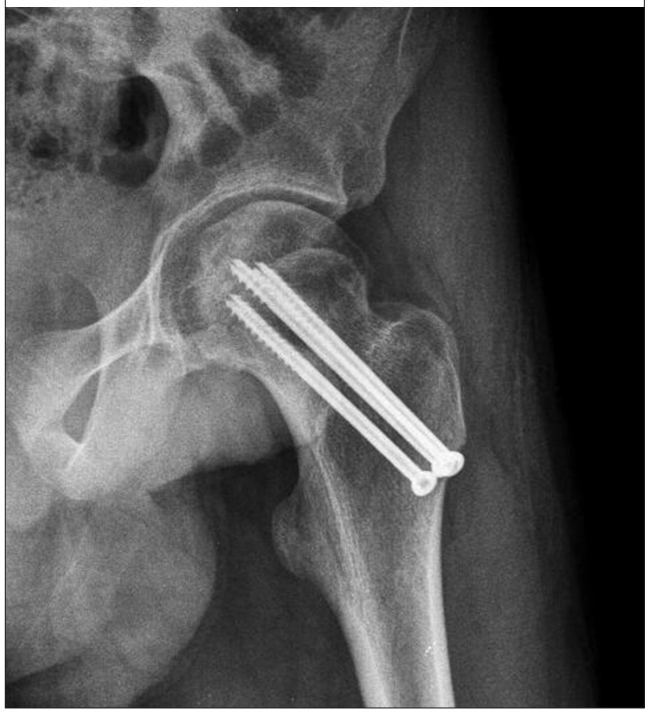


Figure 6. Postop 4th months Lat x-ray



tients who underwent surgery after 8 hours and whose fracture could not be reduced anatomically (Table 2a, 2b). After statistical analysis, no significant differences were found among these four groups in terms of operation time, fracture reduction quality, and development of avascular necrosis ($p>0.05$).

DISCUSSION

Avascular necrosis of the femoral head and nonunion after femoral neck fractures are the most common complications (1-4). Due to these complications, total hip arthroplasty may be a solution in elderly patients; however, this method is not

Figure 7. Postop 2 years AP x-ray

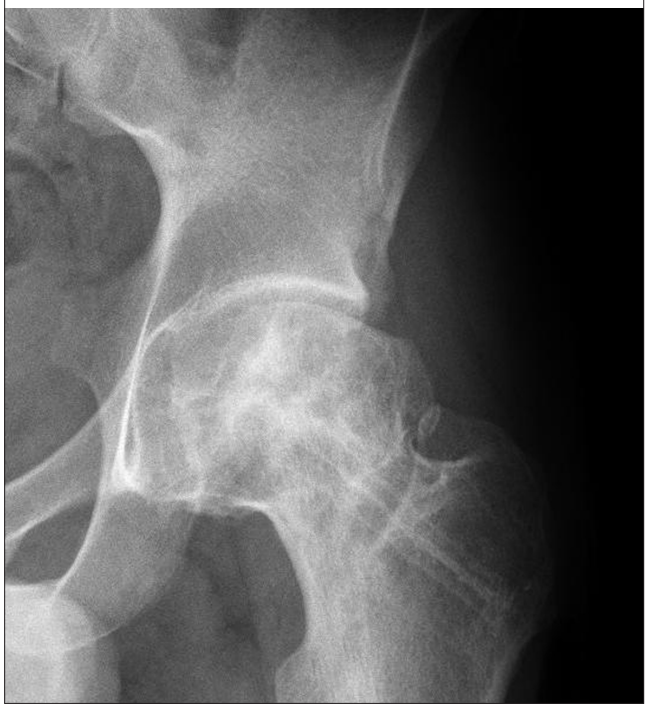


Figure 8. Postop 2 years LAT x-ray

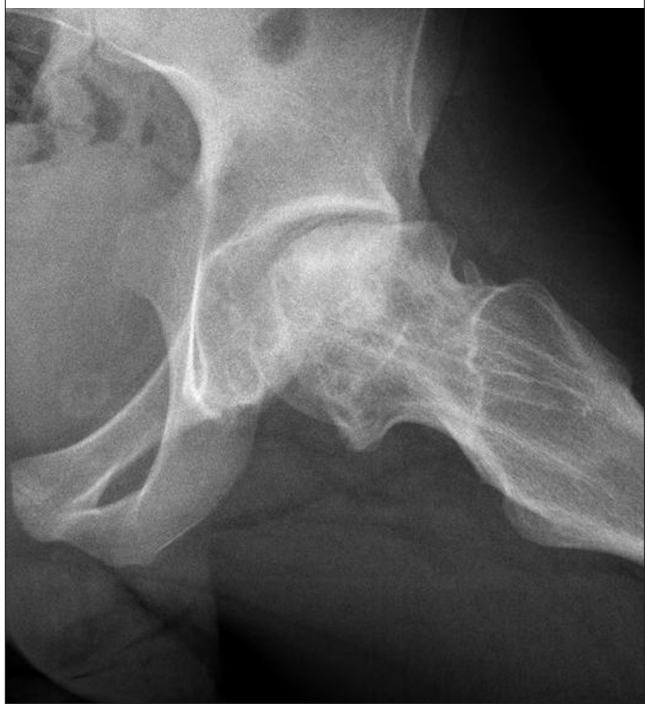


Table 1a. Group of Patients Who Underwent Surgery in the First 8 Hours and Whose Fractures Reduced Anatomically

Group of Patients Who Underwent Surgery in the First 8 Hours and Whose Fractures Reduced Anatomically:										
Patient No:	Age	Gender	Side	Classification (Garden Classification)	Time To Operation (minute)	AP Reduction Quality (Garden Alignment Index)	LAT Reduction Quality (Garden Alignment Index)	Development of Avascular Necrosis (Ficat Arlet Classification)	Follow-Up Period (day)	Development of Avascular Necrosis (day)
1	60	e	Right	3	143	GR	GR	3	734	517
2	58	k	Left	4	150	GR	GR	1	1227	–
3	28	e	Left	3	188	GR	GR	2	1404	815
4	15	e	Left	4	189	GR	GR	3	770	287
5	50	e	Right	3	274	GR	GR	1	1884	–
6	32	e	Right	3	314	GR	GR	3	1025	816
7	16	k	Right	4	323	GR	GR	1	1001	–
8	43	e	Left	3	351	GR	GR	3	1189	759
9	15	e	Right	3	389	GR	GR	1	898	–
10	56	e	Left	3	396	GR	GR	1	987	–
11	35	e	Left	3	476	GR	GR	1	1038	–

GR: Good Reduction

suitable for younger patients. Studies have shown the importance of anatomic reduction, stable fixation, and the timing of surgery to prevent complications and achieve successful treatment results (5, 6). In our study, since we aimed to investigate the effect of the reduction quality and early surgery on the development of avascular necrosis in displaced femoral neck fractures, we included patients with bony union and excluded patients with nonunion.

The blood supply to the femoral head is mainly from the medial femoral circumflex artery (10). The terminal branches of this artery are intracapsular and are at risk in displaced femoral neck

Table 1b. Group of Patients Who Underwent Surgery in the First 8 Hours and Whose Fractures Not Reduced

Group of Patients Who Underwent Surgery in the First 8 Hours and Whose Fractures Reduced Anatomically:										
Patient No:	Age	Gender	Side	Classification (Garden Classification)	Time To Operation (minute)	AP Reduction Quality (Garden Alignment Index)	LAT Reduction Quality (Garden Alignment Index)	Development of Avascular Necrosis (Ficat Arlet Classification)	Follow-Up Period (day)	Development of Avascular Necrosis (day)
1	45	e	Right	3	33	AR	AR	3	928	928
2	52	e	Left	3	241	AR	AR	2	740	181
3	33	k	Left	4	282	AR	AR	3	923	575
4	29	e	Right	3	291	AR	AR	3	924	538
5	46	e	Right	4	306	AR	AR	3	1236	399
6	18	e	Left	4	318	AR	AR	1	732	–
7	19	e	Right	3	352	AR	AR	4	786	264
8	16	e	Right	3	357	AR	AR	1	804	–
9	58	k	Left	3	396	AR	AR	4	1255	775
10	26	e	Right	3	411	AR	AR	1	990	–
11	16	e	Right	3	444	AR	AR	1	804	–

Anatomically
AR:Acceptable Reduction

Table 2a. Group of Patients Who Underwent Surgery After 8 Hours and Whose Fractures Reduced Anatomically

Group of Patients Who Underwent Surgery After 8 Hours and Whose Fractures Reduced Anatomically										
Patient No:	Age	Gender	Side	Classification (Garden Classification)	Time To Operation (minute)	AP Reduction Quality (Garden Alignment Index)	LAT Reduction Quality (Garden Alignment Index)	Development of Avascular Necrosis (Ficat Arlet Classification)	Follow-Up Period (day)	Development of Avascular Necrosis (day)
1	17	e	Left	3	510	GR	GR	3	1822	228
2	54	e	Left	3	718	GR	GR	1	754	–
3	49	k	Left	3	962	GR	GR	1	765	–
4	60	e	Right	3	1170	GR	GR	1	1004	–
5	60	k	Right	3	1266	GR	GR	3	977	400
6	52	e	Right	3	1267	GR	GR	1	1810	–
7	61	k	Left	4	1433	GR	GR	3	738	553
8	51	e	Right	3	2481	GR	GR	3	1167	783
9	40	e	Left	4	3996	GR	GR	3	832	625
10	35	e	Left	4	3291	GR	GR	3	2352	697
11	60	e	Right	3	2122	GR	GR	1	1875	–
12	30	e	Right	4	1440	GR	GR	4	1056	394

GR:Good Reduction

fractures. This may be the most important factor for the development of osteonecrosis (11). Providing anatomic reduction and stable fixation as early as possible will protect these arteries that supply blood to the femoral head (12-14). It is widely accepted that anatomic reduction and stable fixation in femoral neck fractures reduce the risk of femoral head avascular necrosis (15-17). Treatment of femoral neck fractures is a traditionally urgent surgery. Swiontkowski et al. (18) reported successful results in pa-

Table 2b. Group of Patients Who Underwent Surgery After 8 Hours and Whose Fractures Not Reduced Anatomically

Group of Patients Who Underwent Surgery After 8 Hours and Whose Fractures Not Reduced Anatomically										
Patient No:	Age	Gender	Side	Classification (Garden Classification)	Time To Operation (minute)	AP Reduction Quality (Garden Alignment Index)	LAT Reduction Quality (Garden Alignment Index)	Development of Avascular Necrosis (Ficat Arlet Classification)	Follow-Up Period (day)	Development of Avascular Necrosis (day)
1	31	e	Left	3	799	AR	AR	3	1331	473
2	35	e	Left	3	849	AR	AR	2	1961	1961
3	21	e	Right	3	976	AR	AR	1	2018	–
4	46	e	Left	3	1193	AR	AR	1	874	–
5	23	k	Right	3	2241	AR	AR	1	2329	–
6	17	k	Right	3	4022	AR	AR	1	933	–
7	25	e	Right	4	2260	AR	AR	1	1203	–
8	28	k	Right	4	2001	AR	AR	3	1677	699

AR: Acceptable Reduction

tients who underwent surgery within 8 hours after injury, and Jain et al. (19), Zetterberg et al. (20), and Bray et al. (21) showed in their studies that the timing of surgery after injury was an important factor for the results. In contrast, Karaeminoğulları et al. (22) and Gumustas et al. (23) showed no correlation between the timing of surgery and avascular necrosis of the femoral head. Papacostidis et al., in their meta-analysis, could not prove a relationship between the timing of internal fixation and the incidence of avascular necrosis (24). However, they have indicated that a delay in internal fixation of more than 24 hours may increase the probability of nonunion. While surgery can be delayed for Garden I–II nondisplaced fractures, Garden III–IV fractures may require immediate treatment (4). We included displaced femoral neck fractures (Garden III–IV) with higher complication rates in our study. We thought that this would create stronger evidence to evaluate the timing of the surgery and the effects of anatomical reduction on avascular necrosis.

In our study, no differences were found among the four groups in terms of the development of avascular necrosis. The fact that no difference was found in terms of avascular necrosis development between the patients who underwent surgery after 8 hours and whose fracture could not be reduced anatomically, which was the worst scenario, and the patients who underwent surgery within the first 8 hours and whose fracture was reduced anatomically, which were the best scenario, demonstrates that avascular necrosis develops independently of surgical timing and anatomic reduction, especially in patients with grade 3 and 4 instable femoral neck fractures. No statistically significant association was found between the rate of avascular necrosis of the femoral head and timing of surgery in our patients.

As we have shown in our study, in 52% of the patients, avascular necrosis can develop after an average of 1.67 years, although postoperative union is provided for displaced femoral neck fractures. The development of avascular necrosis in 45% of the patients who underwent early surgery, anatomic reduction, and developed union indicates the difficulty in treating displaced femoral neck fractures. According to the results of our study, the timing of surgery and anatomical reduction have no effect on the development of avascular necrosis in the treatment of displaced femoral neck fractures fixed with three cannulated screws. In the treatment of these patients, we believe that changing the treatment method or turning to new treatment methods may increase the success rate.

A limitation of our study is the retrospective design. On the other hand, we think that evaluating a specific patient group (patients who have Garden grade III and grade IV femoral neck fracture, who develop union and who are followed up at least for two years) and having a large number of patients compared to the literature are the strengths of our study.

CONCLUSION

According to the this study the timing of surgery and anatomical reduction have no effect on the development of avascular necrosis in the treatment of displaced femoral neck fractures fixed with three cannulated screws.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University Medical Faculty (13.11.2019, 2019/443).

Informed Consent: Written consent was obtained from the patients for the surgery.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - B.K.; Design - B.K., N.G.; Supervision - B.K., N.G.; Resources - B.K., N.G.; Data Collection and/or Processing – B.K., N.G.; Analysis and/or Interpretation – B.K., N.G.; Literature Search - B.K., N.G.; Writing Manuscript - B.K., N.G.; Critical Review - B.K., N.G., O.B.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Research

Traumatic Injuries From Sheep Sacrifice During the Eid Al-Adha Holiday: A Prospective Multicentered Study

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ABSTRACT

Objective: We sought to describe injuries related to the sacrificial slaughtering of animals during the Eid holiday.

Methods: We conducted a centered prospective observational cohort study during the Eid (August 21-24, 2018) at 5 emergency departments in Gaziantep, Turkey. Descriptive statistics of injuries collected included the injury location, involvement of dominant or non-dominant hand, cause of injury (instrument vs animal), type of instrument causing injury, surgical interventions performed, and professional occupations of patients.

Results: We included two hundred seventy-seven patients with injuries who fulfilled the criteria and excluded injuries not related to animal slaughter. Most injuries (91%) occurred in people who were not professional butchers (n=252) and simple laceration (not involving vessels or tendons) was the most common injury type (95.3%; n=265). Those who were injured and had no experience were mostly injured during the processing of the meat (butchering) and while helping others. Lacerations were most commonly observed in the upper extremity (83.4%; n=231), on the non-dominant side (67.5%; n=187), in the hand (78.7%, n=218), and specifically in the index finger (23.1%; n=64). A surgery was performed on 8 patients.

Conclusion: The first day of Eid is associated with an increase in mostly non-dominant upper extremity injuries among inexperienced people slaughtering animals. Further education and safety measures may reduce such injuries. Emergency departments serving larger Muslim communities may benefit from anticipating an uptick in these injuries.

Keywords: Sacrifice, Eid Al-Adha, injuries, emergency medicine, sheep

INTRODUCTION

The annual Eid holiday (Festival of Sacrifice) is one of the two main Islamic holidays celebrated by approximately 1.8 billion Muslims across the world. This is marked by animal sacrifice during the Eid Al-Adha holiday, which is one of the pillars of worship in Islamic belief, and can be performed by professional butchers or laypeople. The Festival of Sacrifice (Bayram or Eid) after the Hajj pilgrimage is celebrated duringfour days starting from the 10thday of the month "Zul-Hijjah" of the lunar Islamic calendar. Muslims sacrifice bovines or sheep during any of the four days of this celebration, generally on the first day of the Eid holiday (1). In the 2018 Eid, an estimated 3.6 million animals were prepared for sacrifice in Turkey (2). Injuries from sacrificing and processing of meat can range from mild skin lacerations to deadly accidents. Blunt trauma may be observed as a result of an animal attack. The person or another person sacrificing orprocessing the meat may be injured during the sacrifice (3-7). Recently, a number of precautions have been placed in Turkey to provide a safer, easier and healthier sacrifice. Throughout the holiday, municipalities facilitate venues for the sacrifice of animals. These well-equipped sacrifice centers provide professional butchers. However, injuries are observed despite these precautions. Although animal slaughter and meat processing are typically reserved for the butchers, many people prefer to perform the sacrifice themselves. Despite these precautions, serious injuries are incurred.

The purpose of this study was to quantify and describe the incidence, causes and types of injuries related to the sacrificial

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slaughtering of sheep during the Eid Al-Adha holiday from patients presenting to five emergency departments (ED) in the city of Gaziantep, as well as the medical and surgical interventions performed. Few retrospective studies have been performed on this topic,and this prospective, multicentered design is of great value. The aim of the study is to provide knowledge about the frequency and types of injuries observed in animals sacrificed during the Eid holiday to anticipate a need for certain consultant services (such as hand surgery) and to raise awareness of such injuries in hopes that it will lead to preventive safety measures to reduce such injuries in the future.

METHODS

Ethics committee approval was received for this study from the ethics committee of Hasan Kalyoncu University (Date: 06/06/2018; No:2018-05). This study was conducted during the 4 days of the Eid Al-Adha holiday (August 21-24, 2018) in the emergency departments of 5 hospitals in Gaziantep, Turkey. The patients presented with laceration or blunt trauma to the EDs (864). We included consenting patients who met the inclusion criteria.

Inclusion criteria in research: Patients

- With injury during the slaughtering and/or meat processing
- Who agree for a return visit to the ED for wound/injury recheck one month post-injury

Exclusion criteria in research:

- Injury not related to sacrifice processing
- Injury occurring outside specified days

Process:

Patients presenting to the ED with injury after slaughtering of animals or processing of meat were registered using previously prepared forms that included questions on:

- Demographics: age, gender, contact information
- The day and time during the Bayram in which the injury occurred
- Occupation: professional butcher or non-butcher
- Experience (individuals believed to be experienced other than professional butchers were those who had attended at least one animal slaughter)

Main Points:

- Most of the patients who present to the emergency department due to the injury during the Eid of sacrifice are non-butchers.
- There is a sudden spike in mostly hand surgery cases in emergency departments during the first day of Eid. Emergency departments should anticipate this increase and prepare on call coverage.
- The first finger of the upper extremities were most commonly injured and injuries observed had been during the first day of Eid.
- Education and safety precautions may help reduce the number of injuries

- Type of injury: penetrating or blunt trauma
- Type of laceration: simple (cutaneous, subcutaneous) or complicated (muscle, tendon, nerve, artery, bone injuries)
- Causes of injury: knife, meat chopper, meat grinder, axe, skewer, animal attack
- Role of person: *sacrificer* who uses the sharp object (knife, axe), *helper* who helps the sacrificer during the procedures, or *others* who observe the procedures during sacrificing or meat processing
- The stage at which the injury was incurred, either during sacrificing or meat processing (sacrificing: livestock slaughtering process; meat processing: processing of meat products after sacrificing)

All patients were called after one month for check-up and the following information was collected:

- Sequelae from injury
- Workforce loss post-injury (called-in sick)
- Reason for animal sacrifice(religious, economic or others)

Measurements:

- Descriptive statistics
- Comparison of types of injury, location of injury, types of laceration, severity of injury (simple/complicated), and sequelae between butcher and non-butcher patients.
- Comparison of types of injury, location of injury, types of laceration, severity of injury (simple/complicated), and sequelae between experienced and inexperienced patients.

We initially included 330 patients who had presented with injuries in the study. We subsequently excluded 53 patients who did not returnfor follow up and those who could not be reached by phone.The study was completed with 277 patients who fulfilled the inclusion criteria.

SPSS version 13 was used for the statistical analysis of the data. Descriptive statistics were used to summarize data with absolute and relative frequencies for categorical data, and means and standard deviations for continuous variables. The Pearson Chi-square test was used to investigate the relationship between the categorical variables.

RESULTS

We included 277 patients in the study. Among these, 227 (81.9%) were men and 50 (18.1%) were women. The mean age was 36.9 ±14.2 (SD) (range 3-76) years. The youngest injured person was a three-year-old boy injured while playing with a knife. Among the injured persons, only 9% were professional butchers (n=25) and 57% (n=158) were experienced. Among the patient, 190 (68.6%) did primary school,and 230 (83%) were evaluated during the first day of Eid and most of them (n=140, 50.5%) were seen between 06:00 and 12:00. The most common injuries were simple lacerations (92.1%; n=255), mostly with knives (n=227; 81.7%). Injuries occurred mostly during the processing of meat (n=144, 52%) and while helping (n=193, 69.3%). Among the patients surveyed, 82.7% (n=229) felt obligated to personally perform the sacrifice due to their religious beliefs (Table1).

Concerning the types of laceration, simple lacerations were detected in 255 (92.1%) cases. These injuries most frequently involved the non-dominant side (n=187, 67.5%), hand (n=218, 78.7%), and index finger (n=64, 23.1%) (Table 2). Five patients with complicated laceration had isolated tendon lacerations, two had an isolated arterial injury, one had an isolated nerve injury, one had tendon and nerve injury and one had injuries to a tendon, nerve and artery.

Among the 12 patients who presented with blunt trauma, one had a nasal fracture, two had intraocular bleeding, one had soft tissue injury of the upper lip, three had soft tissue injury of the hand, two had a distal fibula fracture, one had a distal tibia and fibula fracture, one had a 5th metatarsal fracture and one patient had a soft tissue injury of the foot.

Eight patients required surgery by otolaryngologists (n=1), orthopedics (n=1), and plastic surgery (2.2%, n=6). One of the two patients with amputation lost the distal phalanx of the first finger of the left hand due to chopping injury, and another lost the distal phalanx of the second finger of the right hand due to use of the mincer. Both patients underwent primary closure in the ED.

The patients were called for a recheck after one month; 222 (80.1%) patients recovered without any sequelae. We identified paresthesia in 17 patients (6.1%), pain in 15 (5.4%) patients, movement limitation in 13 (4.7%) patients, persistent swelling of injury site in 7 (2.5%) patients, amputation in 2 (0.7%) patients and wound side infection in one patient (Table 3).

Table 1. Descriptive Statistics

		Mean	Minimum	Maximum
Age		36.86±14.17	3	76
			Count (N)	Percent (%)
Gender	Male		227	81.9
	Female		50	18.1
Education Degree	None		2	0.7
	Primary school		190	68.6
	High school		60	21.7
	University		25	9
Occupation	Butcher		25	9
	Non–butcher		252	91
Experience	Yes		158	57.0
	No		119	43.0
Day of Bayram ED Visit Occurred	1st day		230	83
	2nd day		39	14.1
	3rd day		5	1.8
	4th day		3	1.1
Time of admission	600–1200		140	50.5
	1200–1800		105	37.9
	1800–2400		32	11.6
Type of injury	Laseration		265	95.7
	*Simple		255	92.1
	*Complicated		10	3.6
	Blunt trauma		12	4.3
Causes of Injury	Knife		227	81.9
	Meat chopper		33	11.9
	Mean grinder		1	0.4
	Axe, skewer, animal attack, etc		16	5.8
Sacrificing & Meat Processing	Sacrificing		133	48
	Meat Processing		144	52
Role of Injured Person	Sacrifier		82	29.6
	Helper		193	69.7
	Others		2	0.7
Reason for animal sacrifice	Religious		229	82.7
	Financial		45	16.2
	Other		3	1.1

Table 2. Anatomic Location of the Laceration

		Count (N)	Ratio (%)
Type of laceration	Simple	255	92.1
	Complicated	10	7.9
Side	Dominant Side	90	32.5
	Non–Dominant Side	187	67.5
Localization of injury	Upper Extremity	231	83.4
	·Hand	218	78.7
	Thumb	63	22.8
	Index finger	64	23.1
	Middle finger	25	9
	Ring finger	8	2.9
	Little finger	13	4.7
	Volar side	19	6.9
	Dorsal side	26	9.3
	·Forearm	10	3.6
	·Others	3	1.1
	Lower Extremity	38	13.7
	·Foot	16	5.8
	·Cruris	14	5
	·Others	8	2.9
	Non– Extremity	8	2.9

No significant differences were found between the experienced and inexperienced groups in terms of the type of injury, and the type of laceration and sequelae (p>0.05). Injuries among the experienced patients happened more often during the sacrifice, while inexperienced people were more likely to be injured during meat processing (p=0.001). While injury patterns among experienced patients were more often during the active use of a knife, the inexperienced were injured more often when they were helping (p=0.001) (Table 4).

In analyzing differences between butchers and non-butchers, there was no statistically significant relationship between the

Table 3. Comparison of Experiences

	Experience	Yes	No
Type of injury	Laceration	149	116
	Blunt trauma	9	3
	x2=1.651 P=0.199		
	Upper Extremity	124	107
	Lower Extremity	28	10
	Non-extremity	6	2
Localization of injury	x2= 6.414 P=0.040		
	Hand	115	103
	Food	9	7
	Forearm	7	3
	Cruris	13	1
	Others	14	5
	x2= 11.802 P=0.019		
Type of laceration (n=265)	Simple	142	113
	Complicated	7	3
	x2= 0.801 P=0.371		
Sacrificing & Meat Processing	Sacrificing	98	35
	Meat Processing	60	84
	x2=28.925 P=0.001		
Duty of Person	Active sacrifier	72	10
	Helper	86	107
	Others	0	2
	x2=46.596 P=0.001		
Sequel	None	129	93
	Limited motility	8	5
	Paresthesia	9	8
	Pain	7	8
	Bump/edema	3	4
	Infection	0	1
	Amputation	2	0
	x2=4.395 P=0.623		

Table 4. Comparison of Butchers and Non-Butchers

	Occupation	Butcher	Non-butcher
Localization of injury	Upper Extremity	19	212
	Lower Extremity	5	33
	Non-extremity	1	7
	x^2= 1.088 p=0.580		
Location	Hand	17	201
	Food	2	14
	Forearm	2	8
	Kruris	2	12
	Others	2	17
	x^2= 2.626 p=0.622		
Type of Injury	Laceration	24	241
	Blunt	1	11
	x^2= 0.007 p=0.932		
Type of laceration (n=265)	Simple	24	231
	Complicated	0	10
	x2=1.035 p=0.309		
Sequelae	None	25	197
	Limited motility	0	13
	Paresthesia	0	17
	Pain	0	15
	Bump/edema	0	7
	Infection	0	1
	Amputation	0	2
	x2=6,808 p=0,339		

Table 5. Injury Sequelae

Sequel	Count (N)	Ratio (%)
None	222	80.1
Limited motility	13	4.7
Paresthesia	17	6.1
Pain	15	5.4
Contusion/edema	7	2.5
Infection	1	0.4
Amputation	2	0.7

anatomic location of the injuries, the nature of the lacerations (simple or complicated), and injury sequelae (p>0.05) (Table5).

DISCUSSION

The Eid sacrifice is a ritual involving family members of all ages. The young injured patients were reported in the study by Avşaroğluları (4) and Ersen (8). In the literature, there are studies reporting mean ages of 32±14, 35±15 and 39 years (4, 6, 9). These reports are similar to our report and generally comprise adult age groups. In line with previous studies, the gender distribution of the cases demonstrated that injured patients were mostly men (3, 4, 6, 7) demonstrating a predominately male population with rates of 85%, 86%, 85.5%, and 84.2%. The male preponderance was 7:1 in the study by Rahman et al. (10). We believe that this was due to the traditional role of adult males in the sacrifice procedure and the role of female members of the family in the meat processing procedure in Muslim traditions.

In our study, we found that only 9% of the injured people were professional butchers (n=25). Other studies in the literature show rates around 3.7%, 3.3%, and 8% (3, 7, 10). This suggests that injuries might be minimized by relegating the animal slaughter to professionals.

In our study, only 4.3% of all cases had blunt traumas (n=12), compared with 16.1% in the study by Baştürk et al. (2). Similar to our results, other studies (3-7) show that most injuries observed had been during the first day of Eid, especially in the morning.We believe this was due to the fact that the sacrifice is most frequently performed early on the first day of Eid. Similar to the study by Avşaroğulları (4), we found that most injuries were observed among the non-experienced family members who had assisted in the animal slaughter. Experienced individuals in sacrifice were more likely to be injured during the animal slaughter (p=0.01). Those inexperienced in sacrifice were mostly injured while assisting others in butchering/processing of the meats (p=0.001). In the study by Bildik et al. (7), 96% of the injured patients had almost no prior experience slaughtering or butchering animals. Avşaroğlu suggested that this likely stems from the prevalent belief that the sacrifice should be performed by a family member (4). We found that 82.7% of the injured people were not professional butchers but did share this belief.

Similar to the outcomes observed in our study, other studies have reported that most injuries were simple lacerations. Simple lacerations comprised 89.2% of injuries in the study by Bildik (7), 78.6% in the study by Baştürk (3), 60.6% in the study by Dizen (6), and more than 50% in the study by Sarıfakioğlu (5). In all these studies, injuries were mostly observed in the upper extremity and the hands (3-7). These outcomes are consistent with thos of our study. In the study by Avşaroğulları, 91% of patients actively used their right hands, but the injuries were observed at equal rates in each hand (4). In the study by Rahman, right hand dominant injuries were observed at a rate of 73.5% (10). In studies comparing both hands, injuries were more common in the left hand (5,6). In our study, 67.5% of the injuries were observed in the non-dominant hand and the most common hand injuries were those in the index fingerfollowed by those on the thumb. In the study by Sicca, the dorsal face of the first finger of the non-dominant hand was most commonly injured (9). In the study of Ersen et al. (8), the most commonly injured finger was the second (33%), followed by the first. In our study, the injuries were most commonly observed in the hand, the index finger and the thumb of both experienced and non-experienced individuals (p=0.019). This is due to the fact that the hands and fingers are exposed to the lacerating tool during active cutting and supportive help.

In our study, only 3.6% of all cases had complicated lacerations involving the tendons, nerves or arteries. In the study by Baştürk et al. (3), 1.8% of the cases had muscle, nerve, tendon or artery injuries. These rates were very high in the study conducted by Dizen et al. (6) in 2009 with rates of injury to tendons of 27%, arteries and nerves of 5.8%, and amputations of 6.6%. In another study, the rate of tendon laceration was 25%, whereas the rate of vessel and nerve injury was 4.2% (7). The complicated injury rates observed in our study were lower compared to those in the afore-mentioned studies. Between 2011 and 2014, the most common artery injured was the radial artery in 195 patients hospitalized in the plastic surgery clinics, whereas distal amputation was observed in 26 patients (8). In our study, one patient had arterial injury, which included the radial artery, and amputation was observed in the distal end of the upper extremity.

In the study by Baştürk et al., 79.6% of the patients were discharged from the EDs with primary repair and wound dressing (3). In other studies, the rates were 82.5% and 92.3% (7, 10). In another study, 52 of 98 patients were discharged from the EU with primary closure (5). In our study, 97.8% of patients were treated in the ED and discharged. Surgical intervention was only necessary in 8 patients (1 by an otolaryngologist, 1 by an orthopedic, and 6 by a plastic surgeon). The rates of patients requiring surgery for injuries including complete tendon lacerations, finger amputations, extremity fractures, or ocular trauma, and hand injuries, were 11.7% and 7.7%, respectively (7, 10). In our study, we performed a control visit in order to determine the rate of sequelae, and the most common sequela was paresthesia. We believe that this was due to the partial damage of the superficial nerves observed in the distal part of the extremities.

A portion of the injuries observed during the sacrifice may be preventable. Non-butchers incurred 91% of injuries in this study, even though a butcher could sacrifice 40-50 animals in a day while a non-butcher could sacrifice 1-2 animals. Precautions should be taken in the EDs, especially on the first day of the Bayram holiday, in anticipation of an increased number of patients with laceration injuries involving the upper extremities and sometimes blunt trauma in localities where sheep sacrifice is prevalent. The number of professional sites for sacrifice should be increased, especially by the local managements, in order to provide proper service to the public.

CONCLUSION

The first day of the Bayram (Eid Al-Adha) holiday is associated with an increase in non-dominant upper extremity injuries among most inexperienced people slaughtering sheep. The rit-

ual slaughter of animals, usually sheep, is a central part of the Bayram (Eid Al-Adha) religious holiday for Muslims. This slaughter is sometimes performed by novices instead of professional butchers. Compared to the following 3 days of the holiday, we found an increase in the rates of injury during the first day of Bayram, particularly in the morning hours when the animal sacrifice is often performed. Injury patterns noted during the first day of the Bayram holiday usually consisted of simple lacerations often involving the upper extremity, specifically the non-dominant hand and the first finger. While government measures have been taken to reduce the chance of injury from the animal slaughter, further education and safety precautions may help reduce the number of injuries. EDs serving communities with larger Muslim populations where nonprofessionals engage in sheep slaughtering may benefit from anticipating an uptick in such injuries.

Limitations of Our Study

Although the EDs where our study was conducted likely captured many of the injuries experienced in Gaziantep, Turkey, the results may not be generalizable to the rest of Turkey or the world. Patients may have been injured but not received care in the ED, potentially leading to an underestimation of the actual rate of injury. Sixteen percent of patients were lost to follow up and therefore excluded. Although 19% of people 25-64 years old in Turkey attained at least an upper secondary education level, 68% of patients in this study had a primary school education level, which likely reflects a disproportionate amount of rural people being included in this study (11).

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Hasan Kalyoncu University (Date: 06/06/2018; No:2018-05).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Original Research

Comparison of Therapeutic Effectiveness between Kinesio Taping Technique and Static Resting Splint in Carpal Tunnel Syndrome

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ABSTRACT

Objective: We aimed to compare the kinesio taping (KT) technique and static wrist resting splint therapy in terms of clinical symptoms, hand grip strengt and daily living activities in patients with carpal tunnel syndrome.

Methods: In this study, 25 of 42 patients with mild/moderate carpal tunnel syndrome received KT for 4 weeks, while the remaining 17 patients used a static wrist resting splint for the same period. For all the patients, details on age, sex, occupation, and degree of carpal tunnel syndrome were recorded. Tinel's test, Phalen's test, hand grip strength, and visual analogue scale (VAS) score for pain during the day and night were assessed before the treatment and in the first and third months after the treatment. The patients also completed a QuickDASH questionnaire.

Results: No significant differences were found between the groups in terms of age, sex, and degree of carpal tunnel syndrome. In both groups, the VAS-day, VAS-night, and QuickDASH scores significantly decreased, while hand grip strength significantly improved after the treatment as compared with their pretreatment values. These effects were maintained for 3 months ($p<0.005$). The decrease in VAS-night score in month 1 and the improvement in hand grip strength in months 1 and 3 were statistically significant in the group who received KT, while the decrease in QuickDASH score was statistically significant in the group who received static wrist resting splint therapy ($p<0.005$).

Conclusion: This study shows that treatment with KT and static wrist resting splint therapy improved the symptoms, daily living activities, and hand grip strength of the patients with mild/moderate carpal tunnel syndrome. In conclusion, we think that KT should be kept in mind as an alternative to conservative therapies in the treatment of carpal tunnel syndrome.

Keywords: Carpal tunnel syndrome, kinesio taping, splint

INTRODUCTION

Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy and occurs when the median nerve is compressed in the wrist (1). The entrapment neuropathy may cause a decrease in hand grip strength and loss of hand function in addition to complaints such as paresthesia, pain, and stiffness that are more frequent especially at night (2).

The disease is diagnosed on the basis of present symptoms, provocative test results (Tinel's sign and Phalen's test), and nerve conduction studies, and treated either conservatively or surgically. A literature review revealed that single or combined conservative treatment methods are used in the treatment of mild/moderate CTS. Statistic wrist resting splint, corticosteroid infection, nonsteroidal anti-inflammatory drugs, physiotherapy agents, activity modification, and tendon/nerve glide exercises are among the treatment methods (3, 4).

Recently, a limited number of studies have used the kinesio taping (KT) technique for various musculoskeletal system diseases in CTS. The use of the KT technique has increased in the treatment of several diseases such as shoulder pain (5), patellofemoral pain syndrome (6), subacromial impingement syndrome (7), plantar fasciitis (8), and spasticity (9). This prominent effectiveness of KT can be explained by the fact that it has some advantages different from other conventional techniques (10). Regulating the weak muscle function, subcutaneous edema, and impaired circulation by stimulating the lymphatic and blood circulation systems; facilitating the movement of the fascia and tendons; and reducing pain by alleviating the abnormal muscle

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tension, repositioning the subluxated joints, and increasing proprioception through cutaneous mechanoreceptors are among these effects (11-14).

The use of a static wrist resting splint in a neutral position is the first step in CTS therapy in clinical practice. The aim of splinting the wrist in a neutral position is to increase the volume of the carpal tunnel and reduce the pressure on the median nerve. Studies revealed that the use of a wrist resting splint in CTS improved symptoms and functions (15, 16). However, only few studies have reported the effect of KT on CTS (17).

This study was aimed at comparing the KT technique and static wrist resting splint therapy in mild/moderate idiopathic CTS in terms of their effectiveness for improving symptoms, hand function, and grip strength.

METHODS

A total of 42 patients (50 hands) who were admitted to the physiotherapy outpatient clinic of the Kayseri Training and Research Hospital, who underwent electroneuromyography, who were diagnosed with mild/moderate CTS, and whose informed written and verbal consents for participation in the study were obtained, were retrospectively included in the study. While patients aged >18 years, who did not receive any treatment; and who were diagnosed as having mild/moderate CTS were included in the study; those who had a metabolic disease (diabetes mellitus or thyroid diseases), systemic or malign diseases, trauma history in the etiology (fracture), and CTS therapy before (corticosteroid injection, splint, and taping therapies); pregnant and breastfeeding women; patients aged <18 years; and patients with thenar atrophy (to exclude severe CTS) were excluded from the study. The first group consisted of 25 patients (25 hands) who received KT, and the second group consisted of 17 patients (25 hands) who used a static wrist resting splint. Eight of the 42 patients were diagnosed as having bilateral CTS, and these patients received the same treatment for both hands. KT was applied four times and renewed each week, and the tape was applied using a neural technique and ligament/space correction technique recommended for CTS (18). First, the skin was cleaned with alcohol-soaked cotton to remove oil and moisture. A Y tape was applied along the line of the nerve for the neural technique, and an I tape was applied around the wrist for the ligament/space correction technique. The Y tape was applied starting from the first and fifth metacarpal joints up to the area 5 cm below the medial epicondyle along the line of the nerve, with 50% stretching, as the wrist was the transition area when the forearm was in supination and the elbow and wrist were in extension. The I tape was applied to the volar side of the wrist with maximum stretching when the wrist was in the neutral position (Fig. 1). The patient was informed to avoid activities that may cause excessive sweating and exposure to water during the process. The splint given to the patients in the splint group had a neutral position and volar support, as it did not allow flexion, extension, and deviation of the wrist but allowed pronation and supination. The patients were recommended to use it for 4 weeks (19) (Fig. 2). All the patients were given a home program consisting of tendon and nerve-shifting exercises.

Main Points:

- We have found statistically significant difference between KT and splint groups.
- KT significantly increase hand grip strength was observed in the first and third months as compared splint group.
- KT significantly decrease QuickDASH score was found in the first and third months as compared with before the treatment.

Figure 1. VAS-Day Score

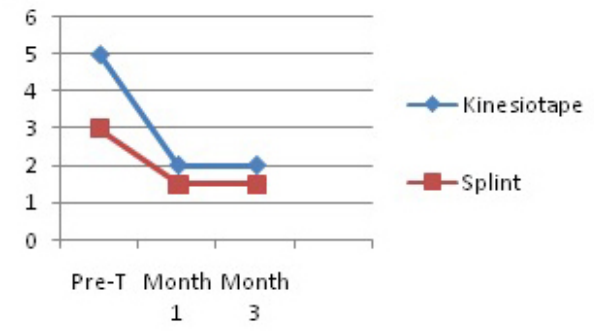
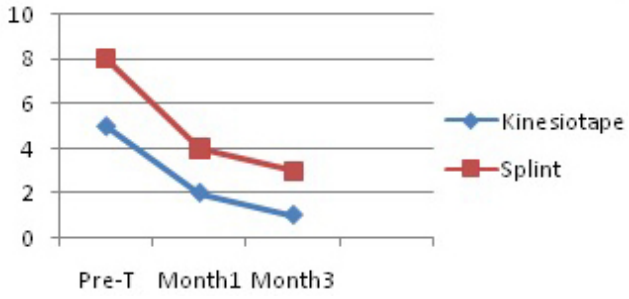


Figure 2. VAS-Night Score



All the patients' demographic characteristics, occupations, injured hand, and dominant hand were recorded at the beginning of the study. Tinel's and Phalen's test scores, hand grip strength, and visual analogue scale (VAS) scores of the patients were evaluated during the day and night before the treatment (pretreatment) and in the first and third months after treatment. The QuickDASH questionnaire was also administered to the patients. The VAS was scored between 0 (no pain) and 10 (worst possible pain) to determine the degree of pain. Hand grip strength was evaluated in kilograms by using the Jamar-type hydraulic hand dynamometer (Saehan Corp. Masan, Korea). All the measurements of the patients were performed in a sitting position, with the shoulder in adduction and neutral position at 90° elbow flexion, the forearm in neutral position, and the wrist at 0–10° of extension and 15° of ulnar deviation. The patients were asked to perform a maximal voluntary grip. The measurements were performed three times, and the mean value was calculated (20).

The QuickDASH (The Quick Disabilities of Arm, Shoulder and Hand Questionnaire) questionnaire consists of 15 questions in total and was validated in Turkish and found to be reliable for evaluating the severity of symptoms and functional capacity of patients (21). The patients in this study completed the questionnaire by themselves. The QuickDASH has 11 titles (each title includes five answer options), and at least 10 of the 11 titles must be answered to calculate the QuickDASH score (0=no disability and 100=the most severe disability). Approval of this issue was obtained from the ethics committee of the Ministry of Health Kayseri City Hospital on March 12, 2020 (Document No. 14).

Statistical Analysis

The data obtained were evaluated with the Statistical Package for Social Sciences 22.0 (IBM SPSS Corp.; Armonk, NY, USA) statistical package software. Descriptive statistics were presented as mean±standard deviation, median (range), frequency distribution, and percentile. For the statistical methods, the chi-square test was used for nominal values, and the Wilcoxon rank-sum test, Kruskal–Wallis analysis of variance, and Mann–Whitney U test were used for non-parametric values. Variables with p values of <0.05 were considered statistically significant.

RESULTS

The mean age of the patients who participated in the study was 47.48±8.13 years in the first group and 45.56±6.55 years in the second group, indicating similar ages between the groups (p=0.354). No statistically significant differences were found between the groups in terms of sex, treated extremity, and degree of CTS. The demographic characteristics of the patients are given in Table 1.

The mean VAS-night score in the first group was 5.0 before the treatment and 2.0 after the treatment. A statistically significant decline in VAS-night score was recorded after the treatment, and this effect was observed to continue for 3 months. While the mean VAS-night score in the second group was 8 before the treatment, it decreased to 4 after the treatment, and this effect was observed to continue for 3 months. The decrease in VAS-night score within 1 month was statistically more significant in the first group. A statistically significant decrease in VAS-day score was recorded in both groups in the first and third months as compared with before the treatment, and no significant difference was found between the groups (Figs. 1 and 2).

A statistically significant increase in hand grip strength was observed in the first and third months as compared with before the treatment in both the groups. A statistically significant greater increase in hand grip strength was recorded in evaluations for the first group as compared with the second group during the first and third months (p<0.05; Table 2).

A statistically significant decrease in QuickDASH score was found in the first and third months as compared with before the treatment in both groups, and the decrease in the third month was statistically more significant in the second group than in the first group (p<0.05; Table 3).

Table 1. Distribution according to the Demographic Characteristics

	Kinesiotape Group (n=25)	Splint Group (n=17)	p*
Age (Mean.±SD)	47.4 ± 8.1	45.6 ± 6.7	0.354
	Number(%)	**Number(%)**	
Gender			
Female	23 (92)	16 (94.11)	0.853
Male	2 (8)	1 (5.8)	
Occupation			
Housewife	21 (84)	14 (82.3)	0.518
Employee	3(12)	3 (17.7)	
Retired	1(4)	0 (0.0)	
Degree			
Mild	15(60)	14(56)	0.554
Moderate	10(40)	11(44)	

Table 2. Hand grip value

		Kinesiotape	Splint	p *
Hand Grip	Pre–Treatment	19 (18.08–23.63)	15 (12.75–18)	**0.003**
	Month 1	23 (19.39–28.44)	16 (12–21.5)	**0.001**
	Month 3	25 (20.4–29.09)	19 (15.5–22.5)	**0.004**
p*		**p<0.05**	**p<0.05**	

Table 3. QuickDASH Value

		Kinesiotape	Splint	p *
QuickDASH	Pre–Treatment	50 (40.9–62.5)	45 (34.6–55)	0.256
	Month 1	38 (18.2–55.6)	29.5 (17–39.3)	0.145
	Month 3	34 (19.3–43.2)	17.5 (7.5–27.5)	**0.008**
p *		**p<0.001**	**p<0.001**	

A statistically significant increase was observed in negative results in Tinel's and Phalen's tests in the first and third months compared with the pretreatment results in both groups and the decrease was statistically more significant in the third month in the second group than in the first group (p<0.05; Tables 4 and 5).

Table 4. Tinel's Test Results

		Kinesiotape	Splint	p
Tinel's	Pre–Treatment	60%	56%	0.177
	Month 1	40%	25%	0.089
	Month 3	35%	20%	**0.004**
p *		**p<0.001**	**p<0.001**	

Table 5. Phalen's Test Results

		Kinesiotape	Splint	p *
Phalen's	Pre–Treatment	72%	80%	0.508
	Month 1	60%	56%	0.382
	Month 3	45%	28%	0.011
p *		**p< 0.001**	**p<0.001**	

DISCUSSION

CTS is an entrapment neuropathy characterized by the loss of hand function accompanied by clinical symptoms such as pain and paresthesia. Our study was aimed at determining the effects of using KT and a splint device on the severity of the symptoms and functional conditions of patients with CTS.

In the study by Kulcu et al., the 22 wrists in the 45 patients included in the study (65 wrists) received KT, 22 received placebo KT, and 21 received splint therapy, and the effectiveness of the therapies were compared. The Boston Carpal Tunnel Questionnaire (BCTQ) was used to evaluate the functional condition in their study. While functional improvement was observed in the KT group, no significant improvement was found in the other two groups (17). In the study by Yildirim et al. (22), an improvement in the BCTQ-S score was observed in the KT group during the third follow-up. In our study, we used the QuickDASH questionnaire, which was developed to evaluate the functional condition. As a result of our study, while significant improvements were found in the first and third months in the KT and splint therapy groups, the improvement was more significant at the end of the third month in the splint group. In the same study, a statistically significant improvement in hand grip strength was found in the splint group; however, no significant difference was found among all the groups (22). In our study, although significant clinical improvements in symptoms, hand function, and grip strength were observed within the first 3 months after treatment in the patients who received KT and splint therapy, the improvements of the symptoms and grip strength in the KT group and hand function in the splint group were more significant.

KT helps improve blood circulation and lymphatic drainage. This effect on the blood circulation helps eliminate the tension and tenderness in the damaged area, and decreases the stimulation of the subcutaneous pain receptors. With these features, KT showed positive effects in the treatment of CTS (23). In our study, an increase in both VAS-day and VAS-night scores were found in the KT group. The study by Kaplan et al. (24) showed a decrease in VAS score in the KT treatment group.

Epidemiological studies on CTS showed that it was more common in the middle-aged female population. In our study, 92% of the patients were female, and their mean age was approximately 47 years; these results were compliant with the epidemiological data (25). Several studies have reported that CTS is an occupational disease that is more common in occupations that require intensive use of the hands (26). In our study, 98.1% of the patients had occupations (housewife, employee, etc.) that require intensive use of the hands and frequent repeated movements.

CTS is diagnosed by performing medical history taking and clinical examination, and its severity is determined with electrophysiological testing. Whereas physical examination has reduced sensitivity and abnormal responses to provocative tests and/or symptoms due to reduced muscle power are detected in patients with CTS, provocative test results are not always positive but are useful in the diagnosis. The sensitivity and specificity of these tests in the diagnosis of CTS are still disputed (27). According to Brüske et al. (28), the sensitivity of Phalen's test was between 42% and 91%, and the sensitivity of Tinel's test was between 38% and 100%. In our study, we used Tinel's and Phalen's provocative tests in physical examinations for patients with symptoms of CTS. In a meta-analysis in which MacDermid et al. evaluated 60 studies, the sensitivity was 68% for Phalen's test and 50% for Tinel's test. In our study, the Phalen's test result was positive in 76% of the patients, and the Tinel's test result was positive in 58%, which is similar to the results of MacDermid et al. (29). In the study by Akturk et al. (30), a decrease in the sensitivity of provocative tests was observed, which is similar to the results of our study.

In the study by Oncu et al. (31) on the effectiveness of KT for CTS, the patients were divided into four groups, and each group consisted of 15 wrists. The first group received KT and exercise therapy; the second group, splint and exercise therapy; the third group, KT, splint, and exercise therapy (KT + Splint); and the fourth group (control group), only exercise therapy. They reported that the use of KT with a night splint showed a significant clinical improvement in symptoms, hand function, grip strength, and hand skill test results within the first 3 months after treatment as compared with the other treatments. The improvement in symptoms in the group that received only KT or splint therapy was significant only in the first 2 months after treatment as compared with that in the control group. In the study by Akturk et al. (30) in which KT and splinting methods were compared in 44 patients (38 female and 6 male patients: 58 hands) with early CTS between the ages of 20 and 65 years, the KT group showed a significant improvement in motor distal latency, sensory latency, sensory conduction velocity, responses to provocative tests, sensory loss, hand function, and post-treatment symptoms, and statistically significant differences in BCTQ-functional disabilities and BCTQ-S scores. In our study, the symptoms improved in both groups, and this effect continued for 3 months in the KT group.

The use of a wrist splint in the treatment of CTS is based on the observation that symptoms increase with wrist movements and

decrease with resting. Studies have shown that the therapeutic role of the wrist resting splint is due to its decompression effect on the carpal tunnel. Pressure on the tunnel plays an important role in the pathophysiology, and as the wrist moves away from the neutral position, the pressure increases (32, 33). In our study, a significant clinical improvement was observed in the splint group in terms of symptoms, hand function, and grip strength. A meta-analysis on the effect of KT on grip strength reported that KT had positive effects on both grip strength and muscle activity (34). In a study in which the effect of KT on grip strength was evaluated in healthy athletes, KT was used for the forearm in 21 healthy athletes, and no long-term increase was observed in the maximal grip strength, while proprioception and hand grip strength improved in the early period. Oncu et al. (31) concluded in their study that KT improved grip strength only in the early period when used alone but improved grip strength over the long term when used with a splint. Guner et al. (35) found that the KT method used with a low-level laser therapy had no additional positive effect in the short term; however, they found an increase in hand grip strength and finger pinch strength in the long term. In our study, hand grip strength increased in both the splint and KT groups, and this increase was higher in the KT group.

As a result of these studies and our study, KT does not prevent daily activities as compared with the splint, and patient compliance with the KT method is high. The use of splints may limit daily activities and housework. According to the observations, KT is easier to use in clinical practice.

Limitations

The limitations of this study are its retrospective design, the fact that the long-term effectiveness of KT in the treatment of CTS was not investigated, and the limited number of patients included in the study. A further limitation is the lack of ultrasonographic images during follow-up.

CONCLUSION

We conclude that KT may be considered as an alternative method to the available conservative therapies for the treatment of CTS. The positive results that were obtained in the intervention group might have resulted from a placebo effect of KT. We think that more comprehensive retrospective placebo-controlled studies investigating the long-term effectiveness of KT are needed.

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Informed Consent: Written consent was obtained from the patients for the application.

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Original Research

Features of Childhood Colorectal Carcinomas and Frequency of K-ras Mutations

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ABSTRACT

Objective: Colorectal carcinoma (CRC) is extremely rare in childhood and has a poor prognosis in young patients. The tumorigenesis of CRC in children and adolescents is still unclear and probably evolves through different stages. There are not enough studies about the rarity of K-ras mutations with childhood CRC. This study aimed to investigate the features and outcomes of childhood CRC as well as examine the frequency of K-ras mutations in CRC among children and adolescents.

Methods: The clinical and pathologic features, prognostic factors, and outcomes of CRC in 28 children and adolescents (ages 10 to 17 years) referred to the Pediatric Oncology Department of Hacettepe University Children's Hospital between 1974 and 2010 were reviewed for this study. Paraffin-embedded tissues of 18 patients were available and these tissues were analyzed by using the "pyrosequencing" method to detect K-ras mutations.

Results: The median age of patients was 14 years and the male/female ratio was 2.5/1. At presentation, the most common symptoms were abdominal pain (57%) and weight loss (43%). The time between symptoms and diagnosis was 4 months. The most common sites of involvement were the rectum (43%) and sigmoid colon (25%). Mucinous adenocarcinoma was the most common histiotype (71%). At presentation, 89% of patients had metastatic disease, especially to the peritoneal surface (39%). Overall survival rates at 3 and 5 years were 10%. Distant stage (p=0.045), incomplete resection, and macroscopic tumor (p=0.000) were poor prognostic outcomes. A K-ras mutation was identified in three of the 18 patients (17%). The most common mutation of the patients was GGT→GAT at codon 12.

Conclusion: Childhood colorectal carcinomas occur in a shorter time than in adults, with different histiotypes and more likely different steps. It seems that K-ras mutation plays a role in this different biology of pediatric CRC. However, further studies are essential to investigate and understand the biology of childhood CRC.

Keywords: Childhood colorectal carcinoma, K-ras mutation

INTRODUCTION

Colorectal carcinoma (CRC) is extremely rare in the pediatric age. It accounts for less than 1% of all cancer cases in children younger than 20 years. The incidence is approximately one case per million in this age group (1, 2). In addition, a recent study by Ferrari et al. (3) has shown that the incidence of epithelial tumor of the colon is 0.3/100.000. Although CRC has a good prognosis in adults when diagnosed early and treated by multidisciplinary approach, it has a poor prognosis in children because of the rarity of the tumor and its high potential for dissemination (4). Further, the pathobiology of pediatric and adult CRC may differ (5). The biology of CRC in adults is well known. In contrast, the tumorigenesis of childhood CRC, which necessarily occurs over a shorter period, is still unclear and most likely evolves through different stages (6).

K-ras is a proto-oncogene located on chromosome 12p12.1, encodes the plasma membrane-bound Guanosine Triphosphate (GTP) binding protein that is a key regulatory component of numerous signal transduction pathways, and is activated by point mutations that occur at the critical hot-spot coding sequences (7, 8). Point mutations in codons 12,13, and 61 in the K-ras gene result in amino acid alterations in the $p12^{(ras)}$ protein and activation of the oncogenic potential (9). However the biology of childhood carcinoma is unclear and the role of K-ras mutations is not known very well in CRC of children and adolescents. The current study aimed to investigate features and outcomes of CRC as well as examine the frequency of K-ras mutations in colorectal among children and adolescents.

METHODS

Patients and Clinical Data

Twenty-eight children and adolescents (aged 10–17 years) who had CRC diagnosed and referred to the Pediatric Oncology Department of Hacettepe University Children's Hospital between

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Table 1. Demographic characteristics of the 28 patients with colorectal carcinoma

		n	%
Sex	Female	8	26.8
	Male	20	71.4
Age	Median=14 (10–17)		
Time between symptoms and diagnosis	Median=4.1 (2.0–6.2)		
Cancer History of relatives		6	21.4
Anemia at diagnosis		17	60.7
Most common symptoms	Abdominal pain	16	57.1
	Weight loss	12	42.8
Location	Rectosigmoid	19	67.8
	other sites	9	32.2
Stage (Modified Dukes*)	A	–	0
	B	3	10.7
	C	18	64.3
	D	7	25
Histology	Mucinous Adenocarcinoma	20	71.4
	Signet– ring cell Adenocarcinoma	4	14.2
	Adenocarcinoma	4	14.2
Metastatic disease at diagnosis		25	89.2
Most common metastatic site	Peritoneal surface	11	39.3
Chemotherapy		26	92.8
Radiotherapy		6	21.4
Overall survival of 3 and 5 years		3	10.7

*Modified Dukes' Classification of Colorectal Carcinoma
A: Lesion confined to the bowel wall
B: Direct extension to serosal fat without lymph node involvement
C: Lymph node involvement
D: Distant metastases (may include extranodal intra–abdominal tumor, lung, brain, bones, etc.)

1974 and 2010 were retrospectively reviewed for this study. Patient data were reviewed for age, sex, presenting symptoms, other chronic medical diseases, second malignancy, familial cancer history, consanguinity, diagnostic procedures, clinical characteristics, hemoglobin levels, body mass indexes, histological type, stage of disease according to the Modified Dukes Staging (10), treatment methods, the interval between CRC diagnosis and recurrence or progression, prognostic factors, frequency of K-ras mutation, and mutation analyses. This study was approved by the institutional review board of the Hacettepe University Faculty of Medicine, and written informed consent was obtained from the patients.

Main Points:

- Colorectal carcinoma (CRC) is extremely rare in childhood and has a poor prognosis in young patients.
- CRCs occurs in a shorter time than in adults, with a different histology and more likely with different stages.
- It seems that K-ras mutations play a role in the different biology of pediatric CRC.

Tumor Tissue Preparation and K-ras Sequencing

Paraffin-embedded tissues of 18 patients were available. Mutations on 12th, 13th, and 61st codons of the K-ras gene were analyzed in colorectal carcinoma sample tissues by using the 'pyrosequencing' method. Study was composed of two analyses, which were performed using the PyroMark K-ras kit. The mutations were searched on the 12th and 13th codons in the first analyses and on the 61st codon in the second analyses. "QIAamp DNA

FFPE Tissue Kit" was used for DNA isolation from the paraffin-embedded tissues, obtained from 10 micron thickness samples that represent the tumor. DNA quantity was 10–20 ng/µl in a sample. Then, K-ras polymerase chain reaction (PCR) protocol was applied. Amplification was done using the "Thermal Cycler 9700" device. K-ras studies were done using the "PyroMark Q24 MDx" device through the "pyrosequencing" method (11, 12).

Statistical Analysis

All data were analyzed using the SPSS 17.0 (SPSS Inc.; Chicago, IL, USA) for Windows package program. Continuous variables that are normally distributed were expressed as mean ± standard deviation, and those that were not normally distributed as median (min-max). Categorical data were expressed as percentages. Normal distribution of continuous data was determined by a histogram and the "Kolmogorov Smirnov Test." The significance of the difference between the normally distributed data was analyzed using the "One Sample t-test"; the significance of the difference between the data that were not normally distributed was analyzed using the "Mann Whitney U-test." The difference between pathologic types was determined by "Kruskal–Wallis Test." Estimation of the duration of survival was done by the "Kaplan–Meier" method. "Log Rank Test" was used in determining the difference of survival duration between groups. The p values less than 0.05 was accepted as significant.

RESULTS

Of the 28 patients, 8(28.6%) were females and 20(71.4%) were males. The male/female ratio was 2.5/1. The median age of patients was 14 years at diagnosis (range 10 to 17 years). The other features are shown in Table 1. All patients had more than one symptom at presentation. The time between symptoms and diagnosis was 4.1 months (range: 2–6.2 months). The predominant symptoms were abdominal pain (n=16,57.1%) and the second was weight loss (n=12,42.8%), followed by abdominal distention (n=9), vomiting (n=9), constipation (n=8), loss-of-appetite (n=7), weakness (n=6), diarrhea (n=5), hematochezia (n=5), melena (n=3), fever (n=3), intestinal obstruction (n=2), and dysuria (n=2).

Five patients had a relevant medical history, including Bloom's syndrome (n=1), chronic glomerulonephritis (n=1) and guatr, hamartomatosis polyposis coli and hypertrophic osteoarthropathy (n=1), non-familial polyposis coli (n=1), and nephrolithiasis (n=1). Regarding familial cancer history, patients had no family cancer history (n=22), family members had a history of colon cancer (n=2) and non-colonic cancer (n=2), some patients has an undetailed cancer history (n=1), and one patient's family cancer history was unclear. In addition, patients had no secondary malignancy. Further, the degree of consanguinity in the parents was evaluated. Eighteen parents of patients had no consanguinity, followed by first-degree relatives (n=7), second degree relatives (n=2), and unknown degree relatives (n=1). Seventeen (60.7%) of 28 patients with hemoglobin data were anemic at presentation, with hemoglobin values of less than 8 g/dL (5.6–7.9 g/dL) (n=2), 9.6 g/dL (n=1), and between 10.1 g/dL and the lower limit of the normal value for age and sex (n=14). The documentation of the fecal occult test results was poor.

Body mass indexes (BMI) were calculated from the charts of 18 patients at diagnosis. The BMI of 16 patients was less than 20, one patient between 20 and 25, and one patient between 25 and 30. Five of the patients with a BMI of less than 20 (31.2 %) had a secondary disease but the other 11 (69%) did not have. Of the 28 patients who had a pathologic diagnosis, the charts of 25 patients reported that the diagnostic evaluation was performed with more than one procedure. The main initial procedure was barium enema (n=13), endoscopy (n=10), abdominal computed tomography (n=9), abdominal ultrasonography (USG) (n=13), and exploratory laparatomy (n=6).

The primary site of the tumor was the rectum in 12 patients, sigmoid colon in 7, descending colon in 2, splenic flexura in 1, transverse colon in 4, hepatic flexura in 1, and cecum in 2 patients. The ascending colon location did not exist. Histopathological findings included mucinous adenocarcinoma in 20 (71.4%), single-ring cell carcinoma in 4 (14.2%), and adenocarcinoma in 4 (14.2%) patients. The other pathologic types did not exist. According to the colon localization, the most common histopathologic type was the mucinous adenocarcinoma in the recto sigmoid and other sites.

Of the 28 patient, only 3 (10.7%) had a localized disease, while the others (n=25, 89.2%) had a metastatic disease. The extent of disease was determined using the Modified Dukes Staging. Although stage A did not exist, stage B was observed in 3 patients (10.7%), stage C in 18 (64.3%), and stage D in 7 (25%). The most common site of metastatic disease was the peritoneal surface (n=11, 39.3%), followed by the close lymph nodes (n=10, 35.7%), distant lymph nodes (n=2, 7.1%), omentum (n=6, 21.4%), mesenterium (n = 7, 25%), lung (n=2, 7.1%), liver (n=5, 17.9), kidney (n=1, 3.6%), bladder (n=1, 3.6%), and stomach (n=1, 3.6%).

Surgical procedures for diagnosis or treatment were biopsy (n=9), colon resection (n=20), colostomy (n=19), expiratory laparotomy (n=7), and anastomosis (n=3). Complete resection (R0) was not preferred for any of the patients. Seventeen patients (60.7%) had incomplete resection and microscopic tumor (R1) and 11 (39.3%) had incomplete resection and macroscopic tumor.

The other treatment procedures were chemotherapy and radiotherapy. Of 28 patients, 26 received chemotherapy, while the other two patients after diagnosis went to another medical center for treatment. The treatment and radiotherapy information were incomplete. However, 13 (46%) patients were diagnosed on or before the year 1990. The treatment choices were as follows: 5-FU, lomustine (CCNU), dacarbazine (DTIC), adriamycin, and mitomycin C. On the other hand, 15 (53%) patients were diagnosed after 1990, and received chemotherapy consisting of 5-FU, levamizole, adriamycin, mitomycin C, irinotecan, bevacizumab, oxaplatin, and interferon. Only six patients (21%) received radiotherapy. There were three known long-term survivors in our study, who received a treatment consisting of 5-FU, lomustine, irinotecan, and oxaplatin, and are still alive.

Paraffin-embedded tissues of 18 patients were available and these tissues were analyzed using the 'pyrosequencing' method for detecting K-ras mutations. K-ras mutation was identified in three of the 18 patients (16.6%). The most common mutation of the pa-

tients was GGT→GAT at codon 12. The patients with K-ras mutations were 13, 16, and 10 years old and the male/female ratio was 2/1. The most common location was sigmoid and the most common histiotype was mucinous adenocarcinoma. Stages were C, B, and D (Modified Dukes), respectively. No one had other illnesses. Survival times were 25, 14.5, and 10 months, respectively.

The twenty-eight patients were evaluated for the survival analysis. Event-free survival (EFS) was evaluated only in 23 of 28 patients as 5 patients did not receive all the treatments in our medical center. Overall survival and EFS rates at 3 and 5 years were 10% and 17%, respectively. Distant stage (p=0.045), incomplete resection, and macroscopic tumor (p=0.000) were poor prognostic outcomes.

DISCUSSION

CRC is primarily a disease of adulthood, and is very rare in children and adolescents (13). There is limited data on childhood CRC in the literature (14). Young colorectal cancer patients have a distinct group of malignancies with various clinical and pathobiological features (6, 5). To our knowledge, the current study is one of the rare CRC case series in children and adolescents, which demonstrates the relationship K-ras mutations and childhood CRC (15).

As was previously noted, the male predominance in childhood CRC is in contrast to the situation for adult patients. Sex differences in adult CRC have been associated with biological, behavioral, and environmental factors; however, the data on gender differences in childhood CRC are limited (16). In a study of 11,071 primary CRC cases diagnosed at the ages of 15–39, Teng et al. found that male gender was associated with an increased risk of death (17). People in their 30s or 40s with positive family histories exhibited a higher relative risk compared to their age-matched peers, as well as older people with the same positive family histories (18-20). The evidence for an elevated risk of bowel cancer in children and adolescents with a family history of the disease is unclear (21). The predisposing genetic diseases, polyposis syndromes, and inflammatory bowel diseases associated with pediatric CRC include familial adenomatous polyposis, Lynch syndrome (hereditary non-polyposis colorectal cancer [HNPCC]), and ulcerative colitis (22, 14). In some series, 10%–30% of reported childhood CRCs had predisposing factors (23, 24, 5); however, most childhood cases occur without known predisposing syndromes (21, 22).

The presenting features of pediatric and adult CRC such as the interval between symptoms and diagnosis, pathological findings, stage, and prognosis are similar, even as observed in this study. The symptoms of childhood CRC can vary from abdominal pain, nausea and vomiting, abdominal distention, changes in bowel habits, and weight loss to bloody stool or rectal bleeding. Patients usually have more than one symptom (25). In accordance with the findings of previous studies, abdominal pain was the most predominant presenting symptom (26). Compared to adults, CRC rarely occurs in children; thus, there is a low index of suspicion. The limited experience with this disease in children often results in delayed diagnosis (27, 14).

A review of the literature suggests that the duration of the symptoms of childhood CRC varies widely, and the median interval is short. Acute presentation is more frequent in young patients (28). The duration of symptoms in our series was 15 days–12 months, with an average of 1.5 months despite the presence of advanced disease in most of the cases. The short duration could confirm the aggressiveness of the tumor biology and the rapid tumor growth besides a delayed diagnosis or both of them (29, 24, 30). Similar to the findings of previous studies, the most common stage in accordance with the modified Duke criteria was stage D, followed by stage C, and the most common sites of metastatic disease in our study were the lymph nodes, peritoneal surfaces, and liver (31). Children and adolescents contributed to the advanced disease at presentation (29, 22, 28, 24). Important CRC studies in which childhood was defined as ages lower than 21 have demonstrated that left colon cancers are more predominant. The differences in presentation, histology, undifferentiation, and outcome are likely to reflect the distinctive features in the tumor biology of pediatric and adult CRCs. This suggests that the disease progresses through different stages in these populations (5, 24). CRC in adults is usually linked to preexisting adenomas. It develops over approximately 10 years through a well-known multistep process. In contrast, premalignant adenomas are rarely seen in relation to the presence of sporadic CRC in children, and the tumorigenesis of childhood CRC occurs over a shorter period (21, 26, 24). The incidence of mucinous adenocarcinoma is higher in children and adolescents (44%–91.7%) in comparison to adults (5%–15%) (32, 29). In the largest cohort of pediatric CRC, the signet-ring cell carcinoma accounted for 15.4% of the CRCs in patients under 21 years old; the incidence for adults was 0.01%–2.6% (33, 22). Mucinous CRC has distinctive clinical and molecular features from non-mucinous CRC (34). In a study of the molecular features of early-age onset (age ≤ 30) CRC, Khan et al. found that microsatellite instability (MSI) was more prevalent in early-onset CRC. Unlike CRC in adults, CRC in children is not tightly linked to MLH1/PMS2 loss, and it has never been associated with BRAFV600E mutations. The MSS/BRAFV600E genotype had a poor prognosis and was more prevalent in early-age CRC (9% vs. 3%) (15).

The K-ras gene is an oncogene, the natural form of which is a proto-oncogene or wild-type oncogene, which is involved in the regulation of cellular responses (35, 36). The K-ras protein is responsible for the transduction of mitogenic signals from the epidermal growth factor receptor (EGFR) on the cell surface to the cell nucleus (37). This short gene sequence is susceptible to point mutations, and GTP activity is lost with mutation (37). K-ras mutations occur during the progression and metastasis of CRCs. In addition, it might be present in other cancers, such as lung adenocarcinomas and pancreatic ductal adenocarcinoma (38-40). K-ras mutations have been found in 30%–50% of colorectal CRCs in adults, and the most frequent point mutation has been found in codons 12, 13, and 61 (35, 41). K-ras mutations have a strong effect on the growth of colonic polyps and early cancers (42); however, the K-ras mutation is not sufficient alone to influence the progression malignant transformation. The associations of the K-ras mutations with other mutations, such as APC, are also important (38, 35). The K-ras mutation is

used in CRC as a useful marker for predicting tumor responsiveness to anti-EGFR antibody therapy, as its key role in EGFR signaling (36, 43). It is also a negative predictor of the response to anti-EGFR therapy (41). The value of the K-ras mutation as a prognostic factor after CRC diagnosis in adults is controversial. It would appear that the K-ras mutation is a useful marker for CRC treatment rather than a prognostic factor (36). The data on K-ras mutations in early-onset CRC are heterogeneous and limited. Previous studies have reported that K-ras gene mutations occur at rates of 4%–27% in early-onset CRC (44-47), as well as a significantly higher proportion (54%), with mean age over 30 years, as reported by Watson et al. (48). Khan et al. reported the rate of K-ras mutations in individuals diagnosed with early age (≤ 30 years) onset CRC as 28% (15). The mean age was not under 18 years in these previous studies; consequently, these results might not reflect the rates of K-ras mutations in childhood CRC. The treatment options for childhood CRC are similar to those for adults (21).

Modified adult CRC protocols are utilized initially because of a lack of standardized protocols for CRC treatment of children (22). Surgery is the mainstay of treatment for CRC, and complete resection is a good prognostic factor (49, 28, 21). However, the complete resection rate is reportedly less than optimal in children because most of the patients present with advanced disease at diagnosis (14). Poorer outcomes might be also associated with the delayed diagnosis and low index of suspicion attributed to the rarity of childhood CRC, nonspecific symptomatology that mimics several common infections, and functional gastrointestinal disorders in childhood, with a shorter time between symptoms and diagnosis. The unfavorable histologic variant presentation with the distant stage and the inapplicability of adult screening tests in children are also possible factors in the poor outcomes (2, 50, 51).

CONCLUSION

Childhood CRCs have a poor prognosis even with new therapies by the reason of the delayed diagnosis, nonspecific symptomalogy, lack of feasibility to use adult screening tests in children, distant stage at presentation, and unfavorable histologic variants. CRCs occurs in a shorter time than in adults, with a different histology and more likely with different stages. It is significant that three of 18 (16.6%) patients showed K-ras mutations at an early age. It seems that K-ras mutations play a role in the different biology of pediatric CRC. K-ras mutation's prognostic significance could not be portrayed due to the small number of patients. Further studies are necessary with larger series of patients to investigate and understand the biology of childhood CRC and the relevance of the K-ras mutations on the prognosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Hacettepe University Faculty of Medicine (FON 11/34-32).

Informed Consent: Informed consent was obtained from the patients who participated in this study.

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Original Research

Estimation of Salivary and Tissue Nitric Oxide Levels in Oral Squamous Cell Carcinoma: A Biochemical Study

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ABSTRACT

Objective: The study was conducted to estimate the salivary and tissue nitric oxide (NO) levels in healthy individuals and subjects with squamous cell carcinoma of the oral cavity.

Methods: In this study, the salivary and tissue NO levels were estimated in 20 healthy subjects and 20 patients with oral squamous cell carcinoma (OSCC).

Results: The mean salivary NO levels in Group I (control group) was 78.59 µM/L (standard deviation=5.91608), while the mean salivary NO levels of Group II (study group) were 115.6765 µM/L (standard deviation=0.9431). The mean tissue NO levels in Group I (control group) was 87.6315 µM/L (standard deviation=1.91631), while the mean tissue NO of Group II (study group) was 172.376 µM/L (standard deviation=0.84774.

Conclusion: Our results illustrated that the increase in the NO levels in the saliva is positively correlated with the NO level in tissues; hence, salivary NO level can be used as a potential diagnostic biomarker in OSCC.

Keywords: Oral cancer, nitric oxide, malignancy, neoplasm

INTRODUCTION

Oral cancer accounts for 2%–4% of all cancer cases across the world. About 90% of all oral neoplasm cases are estimated to be oral squamous cell carcinoma (OSCC) (1). It is categorized as the 12th most frequent cancer worldwide (2). The incidence rate of oral cancer is highest in the Southeast Asian countries and in India. In addition, 90%–95% of all cancers of the oral cavity are OSCC in India (3). The prevalence of cancer has been estimated to rise from 1 million in 2012 to >1.7 million in 2035 by the International Agency for Research on Cancer. This prediction implicates that the mortality due to cancer will also rise from 680000 to 1–2 million during the same period (4).

Cancer deaths mainly occur due to the consumption of tobacco and/or alcohol, sedentary lifestyles unhealthy diet, and infection (5). The overall 5-year survival rate has been evaluated to be approximately 50% (6), and more than half of the cases of OSCC are diagnosed at a later stage (7).

In the initial periods of this disease, the patients are usually asymptomatic; hence, the identification, discovery, and diagnosis of this disease become tough (8). Timely and accurate clinical diagnosis as well as decision-making for treatment plan along with adjuvant therapy are important because of the chances of secondary metastasis and the high recurrence rate (9). The diagnosis is established mainly on extensive clinical and histopathological exploration of the suspicious lesion, but it may remain unnoticed at certain hidden sites (10). Hence, the evolution of non-invasive, reliable, safe, and advantageous diagnostic markers is currently needed.

Biomarker is termed as any structure, substance or process that can be estimated in the body or its products that impacts or predicts the incidence of disease or the outcome (11). Tumor markers are substances that are specific for certain tumors or cancer cells and hence are important for diagnostic and prognostic purposes in oral cancer patients (12).

NO plays a role in the pathological process of cancer (13). It is the outcome of the transformation of L-arginine to L-citrulline and is catalyzed by the nitric oxide (NO) synthase (NOS) enzyme. The components essential for this reaction are oxygen and other cofactors, including heme, flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin (14).

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It has been suggested that several types of cancer-related events are regulated by NO (15). It plays a role in certain genotoxic events and causes several types of DNA damage during the initial stages of carcinogenesis (16). Three isoforms of NO synthase have been demonstrated so far, 2 of which are constitutive NOS (cNOS) and the third form is inducible (iNOS) by cytokines and endotoxins. The generation of reactive nitrogen species (RNS) occurs through a reaction of NO with either oxygen or other free radicals that exert numerous biological effects (17). NO could be either cytotoxic or cytostatic and interacts with several molecular targets present within the cell structure. Varying reactions to NO have been demonstrated in cells within various tissues**.** In chronic inflammation, the overexpression of the NOS leads to genotoxicity. NO causes damage of DNA by manufacturing RNS, generating carcinogenic nitrosamines, as well as inhibiting the mechanism responsible for DNA damage restoration. Hence, NO can be considered as a tumor-initiating factor (13). It also influences other stages of the process of cancer formation. The stretch of effects of NO is quite broad in tumor genesis, which includes its participation in the transformation of the cells, genesis of the neoplastic lesions, and initiation and regulatory mechanisms of the metastatic events (18).

Hence, the current study was planned to evaluate the significance of NO levels as an adjuvant diagnostic marker in OSCC by estimating the salivary and tissue NO levels in the squamous cell carcinoma of the oral cavity.

The objectives of the present study were to assess and compare the salivary NO and the tissue NO levels in healthy individuals and subjects with OSCC. The current study also co-related the levels of NO in the tissue and saliva of healthy and study subjects.

METHODS

The present study was a case-control study conducted on subjects reporting to the Department of Oral Medicine and Radiology. A total 40 patients were included in the study, and the study sample were divided into the following groups:

Control Group (Group I): 20 healthy subjects without any systemic and oral diseases (normal).

Study Group (Group II): 20 subjects diagnosed clinically and confirmed histopathologically with OSCC.

The subjects selected were of age 20–70 years in both the groups. The inclusion criteria of Group I comprised of healthy individuals with no history of oral or systemic diseases, no oral adverse habits such as consumption of tobacco (smoking)/betel nut and alcohol, and no use of any medication. The Group II included subjects who were clinically diagnosed and histopathologically confirmed with OSCC and those with adverse oral habits such as consumption of tobacco (smoking)/betel nut and alcohol. The exclusion criteria comprised of subjects with any systemic diseases, those diagnosed with malignancies at sites other than the oral cavity, those on any long-term medication, those with any other oral mucosal lesions including potentially malignant disorders (PMDs), and those with OSCC without any history of adverse oral habits.

Main Points:

- The current study assessed and compared the salivary NO and the tissue NO levels in healthy individuals and in subjects with OSCC and a positive correlation was obtained.
- The results of the study suggests that salivary nitric oxide can be used as a potential diagnostic marker in OSCC.
- Saliva provides a non-invasive, reliable, cost effective and safe method for screening a large population.

Method of Data Collection:

Collection of samples

Institutional ethical clearance was acquired before the commencement of the study. The goals of the study were described, and informed consent was obtained from individuals involved in the study. Comprehensive case history was documented along with complete inspection of the oral cavity for all subjects.

Collection of saliva

Unstimulated saliva was obtained from the subjects by using the "Spit Technique." Subjects were advised not to eat, drink, or smoke an hour prior to sample collection. They were seated on the dental chair and asked to tilt their head forward. They were then asked to not speak or swallow any saliva. They were also told to expectorate into a sterile graduated container for 8–10 min. The salivary sample collected represented the fluid contents of the whole mouth. The sample obtained was later centrifuged at 3000 rpm for 10 min, and the supernatant was collected and stored at −20°C.

Collection of the tissue

For tissue specimen, a segment of the oral mucosa was resected from the OSCC site of the patients through biopsy.

The tissue samples for the control group were acquired during operculectomy or frenectomy (only non-inflamed tissues were considered).

Fresh tissue samples obtained during surgical intervention were properly cleansed to remove any blood stains or any other necrosed tissues with 0.9% HCl. The samples were later dried on blotting paper and weighed. One gram of the sample was homogenized in 10 mL of 0.1 M cold phosphate buffer (pH 7.4) with 1 mM EDTA for 10 min, and the homogenate obtained was centrifuged at 3000 rpm for 15 min. The analysis was performed using the clear supernatant obtained.

For histopathological investigation, parts of the tissues were fixed in 10% formalin, embedded into paraffin, sectioned, and finally stained with hematoxylin and eosin.

Salivary NO estimation by Griess method (19):

The determination of the NO levels is based on the transformation of nitrate to nitrite with the aid of an enzyme. It is a diazotization reaction in which a nitrosating agent is produced by acetified NO. The nitrosating agent reacts with sulfanilic acid, resulting in the formation of a diazonium ion. This ion is coupled

with N-(1-naphthyl) ethylenediamine to produce the chromophoricazo derivatives, which absorb light at 540–570 nm. The reagents used included N-(1-naphthyl)-ethylene diamine (NED) dihydrochloride and sulphanilamide solutions. The sulphanilamide solution was prepared by mixing 0.5 g of sulphanilamide in 100 mL of 20% v/v hydrochloric acid. The N-(1-naphthyl)-ethylene diaminedihydrochloride solution was prepared by mixing 0.3 g of a solid reagent (NED dihydrochloride) in 100 mL of 1% v/v hydrochloric acid. A standard NO solution was pipetted out into 5 different test tubes in the range of 0.2–1 mL. Distilled water was used to increase the volume of each test tube by 1 mL. A separate test tube was taken with 1 mL distilled water, which served as blank. One milliliter each of the sulphanilamide solution and NED dihydrochloride solution were added to each of the test tube and the solution was mixed. The test tubes were later incubated at the room temperature for 10 min, and the absorbance was read at 550 nanometer. For test sample, 100 µL of the saliva/tissue sample was taken in a test tube. The volume was prepared up to 1 mL with 0.9 mL of distilled water. Then, 2 mL of sulphanilamide solution was included and kept for 5 min. After 5 min, 2 mL of NED dihydrochloride solution was added. After 10 min, the absorbance was measured at 550 nm. The concentration was then calculated from a calibration plot prepared from a series of standard nitrite. The calculation was performed as follows:

0.1 mL of sample contained=------- µM/L of NO.
∴ 1000 mL of sample contained=----- µM/L of NO.

Statistical Analysis

The data was analyzed by using the *SPSS* software version 17 (SPSS Inc.; Chicago, IL, USA). Data obtained were analyzed using independent Student's t-test for comparison between the groups, while Chi- Square Test was used for the association of the age and gender with different parameters. Pearson's correlation was used for analyzing correlations between the groups.

RESULTS

The mean age of Group I subjects was 29.1 years. It included 45% (9/20) and 55% (11/20) of female and male subjects, respectively. The mean age of Group II subjects was 55.45 years. It included 35.0% (7/20) and 65.0% (13/20) of female and male subjects, respectively (Table 1).

The mean salivary NO levels in Group I was 78.59 µM/L, while the mean salivary NO levels of Group II was 115.6765 µM/L. The mean tissue NO levels in Group I was 87.6315 µM/L, while it was 172.376 µM/L for Group II. Comparison of the salivary NO levels between Group I and Group II showed that that salivary NO levels were higher in Group II with a t value of –27.685, which was statistically significant with a p value of <0.001. Comparison of the tissue NO level between Group I and Group II showed that the tissue NO level was higher in Group II with a t value of –180.863, and it was statistically significant with a p value of <0.001 (Table 2).

Fair correlation was noted between the salivary and tissue NO levels in Group I (r=0.42), and excellent correlation was observed between the salivary and tissue NO levels with p<0.001, which is significant at an error of <1% (r=0.99). The overall correlation was found to be excellent between the salivary and tissue NO levels. (r=0. 99 in OSCC) (Table 3, Graph 1).

DISCUSSION

Highly RNS and reactive oxygen species (ROS) are involved in the process of cancer formation of the oral cavity (20). The presumption behind this hypothesis is that these free radicals could damage the cellular materials, resulting in the initiation or transformation of the normal cells into malignant ones (21). The extent

Table 1. Comparison of study participants according to gender

	Group			Chi-Square Test	
Gender	Normal	OSCC	Total	Chi-Square Value	p-value
Male	11	13	24	0.42	0.52(NS)
	55.0%	65.0%	60.0%		
Female	9	7	16		
	45.0%	35.0%	40.0%		
Total	20	20	40		
	100.0%	100.0%	100.0%		

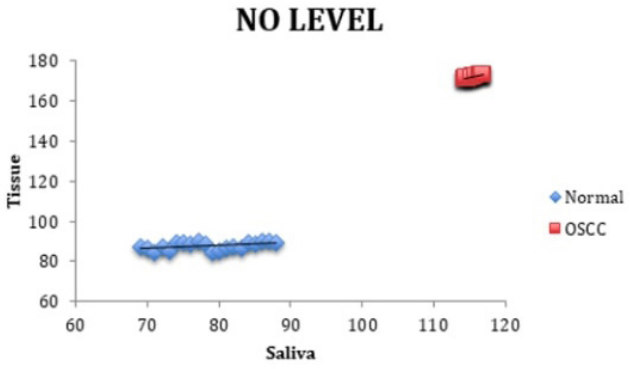
Table 2. Comparison of age and nitric oxide levels between the study groups

	Group	N	Mean	Std. Deviation	Mean Difference	95% Confidence Interval of the Difference		t	df	p-value
						Lower	Upper			
Age	Normal	20	29.10	5.83	–26.35	–31.44	–21.26	–10.48	31.29	<0.001*
	OSCC	20	55.45	9.62						
Saliva	Normal	20	78.59	5.92	–37.09	–39.80	–34.37	–27.69	19.97	<0.001*
	OSCC	20	115.68	0.94						
Tissue	Normal	20	87.63	1.92	–84.74	–85.69	–83.80	–180.86	26.16	<0.001*
	OSCC	20	172.38	0.85						

Table 3. Correlation between tissue and salivary NO levels

	r	p–value
Normal	0.42	0.07
OSCC	0.99	<0.001*

Graph 1. Correlation between tissue and salivary NO levels



of this destruction is also influenced by the defense mechanisms of the body against these free radicals interceded by various other cellular antioxidants. RNS could lead to the activation of pro carcinogens, inactivation of enzyme repair systems, initiation of lipid peroxidation process, and damage to the DNA, thereby affecting the triggering and promotion mechanism of carcinogenesis. The prime source of entire RNS is NO in the biological systems (22). The range of operations of NO are broad, elaborated, and multifarious in cancer biology.

Several studies with serum have been conducted to estimate the NO level in oral cancer patients. However, studies using saliva or tissue samples to estimate the NO levels in OSCC subjects are limited in the existing literature.

The comparison of the salivary NO levels between the groups in our study showed that salivary NO level was higher in the study group. This observation was in line with the research conducted by Bahar et al. (23), which demonstrated an alteration in the salivary composition of patients with OSCC when compared to the healthy subjects. The enhanced salivary NO levels could be attributed to the increased dietary NO from tobacco and its components (24). Dietary NO is absorbed from the upper gastrointestinal tract. It enters the saliva through the salivary glands through an active transport mechanism after getting actively concentrated from the plasma. Furthermore, in oral cancer patients, the NO level is increased due to the overexpression of enzyme inducible NO synthase (iNOS). Malignant epithelium of oral cancer or oral cancer induced inflammatory response are believed to be the possible sources of iNOS (24).

The tissue NO levels between the groups demonstrated that the tissue NO levels were higher in subjects with OSCC than in healthy individuals. This finding was in accordance with the study conducted by Korde et al. (25), where the tissue as well as the serum NO levels were significantly increased in subjects with OSCC. The increase in the NO levels may be described on the basis that there could be a generalized increase in the synthesis of NO throughout the body of the cancer patient or it could reflect elevated degradation of NO (25). This enhanced production of NO products is generally assisted by oxidative stress. The interaction of NO either with superoxide or oxygen leads to the formation of reactive nitrogen oxide species, which in turn can mediate either oxidative or nitrosative stress (25). NO forms peroxynitrite (ONOO) at a higher concentration, which is a potent oxidant that plays a major role in the initiation of oral cancer (25).

Furthermore, the present study was also in accordance with those conducted by Connelly et al. (26), where the authors reported significantly elevated levels of iNOS mRNA and NO production in OSCC cases when compared with oral dysplasias and normal cases. It is believed that the expression of iNOS and NO production play a role in the growth and invasion of oral carcinoma mostly through an angiogenic mechanism. The growth and invasion process of the carcinoma is determined by the proliferation of new vessels arising from the surrounding stroma. This growth of new vessels is mediated by iNOS through NO, which functions as a cellular signal for angiogenesis (26).

Gallo et al. (27) conducted a study that demonstrated significantly increased levels of total NOS in the tissues of the OSCC group when compared with the control group. Their study also demonstrated elevated levels of iNOS and cGMP in the tumor tissue specimens when compared with that in normal mucosa samples. In addition, enhanced total NOS activity was observed in cases where lymph node metastasis occurred. The authors concluded that the NO pathway appeared to stimulate tumor angiogenesis and help spread the tumor in patients with head and neck malignancies (27).

A study by Gokul et al. (28) also showed that the levels of NO and malondialdehyde were significantly increased in OSCC cases in both blood and tissue specimens, specifying elevated oxidative stress in OSCC patients with a restricted antioxidant defense mechanism. The raised oxidative stress in OSCC patients is indicated by the increased levels of MDA and NO. This disproportion in the status of the oxidant–antioxidant can be regarded as one of the elements liable for the pathogenesis of oral cancer.

In the present study, the correlation of salivary NO with tissue NO levels in the 2 groups was also analyzed, which makes this study unique in the current time. Our study highlights the increased expression of NO in the saliva and tissue specimens of subjects with OSCC when compared with those of healthy individuals. Moreover, an excellent correlation was noted between salivary and tissue NO levels in subjects with OSCC. These results suggest that NO could be used as a potential biomarker of OSCC.

Only a limited number of studies have been conducted until date for assessing the role of NO in the pathogenesis of OSCC. Most of the studies have reported the tumor-promoting effects of NO due the damage of the DNA, which is considered as one

of the causative factors for oral cancer development, which is in accordance with our present study as seen in cases of OSCC.

As significant increase was observed in the levels of NO in the saliva and tissue specimens, with excellent correlation in OSCC; this study suggests the use of salivary NO as a potential diagnostic marker in OSCC.

The basic elements for an effective diagnostic technique include the following: ease of use, minimal discomfort to patients, and collection of sufficient evidence for investigation. Basically, a diagnostic procedure should not be complex or time consuming. High sensitivity and potential for automation are other desirable requirements. In addition, avoidance of false-positives results and therefore reducing the anxiety of the patients and the need for additional investigations or unnecessary treatments are also required. Therefore, in the present study, we used saliva and tissue samples for the assessment of the NO levels, which optimally meets all of the above requirements.

Non-invasive methods such as the analysis of saliva and reliable methods such as the analysis of tissues will provide a cost effective approach for screening a large population in the near future.

Extensive studies with larger sample size are required to assess the actual role of NO in the initiation and promotion of OSCC along with testing of the utility of the NOS enzyme inhibitors as chemo-preventive agents in order to minimize the risk of human cancer. Elaborate studies with a larger sample size are also required to establish the effectiveness of using NO as an adjunctive diagnostic marker in OSCC.

CONCLUSION

The encouraging results of present study demonstrate the possible involvement of NO in the pathological process of OSCC as noticeable from elevated NO level in the saliva and tissues of OSCC patients. Thus, the findings of the present study indicate that NO can be used as an adjunctive diagnostic marker by evaluating the salivary and tissue NO levels in OSCC. Furthermore, it could provide a profitable approach for screening huge population groups in the future.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Nitte University (certificate number ABSM/EC/97/2014).

Informed Consent: Informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - R.G.; Design - R.G.; Supervision - S.G.B. Resources - R.L.C.; Materials -R.G.; Data Collection and/or Processing - R.G.; Analysis and/or Interpretation - R.G.; Literature Search - B.B.; Writing Manuscript - R.L.C.; Critical Review - B.B.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Research

Situation of Southeastern Anatolia in Thymus Surgery: To Whom, Why, and How Thymectomy Was Performed

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ABSTRACT

Objective: We sought to obtain information on the number of thymic lesions, surgical method, histopathology. We also investigated the relationship between thymic lesions and myasthenia gravis.

Methods: We retrospectively examined patients who underwent thymectomy for different thymic pathologies in our clinic between February 1998 and April 2014 according to their demographic characteristics, symptoms, surgical method, resection width, and histopathological diagnosis.

Results: The proportion of men and women was similar. The average age was 40.6±14.7 years. The most common symptom was rapid fatigue and the most common surgical method was median sternotomy. During extended thymectomy, we resected and recontructed the anatomical structures. We found a high degree of association between thymoma and myasthenia gravis. The average life expectancy of the patients was 171.8±8.8 months.

Conclusion: Although the indications of thymectomy are not well known, the surgical procedures vary. We would therefore continue developing ourselves as the center where these surgeries are most frequently performed in the Southeastern Anatolia Region.

Keywords: Thymoma, thymectomy, myasthenia gravis

INTRODUCTION

Thymoma, seminoma, and lymphoma are primary malignant tumors occurring in the anterior mediastinum, making up the majority of malignant mediastinal tumors (1). Studies on anterior mediastinal tumors and those of the thymus correlate with those on myasthenia gravis (MG) (2). According to the literature, 30% of patients with thymoma develop MG, while approximately 10-15% of patients with MG develop thymoma (3, 4).

The most common indications for thymectomy are thymic neoplasia and MG (5). The main goal of surgery in thymic neoplasia is complete resection of the tumor, and tumoral invasion and metastasis when necessary (6); this is because complete surgical removal of thymomas is the most important long term prognostic marker of thymic malignancies (7). The main goal of surgery in MG is to eliminate the production of antibodies that cause autoimmune diseases. Currently, thymectomy is indicated in patients with common MG and ocular symptoms that cannot be controlled by anticholinesterase (ACE) (2, 8).

Thymectomy can be performed with median sternotomy (MS), and with less invasive methods such as partial sternotomy, transcervical approach, video-assisted thoracoscopic surgery (VATS), and transcervical-VATS combination (9). There are two schools of thought concerning the form of excision during thymectomy: those who advocate the method that protects the sternum and those who perform sternotomy with resection of the surrounding tissue and thymus to prevent local recurrence (10). The surgery indicated for MG depends on the width of the thymectomy required for complete remission. For this purpose, studies revealing the thymic tissue distribution and presence of ectopic thymic tissue in the anterior mediastinum have been conducted. Regardless of the approach used in MG patients, the aim is complete remission (11, 12).

In this study, we retrospectively examined patients who underwent thymectomy for various reasons according to their demographic data, symptoms, surgical methods, resection width, and histopathological diagnoses, and aimed to obtain information

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on thymic lesions in our region and the connection between the thymic lesions and MG.

METHODS

Cases

We included 80 patients who underwent thymectomy for different thymic pathologies between February 1998 and April 2014 in Gaziantep University, Faculty of Medicine, Şahinbey Research and Practice Hospital Thoracic Surgery Clinic. Gaziantep University Clinical Research Ethics Committee Presidency (ethics committee approval number: 167). All patients provided an informed consent.

The inclusion criteria were as follows:

- Patients with benign or malignant lesions on radiology
- Patients with MG who have thymic lesions or thymus residues in the anterior mediastinum on radiology
- Patients with unresectable thymomas or thymic carcinomas that became resectable after chemotherapy (CT), radiotherapy (RT), or chemoradiotherapy (CRT)

The exclusion criteria were as follows:

- Patients operated for an anterior mediastinal mass and whose pathological diagnosis was out of the thymus (such as lymphoma)
- Patients undergoing thymectomy but whose pathology reports were not available
- Patients aged ≤ 16 years

Preoperative Evaluation

We obtained a detailed anamnesis from all patients and performed physical examinations. We performed routine biochemical tests, chest X-ray, electrocardiography, and chest computed tomography (CT) tests in all patients (Figure 1). In some patients, we performed a mediastinal magnetic resonance imaging to evaluate the relationship between the lesion and anatomical structures, and the resectability of the lesion (Figure 2). We performed CT scan and positron emission tomography (Figure 3) to screen for metastases in patients with thymomas or thymic carcinoma.

We evaluated MG patients in the neurology clinic and requested a surgery upon neurologist approval. While all of this group of patients used ACEs, some of them also used immunosuppressive drugs. Some patients required intravenous immunoglobulin and plasmapheresis.

Main Points:

- When a thymic mass is diagnosed, myasthenia gravis screening should be performed for each patient.
- Thorax computed tomography should be performed in every patient diagnosed with myasthenia gravis to investigate the presence of thymic lesion.
- Median sternotomy may be considered for wide resection in the presence of thymoma or thymic carcinoma.
- Minimally invasive surgery may be considered in patients with myasthenia gravis if thymoma is not suspected.

Figure 1. Axial CT image of a patient with thymic lesion

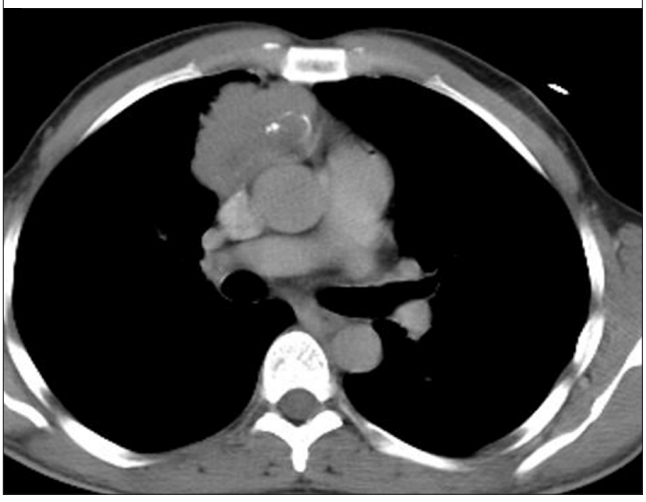


Figure 2. MRI image of a patient with suspected invasion

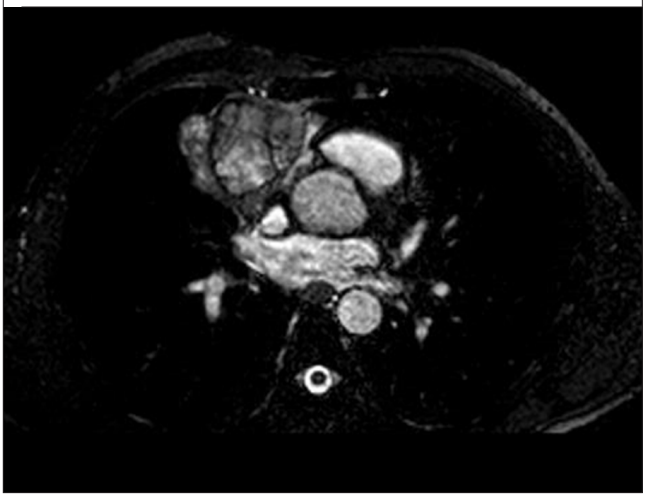
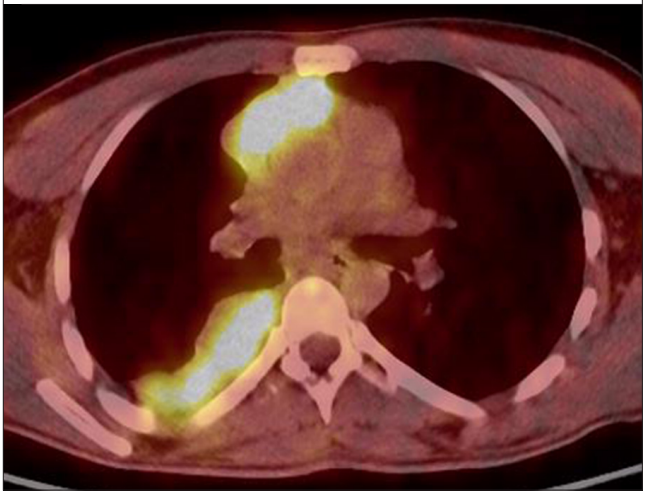


Figure 3. PET-CT image of a patient diagnosed with thymoma with metastasis



Anesthesia Applications
Although standard procedures for thoracic surgery are applied, muscle relaxants are either not used or used in selected patients, according to the anesthesiologist and neurologist.

Surgical Methods
Our basic rules in thymus surgery are: The mediastinum should be well explored, the cervical pole and mediastinal fat tissue should be resected, the phrenic nerve should be preserved, the tumor capsule should not be damaged, and the resection should be extended from the diaphragm to the neck. Therefore, MS has been used frequently. In patients with MG, right VATS was used from 2008 if there was no radiologic image of the thymoma.

Exploration also included the lungs and pleura to determine whether the thymic mass invaded the surrounding tissues and whether there is a metastasis (Figure 4). This was in accordance with the definition of extended thymectomy defined as "block resection of the thymus and adipose tissue in the anterior mediastinum via MS" (Figure 5). Due to invasion, various degrees of resection and reconstruction have been applied to anatomical structures like the pericardium, diaphragm, vascular structures, phrenic nerve, and lung.

Right VATS was done in patients with MG and no thymoma image (Figure 6). Left VATS was done to some patients with masses localized to the left and requiring exploration of the aorticopulmonary window. As a rule, complete thymectomy was performed in VATS (Figure 7). Some patients who started with VATS switched to thoracotomy or MS for various technical reasons, and these patients were not included in the VATS group.

Postoperative Care
All patients were taken to the intensive care unit, and most patients were taken to the service after a day of intensive care follow-up. All MG patients were evaluated by the postoperative neurologist, and ACE and immunosuppressive treatments were continued.

Histopathologically, all patients diagnosed with thymoma or thymic carcinoma presented to the tumor council and the need for an additional oncological treatment was decided. Patients with MG continued their neurological follow-up.

Statistical Analysis
Statistical analyses were conducted using IBM SPSS Statistics for Windows 22.0 (IBM SPSS Corp.; Armonk, NY, USA). Student's t test (for normally distributed variables) and Mann Whitney u tests (for variables without normal distribution) were used to compare numerical variables in groups. Kaplan Meier method was used to calculate survival probabilities. $P < 0.05$ was considered significant.

RESULTS
Regardless of the reasons for performing thymectomy, we examined the demographic data, symptoms, surgical approaches, resection width, and histopathological diagnosis of all patients. In addition, we obtained the survival analyses of all patients who underwent thymectomy. The invasion status of patients undergoing extended thymectomy and the resections and recon-

Figure 4. Exploration of a patient with thymoma by MS approach

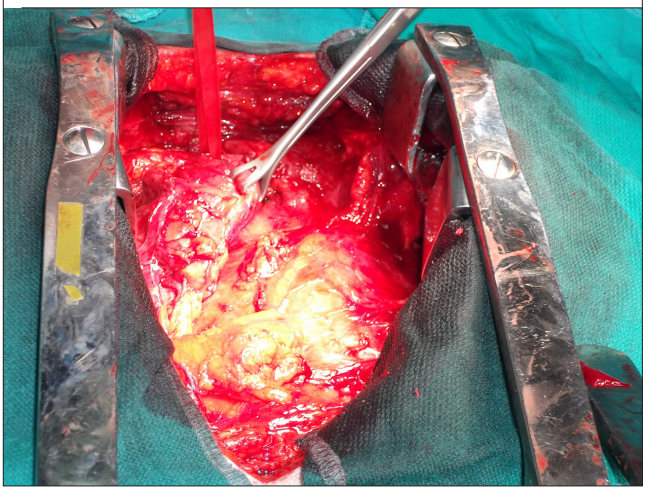


Figure 5. Thymectomy material removed in block with mediastinal adipose tissue in a patient approached with MS

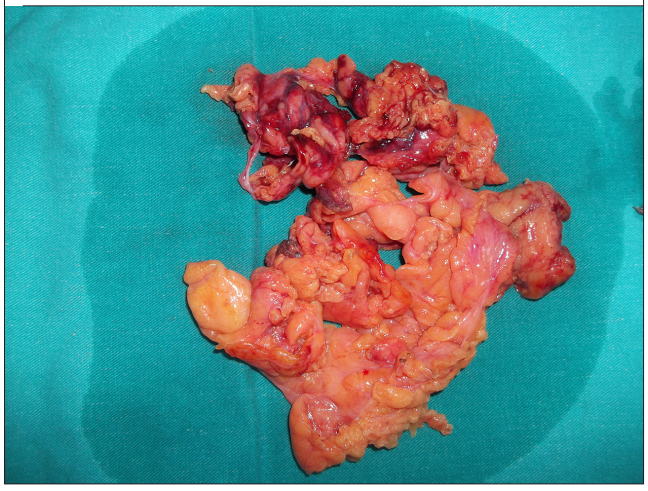


Figure 6. Thoracoport incisions in right VATS approach

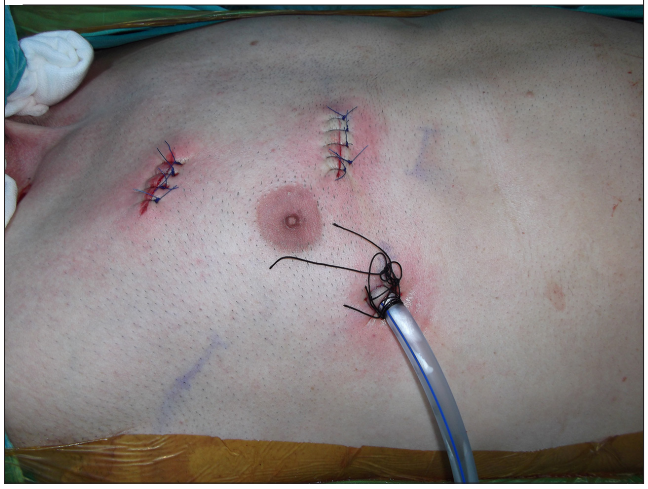


Table 1. Distribution of all patients undergoing thymectomy by age and gender

	Under 20	20–29 years	30–39 years	40–49 years	50–59 years	60 years and over	Total n (%)
Female n (%)	4 (5%)	11 (13,75%)	4 (5%)	6 (7,5%)	8 (10%)	5 (6,25%)	37 (46,25%)
Male n (%)	–	8 (10%)	10 (12,5%)	14 (17,5%)	6 (7,5%)	4 (5%)	43 (53,75%)
Total n (%)	4 (5%)	18 (22,5%)	14 (17,5%)	20 (25%)	14 (17,5%)	9 (11,25%)	80 (100%)

Figure 7. Thymic lesion and mediastinal adipose tissue in the patient approached with right VATS

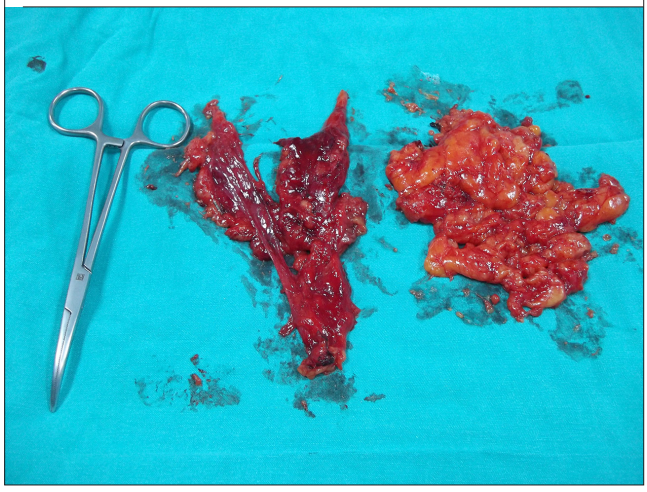
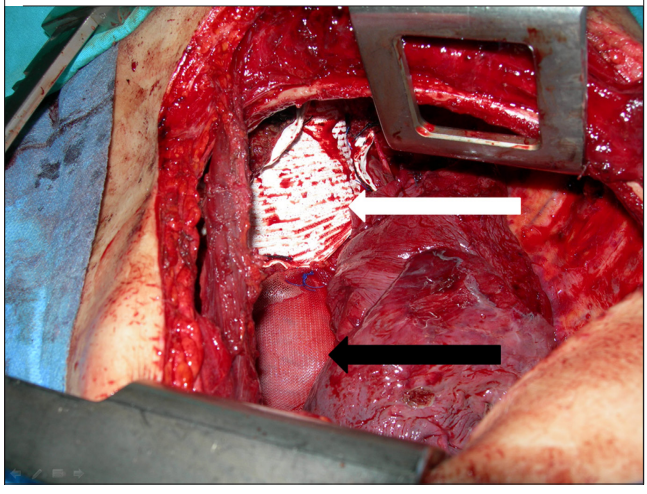


Figure 8. Diaphragmatic graft (white arrow) and pericardial graft (black arrow) after resection in a patient with diaphragm and pericardial invasion



structions applied to anatomical structures depending on this invasion are given in table 3. We delayed the analysis on the improvement in the clinical condition of MG patients because it is seen in the long term. However, we an analyzed the connection between MG and histopathological diagnosis.

Demographic Data

Of the 80 patients included, 38 (47.5%) were women and 42 (52.5%) men. The age range of the patients was 17–74 years and the mean age was 40.6±14.7 years. Concerning the age distribution by gender, the age range of women was 17–72 years (mean 39.1), and that of men was 20–74 years(mean 41.7) (Table 1).

Symptoms

Table 2 shows the distribution of the symptoms, which were mostly associated with MG. None of the 8 asymptomatic patients (10%) had MG.

Surgical Method

MS was most commonly used, followed by right VATS. Table 3 shows the number of malignancy and number of patients with MG according to the surgical approach used.

In our clinic, before 2008, thymectomies were performed by MS. Of the 20 patients who underwent right VATS, 11 (55%) were patients with non-thymomic MG. Only one (5%) of the 20 patients who underwent thymectomy through right VATS had a thymoma.

The Rate Of Surrounding Organ Invasion In Extended Thymectomies

Invasion into the surrounding anatomical structures was detected in 13 (16.25%) patients, 10 with thymoma, and 3 with thymic carcinoma. While 4 (5%) of these patients had macroscopic residues, 9 (11.25%) were completed with no residue (Table 4). Invasion of more than one anatomical structure was frequently seen in the same patient (Figure 8).

Eight (10%) patients had lung invasion in various degrees and localizations (Figure 9).

Six patients (7.5%) underwent various levels of resection and reconstruction of the vascular structures due to invasion (Figure 10).

One of three patients with phrenic nerve invasion had MG, and a residual tumor was left on the phrenic nerve. The phrenic nerve of the other two patients was resected.

One patient with thymoma underwent pleurectomy for pleural invasion, and intracavitary hyperthermic perfusion chemotherapy (HIPEC) was performed after the operation.

Histopathological Evaluation

Histopathological results of the 80 patients who underwent thymectomy showed thymoma and thymic hyperplasia (Table 5).

Survival

Six of the 80 patients who underwent thymectomy died during follow-up, and one died early in the postoperative period. Figure

Figure 9. Lung invasion and wedge resection

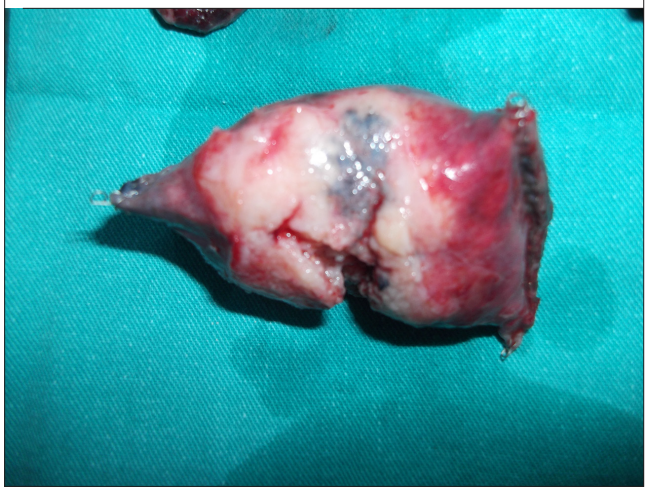


Figure 10. Resection and repair in a patient with right brachiocephalic vein invasion (black arrow)

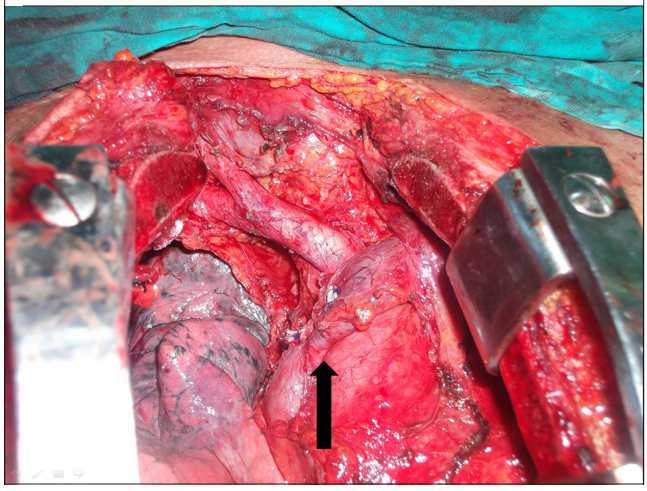


Figure 11. Graph showing survival analysis of patients undergoing thymectomy

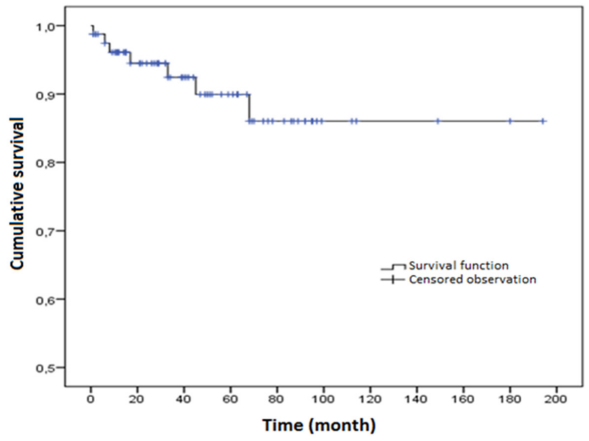


Table 2. Application symptoms of patients undergoing thymectomy

Symptoms	number	%
Rapid fatigue	37	46,3
Drooping of eyelides	26	32,5
Difficulty swallowing	19	23,8
Chest pain	17	21,3
Difficulty breathing	14	17,5
Difficulty speaking	7	8,8
Cough	2	2,5
Swelling of the face and neck	2	2,5
Hemoptysis	1	1,3
Frequent expectoration	1	1,3
Hoarseness	1	1,3
Arm pain	1	1,3
Backache	1	1,3
No symptoms	8	10

Table 3. Malignancy and MG rates according to the surgical approach

Surgical Approach	Number of Cases	Malignant	MG
Median Sternotomy, n (%)	50	34 (68%)	30 (60%)
Right VATS, n (%)	20	1 (5%)	11 (55%)
Left VATS, n (%)	4	2 (50%)	0 (0%)
Right Thoracotomy, n (%)	4	3 (75%)	0 (0%)
Left Thoracotomy, n (%)	2	1 (50%)	1 (50%)

11 shows the survival analysis of patients undergoing thymectomy. The causes of death of patients are:

- Myocardial infraction on postoperative day 11 in 1 patient
- Heart failure in two patients, one at the 6th postoperative month and the other at the 68th postoperative month
- Myasthenic crisis in two patients, one at the 8th postoperative month and the other at the 17th postoperative month
- Thymoma in one patient at the 38th postoperative month
- Non-hodgkin lymphoma in one patient at the 45th postoperative month

The average life expectancy of the patients was 171.8±8.8 months.

MG-Histopathology Relationship

Histopathologically, 54% of patients diagnosed with thymoma had MG, while 47.6% of patients with MG had a thymoma. The number of non-MG and non-thymoma patients was 17 (21.25%).

Table 4. Invasive structures and resection rates in patients approached with MS

	İnvasion n (%)	Resection n (%)	Grafting n (%)	Residue n (%)
Pericardium	10 (%12,5)	10 (%12,5)	5 (%6,25)	1 (%1,25)
Lung	8 (%10)	7 (%8,75)	–	1 (%1,25)
Vascular	6 (%7,5)	4 (%5)	3 (%6)	2 (%2,5)
Phrenic nerve	3 (%3,75)	2 (%2,5)	–	1 (%1,25)
Diaphragm	1 (%1,25)	1 (%1,25)	1 (%1)	0 (%0)
Pleura	1 (%1,25)	1 (%1,25)	–	0 (%0)
Atrium	1 (%1,25)	0 (%0)	–	1 (%1,25)

Table 5. Histopathological diagnoses after thymectomy

Histopathological diagnosis	Number	%
Thymoma	37	46,25
Thymic hyperplasia	29	36,25
Thymic carcinoma	4	5
Thymus tissue	4	5
Thymolipoma	4	5
Thymic cyst	2	2,5
Total	80	100

Table 6. The relationship between MG with histopathological diagnosis of patients undergoing thymectomy

Histopathological diagnosis	Patients with MG (n)	Patients without MG (n)	Total (n)
Thymoma	20	17	37
Timic hyperplasia	14	15	29
Timic carcinoma	–	4	4
Normal thymus tissue	4	–	4
Thymolipoma	4	–	4
Thymic cyst	–	2	2
TOTAL	42	38	80

Table 6 shows the relationship between MG and histopathological diagnosis.

DISCUSSION

Mediastinal masses arise from tissues normally located in the mediastinum or which migrated to the mediastinum during their development. The most common lesions in adults are thymomas, which are located in the anterior mediastinum (13, 14). Approximately 25% of all mediastinal tumors in adults and children are malignant (15). We did not include patients aged ≤16 years. Thirty-seven (46.25%) of 80 patients who underwent thymectomy were diagnosed with different histopathological types of thymoma and 4 (5%) were diagnosed with thymic carcinoma (Table 7).

Most patients with thymoma (50–60%) were asymptomatic. Symptoms depend on the compression effect of the lesion on the surrounding tissues or systemic disease accompanied by the thymoma (6, 16, 17). According to our study, the most common symptoms in thymoma patients were those associated with MG.

While 30% of patients with thymomas have MG, approximately 10–15% of patients with MG had thymoma. In our study, 54% of patients diagnosed with thymoma had MG and 47.6% of patients with MG had thymoma. The coexistence of MG and thymoma in our study group was high, because all patients who apply to our clinic with thymic lesions were diagnosed with MG. This increased the number of patients with MG.

Literature on the form of excision had divided opinions some adopt sternotomy and others advocate the method that protects the sternum (11).

Most people advocate the transsternal thymectomy method because it extends out of the capsule (18). Stage I thymomas may relapse or metastasize although they are histologically mild in those who undergo transsternal thymectomy (19).

Advocates of the transcervical approach say that it is possible to see the anterior mediastinum directly with advanced imaging methods (20).

In a study in which minimally invasive thymectomy was recommended, VATS was considered safe and had a comparable resection rate when selectively used in MG and small thymic masses (21).

Minimally invasive approaches such as videothoracoscopic, transcervical, and robotic surgery can be applied in early stage thymomas but no large series exist on this issue. Therefore, MS is still the most valuable approach for complete resection (22).

In our study, MS was used in patients with thymomas radiologically seen before surgery. However, if the tumor was encapsulated, there was no invasion into the surrounding tissue and the tumor could be removed with mediastinal fatty tissue using VATS or thoracotomy. Nevertheless, the results of multi-patient studies are needed to confirm that minimally invasive methods provide complete resection and complete remission.

Among patients with MS, 32% had benign thymic. This rate was 40.9% before the thoracoscopic approach, and decreased to 25% after its start. This may indicate that the minimally invasive approach is more rational in cases that we consider to be histopathologically benign.

Today, there is consensus on the application of surgery to ocular MG patients who cannot be controlled by ACE common MG and

patients (23, 24). Unfortunately, appropriate patient selection criteria for remission have not been defined. Likewise, the size required to perform surgery is not exactly defined (25). In the study by Toker, thymectomy was presented as a reliable surgery in MG patients (26). The current approach of our clinic was used MS only in MG patients who have radiologically thymoma images.

There is no standard treatment approach defined for thymic carcinoma, nor is there a universally accepted staging system. The most common approach is RT, CT, or both following surgical resection. Unfortunately, most tumors are not resectable and the effect of surgery is unclear. Most patients experience local and distant disease recurrence (6, 16).

In our study, there were four patients operated for thymic carcinoma. The symptoms of these patients were due to the lesion pressing on the surrounding tissue. There is no MG in our patients with thymic carcinoma. All patients were subjected to CRT before or after surgery according to the resectability condition.

Thymic benign lesions are thymic hyperplasia, thymic cysts, and thymolipoma. Surgical resection of benign thymic lesions can be performed by many methods depending on the size of the tumor, the body condition of the person, and the experience of the surgeon (27).

The histopathological results of 39 patients included in our study were benign. Among these patients, thymic hyperplasias accounted for 74.3% of benign thymic lesions. No recurrence was observed in the follow-up of patients with benign thymic lesions. Especially non-MG non-thymoma patients are excellent candidates for VATS.

CONCLUSION

Thymectomies can be performed using various methods as curative treatment in malignant or benign thymic diseases and to contribute to the treatment of the disease in MG. Although there is not much discussion on the indication of thymectomy, there are differences in opinion regarding the method used. MG and thymic lesions have important health problems and change the comfort of patients. Because these patients may have a long hospital stay, it is very important for patients who will undergo thymus surgery, especially those with MG, to be treated in a hospital near their home.We would therefore continue developing ourselves as the center where these surgeries are most frequently performed in the Southeastern Anatolia Region.

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Informed Consent: Informed consent was obtained from the patients who participated in this study.

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Original Research

Comparison of Group Eye Movement Desensitization and Reprocessing with Cognitive and Behavioral Therapy Protocol after the 2020 Earthquake in Turkey: A Field Study in Children and Adolescents

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ABSTRACT

Objective: We aimed at comparing the efficiency of "Eye Movement Desensitization and Reprocessing Integrative Group Treatment Protocol (EMDR-IGTP)" with "Cognitive and Behavioral Therapy Based Crisis Prevention Program for Children and Adolescents (CIPCA)" in children who survived the 2020 earthquake in Turkey.

Methods: We randomly divided 56 children and adolescents who were earthquake victims between the ages of 8 and 14 into two groups. Half of the participants underwent EMDR-IGTP, while the other underwent CIPCA. Outcomes were obtained using clinical global impression (CGI) and the subjective units of distress (SUDS) scales before and after therapy.

Results: The median age of the participants was 10 years (range: 8–14) and 53.6% of them were male. The median CGI scores of the EMDR-IGTP group before and after therapy were 7 (3–7) and 1 (1–7), while that of the CIPCA group before and after therapy were 7 (3–7) and 4 (2–7), respectively ($p<0.001$). The median SUDS scores of the EMDR-IGTP group before and after therapy were 10 (5–10) and 1 (0–10), while that of the CIPCA group before and after therapy were 9 (5–10) and 5.5 (3–9), respectively ($p<0.001$).

Conclusion: Both EMDR-IGTP and CIPCA are effective in reducing the acute traumatic stress following the earthquake; however, EMDR-IGTP is relatively more effective. Thus, both methods can be used as a psychosocial intervention in post-earthquake traumatic events.

Keywords: Earthquake, EMDR, CBT, psychosocial intervention, group therapy, PTSD

INTRODUCTION

One of the most frightening natural disasters has been earthquakes since the existence of human beings. Earthquakes are natural events that affect thousands of people at the same time, thereby resulting in a change of daily routines and priorities. In such natural disasters, the primary needs are often eating, drinking, and sheltering. However, one issue that should not be left out is the need for psychological support (1). According to World Health Organization reports, psychiatric disorders such as mild and moderate depression, post-traumatic stress disorder (PTSD) or anxiety disorder occur in an average of 15%–20% of the affected population after a disaster; complex PTSD and high morbidity disorders such as severe depression and anxiety disorder (psychosis) occur in 3%–4% of them (2). When the number of people affected by the earthquake was taken into consideration, these given rates can be considered significant enough to affect social functionality. In addition, previous studies reveal that early psychosocial interventions after disasters (earthquakes and violence attacks) are effective and can prevent future psychiatric disorders (3, 4). For this reason, psychosocial interventions have been developed for children and adolescents in order to preserve the mental health of the survivors. The protocols developed in this direction are short and established on the basis of previously proven therapeutic regimens, and are also suitable for application in groups. The most common methods used following natural disasters include Cognitive Behavioral Therapy (CBT), and Eye Movement Desensitization and Reprocessing (EMDR). CBT have developed protocols that can be applied in several sessions including techniques such as psychoeducation, emotion expression and modulation, cognitive reconstruction, creation of trauma stories, and management of dysfunctional behavior (5).

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Crisis Intervention Program for Children and Adolescents (CIPCA) is a CBT-based intervention developed for this purpose. CIPCA has been shown to reduce symptoms of trauma in a study undertaken in internally displaced children and adolescents caused by war in Iraq (6). EMDR is based on bilateral stimulation while clients' imaging the bad part of the target event. This method is effective especially in reducing the distress caused by traumatic experiences (7, 8). EMDR-based applications have been developed for use after crisis and disasters (9). EMDR-IGTP was used in the study conducted by Tirentini et al. (10) after the 2016 Italy Earthquake. Moreover, we recorded a greater improvement in children who had received a timelier intervention, compared to those with delayed treatment in this study.

In this study, we aimed at demonstrating the effectiveness of EMDR-IGTP and CIPCA protocols in trauma-related symptoms in children and adolescents who survived the earthquake, which occurred on January 24, 2020 in Turkey's Elazig province and lasted about 40 seconds, and the epicenter of which is Sivrice and violence of which is 6.8.

METHODS

This is a pre/post study with a control group. Three weeks after the earthquake in Elazig, during February 15–17, children and adolescents staying in tents because their house was destroyed or damaged were voluntarily visited. We invited 60 participants for the study. However, three children did not want to participate in the study, and one child stopped working due to intense anxiety during EMDR-IGTP intervention. As a result, 56 people were included in the pre-post analysis. Half of children and adolescents between the ages of 8 and 14 interviewed during this visit were applied EMDR-IGTP and the other half were applied CIPCA. In both groups, there were three applications including 10, 9, 9 children/adolescent in each. After determining the groups, therapeutic method was chosen randomly. The application of protocols in each group took about an hour. Psychotherapy applications in both groups were performed by a child and adolescent psychiatrist, who was trained in professional field and had field study permits. In addition, Informed Consent Form was signed by families and child-adolescents before therapeutic applications. Approval was obtained from Gaziantep University Ethics Committee for this study (Date: 02.04.2020, Number: 2020/135) In order to evaluate the effectiveness of treatment methods before and after the therapy, an instruction was given to children/adolescents such as "when you think about the earthquake moment, if you score the level of discomfort it creates in you, how many points do you give from 0 to 10 (0 is none … 10 is too much)?" The assessments before and after therapy were based on the clinical Global impression (CGI) and the subjective units distress (SUDS) scales. Children and adolescents whose psychological problems continue are referred to the child and adolescent psychiatry outpatient departments in Elazig for individual treatment.

Main Points:

- Individual psychotherapy might not be possible in such events where large masses are affected. Therefore, the role of psychosocial interventions in groups is very important.
- In this study, we applied the group protocols of the two most important psychotherapy models (EMDR and Cognitive Behavioral Therapy (CBT)) that can be used in traumatic events.
- We found that both protocols are effective in improving traumatic effects in children.
- EMDR is more effective than CBT for acute stress disorder after earthquake.

Clinical Global Impression Scale

CGI is a measurement tool that evaluates the severity of any disease as well as improvement of its symptoms. The clinician uses the scale, in the light of his knowledge and experience about the disease, on a Likert type rating ranging from 1 to 7 (1- normal, not patient, 2- borderline patient, 3- mild patient, 4- moderately ill, 5- prominently ill, 6- advanced, 7- most advanced) (11).

Subjective Units Distress Scale

SUDS is a qualitative assessment tool with a value within 0–10 to determine the subjective distress of clients in psychotherapies: "0" does not feel any distress; 10 indicates the highest level of distress felt. As the number increases, the level of distress felt increases as well (12).

Statistical Analysis

IBM SPSS Statistics 23 program was used for data analysis (IBM SPSS Corp.; Armonk, NY, USA). As descriptive statistics; median (min–max) values for continuous variables, frequency and percentages for qualitative variables were given. The normality of continuous variables was evaluated by Kolmogorov–Smirnov test. For group comparisons; Mann–Whitney U test was used in comparing continuous variables and chi-square test for qualitative variables. For pre-post comparisons within groups, Wilcoxon signed-rank test was used. P-values less than 0.05 was considered as statistically significant.

Eye Movement Desensitization and Reprocessing Integrative Group Treatment Protocol for Children [EMDR-IGTP] (13)

EMDR-IGTP was developed by the Mental Health Support Association in 1997 after Hurricane Pauline, in order to be implemented in the Mexico Crises. This model was first created by combining standard EMDR protocols developed by Francine Shapiro and group therapy protocols (14). It is suggested that EMDR-IGTP could be a treatment alternative in crises where there are many individuals who need to be reached in a short time (15). In our study, EMDR-IGTP for children was applied as a protocol consisting of eight stages in the place of the original protocol. The stages are listed below.

Stage 1, Client Story and Treatment Planning: At this stage, the therapist collected detailed medical and psychiatric history of children and adolescents who were victims of the earthquake, and organized treatment for them.

Stage 2, Preparation Stage: At this stage, the therapist explained trauma, PTSD, and their effects on child adolescents. Then, a warming technique was applied to attract the attention of the

group members and increase their harmony. In order to make it easier for them to recognize their own emotions, they were asked to imitate their "happy/sad/scared/surprised/angry" emojis, and to recognize these emotions by thinking about some of the events that they experienced in the past. Then, abdominal breathing technique was taught. Moreover, they were taught the Butterfly Hug technique (14). By means of the latter, individuals gained the ability to make two-way stimulation by themselves. The therapist then said "Close your eyes, move your hands diagonally to your shoulders and move them like a butterfly flapping. Breathe deeply and slowly while trying to recognize all the changes in your mind and body such as thoughts, images, sounds, smells, emotions, and sensations. You can be as comfortable as if you are moving above the clouds" and encouraged them to combine Butterfly Hugging with abdominal breathing. After these steps, he said, "Now, please close your eyes, and imagine you are in a place where you feel safe or calm." Then he gave the following instruction "draw this Safe/Quiet Place on the A4 paper in front of you." He made the Safe/Calm Place placement with the "Now focus on what you see in your Safe/Calm Place, smells, sounds, and do Butterfly Hugging 6–8 times slowly" instruction. It was stated that individuals with satisfactory feelings after the study could take the Safe/Calm Place study with them and implement whenever they want, while going to the next stage.

Stage 3, Evaluation: They were asked to divide an A4 sheet of paper by four. Each part was named with the letters A, B, C, D. In addition, they were asked to consider the worst part of the earthquake event they experienced in a similar way to the standard EMDR protocol. Then, they were asked to draw this part of the event on part A on A4 paper. They were then asked to look at the paper and score the distress felt between 0 and 10 (SUDS).

Stage 4, Desensitization: At this stage, participants were asked to drop their pens, and do Butterfly Hug for about 60 seconds, while looking at what they drew. After the Hug, the "Try to notice how you feel, and draw whatever you want to draw on part B" instruction was given. Moreover, they were asked to re-score their distress as they looked at their drawings (SUDS). Again, they were asked to drop their pens, and do Butterfly Hug for about 60 seconds. The same process was repeated for parts C and D. This stage was completed following scoring SUDS for the last time.

Stage 5, Vision for the future: At this stage, they were told to draw/write whatever they wanted about how they visualize themselves in the future. After the drawing was completed, placement was done with a Butterfly Hug.

Stage 6, Body scan: "Think about the event and scan your body thoroughly. If there is any discomfort, do a butterfly hug" instruction was given.

Stage 7, Closing: The following instruction was given: "Close your eyes, remember your safe/calm place and do a butterfly hug for about 60 seconds. Then, take three deep breaths and open your eyes."

Stage 8, Reassessment and follow-up: At the end of the group intervention, the therapist determined the children who needed more help. The therapist then directed participants in need for individual interventions.

Crisis Prevention Program for Children and Adolescents (CIPCA) (16)

This intervention was developed by Metin Health House for early intervention in children and adolescents with post-traumatic psychopathology. It was first used in children and adolescents who were forced to migrate, and started being used to prevent other post-traumatic psychopathologies. In this study, the questions were revised, in accordance to the original, for the earthquake. This method is a time and cost effective application. It can be applied with 10–30 children and adolescents, and each application takes an average of one hour. The group leader follows the crisis intervention guide and performs the application. The purpose of this method is to help children and adolescents play an active role in verbalizing their negative feelings and thoughts, and supporting them to create positive emotions instead. CIPCA is a semi-structured interview covering several models such as information, group therapy, cognitive reconstruction, systemic theory, attachment theory, salutogenesis, and post-traumatic growth. During the application, the group leader asked the children and adolescents 12 questions in the Crisis Expression Guideline and waits for them to express themselves. After the questions, those who still had a high level of distress were referred to child and adolescent psychiatry outpatient clinics in health institutions to undergo individual interventions.

RESULTS

We included 56 children in the study. There were 15 (53.6%) males and 13 (46.4%) females in each group. The average age of EMDR-IGTP group was 10.3 years (±2.02), and the average age of CIPCA group was 10.4 years (±2.09). There was no \significant difference between the groups in terms of gender and age (p=1.000, p=0.940, respectively). (Table 1)

There was no significant difference between SUDS levels across both groups before the therapy (p=0.485). When the changes within groups are examined, both methods provided a significant improvement in SUDS level of the children (p<0.001). The median level of SUDS of the EMDR-IGTP group after therapy was found to be 1 (min:0–max:10) and the CIPCA group was 5.5 (min:3–max:9). It was found that both applications decreased the SUDS level after the earthquake, and EMDR-IGTP decreased relatively more. Similarly, there was no significant difference between CGI severity of both groups before the therapy (p=0.831).

Table 1. Gender and age comparison of groups

		EMDR	CIPCA	p
Gender	Boy n (%)	15 (54%)	15 (54%)	1.000
	Girl n (%)	13 (46%)	13 (46%)	
Age*		10 (8–14)	10 (8–14)	0.940

*The data are shown as median(min–max).

Table 2. Comparison of pre– and post–therapy SUD and CGI scoring

	Pre–therapy	Post–therapy	P**
CGI scores			
EMDR	7 (3–7)	1 (1–7)	<0.001
CIPCA	7 (3–7)	4 (2–7)	<0.001
P*	0.831	<0.001	
SUD scores			
EMDR	10 (5–10)	1 (0–10)	<0.001
CIPCA	9 (5–10)	5.5 (3–9)	<0.001
P*	0.485	<0.001	

* Between–group comparison, **Within–group pre–post comparison
*** The data are shown as median(min–max).

Nevertheless, it was found that there was a significant difference between the two groups with respect to the CGI values. The improvement rate was higher in the EMDR-IGTP group. There was a significant decrease in CGI severity after both group underwent therapies (p<0.001). (Table 2)

DISCUSSION

In our study, EMDR-IGTP and CIPCA has been applied to children who survived the earthquake in Turkey's Elazig province. As a result, it was concluded that both groups benefited from these psychosocial interventions, and the level of improvement in the EMDR-IGTP group was higher than the other group.

In a study using CBT-based group intervention, CBT was shown to be more effective than the general supportive intervention and controls adolescents who lost their parents in the earthquake, in increasing psychological resistance, and decreasing the symptoms of PTSD and Depression (17). Our research supports these findings, as there was a significant decrease in SUDS level before and after treatment. CBT-based early intervention protocols have been used not only in natural disasters but also in war-related traumas. In a study with Congolese young people, Trauma Focused CBT group, a non-trauma based psychosocial intervention (Child Friendly Spaces) group and waiting list group were compared (18). In the pretest, post-test, and 6th month follow-up evaluation, both methods reduce depressive symptoms and behavioral problems better than the waiting list. Psychosocial interventions based on CBT are also effective in minimizing emotional problems (19). Although the effectiveness of CBT-based CIPCA used in our study has been shown in children suffering from war and migration, it has been used for the first time in earthquake-related trauma, and is thought to be effective in reducing trauma-related symptoms.

In our study, in the pre-post comparison of the group that applied EMDR-IGTP, the level of SUDS decreased significantly. Findings consistent with ours have been demonstrated in both field and pilot studies (15, 20). Despite similar basis, there are different group EMDR applications other than EMDR-IGTP. In another study conducted in Taiwanese adolescents, another EMDR-based group application was used and was shown to be effective (21). Proudlock et al. (22) suggest that EMDR could be an effective treatment for patients experiencing a mental health crisis having a trauma picture, resulting in significant improvements in their mental well-being and substantial cost savings for the National Health Service.

According to our findings, EMDR-IGTP application is more effective than CIPCA. In the meta-analysis conducted in 2017 including 36 studies, CBT, EMDR, narrative exposure therapy for children and school-based applications were compared. All therapy models are effective compared to controls and can be recommended as a psychosocial intervention after disaster. However, the methods with the highest effect size proved to be CBT (d=1.25), exposure therapy (d=1.56) and EMDR (d=2.15) (3). These results correspond exactly to our study. There are also studies with different results in terms of effect size. In a study conducted after the 1999 Athens earthquake, CBT-based group intervention was used and the effect size of the method was found to be 3.74. Differences between the effect sizes of the studies were affected from the professionalism and the level of education of the practitioner (23).

CONCLUSION

We encountered some limitations in our study. First, a structured questionnaire was not used and only the pre-post results were included. In addition, small sample size can be considered as a limitation. In order to make more accurate judgments about how much therapies improve functionality, long-term follow-up studies with larger sample sizes are required. In summary, both EMDR-IGTP and CIPCA are effective (but EMDR-IGTP is more effective) in reducing the acute stress problem in children and adolescents after the earthquake.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Date: 02.04.2020, Number: 2020/135).

Informed Consent: Informed consent was signed by families and child-adolescents before therapeutic applications.

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Original Research

Morphometric Analysis and Clinical Importance of Foramen Ovale

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ABSTRACT

Objective: This study aimed to reveal the length, width, area, and perimeter measurements of foramen ovale (FO) with excessive bone count and correlation status.

Methods: On basis cranii externa of crania, a metric scale was put in a place near to FO. Each FO was photographed with a metric scale, and length, width, area, and perimeter of FO were calculated by using the Image J software program.

Results: The average values of the length, width, area, and perimeter of the FO are 7.05±1.43 mm, 3.30±1.24 mm, 15.68±9.51 mm^2, and 20.89±6.16 mm on the right side, and 6.83±1.53 mm, 3.30±1.34 mm, 15.61±8.73 mm2, and 21.00±6.41 mm on the left side, respectively.

Conclusion: The fact that the number of measured bones in this study is quite high and the perimeter and area measurement parameters in the study can make this study unique compared with other studies. Thus, this study can shed light on clinical studies.

Keywords: Basis cranii, foramen ovale, morphometry, percutaneous approach, photogrammetric measurement.

INTRODUCTION

Foramen ovale (FO) is a large opening that localizes posteromedially in the sphenoid bone (1) and it transmits the mandibular nerve, the accessory meningeal artery, a lesser petrosal nerve, and an emissary vein. It also connects the infratemporal fossa and the middle cranial fossa (2, 3).

Length of the FO was found to be about 3.85 mm in the new born and 7.2 mm in adults in the study of Yanagi (4). Morphometric analysis of the FO is mentioned in many studies worldwide for why anatomical variations in dimensions and shape of the FO are important for surgeons, radiologists, and anatomists (2, 5).

Abnormal position of the FO also has great importance. Skrzat et al. (6) mentioned that an atypical location of the FO and neighboring osseous structures may influence the anatomical organization of the nerves, which run through this hole. Especially, mandibular nerve and its divisions (lingual and inferior alveolar nerves) may have an abnormal course. That is why, it may be possible for the nerves to become entrapped or compressed between the osseous structures and muscles, causing neuralgia (6).

In this study, length, width, area, and perimeter of the FO were analyzed using the Image J software program. Besides this, an asymmetrical location between the right and left sides of the holes was found. Not many studies mention these parameters that are analyzed by the Image J software program. In addition, perimeter analysis of the FO is not usual data that are studied before. It is thought that the data obtained in this study will contribute more to the literature since it is made in a large bone population.

METHODS

This study was carried out in compliance with the Helsinki Declaration, and Ethics Committee approvals were obtained where appropriate. In this study, FO was examined in 100 cranium and 33 basis cranii of the Anatomy Department of Ege University Medicine Faculty. In norma basilaris regions of crania, a metric scale was put in a place near to FO. Then, each FO was photographed with a camera (Nikon coolpix P610; 60´ wide optical zoom ED VR, 4.3–258 mm, f: 3.3–8.2). The photographs have been uploaded to the software program Image J, and metric calibration of each photograph has been achieved. After calibration, the length (FOL), width (FOW), area (FOA), and perimeter (FOP) of each FO were measured. After the measurements, the correlation status of these parameters with each other and the comparison of the right and left sides with each other were calculated with the SPSS (version 20.0) statistical package program (IBM SPSS Corp.; Armonk, NY, USA). In addition, the symmetry and asymmetry of the hole on the right and left sides were analyzed.

RESULTS

The average values of the FOL of the FO are 7.05±1.43 mm (max/min: 11.29/3.34 mm) on the right side and 6.83±1.53 mm (max/

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Table 1. Mean, Standart deviations (SD), t and p values for foramen ovale parameters

parameters	Side	N	Mean	Std. Deviation (SD)	t	p
FOL	right	133	7.05	1.43	–0.06	0.94
	left	133	6.83	1.53		
FOW	right	133	3.30	1.24	–0.14	0.88
	left	133	3.30	1.34		
FOA	right	133	15.68	9.51	1.21	0.22
	left	133	15.61	8.73		
FOP	right	133	20.89	6.16	0.01	0.99
	left	133	21.00	6.41		

FOL: Length of foramen ovale, FOW: Width of foramen ovale, FOA: Area of foremen ovale, FOP: Perimeter of foramen ovale.

min: 10.78/2.91 mm) on the left side. The average values of the FOW of the FO are 3.30±1.24 mm (max/min: 9.96–0.67mm) on the right side and 3.30±1.34 mm (max/min: 6.82/0.66 mm) on the left side. The average values of the FOA of the FO are 15.68±9.51 mm^2 (max/min: 48.12/0.91 mm^2) on the right side and 15.61±8.73 mm^2 (max/min: 47.25/0.8 mm^2) on the left side. The average values of the FOP of the FO are 20.89±6.16 mm (max/min: 48.07/5.24 mm) on the right side and 21.00±6.41 mm (max/min: 47.82/6.34 mm) on the left side. The test used for normality analysis is "Kolmogrov Smirnov". According to this test, only the mean length of the right side and the mean width of the right side (FOWR) fit the normal distribution. The mean area of the right sides (FOAR) and the mean perimeter of the right sides (FOPR), whose Skewness and Kurtosis are between –2 and +2, are considered to be in accordance with the normal distribution, and an independent sample t-test, which is a parametric test, was used. According to the t-test of the FO on the right and left sides, the "p" values of FOL, FOW, FOA, and FOP measurements are given in Table 1. According to the resulting "p" values, there is no significance between the right and left sides' values (p> 0.05). In both right and left sides, correlation status of the parameters (FOA, FOP, FOL, and FOW) with each other was also examined. According to Table 2, the weakest correlation is between the average values of the FOWR and the average values of the length of the left side. Although there is a weak correlation between FOWR and FOPL (mean perimeter of the left side), as shown in Table 2, it may not be considered because the "p" value is not significant. There is a strong correlation between the average values of the FOAR and the average values of the FOPR. In the comparison of right and left sides of the skull base regions, "73" FOs were found to be asymmetrical.

Main Points:

- In this study, the morphometry of the oval foramen was investigated using the Image J software program.
- The high number of bones used in the study may present meaningful data to the literature in terms of the results.
- In contrast with other studies in the literature, the circumference measurement (20.89±6.16 mm on the right sides and 21.00±6.41 mm on the left sides) of the oval foramen was examined, and the correlation of this measurement with the area measurement (15.68±9.51 mm^2 on right sides and 15.61±8.73 mm^2 on left sides) was observed.
- These parameters can be useful for percutaneous interventions planned to the foramen ovale.

DISCUSSION

Anatomical features of FO have great importance for surgeons who are interested in FO and related structures. Size and shape of FO are variable in each individual (7). In some cases, pterygospinous and ptergoalar ligaments may localize around the FO. In such conditions, mandibular nerve and its branches can be compressed by these bars and lead to trigeminal neuralgia (TN). The presence of these bars around the FO complicates trigeminal ganglion block by the transovale approach (8, 9).

TN is the painful condition of the face and is the most common occurrence of craniofacial neuralgias (10). Many anatomical and radiological studies have been performed to research relationship between the skull foramens and the incidence of the TN (11, 12). Liu et al. investigated narrow FO and its role in etiology of the TN. They studied the size of FO in patients with pain and patients with nonpain. The results were statistically significant. They suggested that narrow FO may be etiologically important in a small percentage of TN patients (13).

In this study, we studied short and long diameters, area, and perimeter of the FO.

It was observed that FO localized asymmetrically in 73 skull base. The study closest to this study in terms of length and width measurements was performed by Osunwoke et al. (11) (Table 3). There are a number of studies on FO in the literature, but there are almost none, including both area and perimeter measurements of FO with their correlation status. This study is similar in terms of area measurement and correlation values by Somesh et al. In the study by Somesh et al., area measurements are more than those in this study. When the study is examined, FOs are considered as ellipse and short and long diameters of the hole

Table 2. The pearson correlation coefficient 'r' and 'p' value of the continuous variables

Parameters	'r' and 'p' values	FOAR	FOAL	FOPR	FOPL	FOLR	FOLL	FOWR	FOWL
FOAR	r	–	.702	.739	.517	.565	.462	.530	.424
	p	–	.000	.000	.000	.000	.000	.000	.000
FOAL	r	.702	–	.563	.656	.412	.603	.252	.543
	p	.000	–	.000	.000	.000	.000	.003	.000
FOPR	r	.739	.563	–	.591	.511	.367	.408	.314
	p	.000	.000	–	.000	.000	.000	.000	.000
FOPL	r	.517	.656	.591	–	.244	.379	.123	.295
	p	.000	.000	.000	–	.004	.000	.152	.000
FOLR	r	.565	.412	.511	.244	–	.528	.505	.347
	p	.000	.000	.000	.004	–	.000	.000	.000
FOLL	r	.462	.603	.367	.379	.528	–	.190	.544
	p	.000	.000	.000	.000	.000	–	.025	.000
FOWR	r	.530	.252	.408	.123	.505	.190	–	.450
	p	.000	.003	.000	.152	.000	.025	–	.000
FOWL	r	.424	.543	.314	.295	.347	.544	.450	–
	p	.000	.000	.000	.000	.000	.000	.000	–

FOAR: area of right side, FOAL: area of left side, FOPR: perimeter of right side, FOPL: perimeter of left side, FOLR: length of right side, FOLL: length of left side, FOWR: width of right side, FOWL: width of left side.

Table 3. The table shows the morphometric findings of the length and width of the foramen ovale of each author

	Mean length (mm)		Mean width (mm)	
Study/Populationn (skull)	right	left	right	left
Osunwoke et al[25] (2009), n=87	7.01	6.89	3.64	3.60
Somesh et al[3] (2011), n=82	7.56	7.64	5.24	5.12
Khan et al[26] (2012), n=25	7.46	7.01	3.21	3.29
Wadhwa et al[27] (2012), n=30	6.50	6.80	3.70	4.00
Desai et al[20] (2012), n=125	8.14	7.98	5.26	5.88
Gupta et al[28] (2013), n=35	7.22	6.48	3.57	3.50
Patil et al[29] (2013), n=52	7.00	6.80	5.00	4.70
Patel et al[30] (2014), n=100	6.60	6.50	3.60	3.50
Ahmed et al[31] (2015), n=100	5.25	4.84	4.87	5.18
Rao et al[21] (2017), n=50	7.24	7.11	3.75	3.75
Bokhari et al[2] (2017), n=55	7.04	7.18	4.15	3.99
Current study (2020), n=133	7.05	6.83	3.30	3.30

are taken into account instead of radius (3). In this study, area and perimeter were calculated with the Image J software program photogrammetrically with the help of metric scale.

This study is ahead in terms of the number of measurements. In addition, the perimeter measurement of FO in this study may bring innovation according to other studies in the literature.

Considering that the area measurement takes place in an almost nonexistent literature, this study is quite up to date and different, including both area and perimeter measurements of the FO and comparing them with the correlation graph.

In recent years, studies on the controlled percutaneous approach to the oval FO have started to increase (14, 15). The major risk associated with the percutaneous approach is the serious complexity that can occur in neighboring structures (16, 17). In this regard, it may be beneficial for morphometric anatomical studies to support percutaneous approaches. Therefore, it is predicted that this study carries data that can be taken into account in the percutaneous approaches planned in the FO.

Information about morphology and morphometry of FO is essential for various invasive surgical and diagnostic procedures such as electroencephalographic analysis of the seizure for patients undergoing selective amygdalohippocampectomy, microvascular decompression by percutaneous trigeminal rhizotomy for TN, and percutaneous biopsy of cavernous sinus tumors (3, 18-21). Not only the morphological structure of the FO but also the t region of the hole with its adjacent anatomical structures is important (22-24). The distance of FO with its neighboring structures can also be considered in other studies and may carry information guiding percutaneous interventions.

CONCLUSION

According to other studies in the literature, the number of bones in this study is quite high, and at the same time, perimeter measurement is included in this study, unlike other publications. The high number of bones in this study and the use of photogrammetric measurement as a method can make the study take an important place in the literature.

Limitations

Difficulties in knowing the characteristics of dry bones such as age, gender, or race have been reported in the literature (32). The limitation of this study is that information about the age, sex, and race the examined bones is not known.

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Original Research

The Cytotoxic Effect of *Polygonium cognatum* and Chemotherapeutic Effect of Doxorubicin on Glioblastoma Cells

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ABSTRACT

Objective: Glioblastoma multiforme (GBM) is an aggressive malignant brain tumor common in adults. Owing to the present difficulty in treating GBM, developing alternative methods is of utmost importance. Recently, the efficacy of various plant extracts in cancer treatment have been evaluated. *Polygonum cognatum (P. cognatum)* known as 'Madımak' is used in herbal medicine in Turkey.

Methods: In this study, we investigated the cytotoxity of *P. cognatum* in the treatment of glioblastoma and its contribution to the effectiveness of doxorubicin (DXR). In the U87 cell line of the *P. cognatum* and doxorubicin administered at different doses, IC50 doses were determined using the 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) method and the effects of combined administration at these doses were examined (respectively 10–125 µg/ml and 0.1–10 µg/ml at 24, 48, and 72 h).

Results: *P. cognatum* extract decreased the cell viability of U87 cells in a time and concentration-dependent manner. It also increased the apoptotic effectiveness of DXR in U87 cells.

Conclusion: This is the first preliminary study that investigates the treatment of *P. cognatum* on glioblastoma *in vitro*. Further studies are required to investigate the effect of the extract on healthy human cells and to understand signaling pathways.

Keywords: Glioblastoma, *P. cognatum*, doxorubicin

INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most common type of brain tumor with an aggressive progression. It is noted in two to three incidents per million adults per year (1). GBM is a lethal human tumor, with a current standard-of-care therapy involving surgical resection, radiotherapy, and chemotherapy (2). The survival time after usual treatment ranges from 12 to 15 months. This is due to the limited ability of the many drugs to penetrate the blood-brain barrier. For this reason, more studies on binding the drugs on nanoparticles have been undertaken (3). However, no satisfactory evidence have been encountered.

Drugs such as vincristine, vinorelbine, vinblastindocetaxel, etoposide, paclitaxel, camptothecin, topotecan, doxorubicin (DXR) and irinotecan are used in the treatment of tumors. The basic feature of these drugs are low safety levels and many side effects (4). DXR is an anthracycline anti-cancer drug that can be used for many malignancies such as lung, breast, ovaries and multiple myeloma (5). These chemotherapeutic agents induce cell death via multiple mechanisms such as blocking nucleic acid synthesis by preventing topoisomerase II activity (6). Clinical studies involving systemic administration of the drug show that its effectiveness in the treatment of gliomas is limited (7). Very high doses of DXR are required to achieve therapeutic levels, and these doses are highly neurotoxic. This is due to its low ability to traverse the blood-brain barrier (8). In recent years, many studies to improve the efficacy of DXR in GBM are ongoing, thereby employing techniques such as local drug delivery to the tumor resection site or using DXR-loaded nanomicelles (9).

Complementary and alternative treatments commonly used in GBM include vitamin and nutritional supplements, and herbal extracts. Some studies show that treatment with herbal extracts can reduce mortality in GBM (10, 11). In order to increase the

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effectiveness of anti-cancer therapy, new approaches are being investigated for the effect of plants on cancer cells (12).

Polygonum cognatum (*P. cognatum*, Madımak) is a member of the *Polygonaceae* and is a perennial plant found in Turkey (13). Previous studies on *P. cognatum* have reported that it contains vitamin C and carotenoids. It also has antioxidant, antimicrobial, diuretic, and antidiabetic properties. It is known to be used for the treatment of various diseases for medical purposes (14).

In this study, we aimed to investigate the cytotoxic effect of *P. cognatum* (collected in the Tokat region of Turkey) on GBM cells. Moreover, we sought to investigate the combined effect of DXR and *P. cognatum* on cell proliferation in the U87 cell line.

METHODS

Plant materials

We obtained *P. cognatum* plants from the wild flora at an altitude of 800 m (40.322796 latitude 36.059895 longitude of Tokat) in North Anatolia. Plant materials were identified by Botanical and Herbarium Application and Research Center. The herbarium specimen of *P. cognatum* (EGE 43193) have been deposited at the Herbarium of Faculty of Science, Ege University.

Preparation of methanol extraction of *P. cognatum*

We crushed the samples leaves with a blender. Then, we dissolved 5 g of powder in 50 mL of methanol for 24 h while shaking intermittently. We collected the extract using a Whatman filter paper. Then, the filtrate was dried with a rotary evaporator at 80°C under reduced pressure and this procedure was repeated three times. The filtrate obtained was stored at –20°C (15). Extracts were dissolved in dimethylsulfoxide (DMSO) to prepare stock solutions as a final concentration under 0.1%. Then, we further diluted the extract in culture medium with four different concentrations.

Cell culture of U87 cell line

Human GBM cell line (U87) was obtained from Leibniz-Institut DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 1 µg/mL penicillin/streptomycin and 2 mM L-glutamine in 5% CO_2 saturated incubator at 37 °C. The cell medium was changed every two days when they attained enough confluence.

Cell treatment

U87 cells were seeded in 96-well plates at a density of 5,000 cells per well in 100 µL growth medium. Cells were incubated overnight before been treated with the extract and DXR. U87 cells were treated with various doses (10, 50, 100, 125 µg/mL, and 0.1, 0.2, 0.5, 1, 5, 10 µg/mL, respectively) of *P. cognatum* extract and DXR (Adrimisin, Saba Ilac, Istanbul, 2020) in triplicates. Moreover, cells were incubated for 24 h, 48 h, and 72 h and then analyzed. Median inhibitory concentration (IC50) was estimated using Microsoft excel version 14.0.4. After the IC50 dose was determined, the plant extract and DXR were applied in combination at these doses and analyzed again.

Main Points:

- *P. cognatum* extract has anti-carcinogenic effect on the U87 cell line.
- *P. cognatum* extract had a decreasing effect on cell viability on the U87 cell line.
- *P. cognatum* extract may increase the effect of DXR on the U87 cell line.

Cell viability assay (XTT)

The anti-proliferative activity of *P. cognatum* and DXR was evaluated using a commercially available proliferation kit XTT (Biological Industries, Israel). XTT reagent solution and the activation solution was prepared with a reaction solution sufficient for one plate, followed by the addition of 0.1 mL activation solution to 5 mL XTT reagent. We then added 50 µL of the reaction solution to each well and the plate was incubated at 37°C in 5% CO_2 for 4 h. After incubation, the plate was swirled gently and the absorbance of the samples was measured using a spectrophotometer (ELISA reader PG Instruments) at a wavelength of 450 and 630 nm. DMEM (0.1% DMSO) was used as a negative control.

Statistical Analysis

We performed overall analyses using SPSS (Statistical package for social science for Windows, version 25.0, IBM SPSS Corp.; Armonk, NY). According to the results of ANOVA, we observed that the groups in which DXR, *P. cognatum* and both were administered in 48 hours were significantly different from the untreated controls ($p<0.05$).

RESULTS

Extraction of the plant material

P. cognatum was extracted with methanol, as illustrated above. Plant materials were dissolved in DMSO (Sigma-Aldrich). The final concentration of DMSO in cell lines was less than 0.1% to prevent any possible effect on the cytotoxicity levels.

Cytotoxiticity

Here, we investigated effect of *P. cognatum* extract on the cell viability of U87 cells. For this aim, we treated U87 cells with increasing concentrations (10–125 µg/mL) of *P. cognatum* extract for 24, 48, and 72 h. We evaluated the *in vitro* cytotoxic effect of *P.cognatum* methanolic extracts on U87 cell lines using the XTT assay. According to the assay, *P. cognatum* extract decreased the cell viability of U87 cells in a time and concentration-dependent manner, as compared to untreated controls. The most effective time for this was 48 hours.

As shown in Fig. 1A, decreases in the cell viability of U87 cells exposed to 10, 50, 100, and 125 µg/mL of the extract were 72.96%, 68.62%, 48.82% and 33.43% respectively, as compared to untreated controls at 48 h ($p<0.05$). The IC50 values was determined at 100 µg/mL for the methanolic extract of *P. cognatum* at 48 h for U87 cells.

Figure 1. a, b. Cell viability percentages of U87 cells after application of *P. cognatum* at different concentrations analyzed by XTT (a), cell viability percentages of U87 cells after application of DXR at different concentrations analyzed by XTT (b)

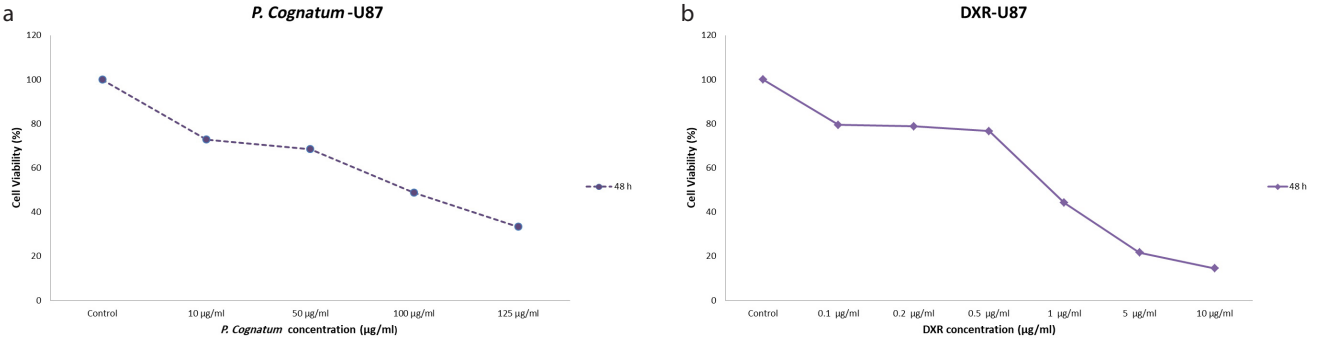
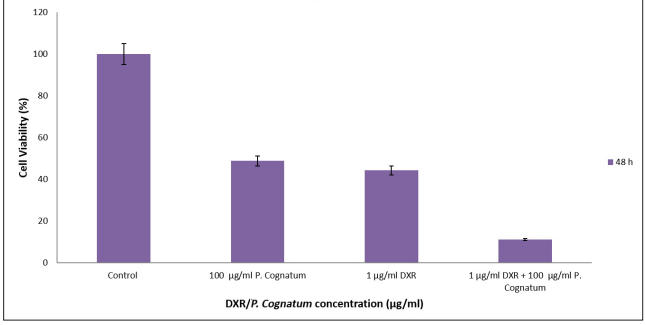


Figure 2. Percentage of cell viability of U87 cells induced by combination of DXR-*P. cognatum* IC50 concentrations, 48 h analyzed by XTT



Furthermore, we evaluated *in vitro* cytotoxicity activity of DXR on U87 cell line using the XTT assay and the results are given in Figure 1B. Results showed that DXR extract has a significant anti-cancer effect on glioblastoma cell in a concentration-dependent manner. DXR also exhibited a time-dependent inhibition of cell proliferation (Fig. 1B).

While U87 cells were exposed to 0.1, 0.2, 0.5, 1, 5, 10 µg/mL of extract, the cell viability for 48 h were 79.51%, 78.93%, 76.67%, 44.25%, 21.70% and 14.55% respectively, The IC50 values of DXR was 1 µg/mL.

While 100 µg/ml *P. cognatum* treatment for 48 h showed a 51.18 % cell death, 1 µg/mL DXR treatment showed a 55.75 % cell death. However, the combined treatment of 100 µg/mL *P. cognatum* and 1 µg/mL DXR for 48 h showed an 88.85% cell death (Fig. 2). This proves that a combined treatment of methanolic extract of *P. cognatum* and DXR inhibits U87 cell proliferation. Thus, *P. cognatum* increases the anti-cancer effect of DXR (Fig 2).

DISCUSSION

Heterogeneity, high motility of cell types, and ability to switch in proliferative/non-proliferative phases render the treatment of GBM very difficult (16). Radiotherapy and temozolomide (TMZ) are used in the treatment of GBM (17). However, TMZ resistance and unresponsiveness can be developed by some patients (18, 19). It was hypothesized that apoptosis-regulating genes and proteins such as *p53*, *p21*, *p16*, and *PTEN* are influenced by dysregulation (20). For these reasons, alternative treatments need to be developed.

One of the promising drugs in the treatment of GBM is DXR. DXR is known to inhibit biosynthesis through intercalation. It prevents the progression of the topoisomerase II enzyme, which enables the unfolding of DNA during transcription, stabilizes the topoisomerase-DNA complex during DNA duplication, thereby preventing the recombination of the DNA double helix (21). DXR cannot be applied systemically against GBM because it cannot reach therapeutic levels in the brain (8). Studies showed that local DXR application can prolong median survival and delay tumor growth, though complete remission cannot be achieved (22). TMZ and DXR are known to induce DNA damage by different mechanisms. A study hypothesized that TMZ-resistant GBM cells will remain sensitive to the cytotoxic effects of DXR. From other preliminary studies, DXR was shown to be an effective cytotoxic agent in TMZ-resistant GBM cells (23).

In recent studies, synergistic effects have been investigated with different plants in order to decrease the resistance of cancer cells to DXR (24). The combined use of different plants with DXR proves to increase its chemotherapeutic effects or reduce the resistance to DXR (25-27).

P. cognatum is used for adjuvant medicinal purposes in Turkey (28), reason why it is gaining grounds in research. An attempt was made to demonstrate its anti-cancer activity on different cell lines and direct them to define its content. There are studies showing that its extract has significant inhibitory effects on breast cancer. Eruygur et al. (29) showed that this extract prepared with ethanol is effective in MDA-MB-231 breast cancer cell line. Their results show that it has significant anti-cancer effects. Moreover, Sarac et al. (28) stated that *P. cognatum* decreased cell viability on the MCF-7 breast cancer cell line, with a little effect on the human umbilical vein endothelial cell line (HUVEC). Furthermore, Pekdemir et al. (30) stated that the methanol, acetone, and hexane extracts of the *P. cognatum Meissn* plant showed a significant decrease in cell viability of the MCF-7 cancer cell line compared to control, but not all doses of the ethanol extract

caused a significant decrease in cell viability. In addition, the plant is the most effective on MKN-45 gastric cancer cell line based on previous data and IC50 values.

This study supports the hypothesis that *P. cognatum* has anti-carcinogenic properties. Our study showed that the plant extract is effective in the U87 cell line and possibly potentiates the effectiveness of DXR in cancer treatment. Thus, we presume that it is beneficial to combine DXR with plants known to have anti-cancer effects in order to increase effectiveness against GBM. Previous reports also show that the combined application of the methanol extract of *P.cognatum* decrease the toxicity of DXR on GBM cells via decreasing IC50.

CONCLUSION

Methanolic extract of *P. cognatum* and DXR was tested for their potential anti-proliferative effect on the U87 cell line. The results showed that *P. cognatum* may have potential could be considered as alternative therapy in the future. In addition, it is presumed that *P. cognatum* increases the effect of DXR. Therefore, the results obtained in the present study form a basis for future clinical studies. The anti-cancer effect of the plant should be studied in detail as well as its mechanisms and signaling pathways.

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Informed Consent: N/A

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Original Research

Investigation of the Coeliac Trunk Morphometry with Multidetector Computed Tomography Angiography

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ABSTRACT

Objective: Accurate knowledge of vascular anomalies is critical in surgical interventions, radiology, and organ transplantation procedures. Vascular variations during these procedures can cause serious complications. This study aimed to evaluate the coeliac trunk (CT) and its branches morphometrically, to examine possible variations with multidetector computed tomographic angiography (MDCTA), and to compare the obtained data with the findings in the literature.

Methods: In this study, abdominal MDCTA images of 126 people taken between April 2014 and April 2016 at Necmettin Erbakan University University Meram Medical Faculty Hospital were analyzed retrospectively. Variation and morphometric analysis of CT and its branches were performed. In the morphometric analysis, diameter measurements were made in centimeters (cm) and compared in terms of sex of the patients. Variation analysis was performed per a useful and simple classification we developed through a comprehensive literature review.

Results: Diameter measurements of CT (0.73±0.13, p=0.002), splenic artery (0.69±0.1, p=0.0004), common hepatic artery (0.66±0.1, p=0.042), and left gastric artery (0.27±0.11, p=0.0001) were statistically significant in men than in women (p<0.05). In our study, type I (normal trifurcation pattern - complete) was detected in 111 (88.09%) cases, and variation was detected in 15 (11.91%) cases. The distribution of these variations is from the most common to the least; type II- (bifurcation-incomplete) 8 (6.34%), type V (additional branches) 5 (3.96%), type IV (coeliomesenteric trunk) 1 (0.79%) and 1 (0.79%) unidentified case. No type III (no CT) variation was found.

Conclusion: Variations and anatomy of CT and its branches should always be taken into consideration in clinical studies, angiographic methods, and surgical interventions against possible complications.

Keywords: Anatomic variation, celiac artery, hepatic artery, multidetector computed tomography

INTRODUCTION

Vascular structure is the result of a complex biological process that is genetically programmed and controlled. Various triggers during embryological development can cause anomalies that are often seen as abnormalities (1). However, accurately identifying, understanding, and interpreting the normal arterial pathway and possible changes are of paramount importance before any external intervention. In addition, some anatomical variations may have a negative effect on blood reaching the target organ or tissue (2).

The coeliac trunk (CT) emerges as the first anterior branch of the abdominal aorta (AA) just below the aortic hiatus at the level of the intervertebral disc between the T12 and L1 vertebrae. From this root, a 1.5–2 cm length of the left gastric artery (LGA), common hepatic artery (CHA) and splenic artery (SA) branches are separated (3). Since these 3 branches were first defined by Haller, they are also referred to as "Haller Tripus," and their classical structure is accepted as such (4).

Previous research has shown that CT anatomy is not the same for all humans, and approximately 15% of the population may differ significantly from the typical branching pattern. Anatomical variations of CT are not uncommon; therefore, knowledge of the variations is mandatory for planning and conducting interventional radiology or surgical procedures. Knowledge of the anatomical structure and variations in CT is very important for liver transplants, appropriate vascular ligation or anastomosis, surgeries in the pancreatic head, and radiological procedures (5).

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Previous studies have generally employed the cadaver dissection method (4, 6-8). In recent studies, radiological methods such as multidetector computed tomographic angiography (MDCTA), spiral CT, and digital subtraction angiography are more common (5, 9-23). However, new technological developments have made MDCTA an extremely sensitive and valuable imaging method in the evaluation of vascular anatomy and pathology. MDCTA minimizes artefacts that may occur and is minimally invasive as it allows the trunk to be spirally scanned with high-contrast resolution in only 1 breath holding time. It provides a very good anatomical orientation with axial and 3-dimensional (3D) images (10, 13, 24).

Thus, detailed information about vascular structures, organs, and their interrelationships can be obtained. Angiographic information required to prevent complications that may occur owing to vascular variations during surgical or interventional procedures and determine the treatment protocol can be easily provided by MDCTA (9, 12). This study aimed to examine the CT vascular pattern according to a simple and useful classification and to perform its morphometric analysis using the MDCTA method.

METHODS

Patients

The study was carried out in Necmettin Erbakan University Meram medical faculty hospital. Images of patients who requested an abdominal MDCTA in the Department of Radiology for various reasons between April 2014 and May 2016 were retrospectively analyzed. The necessary permissions for the study were taken from Gaziantep University clinical research ethics committee with the protocol number #2016/210. A total of 250 patient images were examined. Of these images, 126 (60 women, 66 men) suitable for our study were selected, and CT was evaluated morphometrically and in terms of variation.

Exclusion Criteria

Patients with: a) a history of major upper abdominal surgery, b) pathological conditions that may affect normal vascular anatomy such as hepatic segmentectomy or CT aneurysm, and c) technically inadequate CT examinations were excluded from the study.

Main Points:

- Knowledge of vascular anomalies is of great importance in modern surgery, radiology, and organ transplant procedures. Vascular variations during these procedures can cause serious complications.
- MDCTA is an excellent imaging technique. It is a fast, noninvasive tool that provides highly accurate and detailed evaluations of normal vascular anatomy and variants.
- Knowing the diameter and length of CT and its branches, anatomical structure, and variations are vital for liver transplants, placement of arterial stents, appropriate vascular ligation and anastomosis, as well as surgical and radiological procedures in caput pancreatis.

MDCTA Protocol

In the study, images obtained with a 64-channel MDCT (Siemens Somatom Sensation 64, Earlanger, Germany, 2005) device were used. The patients were placed in the supine position, and 22 Gauge branules were inserted into their visible veins in the antecubital region, and a total of 100 cc intravenous iodinated contrast material was administered at 3–4 cc per second. Following this procedure, a cranio-caudal scan was performed in the axial plane containing the abdominal area (from diaphragm to regio pubica) using the bolus technique for timing. Images were taken in the portal phase (60–65 seconds after the initiation of the contrast agent) with the following parameter settings; 120 kV, 86 mAs, effective mAs 50–170, detector area 1.2 mm, section thickness 1.5 mm, pitch 1.4, rotation speed 0.5 sec.

CT Morphometric Measurements

Transverse diameter measurements of AA, CT, LGA, CHA, and SA at the level of CT in axial reformatted and Inspace (multiplanar) images and CT root length measurement were performed by the researcher with the support of a radiologist. The root length was measured as the distance from AA to the part with bifurcation or trifurcation was measured (Figure 1).

CT Variation Analysis

CT branches were detected in the coronal, sagittal, and axial planes with the Inspace imaging technique, and possible variations were evaluated. Images in which a variation was detected were categorized according to a new classification we developed after a thorough examination of the literature (Table 1) (18, 25).

Type I: This type is accepted as the normal branching pattern of CT. Classically, all forms that include LGA, CHA, and SA and arising from a single root correspond to this type. This pattern has 2 subforms, type Ia and type Ib. Type Ia is the form in which LGA, CHA, and SA arise from a common point on the same trunk. Type Ib is the form in which LGA arises from CT as the first branch, and CHA and SA make a bifurcation at a common point from CT (Table 1).

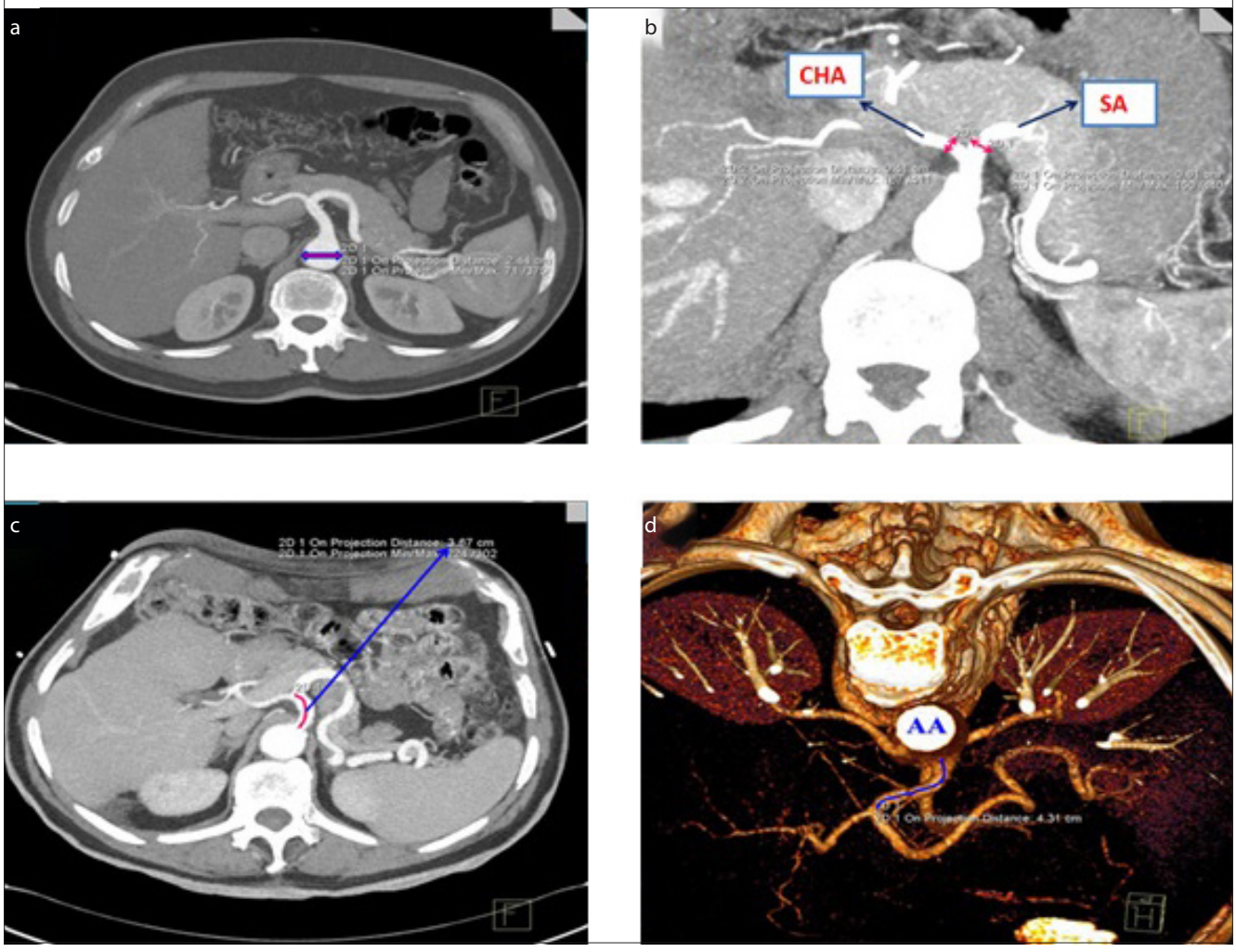
Type II: This pattern can be defined as incomplete CT, where 2 of the 3 classical branches arise from a common point by making a bifurcation, and the third branch arises from AA or another artery. This pattern has 3 sub-forms; type IIa, type IIb, and type IIc (Table 1).

Type IIa is also called the hepatosplenic trunk. Although CHA and SA arise from a common trunk, LGA arises from a place outside this trunk. Type IIb is also called the gastrosplenic trunk, where SA and LGA arise from a common trunk, and CHA arises from a place outside this trunk. Type IIc is also called hepatogastric trunk, where CHA and LGA arise from a common trunk, and SA arises from a place outside this trunk.

Type III: It is the pattern in which CT never occurs. None of the 3 classical branches separate from a common trunk. It is the pattern in which all of them independently arise from AA (Table 1).

Type IV: In this type, the superior mesenteric artery (SMA) and CT arise from a common trunk, also called the coeliomesenteric trunk (CMT) (Table 1).

Figure 1. a-d. Coeliac trunk (CT) morphometric measurements. (a) Transverse diameter measurement of abdominal aorta in axial reformatted image at the CT level. (b) Common hepatic artery and splenic artery transverse diameter measurements on axial reformatted images. (c) Measurement of CT root length on axial reformatted images. (d) Measurement of CT root length by Inspace imaging technique.



Type V: This pattern is the quadrifurcation form in which any 4th branch, in addition to the LGA, CHA, and SA branches and other than the ventral main branches, arises from the main trunk. Some do not have CHA and instead have left hepatic artery (LHA), right hepatic artery (RHA) arising from the main trunk.

Variations other than types I, II, III, IV, and V that could not be categorized in any class were classified as "other."

Image Interpretation and Statistical Analysis

Axial images were transferred to the workstation (Leonardo, Siemens, 3D and Inspace programs, Germany) for processing in 3D maximal intensity projection, multiplanar reformation, and volume rendering technique (VRT) format by multiplanar imaging method. VRT images were acquired with the Inspace software. The data obtained by radiological methods were analyzed with the statistical analyses were performed using the the Statistical Package for Social Sciences version 15.0 software for Windows (SPSS Inc.; Chicago, IL, USA). and compared according to sex by the independent samples t test. Summary of data are expressed as mean, standard deviation, and percentage.

RESULTS

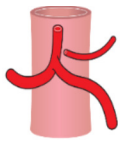
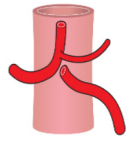
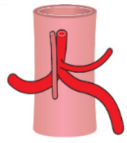
Morphometric Findings

The mean age was 62.5±11.75 (40–84) years in men and 60±10.1 (40–83) years in women. Morphometric measurements are given in Table 2 and Graphic 1. In our morphometric evaluation, AA (p=0.000041), CT (p=0.002), SA (p=0.0004), CHA (p=0.042), and LGA (p=0.0001) transverse diameter measurements were found to be statistically significantly larger in men than in women (p<0.05). No significant difference was found between CT root lengths (p=0.067) in terms of sex (p>0.05).

Variation Results

Type I: In our study, this type was found in 111 (88.09%) patients. This pattern was the most common type; 2 sub-forms of this pattern, type Ia and type Ib, were also observed in our study. Type Ia

Table 1. CT classification used in this study

Types		Subtypes		
Type I	Complete CT. LGA, CHA and SA present (trifurcation)	Type Ia (LGA, CHA and SA are arised from common origin) 	Type Ib (LGA is arised as the first branch) 	
Type II	Incomplete CT (bifurcation)	Type IIa (Hepatosplenic trunk) 	Type IIb (Gastrosplenic trunk) 	Type IIc (Hepatogastric trunk) 
Type III	Absence of CT (LGA, CHA and SA arise from the AA)			
Type IV	Coeliomesenteric trunk (CT+SMA)			
Type V	Additional branc other than LGA, CHA and SA (quadrifurcation)			

CT- Coeliac trunk, LGA-left gastric artery, CHA-common hepatic artery, SA-splenic artery, SMA- superior mesenteric artery AA-abdominal aorta

Table 2. Comparison of AA, CT, SA, CHA, LGA transverse diameters and CT length by gender (mean ± standard deviation, cm)

Measurements	Male	Female	Mean±SD	p
AA diameter	2.58±0.37	2.25±0.41	2.41±0.42	0.000041
CT diameter	0.77±0.14	0.68±0.11	0.73±0.13	0.002
SA diameter	0.62±0.13	0.53±0.10	0.57±0.12	0.0004
CHA diameter	0.47±0.10	0.42±0.11	0.45±0.11	0.042
LGA diameter	0.3±0.07	0.24±0.56	0.27±0.11	0.0001
CT length	3.2±0.75	2.9±0.66	3.12±0.71	0.067

SD: Standard Deviation

was found in 63 (50%) patients, this was the most common subform. Type Ib was detected in 48 (38.09%) patients (Figure 2) (Table 3).

Type II: This type was detected in 8 (6.34%) patients (Table 3). The most common variation was this pattern. Type IIa was found in 5 (3.96%) and type IIb in 3 (2.38%) patients (Figure 3). Type IIc (hepatogastric trunk) pattern was not found.

Type III: It is the pattern in which CT never occurs. None of the 3 classic branches arise from a common trunk. It is the pattern

Figure 2. a, b. Type I sub-forms. (a) Type Ia; left gastric artery (LGA), common hepatic artery (CHA), and splenic artery (SA) arise from a common point on the same trunk. (b) Type Ib: LGA arises from the coeliac trunk (CT) as the first branch, and CHA and SA make a bifurcation at a common point from CT.

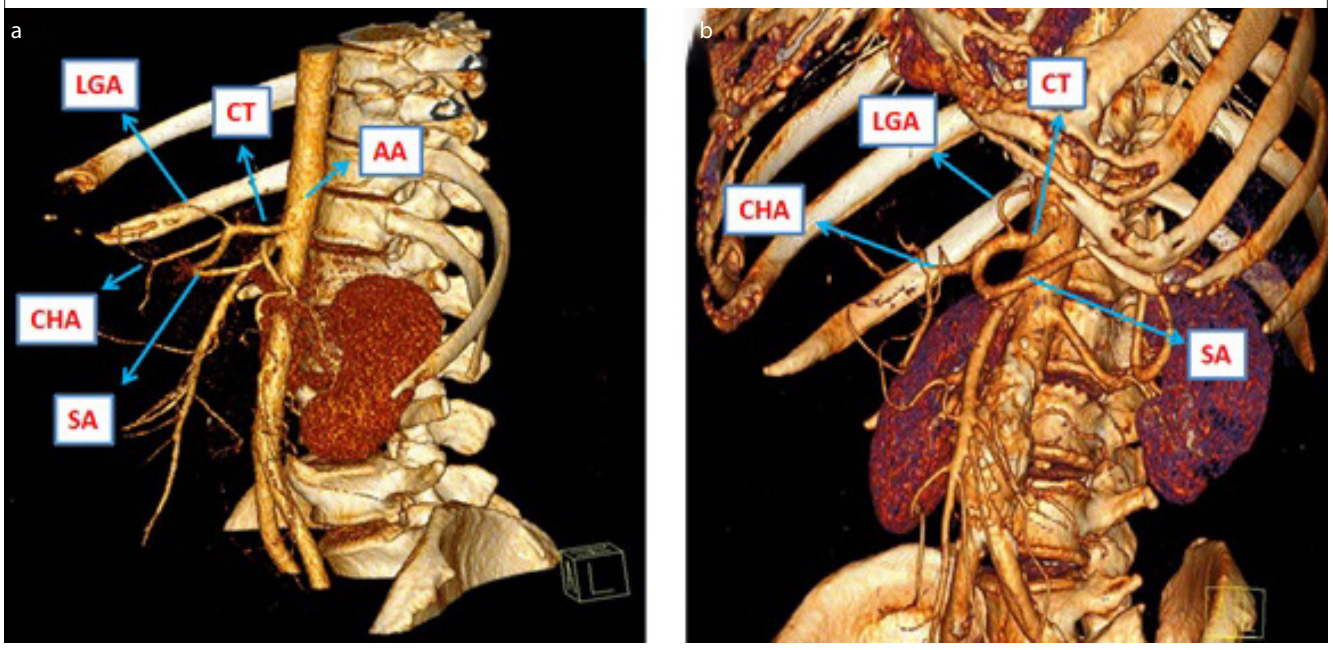
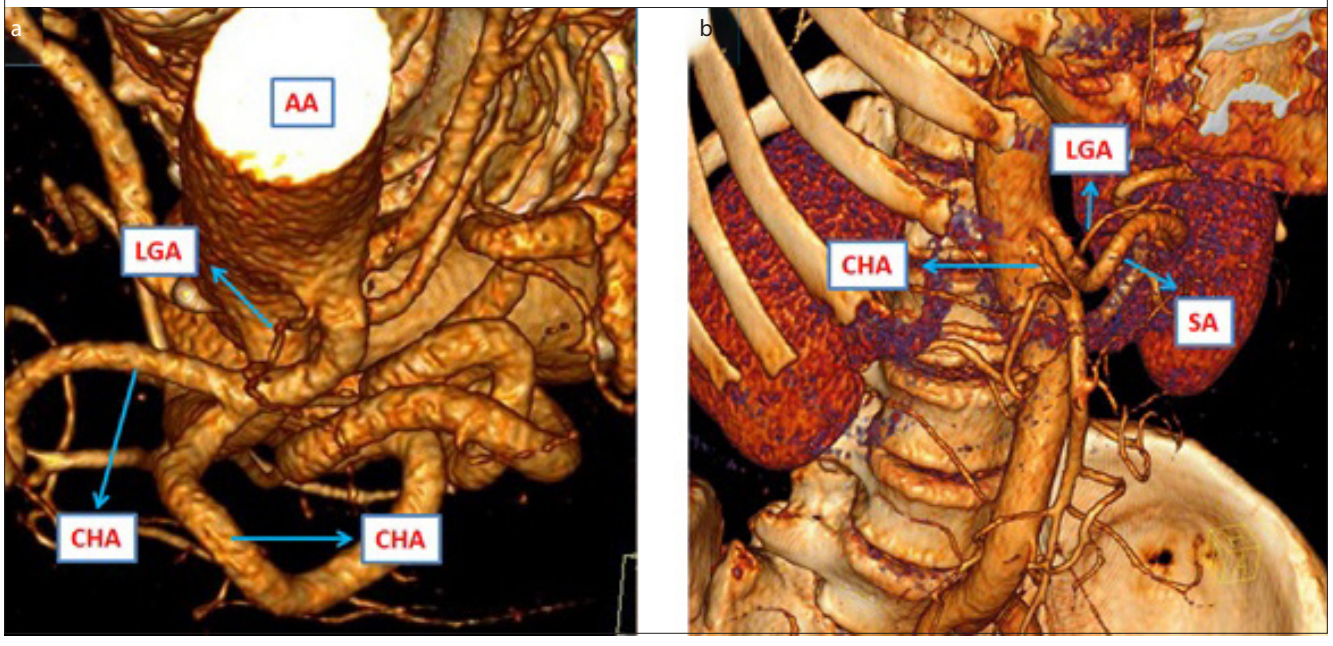


Figure 3. a, b. Type II forms encountered in the study. (a) Left gastric artery (LGA) arising from abdominal aorta. Common hepatic artery (CHA) and splenic artery (SA) making a bifurcation (type IIa - hepatosplenic trunk). (b) Type IIb CHA arising from AA. LGA and SA making a bifurcation (Type IIb-gastrosplenic trunk).



in which all of them independently arise from AA. This variation pattern was not found in our study (Table 3).

Type IV: In this type, the SMA and CT arise from a common trunk. It is also called CMT. In this study, type IV was found in 1 (0.79%) patient (Figure 4a) (Table 3).

Type V: This type was detected in 5 (3.96%) patients in our study (Figure 4b). In these variations, the gastroduodenal artery (GDA) (2 patients), left inferior phrenic artery (LIPA), and right inferior phrenic artery (RIPA) branches were identified as the 4th branch. In one patient, LHA and RHA branches were detected instead of CHA.

Figure 4. a, b. Type IV and type V forms encountered in the study. (a) Superior mesenteric artery arises from the coeliac trunk (CT), type IV coeliomesenteric trunk (CMT). (b) Type V: There is a fourth branch (quadrifurcation). Right inferior phrenic artery leaves the CT.

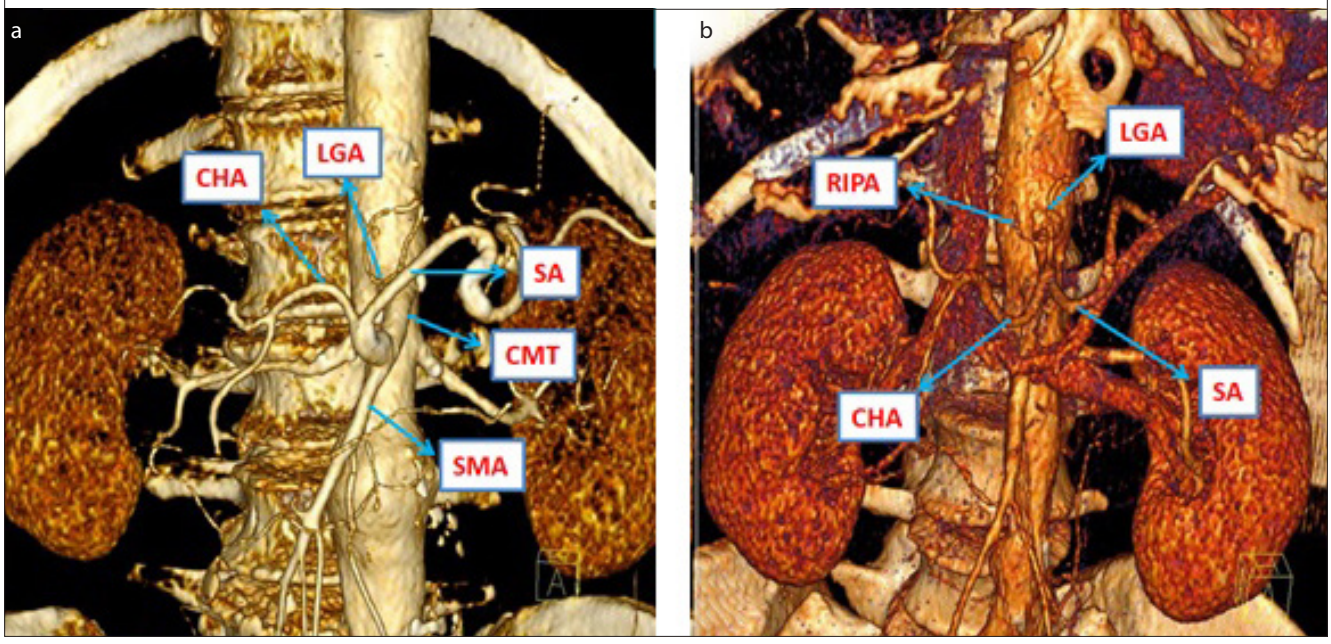


Table 3. Type and distribution of CT variation in our study

Types	Subtypes	n (%)	Total
Type I Complete CT. LGA, CHA and SA present (trifurcation)	Type Ia (LGA, CHA and SA arising from common origin)	63 (50%)	111 (88.09%)
	Type Ib (LGA arising from as the first branch)	48 (38.09%)	
Type II Incomplete CT (bifurcation)	Type IIa (hepatosplenic trunk)	5 (3.96%)	8 (6.34%)
	Type IIb (gastrosplenic trunk)	3 (2.38%)	
	Type IIc (hepatogastric trunk)	–	
Type III The absence of CT		–	–
Type IV Coeliomesenteric trunk (CMT)		1 (0.79%)	1 (0.79%)
Type V Presence of a branch except for LGA, CHA and SA. (quadrifurcation)		5 (3.96%)	5 (3.96%)
Other		1 (0.79%)	1 (0.79%)
Total		126 (100%)	126 (100%)

In our study, we could not define the variation for 1 (0.79%) patient and categorized it as "other." CT had 5 branches (pentafurcation), including SA, LHA, RHA, RIPA, and LGA; and LIPA separated from a common trunk (Table 3) (Figure 5).

DISCUSSION

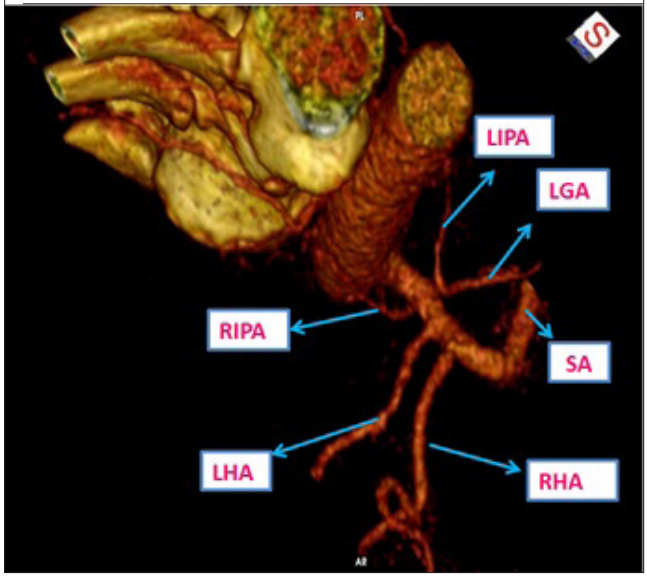
Morphometric Measurements

Knowledge of CT variations reduces the risk of trauma to the vessels in both upper abdominal surgical procedures and angio-

Table 4. Studies on CT diameter and length measurements

Researches	Method	n	CT length (cm)	CT Diameter (cm)	SA Diameter (cm)	CHA Diameter (cm)	LGA Diameter (cm)
Gosai et al (2013)	Cadaver	100	1.70±0.32	0.62±0.14			
Khan et al (2017)	MDCT	160	2.75±0.79	0.7±0.11			
Malnar et al (2010)	Cadaver	90	1.90±0.08	0.78±0.08	0.61±0.05	0.57±0.04	0.38±0.03
Neto et al (2015)	MDCT	60	2.33±0.65	0.80±0.13			
Silveira et al (2009)	Cadaver	21		0.79±0.04	0.53±0.03	0.50±0.04	0.38±0.03
Yadav et al (2014)	Cadaver	50	2.86±0.54	0.8±0.14	0.69±0.1	0.66±0.1	0.5±0.11
Panagouli et al (2011)	Cadaver	62	2.6 (±0.66)				
Pant et al (2013)	Cadaver	40	1.86	0.98			
Petrella et al (2007)	Cadaver	89	1.74 (0.35–4.0)	0.65 (0.40–0.95)			
Pinal–Garcia et al (2018)	Cadaver	140		0.72±0.13			
Seghal et al (2013)	MDCT	50	0.6–2.2	0.4–1.0			
Singh et al (2014)	Cadaver	40	1.71	0.66		0.51	
Tiwari et al (2013)	Cadaver	50	1.20±0.56				
Venieratos et al (2013)	Cadaver	77	2.8±0.8				
Current study	MDCT	126	3.12±0.71	0.73±0.13	0.69±0.1	0.66±0.1	0.27±0.11

Figure 5. Coeliac trunk has 5 branches (pentafurcation). These branches are the splenic artery, right hepatic artery, left hepatic artery, right inferior phrenic artery, and the left gastric artery with left inferior phrenic artery arise from a common trunk. In this variation, hepatic branches emerge separately instead of the common hepatic artery.



graphic examinations. The diameters of the branches of CT are particularly important in organ transplant surgery and definitive radiographic diagnosis of arterial aneurysms (26). In addition, knowledge of the diameter and length of the vessels is essential for placement of arterial stents in surgery and also useful for professionals designing and developing stents (16). Awareness of the mean values of normal artery diameters specific to a particular population is very important for accurate and definitive radiological diagnosis of arterial aneurysms. In addition, evaluation of arterial diameters is essential for the follow-up of liver transplantations. However, if the diameters of the arteries are less than 0.3 cm, the patients are considered as high risk for liver transplantation. For liver transplantation, diameter of the relevant arteries of the donor >0.2 cm is stated as an absolute exclusionary criterion, and diameter between 0.2–0.3 cm is a relative exclusion criterion. Previous knowledge of a particular artery can be helpful in situations such as early diagnosis, radiological examination of an arterial stenosis, and even low arterial flow with clinical symptoms (27).

Although the anatomical features and variations of CT have been well researched in the literature, there is not much information about artery diameters, which is very important for surgical procedures and angiographic interventions. In recent years, there have been some studies about CT and its main branches, mainly by the cadaver dissection method (14, 21, 26, 28-35). In these studies, among the main branches of CT, SA was reported as the largest in diameter, and LGA was the smallest.

Seghal et al. (14) and Tiwari et al. (32) measured the distance from AA until the first branch arose when measuring CT length. Sing et al. (31) measured the distance from AA to CHA. In oth-

Table 5. Adaptation of the studies in the literature according to the new classification we use

			Distribution of types (%)										
Researches	Method	Number (n)	Type I	Type Ia	Type Ib	Type II	Type IIa	Type IIb	Type IIc	Type III	Type IV	Type V	Other
Adachi (1928)	Cadaver	252	89	–	–	10.8	6.4	2	2.4	–	–	–	–
Araujo et al (2015)	MDCT	60	90	–	–	10	8.3	–	1.6	–	–	–	–
Aslaner et al (2017)	MDCT	1000	89	–	–	8.2	5.4	2.8	0.01	0.01	–	–	2.2
Chen et al (2009)	Cadaver	974	89.8	66.6	23.2	8.5	4.4	3.9	0.2	–	1.5	–	–
Clement et al (2016)	MDCT+ Cadaver	639	90.5	57.6	32.1	9.5	4.5	5	–	–	–	–	–
Iezzi et al (2008)	MDCT	524	72.1	50.4	19.4	10.9	5	2.3	3.6	0.6	0.4	–	–
Lipshutz (1917)	Cadaver	83	73.4		25.3	24.1	13.3	4.8	6	–	2.4	–	–
Mburu et al (2010)	Cadaver	123	61.7			17.9	13.1	4.9	–	–	–	20.3	–
Michels (1955)	Cadaver	200	89	–	25	11	4	5.5	1.5	–	–	–	–
Osman and Abdrabou (2016)	MDCT	1000	90.5	–	–	7.7	2.8	4.3	0.6	1	0.6	–	0.2
Pinal–Garcia et al (2018)	Cadaver	140	43.6	7.1	36.4	7.1	2.8	2.8	1.4	–	–	47.9	1.4
Song et al (2010)	Spiral CT and DSA	5002	89.1	–	–	7.5	4.4	2.9	0.2	–	1.8	–	–
Sureka et al (2013)	MDCT	600	91	–	–	4.3	2.8	1.5	–	–	0.6	–	3.5
Torres et al (2015)	MDCT	1569	92.7	–	–	6.5	2.2	4.1	0.2	0.1	0.5	–	0.1
Uğurel et al (2010)	MDCT	100	89	–	–	8	3	4	1	1	2	–	–
Vandamme and Bonte (1985)	Cadaver	156	85.9	–	–	12.8	–	–	–	1.3	–	–	–
Venieratos et al (2013)	Cadaver	77	90.9	74	16.9	1.3	–	1.3	–	2.6	–	–	5.2
Wang et al (2014)	MDCT	1500	89.8	–	–	0.93	0.27	0.53	0.13	0.2	3.4	–	–
Wysiadecki et al (2017)	Cadaver	40	62.5	–	–	10	10	–	–	2.5	–	20	5
Current Study	MDCT	126	88.1	50	38.1	6.34	3.96	2.38	–	–	0.79	3.96	0.79

er studies, this part was not explicitly reported. In our study, the distance from AA to the part with bifurcation or trifurcation was measured regardless of where the first branch arose (Figures 1b and 1d). The higher CT length in our study compared with the studies in the literature may be related to this difference. As the difference of even a millimeter can change measurements, the methods should be clearly explained and be sensitive.

There was no statistically significant difference between the normal and variation groups in the studies examining diameter differences (26, 27). Tiwari et al. (32) have suggested that CT length is related to the branching pattern of CT and reported that there is a significant relationship between the short CT length and the probability of CT variation. The correlation between diameter, length, and sex was examined in our study. Diameter measurements were found to be statistically significantly larger in men than in women ($p < 0.05$). However, there was no significant difference in terms of sex between CT root lengths.

Variations

Many studies have examined the anatomical structure of CT and its branches using different methods and reported many variations. Some of these have classified the variations of CT (4, 6-8, 36). Although the first classifications consisted of several different types, with the newly detected variations, the number of types and sub-forms have gradually increased. The classifications put forward in the past did not cover some important variations; however, current classifications, including most of the variations

reported in the literature, are quite complicated (4, 6-8, 23, 25, 36-39). Therefore, we used a new classification that we believe is simpler and more useful in our study.

In the literature, the anatomical variations of CT have been reported as bifurcation structure, presence of add-on branches, emergence with mesenteric arteries (usually SMA), or absence of CT (4-9, 11, 12, 15-20, 22-28, 36-38, 40-43). In their review, Panagouli et al. (25) have reported all CT patterns with 3 main branches as type I, regardless of whether the three main branches were of common origin or whether a branch arose from other branches. They considered all patterns outside of this structuring as variation (25). In our study, type I and its variations were accepted in this way.

The most common subform of type I in the literature is controversial. Some studies have described the form we call type Ia as a "true tripod" (true triple branching) or "classical pattern," and the form we call type Ib as "false tripod" (false triple branching) or "nonclassical" (25, 28, 36). Clement et al. (18) have accepted the type Ia form as the first form in which LGA arose from the body and type Ib as the form in which the three main branches arose from a common point, and reported that type 1a was the most common form in their studies. Similarly, Higashi et al. (39), in their study, have argued that a standard CT structure was not included in anatomy books, and they most frequently found the form in which LGA arose from the common body as the first branch, and this form was the standard CT model. However, Uflacker (36) accepted the form in which the three main branches arose from a common point as the "classical pattern" and the form in which LGA arose early as the "nonclassical pattern." Venieratos likewise named these two forms as "true tripod" and "false tripod" likewise (28). Panagouli et al. (25) have accepted the "false tripod" as the form in which one of the three main branches arose from the trunk early and divided it into three sub-forms.

When we look at the forms in the literature in which one of the three main branches arose earlier than the others, we see that LGA is the most common, and even the number of forms in which other branches arose early is much less or not at all existent (6, 12, 18, 25, 28, 36, 38, 39). Therefore, to keep the classification simple and useful, we hypothesize that type I sub-forms should be classified as the type in which only three main branches arise from a common origin or LGA arises from the other two branches earlier. In our study, type I pattern was found in 88.09% of the cases, whereas type Ia sub-forms of this pattern were found in 50% and type Ib in 38.09% of the cases. Whitley et al. (23) have reported the occurrence of the CT classic pattern in 40.62% and the nonclassic pattern in 60.41% of the cases in their review. When we examine the literature, we see that type II is the most common variation (4-9, 11, 12, 18, 19). This pattern is also referred to as the "bifurcation" structure of CT or "incomplete CT" in some studies (18, 23, 25, 33). In this, two of the three main branches arise from a common trunk, and the other arises from any other point (usually SMA or AA). Lipschutz (6), Michels (7), Adachi (8), and Uflacker (36) have classified the sub-forms of this type in different ways as if they were a separate type. These classifications can still be used by researchers today. This situation causes confusion and an increase in the number of types used in the classification. Some studies that put forward a new classification for CT named this pattern as type II and categorized this type into its sub-forms (18, 23, 25, 37, 40). We used this classification as we thought it was more accurate. In our study, the most common variation was type II with an occurrence rate of 6.34%. Whitley et al. (23) have reported this variation as the most common variation in the literature at an occurrence rate of 7.58%. Type III, or the absence of CT, is a rare variation. Panagouli et al. (25) have reported that only Morita used this variation in previous CT classifications. However, Vandamme and Bonte (4), Uflacker (36), and Gielecki et al. (37) also included this variation in their classification. Apart from this, in recent studies Babu and Khrab (40) and Clement et al. (18) have included this variation in their classifications. Vandamme and Bonte reported the prevalence of this variation as 1.7%, Panagouli et al. as 1%, Osman and Abdrabou as 1%, Uğurel et al. as 1%, Iezzi et al. as 0.06%, Aslaner et al. as 0.01%, Wysiadecki et al. as 2.5%, Wang et al. as 0.02%, Torres et al. as 0.1%, Venieratos et al. as 2.6%, and Whitley et al. as 0.28% (4, 9, 12, 15, 17, 19, 20, 22, 23, 25). In our study, this variation was not found. Type IV is referred to as CMT in the literature; Adachi, Michel, Uflacker, Clement, and Babu and Khrab have included this variation in their classifications (7, 8, 18, 36, 40). In our study, we encountered this variation in 1 (0.79%) patient. In the literature, Adachi reported CMT incidence as 2.4%, Song et al. as 1.06%, Osman and Abdrabou as 0.6%, Gielecki et al. as 1.5%, Panagouli et al. as 0.76%, Malnar et al. as 1.7%, Wang et al. as 3.4%, Whitley et al. as 0.09%, and Torres et al. as 0.5% (8, 11, 15, 17, 19, 23, 26, 35, 37). In these studies, CMT is defined as the variation in which the 3 main branches of CT and SMA are together. Wang et al. (15) defined CMT as any 2 major branches of SMA and CT and their combinations. Thus, Wang et al. (15) reported a higher incidence of CMT than other studies. Type V was classified as a variation with any collateral branch to the 3 main branches of CT except the main branches. These add-on branches often take the form of RIPA or LIPA branches. Apart from these, these add-on branches (collateral) may be 1 of the branches of GDA, dorsal pancreatic artery, middle colic artery (MCA), accessory hepatic artery (AHA), RHA, or LHA (16).

Collateral branches are divided into parietal (PIAs) and visceral branches (MCA, hepatic, pancreatic, gastroduodenal arteries, and so on) (23). In our study, after the bifurcation structure, this variation was found most frequently at an occurrence rate of 3.96%. Some studies have not evaluated this type as a variation. Adachi did not accept this type as a variation as it contained three classical main branches, but accepted it as a subform of type I (8). Similarly, Clement et al. (18) accepted this pattern as a subform of type I in their classification. Mburu et al. have reported the prevalence of this variation as 20.3%, Pinal-Garcia et al. as 47.9%, Wysiadecki et al. as 20%, Panagouli et al. as 1.06%, Venieratos et al. as 5.2%, and Clement et al. as 0.8% (18, 22, 25, 28, 33, 42). Some studies have evaluated variations in which CT has not just four but five, six, or even seven branches as this type. Therefore, these studies have reported higher rates of variation in this type compared with the literature (22, 33, 42).

There was a variation in our study that we could not classify. As far as we know, no such variation has been reported in the literature. The CT had five branches (pentafurcation). RHA and LHA

were present instead of CHA, and PIAs were also available as add-ons. RIPA left CT directly, but LIPA had a common trunk with LGA. We classified this variation as "other" in our classification.

Studies on CT diameter and length measurements are given in Table 4, and the adaptation of the studies in the literature according to the new classification we used is given in Table 5.

Limitations

This study had some limitations. First, the study was retrospective. Second, the age distribution of the patients was not homogeneous (40–83 years). There were very few young individuals. In addition, as our study was based on the analysis of radiological images, the depiction of very thin arterial networks may have been overlooked.

CONCLUSION

Surgeons must be aware of the length and origin of the vessels to predict the possible area where arteries can be found. Such data are important not only in vascular surgery but in any surgery to prevent iatrogenesis. Data obtained from this study will be useful in laparoscopic and robotic surgeries. Knowing the anatomical structure and variations of CT and its branches is very important for liver transplantations, intra-arterial chemotherapy, hepatopancreatobiliar surgery, vascular ligation, and anastomoses in the relevant region, interventional radiology, and surgical procedures.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (2016/210).

Informed Consent: The study was not required as it was a retrospective study.

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Original Research

Relationship between Serum Total Bilirubin Level and Cardiac Outcomes in Patients with Isolated Coronary Artery Ectasia

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ABSTRACT

Objective: This study aimed to investigate the association of admission serum bilirubin concentrations with major adverse cardiac event (MACE) in isolated coronary artery ectasia (ICAE).

Methods: This study enrolled 75 consecutive patients (42.7% women with the mean age of 52.3±11.4 years) with ICAE. MACE, basal demographic, laboratory, angiographic parameters, and admission serum bilirubin concentrations were recorded on follow-up forms. MACE was defined as heart failure, nonfatal myocardial infarction, and cardiovascular death.

Results: During follow-up (median 61±11 months), 19 (25.3%) patients experienced MACE. The patients were assigned into two groups: with MACE and without MACE. Compared with the non-MACE group, the MACE group had significantly lower serum total bilirubin levels. In addition, when the patients were subcategorized into tertiles of serum bilirubin concentrations, MACE were identified in 11 patients in the first tertile, 7 patients in the second tertile, and 1 patient in the third tertile. In the Cox regression analyses, serum total bilirubin and C-reactive protein (CRP) were found as independent risk factors for MACE ($p<0.05$).

Conclusion: We identified that MACE rates were inversely associated with serum bilirubin concentrations and directly associated with CRP in isolated ICAE patients. To the best of our knowledge, this is the first study to report a relation between total bilirubin level and MACE in patients with ICAE.

Keywords: Atherosclerosis, bilirubin, coronary ectasia

INTRODUCTION

Isolated coronary artery ectasia (ICAE) is described as localized or diffuse dilation of a coronary artery diameter to 1.5 times or more that of the adjacent nonectatic segment without concomitant coronary artery obstruction (1-3). ICAE has a low prevalence and occurs at a rate of 0.08%–1% in patients undergoing coronary angiography (4-6). Typical coronary angiographic characteristics of coronary artery ectasia (CAE) include segmental back flow phenomenon, delayed antegrade coronary opaque filling, stasis, and deposition of opaque in dilated segments (7). Ectatic coronary arteries may cause significant acute cardiac events owing to distal embolization caused by stasis in dilated luminal segments, dissection, slow blood flow, thrombus formation, and impaired coronary flow (8).

Serum bilirubin, the product of heme catabolism, is an important marker of hepatic function with biliary excretion. Bilirubin has been described as a natural antioxidant that inhibits lipid peroxidation (9). Studies indicated that serum bilirubin levels are inversely correlated with the risk of premature coronary artery disease (PCAD), metabolic syndrome, hypertension (HT), and diabetes mellitus. In addition, a lower risk of the cardiovascular events was shown at elevated serum bilirubin levels (10, 11).

The significant relation between serum bilirubin concentrations and ICAE was previously demonstrated (12). However, although bilirubin is associated with a number of cardiovascular endpoints, no data exist in current literature regarding its effect on major adverse cardiac events (MACE) in ICAE over long-term follow-up. Therefore, in this study, we aimed to explore the association between serum total bilirubin concentration and MACE in the ICAE patients.

METHODS

A total of 75 consecutive patients (32 females [42.7%], with the mean age of 52.3±11.4 years) with ICAE between 2010 and 2014 were included in this study. Coronary angiography was considered based on positive noninvasive stress tests or high clinical suspicion for coronary artery disease (CAD). Health Sciences University, Adana Research and Training Hospital ethics committee

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approved the study protocol (486/2019). Medical therapy was standardized according to the clinical indications irrespective of the presence of ICAE. The demographic characteristics of the patients were recorded in accordance with current guidelines. A detailed medical history of the patients was retrieved from the medical files and recorded on forms. Serum total bilirubin levels were obtained with colorimetric method on an Aeroset System (Abbott Laboratories, Chicago, IL, USA). Basal laboratory parameters were analyzed for all patients.

The study exclusion criteria were history of coronary bypass grafting, coronary syndromes, presence of ≥20% stenotic coronary lesions, heart failure, severe or moderate valvular heart disease, congenital heart disease, pre-existing inflammatory/autoimmune/infectious diseases, chronic renal failure, acute or chronic hepatic disease, cholestatic jaundice, gallbladder and bile duct diseases, chronic obstructive pulmonary disease, hematologic disorders, known malignancy, and thyroid dysfunction.

Coronary Angiography

Coronary angiography was performed on a basis using the Judkins technique with 6/7 French catheters. Angiograms were recorded in DICOM format at 15 frames/s and were evaluated by 2 experienced cardiologists. In this study, ICAE was described as dilation of a coronary artery luminal diameter to 1.5 times or more than that of the adjacent nonectatic segment without concomitant coronary artery stenosis (≥ 20%) (2). ICAE was categorized into 4 types based on the Markis classification (4).

Outcomes and Follow-up

The primary endpoint was MACE defined as heart failure, nonfatal myocardial infarction (MI), and cardiovascular death. To evaluate clinical status and adverse events, patients returned to the outpatient clinic at the 6th month and first year after the coronary angiography. Clinical follow-up was carried out by telephone in the 2nd, 3rd, 4th, 5th, and 6th years.

Statistical Analysis

Continuous variables were analyzed for data normality using the Kolmogorov–Smirnov test. The variables following a normal distribution were presented as mean (±standard deviation), and those without a normal distribution were presented as median (interquartile range). The variables that follow normal distribution among groups were compared using the Student's t-test, and the Mann–Whitney U-test was used to compare the variables without normal distribution. Categorical variables were summarized as numbers and percentages and compared between the groups using the Chi-square test or the Fisher's exact test. The receiver operating characteristic (ROC) curve was used to demonstrate the sensitivity and specificity of bilirubin cutoff values for MACE development. Univariate and backward stepwise multivariable Cox regression analyses were performed to determine independent predictors of MACE. Variables with an unadjusted p-value <0.05 in univariate analysis were included in the multivariate analysis. A survival analysis of MACE between tertiles of bilirubin (T1, T2, and T3) was conducted using the Kaplan–Meier method with a log-rank analysis. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 20.0) for Windows (IBM SPSS Corp.; Armonk, NY, USA). The p-value <0.05 was considered statistically significant.

Main Points:

- We identified that serum total bilirubin value was inversely associated with MACE rates.
- CRP was directly associated with MACE rates in ICAE patients over a long-term follow-up period.
- This is the first study in the literature to report a relation between serum total bilirubin value and MACE in patients with ICAE.
- We identified that serum bilirubin values might be used to predict MACE in ICAE patients.

RESULTS

A total of 75 ICAE patients were included in this study. In the follow-up period (median 61±11 months), MACE was observed in 19 (25.3%) patients, of whom 7 died owing to cardiovascular causes. All of these deaths occurred in hospitalized patients.

The study patients were assigned into 2 groups: with MACE and without MACE. Baseline demographic, laboratory, and angiographic data are presented in Table 1. The groups were similar in terms of demographic and laboratory parameters (p>0.05). Only serum total bilirubin level was statistically significantly lower in the MACE group than in the non-MACE group (p=0.001). In MACE subgroup analysis, serum total bilirubin level was statistically significantly lower in the nonfatal MI and cardiovascular death group than in the non-MACE group (p=0.005 and p=0.021, respectively). However, there was no statistically significant difference between the heart failure group and serum bilirubin levels (p=0.727).

On angiographic assessment, the right coronary artery (n=47, 62.7%) was the most commonly affected vessel. Using the Markis classification, type IV CAE (30.6%) was most common, whereas type III (18.7%) was the least common. There was no significant difference between groups with respect to involved vessels and Markis classification (p>0.05).

The patients were also subcategorized into three groups according to the tertiles of the total bilirubin values at baseline. Table 2 shows a comparison of demographic and laboratory parameters based on total bilirubin tertiles. MACE was found in 11 (57.9%) patients in T1, 7 (18.9%) patients in T2, and 1 (5.3%) patient in T3 (p<0.001). Moreover, we have evaluated the effect of major cardiovascular risk factors on serum bilirubin values, and a statistical relationship was found only between HT and bilirubin values (p=0.034).

To determine the independent risk factors for MACE, univariate and multivariate cox regression analysis was performed (Table 3). In univariate analysis, hyperlipidemia, CRP, and serum total bilirubin were independent predictors of development of MACE. In multivariate Cox regression analysis, CRP (p=0.002) and total bilirubin (p=0.025) were independent predictors of MACE.

Table 1. Demographic, laboratory, angiographic parameters and medications of study population and patients with or without MACE

	All Patients (n=75)	MACE (n=19)	Non-MACE (n=56)	p
Demographic parameters				
Age (years, mean)	52.3±11.4	48.8±12.6	53.5±10.8	0.116
Female, n, (%)	32 (42.7%)	6 (31.6%)	26 (46.4%)	0.258
Body mass index (kg/m^2)	28.8±4.09	27.9±4.00	29.1±4.11	0.283
Left ventricular ejection fraction (%)	60.0±5.66	59.5±5.98	60.2±5.59	0.659
Diabetes mellitus, n, (%)	20 (26.7%)	4 (21.1%)	16 (28.6%)	0.522
Hypertension, n, (%)	34 (45.3%)	10 (52.6%)	24 (42.9%)	0.460
Hyperlipidemia, n, (%)	41 (54.7%)	8 (42.1%)	33 (58.9%)	0.203
Family history of CAD, n, (%)	29 (38.7%)	8 (42.1%)	21 (37.5%)	0.722
Current smoker, n, (%)	37 (49.3%)	12 (63.2%)	25 (44.6%)	0.163
Medications				
Aspirin, n (%)	54 (72.0%)	13(68.4%)	41 (73.2%)	0.688
ACE-I/ARB, n (%)	29 (38.7%)	8 (42.1%)	21 (37.5%)	0.722
β-Blocker, n (%)	18 (24.0%)	4 (20.1%)	14 (25.0%)	0.728
Calcium channel blockers	26 (34.5%)	6 (31.6%)	20 (35.7%)	0.743
Statin/fenofibrat, n (%)	22 (29.3%)	5 (26.3%)	17 (30.3%)	0.738
P2Y12 inhibitors, n (%)	17 (22.7%)	6 (31.5%)	11 (19.7%)	0.283
Laboratory parameters				
Hemoglobin (g/dL)	12.9±1.64	13.3±1.71	12.7±1.61	0.218
Leukocyte ($\times10^3$ /μL)	7.3±1.55	7.7±1.33	7.2±1.61	0.204
Monocyte ($\times10^3$ /μL)	0.77±0.19	0.62±0.20	0.82±0.16	0.410
Platelet ($\times10^3$ /μL)	285±86.4	296±79.0	281±89.0	0.537
Plasma fasting glucose(mg/dL)	117.3±50.2	109.4±47.5	120.0±51.1	0.433
Creatinine (mg/dL)	0.71±0.21	0.71±0.25	0.71±0.20	0.985
Serum uric acid (mg/dL)	5.7±1.50	6.0±1.32	5.6±1.55	0.299
C-reactive protein (mg/ dL)	1.3±0.69	1.6±1.28	1.2±0.24	0.189
LDL-Cholesterol (mg/ dL)	139.6±33.6	141.0±34.5	139.2±33.5	0.849
HDL-Cholesterol (mg/dL)	40.4±11.7	41.0±12.3	40.2±11.6	0.812
Total cholesterol (mg/ dL)	224.1±43.3	222.2±43.6	224.7±43.6	0.832
Triglyceride (mg/ dL)	220.1±105.9	201.7±87.5	226.3±111.5	0.384
Alanine aminotransferase (U/L)	20.2±13.63	18.3±7.36	21.1±15.0	0.289
Aspartate aminotransferase (U/ L)	21.2±16.91	17.3±5.93	22.4±19.1	0.261
Total bilirubin (mg/dL)	0.59±0.23	0.45±0.16	0.64±0.22	0.001*
Angiographic parameters				
Left anterior descending,(n,%)	35 (%46.7)	9 (%25.7)	26 (%74.3)	0.943
Circumflex artery, (n,%)	33 (%44)	5 (%15.2)	28 (%84.8)	0.072
Right coronary artery, (n,%)	47 (%62.7)	15 (%31.9)	32 (%68.1)	0.090
Markis Type I (n,%)	21 (%28)	5 (%23.8)	16 (%76.2)	0.961
Markis Type II (n,%)	17 (%22.7)	5 (%29.4)	12 (%70.6)	
Markis Type III (n,%)	14 (%18.7)	3 (%21.4)	11 (%78.6)	
Markis Type IV (n,%)	23 (%30.6)	6 (%26.1)	17 (%73.9)	
Tertile 1	19 (%25.3)	11 (%57.9)	8 (%42.1)	<0.001*
Tertile 2	37 (%49.4)	7 (%18.9)	30 (%81.1)	
Tertile 3	19 (%25.3)	1 (%5.3)	18 (%94.7)	

CAD: Coronary artery disease, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. ACE-I: angiotensin-converting enzyme inhibitör, ARB: angiotensin receptor blocker *p<0.05 is significant.

Table 2. Comparison of demographic and laboratory parameters among tertiles of total bilirubin levels.

	T1 (n=19)	T2 (n=37)	T3 (n=19)	p1	p2	p3
Demographic parameters						
Age (years, mean)	48.4±11.9	53.5±11.2	54.0±10.8	0.115	0.136	0.884
Female, n, (%)	7 (36.8%)	15 (40.5%)	10 (52.6%)	0.788	0.328	0.389
Body mass index, kg/m^2	28.4±4.24	28.7±3.54	29.4±5.00	0.790	0.513	0.542
Diabetes mellitus, n, (%)	3 (15.8%)	11 (29.7%)	6(%31.6)	0.254	0.252	0.887
Hypertension, n, (%)	8 (42.1%)	13 (35.1%)	13(%68.4)	0.610	0.103	0.018*
Hyperlipidemia, n, (%)	7 (36.8%)	24 (64.9%)	10(%52.6)	0.046*	0.328	0.375
Family history of CAD, n, (%	7 (36.8%)	15 (40.5%)	7(%36.8)	0.788	1.00	0.788
Current smoker, n, (%)	10 (52.6%)	15 (40.5%)	12(63.2)	0.389	0.511	0.109
Left ventricular ejection fraction (%)	60.0±5.4	60.3±5.2	59.4±6.8	0.816	0.774	0.573
Laboratory parameters						
Hemoglobin (g/dL)	13.6±1.58	12.8±1.59	12.3±1.60	0.080	0.014*	0.236
Leukocyte count (×10^3/μL)	7.5±1.48	7.6±1.54	6.8±1.59	0.777	0.172	0.068
Monocyte count (×10^3/μL)	0.64±0.25	0.98±1.27	0.48±0.18	0.246	0.037*	0.094
Platelet count (×10^3/μL)	272.9±64.8	288.6±85.8	291±107.2	0.487	0.514	0.903
Plasma fasting glucose(mg/dL)	104.2±42.9	119.4±46.1	126.2±63.1	0.235	0.216	0.649
Creatinine (mg/dL)	0.77±0.26	0.70±0.21	0.67±0.17	0.317	0.184	0.567
Serum uric acid (mg/dL)	5.45±1.64	5.61±1.44	6.17±1.45	0.707	0.161	0.175
C-reactive protein (mg/dL)	1.61±1.21	1.17±0.35	1.08±0.23	0.137	0.081	0.379
LDL-Cholesterol (mg/dL)	136.3±33.0	140.7±37.4	140.8±27.0	0.662	0.647	0.995
HDL-Cholesterol (mg/dL)	36.0±8.7	41.1±12.8	43.5±11.2	0.125	0.028*	0.497
Total cholesterol (mg/dL)	214.6±40.8	227.4±48.7	227.2±34.7	0.329	0.311	0.988
Triglyceride (mg/ dL)	211.4±78.9	227.5±126	214.3±89.2	0.613	0.915	0.685
Alanine aminotransferase (U/L)	17.3±7.08	24.1±17.9	18.3±5.6	0.119	0.616	0.180
Aspartate aminotransferase (U/ L)	17.8±5.9	23.6±23.3	19.6±5.2	0.299	0.344	0.466

T: Tertile, CAD: Coronary artery disease, LDL-C: Low-density lipoprotein cholesterol, HDL: High-density lipoprotein cholesterol. Total bilirubin level T1: < 0.44 mg/dL, T2: 0.44–0.76 mg/dL, T3: > 0.76 mg/dL p1: T1 vs. T2, p2: T1 vs. T3, p3: T2 vs. T3. *p<0.05 is significant,

In the ROC analyses (Figure 1), a cutoff value of ≤0.465 serum total bilirubin had a 78.6% sensitivity and 68.4% specificity for predicting MACE (p=0.001).

The Kaplan–Meier survival analysis showed that patients at the lowest tertile (T1) of total bilirubin levels were more likely to develop MACE compared with patients at the highest tertile (T3) (p=0.002) (Figure 2).

DISCUSSION

We found that, irrespective of conventional cardiovascular risk factors, serum total bilirubin and CRP values were predictors of development of MACE in ICAE patients on long-term follow-up. To the best of our knowledge, this is the first study to establish a link between serum total bilirubin value and MACE in this specific population.

The exact pathophysiological mechanisms of CAE are yet to be defined. A variety of etiologies, including congenital defects, inflammation, endothelial dysfunction, vasculitis, and atherosclerosis, are associated with the development of CAE, but atherosclerosis is the main cause in nearly half of all CAE cases (13). Patients with CAE are at risk of MI and sudden cardiac death because of coronary vasospasm, dissection, intracoronary thrombosis, and slow flow secondary to dilation of coronary arteries (14).

Table 3. Univariate and multivariate Cox regression analyses

	Univariate analysis		Multivarite analysis	
Parameter	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Total bilirubin	0.021 (0.001–0.89)	0.010	0.035 (0.02–.0.652)	0.025*
Hyperlipidemia	0.353 (0.135–0.922)	0.033		
Current smoker	1.845 (0.726–4.694)	0.198		
Hemoglobin	1.166 (0.868–1.567)	0.308		
Leukocyte	1.079 (0.794–1.467)	0.628		
C–reactive protein	2.437 (1.551–3.829)	<0.001	2.041 (1.288–3.233)	0.002*
Circumflex artery	0.546 (0.194–1.533)	0.251		
Right coronary artery	1.623 (0.522–5.048)	0.402		

HR: hazard ratio; CI: confidence interval. *p<0.05 is significant.

Figure 1. Receiver operating characteristic (ROC) curves for MACE

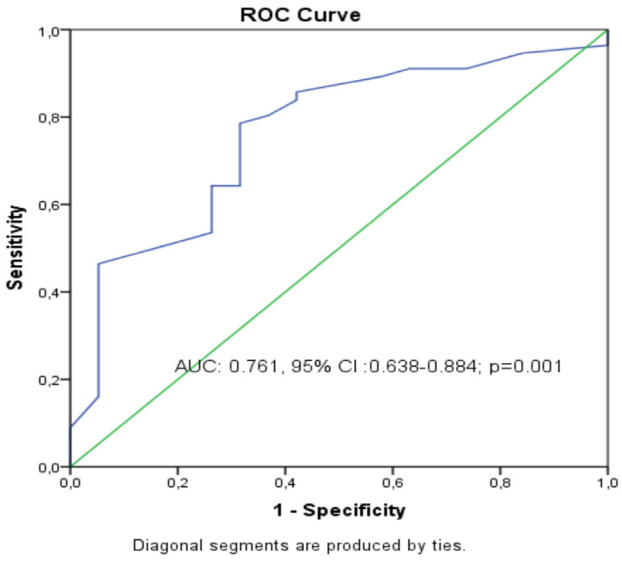
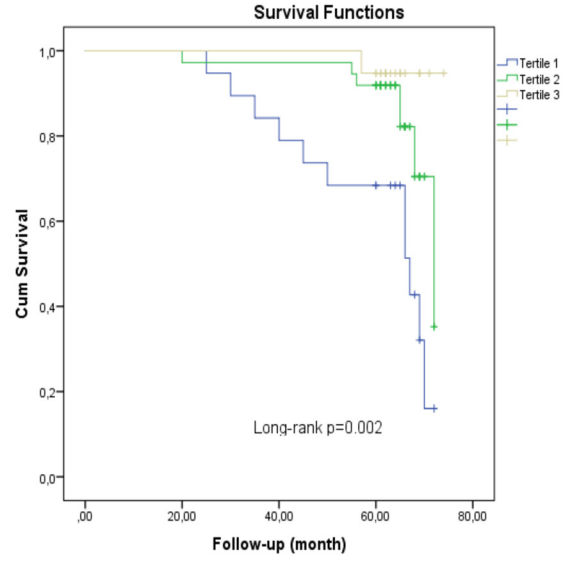


Figure 2. Kaplan-Meier survival plot of patients according to tertiles of total bilirubin levels



Cases of ventricular arrhythmias, nonfatal MI, and sudden cardiac death have been reported with CAE (15, 16). In a study, Boles et al. (17) presented data from a relatively long-term follow-up of patients with CAE (11.4 years). In this study, overall and cardiac death rates were significantly higher in CAE patients compared with non-CAE. The period of follow-up was 61±11 months in our study, during which 19 (25.3%) patients experienced MACE.

Studies have shown that CRP, an inflammation marker, predicts chronic systemic inflammation that occurs in atherosclerotic progression. Furthermore, CRP has been shown to be elevated markedly in many cardiovascular disorders and is related to poor prognosis (18-20). Similarly, in our study, we also found a positive association between CRP level and MACE in ICAE during follow-up.

Bilirubin scavenges reactive oxygen species and reduces the uptake of oxidized low-density lipoprotein, which is an essential feature of the atherosclerotic process (21). Several studies have been published demonstrating the association between serum bilirubin concentrations and oxidative stress mediated disorders, especially atherosclerotic disease (22). Recent studies have found that higher serum bilirubin values confer significant protection against atherosclerotic cardiovascular disorders, and subnormal serum bilirubin values are associated with cardiovascular morbidity and PCAD (23).

Chang et al. (24) reported that bilirubin was associated with the complexity and severity of CAD evaluated by the SYNTAX score and 1st MACE in patients with stable angina pectoris undergoing coronary revascularization. In a study by Demir et al. (12),

serum bilirubin values were negatively correlated with ICAE. In the study by Şahin et al. (25), a strong association between serum bilirubin values and the SYNTAX score in patients with STEMI was found. Consistently, we found a negative association between serum total bilirubin concentrations and MACE in this study.

Study Limitations

The main limitations of our study include small sample size and inclusion of patients from a single center. In addition, bilirubin values were only obtained at the time of diagnosis. Changes in bilirubin levels of the patients during follow-up were not investigated, and this is another limitation of our study.

CONCLUSION

We identified that serum total bilirubin values were inversely associated with MACE rates and directly associated with CRP in ICAE patients over a long-term follow-up period. To the best of our knowledge, this is the first study in the literature to report a relation between serum total bilirubin level and MACE in patients with ICAE. We believe that serum bilirubin values might be used to predict MACE in ICAE patients. Larger, multicenter studies are needed to further evaluate long-term prognosis in patients with ICAE.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Adana Research and Training Hospital (486/2019).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - F.Y.; Design - F.Y.; Supervision - M.K.; Resources - F.Y., M.K.; Materials - F.Y., M.K.; Data Collection and/or Processing - F.Y., M.K.; Analysis and/or Interpretation - F.Y., M.K.; Literature Search - F.Y., M.K.; Writing Manuscript - F.Y.; Critical Review - F.Y.

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Original Research

Whole Blood Viscosity as a Marker of Thrombosis in Cushing's Disease: An Actor or Ineffective Factor

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ABSTRACT

Objective: There is a tendency for thromboembolic events (TE) in Cushing's disease (CD) because of the cortisol excess itself and associated risk factors for TE. Whole blood viscosity (WBV) as a measure of hemorheological features may impact the development of TE. However, limited data are available on the status of these changes in CD. Herein, we aimed to compare WBV between patients with CD and the control group and evaluate the impact of CD treatment on WBV.

Methods: A total of 34 patients with CD without prior TE history and 30 subjects as the control group were enrolled between 2015 and 2020. Demographic, clinical, and laboratory characteristics of the study groups were recorded. WBV was calculated using the de Simone formula.

Results: Among the corticotroph pituitary adenomas of the CD group, 32 of 34 were microadenomas, and 2 were macroadenomas. Postoperative remission was achieved in all patients. However, a recurrence was observed in 10 patients at 5.8±3.2 year follow-up. There was no difference between baseline WBV, at both low and high shear rates, between the CD and control groups ($p>0.05$). Furthermore, the WBVs at both low and high shear rates were also similar before and after treatment in the CD group (16.3±1.8 versus 15.4±1.7, $p=0.2$, for WBV at the high shear rate; 40.5±38.2 versus 25.4±35.2, $p=0.23$, at the low shear rate).

Conclusion: In this small-sized preliminary study, the WBV at both shear rates revealed no difference between the CD and control groups. There was also no impact of CD treatment on WBV at follow-up. However, further large-scale studies are necessary to confirm our study findings.

Keywords: Cushing's disease, hypercoagulability, shear stress, whole blood viscosity

INTRODUCTION

Thromboembolic events (TE) occur 4 times more frequently in Cushing's syndrome (CS) (1). CS is associated with the risk factors of thromboembolism, such as obesity, hypertension, and diabetes. Although increased coagulability and reduced fibrinolysis because of cortisol excess are believed to be the cause of prothrombotic tendency, other pathogenetic mechanisms have not been determined (2).

The thrombosis process is classically triggered by Virchow's 3 factors, including endothelial damage, impaired blood flow, and increased blood clotting tendency. A direct relationship is present among impaired blood flow, blood viscosity, and thrombosis susceptibility (3). High blood viscosity, the inherent resistance of blood in vessels to flow, is the only rheological factor linked with major cardiovascular risk factors (4). Endothelial damage and thrombosis also occur as a result of increased shear stress in hyperviscosity. Therefore, there is a close and dynamic association among these three mechanisms. Blood viscosity can be measured with various techniques. Whole blood viscosity (WBV) can be quickly and accurately calculated using the de Simone formula (5).

However, there is no study regarding the role of blood viscosity and rheology in patients with the CD. Hemorheological alterations should be considered, especially for venous TE, for better knowledge in such a patient group. Therefore, we aimed to evaluate (i) the baseline WBV at both low and high shear rates in the CD and control groups, and (ii) the WBV at both low and high shear rates before (hypercortisolism state) and after (normal cortisol state) CD treatment.

METHODS

Study Participants

Our study has a cross-sectional retrospective design. The study was conducted at Ankara University School of Medicine, Depart-

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ment of Endocrinology, Ankara, Turkey. A total of 34 patients diagnosed with CD and who had a remission after successful transsphenoidal surgery were enrolled between 2015 and 2020. Among the corticotroph pituitary adenomas of the CD group, 32 of 34 were microadenomas, and 2 were macroadenomas. The diagnosis of CD was based on clinical features, loss of diurnal rhythm of cortisol secretion, elevated urinary free cortisol levels, and absence of cortisol suppression after low-dose oral dexamethasone and high-dose oral dexamethasone suppression tests, imaging pituitary adenomas at magnetic resonance imaging, and inferior petrosal sinus sampling if necessary. Remission was determined as the regression of the clinical features and normalization of hypercortisolism in terms of Adrenocorticotropic hormone ACTH, cortisol, and urinary free cortisol levels (morning serum cortisol values 5 mg/dL and UFC 5-55 µg/day within 7 days of selective tumor resection) (6). A total of 30 healthy subjects were randomly selected from the outpatient clinic admissions for the control group.

Patients with hematological disease, including anemia, acute illness, and acute/chronic infectious or inflammatory disease, those who underwent invasive procedures/surgery within 6 months of recruitment, those who had active malignancy, previous venous/arterial TE, history of recurrent abortus, ongoing anticoagulant or antiplatelet therapy, diseases with hypoalbuminemia (chronic renal failure, hepatic insufficiency, and heart failure), lymphatic or venous system-mediated chronic stasis, and cigarette smoking were all excluded.

None of the patients received anticoagulant therapy during the preoperative or perioperative period. All of the coagulation parameters (prothrombin time, activated partial thromboplastin time (aPTT), and thrombin time) were in the normal reference ranges for all patients.

Laboratory Evaluation

Demographic, clinical, and laboratory data were recorded for each participant. Blood samples were obtained from each patient at 8.00 A.M. in an 8-h fasting state to do a complete blood count analysis and biochemistry panel, including serum cortisol and ACTH levels before surgery after surgery. Serum cortisol concentrations were ascertained by immunoenzymatic assays Access cortisol assays, Beckman Coulter, Brea, CA, USA). The intra-assay coefficients of variation (CVs) were less than 5%. Serum ACTH concentration was measured by the electrochemiluminescence immunoassay (Roche Elecsys 2010 analyzer, Roche Elecsys-ACTH, Roche Diagnostics, Indianapolis, IN, USA), and the intra-assay CV was less than 6%. We ascertained the urinary free cortisol by radioimmunoassay (DIAsource Immunoassays S.A., Louvain-la-Neuve, Belgium), and the intra-assay CV was less than 7%. Normal values used in our laboratory were as follows: early morning cortisol: 5–19.4 mg/dL; ACTH: 25–60 pg/mL; and UFC: 5-55 µg/day.

Main Points:

- Although the linkage between hypercoagulability and Cushing's syndrome is very well determined, the missing mechanism of thrombosis as endothelial dysfunction, stasis, and rheological properties of blood coagulation have not been studied yet.
- This is the first study evaluating the hemorheological features of patients with Cushing's disease as a possible cause of thrombosis.
- We did not find an association between whole blood viscosity and hypercortisolemia parameters.

The estimation of WBV (centipoise, cP) was calculated in both high shear rate (HSR = 208 s^{-1}) and low shear rate (LSR= 0.5 s^{-1}) with previously validated formulas. Hematocrit was calculated by an automated analyzer by multiplying the red cell count by the mean cell volume and given in percent (Coulter counter; Beckman Coulter Diagnostics, Brea, CA, USA). Total protein was measured using a Beckman Coulter AU 5800 analyzer with its commercial kits and given in g/L. For HSR (208 s^{-1}), (0.12×HcT) + 0.17 (TP-2.07) and for LSR (0.5 s^{-1}), (1.89×HcT) + 3.76 (TP-78.42) were the formulas used to calculate WBV (7).

The Institutional Ethics Committee approved the study protocol (12/2020, R451), and the study protocol complied with the principles outlined in the Declaration of Helsinki.

Statistical Analysis

The Shapiro–Wilk test was used for the assessment of normality. Numerical variables with normal distribution are represented as mean±standard deviation, and numerical variables with a skewed distribution are represented as median (minimum and maximum). Categorical variables are presented as numbers and percentages. The paired student t-test was used to compare the measurements at two-time points (at the time of diagnosis and after remission) for ACTH, cortisol, WBV-HSR, and WBV-LSR. Since the 24-h urinary cortisol levels were not normally distributed, nonparametric tests were conducted to compare these parameters. The Wilcoxon test was used to compare the change in 24-h urinary cortisol levels between baseline and after treatment. According to the parameter distribution, normal or nonnormal, the correlation coefficients and their significance were calculated using the Spearman or Pearson tests, respectively. All statistical analyses were performed using SPSS statistical software (IBM Corp., released in 2015, IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., Armonk, NY, USA). A two-tailed p-value <0.05 was determined as statistically significant.

RESULTS

Study Population and Laboratory Features

The median age of the CD group was 48±12 years, and the mean age at CD diagnosis was 42±13.5 years. In the CD group, 79.4% (27 of 34) of patients were female. Table 1 shows the demographic, clinical, and laboratory features of study groups both at baseline and after the CD group treatment**.** Postoperative remission was achieved in all patients. However, a recurrence was observed in 10 patients at 5.8±3.2 years follow-up. Pasireotide treatment was initiated and continued in a 39-year-old female patient after a recurrence in the second year of the reoperation. In addition, 5 patients received steroid replacement therapy. The mean gluco-

Table 1. The demographic, clinical, and laboratory features of Cushings' disease and control group

	Before treatment (a)	After treatment (b)	Control group (c)		
	(n:34)		(n:30)	p A–B	p A–C
Age (years)	48 ± 12		45.5±14.7	–	0.75
Age at Diagnosis (years)	42 ± 13,5				
ACTH levels (25–60 pg/mL)	85,9 ±59,6	54,7 ± 42,8		<0.01*	
Serum cortisol levels (3.7 – 19.4 mg/dl)	26,1 ± 13,9	10 (0,19–20)		<0.01*	
24–hour urinary cortisol (5–55 µg/day)	494 (349–4000)	50 (9–1200)		<0.01*	
Hematocrit (%)	40.8±5.9	38.9±5	40±4.2	0.19	0.22
Total protein (mg/dl)	6.8±0.6	6.6±0.5	7.3±4.6	0.4	0.62
WBV at HSR (208/s)	16.3 ± 1.8	15.4 ± 1.7	16.2±1.9	0.2	0.33
WBV at LSR (0.5/s)	40.5 ± 38.2	25.4 ± 35.2	39.2±30.7	0.23	0.54

ACTH: Adrenocorticotroph Hormone WBV: Whole Blood Viscosity HSR: High Shear Rate LSR: Low Shear Rate

corticoid replacement dosage after surgery in these 5 patients was 5 (2.5–5) mg.

WBV at Baseline and After Surgery

At baseline, there was no difference in WBV-HSR and WBV-LSR between CD and control groups (p=0.33 and p=0.54, respectively). Furthermore, WBV-HSR and WBV-LSR were similar before and after treatment (16.3±1.8 versus 15.4±1.7, p=0.2, for WBV-HSR; 40.5±38.2 versus 25.4±35.2, p=0.23, for WBV-LSR) (Table 1).

Correlation analysis revealed that preoperative cortisol levels were significantly correlated with pretreatment levels (r=0.5, p=0.007), pretreatment 2-mg dexamethasone test (r=0.45, p=0.03), pretreatment 24-h urinary cortisol levels (r=0.73, p<0.001), and posttreatment ACTH levels (r=0.37, p=0.04). Moreover, posttreatment cortisol levels were significantly correlated with the pretreatment 2-mg dexamethasone test (r=0.47, p=0.02), pretreatment 24-h urinary cortisol levels (r=0.41, p=0.04), posttreatment ACTH levels (r=0.71, p<0.001), and posttreatment 24-h urinary cortisol levels (r=0.75, p<0.01). However, neither WBV-LSR nor WBV-HSR results were correlated with cortisol levels.

DISCUSSION

Our small-sized preliminary study findings showed that WBV, at both low and high shear rates, in the CD group was similar to that in the control group. In addition, no significant change was observed in WBV at both low and high shear rates before and after CD treatment. To our knowledge, this study was the first to investigate the role of WBV in CD patients.

CS is associated with a hypercoagulable state and increased risk of venous thromboembolism. TE contribute to high mortality rates in CS with pulmonary embolism, ischemic cardiac disease, and stroke (8). The studies show a shortening of the aPTT and elevated levels of factor VIII, factor IX, and von Willebrand factor in CS (9-12). Moreover, an excess of the fast-activating plasminogen activator inhibitor 1 (PAI-1 or SERPINE1) was shown to lead to the impairment in the fibrinolytic system (2). It was hypothesized that the TE might occur because of cortisol-induced upregulation of mRNA transcription of coagulation parameters and the increased activity of fibrinolysis indexes (13).

This study was conducted to investigate the missing piece of the puzzle for TE. As early as the 1920s, it was suggested that localized or general intravasal aggregation of red cells might contribute to thrombus formation (14). Thrombosis is associated with high blood viscosity owing to increased red cell aggregation (15). Blood is a thixotropic and multiphase fluid, and its viscosity is influenced by the flocculation–deflocculation equilibrium of red cells, the interaction between velocity gradient and red cells, and plasma (14).

Blood viscosity is briefly defined as the blood's internal resistance, contributing to endothelial shear stress (16). Both increased shear stress and viscosity induce endothelial damage and inflammation, and tend to thrombosis (3). WBV was investigated for both venous and arterial thrombosis because blood viscosity was suggested as a useful surrogate measure of perfusion for routine use in the general population. It was higher in patients with deep venous thrombosis, obstructive sleep apnea syndrome, retinal vein thrombosis, coronary slow flow phenomenon, systemic lupus erythematosus, aortic sclerosis, and obese patients (3, 4, 17-20). The Edinburgh Artery Study, the most comprehensive prospective study of WBV, showed a cognitive decline and an increased risk of stroke (21). WBV was also associated with insulin resistance, and these data suggest that elevated WBV is associated with insulin resistance (22).

Although considering that insulin resistance is a very well-known condition in CD and WBV might also be increased, we could not find such an increment in WBV in patients with hypercortisolism compared with the healthy control group.

Supraphysiological doses of glucocorticoids lead to accelerated skeletal muscle protein breakdown, stimulation of proteolysis, and protein synthesis inhibition (23). Several studies have revealed lower serum albumin and total protein levels in patients with CS (24, 25). Moreover, cortisol was believed to enhance the formation and increase the proliferation of erythroid cells (26) and to be associated with slightly increased hemoglobin and hematocrit levels (27). As a result of the prediction equation that has been validated for WBV, increased hematocrit and decreased levels of protein could have resulted in similarities between CD before treatment and the control group for WBV. However, the total protein and hematocrit levels were similar between groups in our study.

Our study should be interpreted with some limitations. First, we did not directly measure WBV with a viscometer. We had no data about other hemorheological parameters such as erythrocyte deformability and aggregation, and plasma viscosity. Apart from these, the lack of comparison with a group with CD with thrombosis because of this condition's rarity was another limitation of the study. The optimal cutoff level of WBV at LSR and HSR levels in predicting thrombosis could not be studied because of the limitation, as mentioned above.

CONCLUSION

Our study results revealed that the WBV, at both low and high shear rates, was similar between CD and control groups and in patients with CD either in hypercortisolemic or eucortisolemic state. We propose that more accurate results can be obtained with large-scale prospective studies with viscometer-based analysis.

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Original Research

Risk Factors in Childhood Intractable Epilepsy

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ABSTRACT
Objective: Intractable epilepsy is defined as a continuation of a seizure although antiepileptic drugs prescribed in accordance with the seizure type has been administered with adequate timing and dosage. Factors such as sex, age at onset of the seizure, family history, previous febrile seizure, mental and motor retardation, type of seizure, electroencephalography, and abnormal findings in neuroradiological imaging may cause resistance in treating epilepsy. Determining the risk factors of intractable epilepsy may be useful in the early diagnosis, prognosis, and treatment of the disease. This study aimed to determine the possible risk factors for the development of intractable epilepsy.
Methods: A questionnaire was completed face to face by the families of 210 patients with childhood epilepsy who applied to the Department of Child Neurology, Gaziantep University School of Medicine between January 2018 and September 2018 and were followed up. Data analysis was performed to determine the risk rates for the development of intractable epilepsy.
Results: Early-seizure onset, abnormal neurological examination, microcephaly, symptomatic and cryptogenic epilepsy, electroencephalography abnormality, pathology, and specific epileptic syndrome were found to be the effective risk factors for the development of intractable epilepsy.
Conclusion: The lack of the clear understanding of the definition of intractable epilepsy in the literature and the inability to clearly define its limits lead to contradicting results on the risk factors for it. Determining the exact criteria to evaluate the risk of developing intractable epilepsy in future studies and patient follow-up will guide the diagnosis, treatment, and prognosis of patients with intractable epilepsy.
Keywords: Epilepsy, intractable epilepsy, intractable childhood, risk factor

INTRODUCTION

Epilepsy is a common childhood neurological disease (1). Risk factors for childhood epilepsy include head trauma, encephalitis, perinatal hypoxia, congenital structural disorders, and febrile convulsions (2, 3). Intractable epilepsy is defined as a continuation of a seizure even after an antiepileptic drug prescribed in accordance with the type of seizure has been administered with adequate timing and dosage. However, there is no consensus on the number of drugs used and the frequency and duration of seizures in the definition of intractable epilepsy (4, 5). Many factors–including sex; age onset of the seizure; family history; previous febrile seizure; neonatal seizure; mental and motor retardation; type of seizure; status epilepticus; presence of specific epileptic syndromes or abnormal findings in electroencephalography (EEG) and radiological imaging; and multiple seizure types–may cause resistance to epilepsy treatment (6-8). It is thought that determining the risk factors for intractable epilepsy may be useful in the early diagnosis, prognosis, and treatment of the disease. This study aimed to determine the risk factors that can be prevented in intractable epilepsy and the possible risk factors that effectively predict the prognosis of epilepsy.

METHODS

In this prospectively planned study, a questionnaire consisting of 21 items was prepared taking previous studies into account (3, 4, 6, 8). The questionnaire was completed by the families of 210 patients with childhood epilepsy who applied to the Pediatric Neurology Department of Gaziantep University Faculty of Medicine between January 2018 and September 2018 and were followed up. Prior verbal consent was obtained from the families. The questionnaire was constructed by examining patient medical records.

Ethics Statement

The study was conducted in accordance with the principles of the Declaration of Helsinki, and previous permission was obtained from the Clinical Research Ethics Committee of the University of Gaziantep (Decision number 2018/23).

Statistical Analysis

The suitability of the data to fit a normal distribution was tested using the Shapiro–Wilk test, and Mann–Whitney U test was used to compare the variables that did not fit the normal distribution

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in 2 independent groups. The chi-square test was used to test the relationships between categorical variables. Binary logistic regression analysis was used to determine the variables that may have had an impact on epilepsy resistance and to estimate the risk ratio and 95% confidence intervals. Descriptive statistics are presented as mean±standard deviation for numerical variables and numbers with percentages for categorical variables. SPSS for the Windows version 22.0 package program was used for statistical analysis (IBM SPSS Corp.; Armonk, NY, USA), and $p < .05$ was considered statistically significant.

RESULTS

In this study, a questionnaire was distributed to 210 patients with epilepsy who applied to the Department of Child Neurology, Faculty of Medicine, University of Gaziantep between January 1, 2018 and September 1, 2018. A total of 122 patients (49 girls and 73 boys) who met the criteria were included in the study. The mean age of the patients was 110.50±48.79 months (minimum-maximum: 1-204). There were 21 girls and 42 boys in the group with intractable epilepsy and 28 girls and 31 boys in the group with epilepsy. There was no significant difference between the sex in both groups ($p = .113$).

We found that early seizure onset, abnormal neurological findings, microcephaly, symptomatic and cryptogenic epilepsy, EEG abnormalities, pathology in radiological imaging of the central nervous system (CNS), and specific epileptic syndrome were effective risk factors in the development of intractable epilepsy. Idiopathic epilepsy, CNS infections, febrile seizures, head trauma, consanguineous marriage, a family history of seizures, neonatal jaundice, maternal pre-eclampsia or gestational diabetes, infection during pregnancy, an appearance, pulse, grimace, activity, and respiration (APGAR) score ≤ 6, exposure to smoking, type and frequency of seizures, and seizure onset were not effective risk factors for the same (Table 1).

DISCUSSION

Epilepsy is one of the common neurological diseases of the CNS that are associated with recurrent seizure tendencies (9). Uncontrolled seizures in intractable epilepsy may cause many health problems, including aspiration, cardiac arrhythmia, renal failure, brain edema, electrolyte imbalance, sudden death from an unknown cause, and resistant status epilepticus (10). When these negative results are considered, it is important to determine the factors that may predict the development of intractable epilepsy in children to reduce future health problems and provide adequate support to families.

Main Points:

- Determining the risk factors for intractable epilepsy may be useful in the early diagnosis, prognosis, and treatment of the disease.
- The lack of a clear definition of intractable epilepsy in the literature and its limits have led to studies on the topic presenting differing findings.
- Early-seizure onset, abnormal neurological examination, microcephaly, symptomatic and cryptogenic epilepsy, electroencephalography abnormality, pathology, and specific epileptic syndrome may be the effective risk factors for the development of intractable epilepsy.

The definition of intractable epilepsy in the literature is controversial. Significant differences in treatment, the number of drugs patients use, seizure frequency, and observation time were noted in different studies (4, 5, 11). Gururaj et al. (12) defined patients with intractable epilepsy as those who used at least 3 antiepileptic drugs individually or in combination, were followed for 2 years, and had at least 1 seizure a month.

There was no statistically significant difference between the 2 patient groups in terms of sex, which was not a significant risk factor for intractable epilepsy. Kwan and Brodie (13) also found that sex was not a risk factor for intractable epilepsy. In contrast, another study from India with 442 patients reported that the male sex was a significant risk factor for developing intractable epilepsy (14).

Several studies have reported that age at onset of seizures is a risk factor for intractable epilepsy (10, 15-18). Kwong et al. (15) found that the onset of seizures before a child reached the age of 1 was a risk factor for intractable epilepsy. Ohtsuka et al. (16) reported that the rate of first seizure in patients with intractable epilepsy who were aged < 1 year was 53%. Similarly, this study found that the age at onset of seizures was a risk factor for intractable epilepsy; each monthly increase in age at onset of seizure decreased the risk of resistance by 0.984 units. In this study, the incidence of the first seizure was found to be 50.8% for patients with intractable epilepsy; in the literature, this rate varies between 50% and 60%, meaning that our finding is consistent with the literature (10). In this study, the rate of first seizure was found to be 32.2% for the group with epilepsy; in contrast, this rate was between 10% and 20% in the literature (10). The difference in the number of patients, different criteria for including patients in the intractable epilepsy group, and the cognitive level of the parents were thought to be responsible for this difference between our study and the literature. Age of seizure onset is likely to be a risk factor for intractable epilepsy because the CNS is damaged more and because the seizures are noticed late in a period of rapid neurological development.

Some studies report that abnormal neurological findings are a risk factor for intractable epilepsy (12, 17, 19). One such study found that neurological deficits and developmental delays were significant risk factors for intractable epilepsy (12). Similarly, this study also found that abnormal neurological findings were a significant risk factor for intractable epilepsy. Abnormal neurological findings indicate a pathology of the CNS in most children. Considering that pathological findings in CNS imaging and symptomatic epilepsy are risk factors for intractable epilepsy, it is considered that intractable epilepsy can be predicted in many patients if a neurological examination is performed correctly.

Other studies report that microcephaly is a significant risk factor for intractable epilepsy (10, 17, 20). In a study by Berg et al. (10) the rate of microcephaly occurrence was found to be 23.7%

Table 1. Distribution of patients according to risk factors

Risk factors	Intractable epilepsy	Epilepsy	Total	RR (CI)	p
Age of seizure onset (month) (mean±SD)	26.98±29.04	48.27±43.5	37.3±38.11	0.984 (0.97–0.99)	.003*
Abnormal neurological examination	40	20	60	3.39 (1.61–7.14)	.001*
Microcephaly	9	1	10	9.66 (1.18–78.86)	.034*
Epilepsy type					
Idiopathic	27	40	67		.737
Symptomatic	23	13	36	3.21 (1.08–9.48)	.035*
Cryptogenic	13	6	19	2.62 (1.13–6.05)	.024*
EEG abnormality	47	31	78	2.65 (1.23–5,69)	.012*
Pathology in the CNS imaging	47	19	66	6.18 (2.81–13.59)	.001*
Specific epileptic syndrome	12	0	12	2.15 (1.76–2.64)	.001*
CNS infection	3	2	5		.704
Febrile seizure	17	25	42		.076
Head trauma	7	9	16		.502
Consanguineous marriage	26	26	52		.750
History of seizure in family	30	27	57		.830
Neonatal jaundice	13	14	27		.681
Pre–eclampsia	2	3	5		.598
Maternal gestational diabetes	2	3	5		.704
Pregnancy infection	3	2	5		.704
Drug using during pregnancy	3	4	7		.634
APGAR score ≤ 6	8	2	10		.080
Smoking exposure	13	14	27		.681
Seizure type					.916
Partial	15	15	30		
Generalized	46	44	90		
Other	2	0	2		
Seizure frequency					.283
Every day	27	0	27		
Once a week	6	0	6		
Once a month	24	0	24		
Once a year	6	0	6		
More than 1 year	0	20	20		
More than 2 years	0	39	39		
Onset type of seizure					.674
Tonic–clonic	39	33	72		
Myoclonic	6	3	9		
Clonic	4	4	8		
Tonic	10	10	20		
Absence	3	6	9		
Atonic	1	3	4		

Asterisks indicate statistically significant differences.

APGAR, appearance, pulse, grimace, activity, and respiration; CI, confidence interval; CNS, central nervous system; EEG, electroencephalography; RR, risk ratio; SD, standard deviation.

in a group with intractable epilepsy and 3.1% in a group with epilepsy. Chawla et al. (17) found that the rate of microcephaly occurrence was 58% in children with intractable epilepsy and 2% in those with epilepsy. In these studies, microcephaly was found to be a significant risk factor for intractable epilepsy. In this study, microcephaly was detected in 10 of 122 patients (8.2%). Of these, 9 patients (14.29%) were in the group with intractable epilepsy and 1 was in the group with epilepsy; therefore, consistent with the literature, microcephaly was found to be a significant risk factor for intractable epilepsy. This shows the importance of physi-

cal examination and anthropometric measurements, particularly during infancy. In the literature, patients with intractable epilepsy and microcephaly also have abnormal neurological findings such as mental retardation. Therefore, it is considered that microcephaly is an important risk factor for intractable epilepsy.

Another parameter that constitutes a risk for intractable epilepsy is the type of epilepsy (16, 17, 21, 22). Ohtsuka et al. (16) reported that the type of epilepsy was a significant risk factor for intractable epilepsy and that there was a 52% rate of symptomatic epilepsy in a group of patients with intractable epilepsy. Chawla et al. (17) found the rate of symptomatic epilepsy to be 80% in a group of patients with intractable epilepsy. In this study, 67 of 122 patients (54.92%) had idiopathic epilepsy, 36 (29.51%) had cryptogenic epilepsy, and 19 (15.57%) had symptomatic epilepsy. A total of 27 of 67 children with idiopathic epilepsy (40.2%), 23 of 36 children with cryptogenic epilepsy (63.9%), and 13 of 19 children with symptomatic epilepsy (68.4%) were in the group with intractable epilepsy. However, those with idiopathic epilepsy were found to be the majority in both groups, and our findings did not agree with the literature in terms of patient distribution. The parameter assessed in this study that is consistent with the literature is symptomatic epilepsy, which was found to be a significant risk factor for intractable epilepsy (15-17). The common feature of both symptomatic and cryptogenic epilepsy is the pathology that causes the epilepsy. Idiopathic epilepsy is defined as epilepsy without pathology but with a genetic predisposition. From the definitions of types of epilepsy, the factors that may constitute risk factors for intractable epilepsy are included in symptomatic and cryptogenic epilepsy. Therefore, we considered it normal to identify these types of 2 epilepsy as risk factors in this study.

A study examining the relationship between EEG abnormality and the development of intractable epilepsy (16) found that EEG abnormality caused the development of resistance. In a study by Ko and Holmes (21) EEG abnormalities were found in 73.6% of patients in a group with intractable epilepsy and 41% of patients in a group with epilepsy. Gururaj et al (12) reported no significant difference in EEG abnormalities between a group of patients with intractable epilepsy and another of patients with epilepsy. In this study, 47 in 63 patients (74.6%) in the group with intractable epilepsy had EEG abnormalities, which–in agreement with the literature–were found to be significant risk factors for intractable epilepsy. One of the main reasons why EEG abnormalities appear to be a risk factor for intractable epilepsy is because pathologies in the CNS–including tumors, scars, Alzheimer's, and metabolic and infective pathologies–are more likely to produce abnormal signals than normal brain tissue.

The literature notes that the presence of pathology in CNS imaging studies has been a significant risk factor for intractable epilepsy (12, 17, 21). Gururaj et al. (12) reported an abnormality in CNS imaging as one of the risk factors for intractable epilepsy. Similarly, CNS pathologies were found to be risk factors in intractable epilepsy in 2 different studies (17, 21). In this study, 66 in 122 patients (54.1%) had a CNS pathology. A total of 47 of these 66 patients (71.21%) were in the group with intractable epilepsy, and the rate of the presence of pathology in CNS imaging was 74.6%. As consistent with the literature, an abnormality in CNS imaging was found to be a significant risk factor for intractable epilepsy. Considering that symptomatic epilepsy and abnormal neurological findings are a risk factor for intractable epilepsy, the presence of pathology on CNS imaging is expected to be a risk factor for intractable epilepsy.

A total of 2 different studies in the literature (17, 23) reported that CNS infections are risk factors for intractable epilepsy. In this study, only 5 of 122 patients (4.1%) had a history of CNS infection, and 3 of these (66.67%) were in the group with intractable epilepsy. In contrast to the literature, it was found that history of CNS infection had no effect on intractable epilepsy. This is thought to be due to the relatively small number of patients in this study.

The relationship between the history of febrile seizure and intractable epilepsy was investigated in several studies, and 2 of these (20, 21) reported that the former was a risk factor for the latter. However, others studies have found that a history of febrile seizures has no significant effect on intractable epilepsy (10, 12, 16, 18, 24, 25). The results of this study, in which the history of febrile seizures was not a risk factor for intractable epilepsy, are more in agreement with the latter.

Some studies have also investigated head trauma as a risk factor for intractable epilepsy (14, 26). Malik et al. (14) reported that prior head trauma is a risk factor for intractable epilepsy while Saygi et al. (26) obtained contrasting results. In this study, head trauma was not seen as a possible risk factor for intractable epilepsy. The difference between these results was thought to be a result of clinical findings on head trauma not being separated by clear limits. An investigation into the relationship between intractable epilepsy and head trauma, in another study that examines these variables in detail, is recommended.

In a study by Gururaj et al. (12) no significant relationship was found between consanguineous marriage and intractable epilepsy; our findings are similar.

Huang et al. (18) found that a family history of seizures was not a significant risk factor for intractable epilepsy; our findings are similar on this issue as well.

Two different studies (27, 28) reported that perinatal risk factors significantly increased the likelihood of intractable epilepsy. However, Russo et al. (29) reported that perinatal risk factors were not a significant risk factor for intractable epilepsy while Sidenvall et al. (30) reported that although perinatal risk factors were not risk factors for intractable epilepsy, a low APGAR score was significant. A study by Cansu et al. (23) found that both perinatal risk factors and an APGAR score ≤ 6 were significant risk factors for intractable epilepsy. In this study, APGAR scoring was performed, in addition to the other factors measured, to evaluate the perinatal risk factors; this was done by examining patient medical records. Our results showed that an APGAR score ≤ 6 was not a significant risk factor for intractable epilepsy but that it

was significant in borderline cases.

In terms of prenatal, perinatal, and postnatal characteristics, Sidenvall et al. (30) reported that problems during pregnancy–such as pre-eclampsia, maternal infection, and exposure to smoking–are risk factors for intractable epilepsy. Cansu et al. (23), however, did not identify a history of maternal infection as a risk factor; however, they found that perinatal problems such as hypertension to be risk factors for intractable epilepsy. Daoud et al. (31) identified the abnormal perinatal history criterion as a risk factor but did not identify subtitles for intractable epilepsy; however, they did state that this finding was debatable due to its subjective nature. In this study, gestational diabetes mellitus; pre-eclampsia; infections or drug use during pregnancy; exposure to smoking (an actively/passively smoking mother); and jaundice were hypothesized as prenatal, perinatal, and postnatal causes of intractable epilepsy. These hypothesized factors were not significant risk factors for the same; the low number of patients with prenatal, perinatal, and postnatal abnormalities in this study could be one way it is different from others in the literature.

There was no relationship between the type of seizure and the development of intractable epilepsy in 3 different studies; (26, 32, 33) however, there are studies in which focal-onset seizures were more common in patients with intractable epilepsy (15, 34). This study found no significant relationship between type of seizure and intractable epilepsy. The literature shows that the impact of seizure type on intractable epilepsy is not clear.

Some studies have reported seizure frequency as a risk factor for intractable epilepsy (10, 15, 18). Berg et al. (10) reported that more than half of patients with intractable epilepsy had seizures every day, and Chawla et al. (17) found that the rate of intractable epilepsy was higher in patients who had frequent seizures. In this study, 42.9% of patients with intractable epilepsy had seizures every day, 9.5% had seizures once a week, 38.1% had seizures once a month, and 9.5% had them once a year. Thus, 90.5% of patients with intractable epilepsy included in the study had seizures at least once a month. However, the frequency of seizures was not taken as a risk factor for intractable epilepsy; it was instead used to identify patients with intractable epilepsy. This was because the seizures that occurred in patients with intractable epilepsy could not be controlled with appropriate drug treatments; therefore, the number of seizures was taken as a criterion when determining which patients presented with intractable epilepsy.

There are also studies showing that the presence of a specific epileptic syndrome is a significant risk factor for intractable epilepsy; (10, 19) for example, Berg et al. (10) reported that West syndrome is a risk factor for intractable epilepsy although Ko and Holmes (21) reported that West syndrome alone did not increase the risk of intractable epilepsy. Yılmaz et al. (19) found that specific epileptic syndromes were a risk factor for intractable epilepsy. Similarly, this study found that the presence of a specific epileptic syndrome was a significant risk factor for intractable epilepsy.

Limitations

The low number of patients included in this study is one of its limitations. Future studies should involve more patient groups.

Conclusion

The lack of a clear definition of intractable epilepsy in the literature and its limits have led to studies on the topic presenting differing findings. Future studies to determine the exact criteria with which to evaluate the risk of patients developing intractable epilepsy and patient follow-ups on the same will play a role in guiding the diagnosis, treatment, and prognosis of patients with intractable epilepsy.

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Informed Consent: Informed consent was obtained from the families of the patients.

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Original Research

How Does Social Media Impact the Number of Citations? An Altmetric Analysis of the 50 Most-Cited MicroRNA Articles

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ABSTRACT

Objective: Altmetric analysis is web-based a metric analysis. Social media platforms affect medical literature over the last few years. The altmetric Attention Score (AAS) is an automatically calculated metric for monitoring social media. This study aimed to determine the correlation between AAS and the number of citations received from important articles published in the last 11 years with microRNAs.

Methods: MicroRNA as a search term was entered into the Web of Science database to identify all articles. The most 50 cited articles were analyzed by Topic, Journal Name, First Author, Publication Year, Citation, Average Citation Per Year (ACPY), Impact Factor (IF), Quartile (Q) Category, H Index, and AAS.

Results: Altmetric explorer identified 45.911 articles as being referred to online. Correlation analysis revealed that there was a weak correlation between AAS and the number of citations ($p<0.15$), while a very strong correlation was found between the number of citations and ACPY ($p<0.01$).

Conclusion: These results give some clues about the articles studied did not lose their currency. They are cited regularly each year so they are very popular in academia. This study provides a detailed list of 50 most cited microRNA articles and social media interest using the Altmetric.com database. miRNAs can be used in the diagnosis, prognosis, or treatment of various diseases.

Keywords: Social media, citation, Altmetric microRNAs

INTRODUCTION

MicroRNAs (miRNAs) are small, noncoding RNAs that are approximately 22 nucleotides in length. The biogenesis of miRNAs begins with the copying of DNA sequences into primary miRNAs, continues with transformation into precursor miRNAs, and is completed with the formation of mature miRNAs. miRNAs exert their effects through their target genes, which are messenger RNAs. In most cases, miRNAs interact with the 3′ untranslated region (UTR) of target messenger RNAs to suppress gene expression (1). MiRNAs have been reported to interact with other gene regions, including the 5′ UTR, coding sequence, and promoter (2). It has also been shown that miRNAs activate gene expression under certain conditions (3). Recent studies have suggested that miRNAs are shuttled between different subcellular compartments to control the rate of translation and transcription (4).

MiRNAs are involved in many cellular processes. These processes are proliferation, differentiation, apoptosis, and developmental process. Dysregulation of miRNAs leads to various diseases, such as cancer, cardiovascular diseases, and neurodegenerative disease (5-7). This dysregulation indicates that miRNAs can be used as potential markers in the diagnosis or prognosis of diseases. In addition, miRNAs are thought to be targets that can be used in the treatment of various diseases, including cancer. Understanding the roles of miRNAs in various biological processes has led to an increase in miRNA studies (8).

It is stated that each miRNA has hundreds of target genes. Various databases are used in the prediction of these target genes. Thus, the functional significance of miRNAs will be shown by the identification of possible target genes (9, 10).

MiRNAs can be secreted into extracellular fluids and transported to target cells through vesicles, such as exosomes, or by binding to proteins, including Argonautes. They can act as extracellular messengers because they can be taken up by new cells, where they potentially regulate gene expression. Extracellular or circulating miRNAs can be found in various body fluids, such as plasma and serum (11, 12). Extracellular miRNAs mediate cell-to-cell communication. Circulating miRNAs can be used as potential biomarkers for various diseases (13-15).

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Citation is one of the most important quality indicators of an article. However, the number of citations alone is not sufficient to determine the quality of the article. Impact factor (IF) is also used to measure the quality of a journal. IF is calculated by dividing the number of citations in the current year by the articles published in the journal during the previous two years (16). Another indicator used to measure journal quality is the H index (17, 18).

Many researchers use citation analysis to identify the most valuable studies in their field. The analyses that include the number of citations are referred to as bibliometric analyses. Bibliometric analyses were first applied by Eugene Garfield, the founder of Eugene Garfield Scientific Information Institute, in the 1970s (19).

The influence of social media platforms on medical literature has started to increase in recent years. Altmetric analyses are metric-based citation analyses. These analyses evaluate the effects of the number of citations received by academicians on social media (Facebook, Twitter, Wikipedia citations, Google+, mainstream media, RSS feeds, and videos) (20, 21). There are several sources used for altmetric analyses. One of them is Altmetric (altmetric.com). Altmetric Institution (Altmetric LLP, London, UK) uses different weighting values for various data sources to calculate the Altmetric attention score (AAS) (22).

Altmetric analyses are known to be very fast compared with traditional citation-based metrics analysis (23). While traditional citation-based metrics are only available for a few years after publishing, altmetric data sources can be updated in a real-time feed (e.g., Twitter and Wikipedia) or daily basis (e.g., Facebook and Google+) (24).

As far as we know, there is no study showing the relationship between the number of citations received by miRNA studies and AAS. Our study aims to show the correlation between the number of citations and the AAS using Web of Science (WoS), a data analysis tool, of the remarkable miRNA articles published in the last 11 years.

Therefore, in the context of the growing demand for the World Wide Web and social media, this study aims to analyze and visualize the knowledge structure of articles in the field of miRNA with a high AAS to explore current issues, active researchers, and journals.

Main Points:

- The term "miRNA" was searched on the Web of Science citation indexing database and the research platform and the articles published in the last 11 years were evaluated.
- This is the first study to evaluate the online attention received by the articles published in the microRNA field.
- Correlation analysis reveals strong correlation between citation and average cite per year (ACPY).
- Articles about miRNAs did not lose their currency, they are cited regularly each year so they are very popular in academia.
- The use of circulating miRNAs as minimal invasive biomarkers for diagnosis, prognosis or treatment monitoring has been explored mainly for cancer and cardiovascular diseases.

METHODS

Database

The citation data were obtained from the WoS database produced by Thomson Reuters. Search results from WoS encompassed entries from the WoS Core Collection, comprising Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index, Book Citation Index– Science, Book Citation Index –Social Sciences & Humanities, Conference Proceedings Citation Index-Science, Conference Proceedings Citation Index- Social Science & Humanities, and Emerging Sources Citation Index.

Search Terms and Methods

The WoS database was searched using the terms miRNA and microRNA with the Boolean operator OR. We reviewed articles on miRNA published in the last 11 years using publication and citation information from the WoS database.

The publication timeframe analyzed encompassed January 2009 to December 2019. The articles provided with full text in English are listed according to the citation numbers. The 50 most-cited articles were selected as previously described by Paladugu et al (25). In these articles, the title of the study, the first author, and the publication year as well as the study subjects were evaluated with AAS. AAS is based on three main factors: the volume, the sources, and the authors. The results obtained from the different sources are shown in altmetric donut colors. The amount of each color in the donut varies according to the sources of research output taken. The use of AAS and Altmetric donuts together is extremely useful to demonstrate an interest in the relevant research topic (22).

Statistical Analysis

WoS data tools were used to perform certain elements of result analysis, for example, generating journal citation reports.

Categorical variables were defined using percentages, and continuous variables were defined using median and interquartile ranges. Data were not normally distributed. Spearman rank correlation coefficient was used to assess the correlation between AASs, citations, average citation per year (ACPYs), postpublication year numbers, journal H indexes, and IFs. Spearman correlation test was interpreted according to r level: $r < 0.19$ was interpreted as very weak, $r = 0.2–0.39$ was interpreted as weak, $r = 0.4–0.59$ was interpreted as moderate, $r = 0.6–0.79$ was interpreted as strong, and $r > 0.8$ was interpreted as very strong. $P < .01$ was considered statistically significant. The statistical analysis was performed using the Statistical Package for the Social Sciences, version 21(IBM SPSS Corp.; Armonk, NY, USA).

RESULTS

Database and Publication Distribution

The number of articles published on miRNAs in the WoS Core Collection database (2009-2019) was 45.911. The first miRNA article was published in 2009. A total of 88% of all miRNA literature (40.401 publications) were published between 2009 and 2012, whereas 12% of the miRNA literature (5.510 publications) were published between 2013 and 2015. The most-cited miRNA publications were in 2009 with 67% publications (30.729) (Table 1).

Table 1. Top 50 cited primary miRNA publications.

Rank	Title	Publication Year	First Author	Citation	Average Citation per Year	Altmetric Attention Score
1.	MicroRNAs: Target Recognition and Regulatory Functions	2009	Bartel DP	11131	1011.91	41
2.	Most mammalian mRNAs are conserved targets of microRNAs	2009	Friedman RC	4302	391.09	10
3.	Origins and Mechanisms of miRNAs and siRNAs	2009	Carthew RW	2657	241.55	30
4.	Mammalian microRNAs predominantly act to decrease target mRNA levels	2010	Guo H	2334	233.40	27
5.	The widespread regulation of microRNA biogenesis, function and decay	2010	Krol J	2317	231.70	6
6.	Non-coding RNAs in human disease	2011	Esteller M	2023	224.78	39
7.	Causes and consequences of microRNA dysregulation in cancer	2009	Croce CM	1937	176.09	21
8.	Circular RNAs are a large class of animal RNAs with regulatory potency	2013	Memczak S	1861	265.86	171
9.	Natural RNA circles function as efficient microRNA sponges	2013	Hansen TB	1853	264.71	104
10.	Regulation of microRNA biogenesis	2014	Ha M	1785	297.50	33
11.	Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma	2011	Arroyo JD	1606	178.44	18
12.	Regulation of mRNA Translation and Stability by microRNAs	2010	Fabian MR	1472	147.20	22
13.	Predicting effective microRNA target sites in mammalian mRNAs	2015	Agarwal V	1419	283.80	15
14.	MicroRNAs in Cancer	2009	Garzon R	1407	127.91	9
15.	MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins	2011	Vickers KC	1376	152.89	20
16.	A Long Noncoding RNA Controls Muscle Differentiation by Functioning as a Competing Endogenous RNA	2011	Cesana M	1295	143.89	42
17.	Therapeutic microRNA Delivery Suppresses Tumorigenesis in a Murine Liver Cancer Model	2009	Kota J	1190	108.18	38
18.	The MicroRNA Spectrum in 12 Body Fluids	2010	Weber JA	1152	115.20	9
19.	Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps	2009	Chi SW	1093	99.36	37
20.	miRWalk - Database: Prediction of possible miRNA binding sites by "walking" the genes of three genomes	2011	Dweep H	993	110.33	6
21.	Secretory Mechanisms and Intercellular Transfer of MicroRNAs in Living Cells	2010	Kosaka N	980	98.00	23
22.	miR-145 and miR-143 regulate smooth muscle cell fate and plasticity	2009	Cordes KR	939	85.36	18
23.	MicroRNAs in Stress Signaling and Human Disease	2012	Mendell JT	931	116.38	9
24.	Characterization of extracellular circulating microRNA	2011	Turchinovich A	930	103.33	13
25.	Targeting microRNAs in cancer: rationale, strategies and challenges	2010	Garzon R	887	88.70	20
26.	miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis	2010	Ma L	849	84.90	11
27.	NON-CODING RNA MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship	2012	Pasquinelli AE	842	105.25	7
28.	MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review	2012	Iorio MV	841	105.13	44
29.	Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening	2009	Ng EK	826	75.09	9

Table 1. Top 50 cited primary miRNA publications. (Continue)

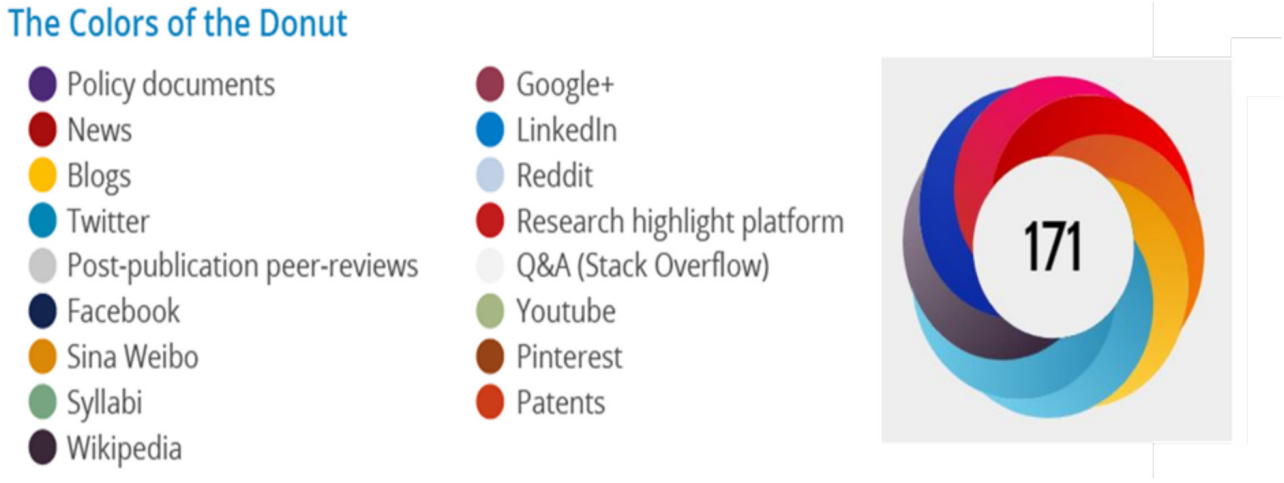
Rank	Title	Publication Year	First Author	Citation	Average Citation per Year	Altmetric Attention Score
30.	miRecords: an integrated resource for microRNA–target interactions	2009	Xiao F	814	74.00	5
31.	Plasma MicroRNA Profiling Reveals Loss of Endothelial MiR–126 and Other MicroRNAs in Type 2 Diabetes	2010	Zampetaki A	806	80.60	10
32.	Unidirectional transfer of microRNA–loaded exosomes from T cells to antigen–presenting cells	2011	Mittelbrunn M	803	89.22	3
33.	Functional delivery of viral miRNAs via exosomes	2010	Pegtel DM	802	80.20	33
34.	miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades	2012	Friedlaender MR	796	99.50	16
35.	Circulating microRNAs, potential biomarkers for drug–induced liver injury	2009	Wang K	792	72.00	15
36.	Modulation of microRNA processing by p53	2009	Suzuki HI	787	71.55	17
37.	starBase v2.0: decoding miRNA–ceRNA, miRNA–ncRNA and protein–RNA interaction networks from large–scale CLIP–Seq data	2014	Li JH	775	129.17	7
38.	MicroRNA profiling: approaches and considerations	2012	Pritchard CC	768	96.00	32
39.	Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis	2010	Kosaka N	761	76.10	13
40.	Downregulation of miRNA–200c Links Breast Cancer Stem Cells with Normal Stem Cells	2009	Shimono Y	761	69.18	9
41.	MicroRNAs in body fluids–the mix of hormones and biomarkers	2011	Cortez MA	743	82.56	31
42.	MicroRNA control of signal transduction	2010	Inui	740	74.00	4
43.	Highly Efficient miRNA–Mediated Reprogramming of Mouse and Human Somatic Cells to Pluripotency	2011	Anokye–Danso F	739	82.11	36
44.	MicroRNA biogenesis pathways in cancer	2015	Lin S	711	142.20	19
45.	MiR–33 Contributes to the Regulation of Cholesterol Homeostasis	2010	Rayner KJ	710	71.00	22
46.	Exosomal MicroRNA: A Diagnostic Marker for Lung Cancer	2009	Rabinowits G	706	64.18	13
47.	Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription–PCR (qRT–PCR)	2010	Kroh EM	704	70.40	7
48.	Induced Pluripotent Stem Cells and Embryonic Stem Cells Are Distinguished by Gene Expression Signatures	2009	Chin MH	697	63.36	19
49.	MicroRNA Control in the Immune System: Basic Principles	2009	Xiao C	690	62.73	6
50.	Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans	2010	Wang GK	682	68.20	6

In this study, the top 50 most-cited miRNA publications were mentioned. According to the information obtained from the WoS Database, miRNA publications are listed according to the number of citations they receive. The publication "miRNAs: Target Recognition and Regulatory Functions" is the most-cited article (11.131), whereas "Circulating miRNAs: A new potential biomarker publication for early detection of acute myocardial infarction in humans" is the least-cited article (682). The first article with the most citations was published by Bartel DP in 2009 (26), and the least-cited article was published by Wang GK (27) in 2010 (Table 1).

The publication with the highest number of ACPYs was published by Bartel DP in 2009 (1011.91) (26). The publication with the lowest number of ACPYs was published by Xiao C in 2009 (62.73) (28). (Table 1).

The publication with the highest AAS (171) was "Circular RNAs, a large animal RNA with regulatory potential," published by Memczak S in 2013 (29). The publication with the lowest AAS (3), "One-Way miRNAs-loaded exosomes are transferred from T cells to antigen-presenting cells" was authored by Mittelbrunn M in 2011 (30) (Table 1). The publication years of these articles, first author,

Figure 1. Altmetric donut shows the article with the highest Altimetric Attention score



Tablo 2. The rank of 50 research categories featuring miRNA publications most frequently, with the the number of publications per research category, and the percentage of overall publication.

Rank	Category	No. Works	%Total Works
1.	Review articles	32	64
2.	Original research articles	14	28
3.	Guidelines and advisory documents	2	4
4.	Editorial material	1	2
5.	Validation study	1	2
Total		50	100

number of citations, ACPYs, and AASs are summarized in Table 1. The colors of the donut show the rate at which the term miRNA appeared on various social media platforms (Research Highlight Platform QA, News, Patents, LinkedIn, and Twitter) (Figure 1).

Document Type

MiRNA publications comprised various document types, including review articles, original research articles, guidelines and advisory documents, editorial material, and validation study. The top 50 most-cited miRNA publications consist of 64% review articles (32 publications), 28% original research articles (14 publications), and 4% guidelines and recommendations (2 publications). The remaining publications comprise 2% editorial material (1 publication) and 2% validation studies (1 publication). Table 2 presents the document types in the top 50 most-cited miRNA publications and the numbers and percentage values of these documents.

Research Categories

MiRNA publications were classified according to research categories and subgroups in the WoS database. These publications consist of 48% cancer and diseases (24 publications), 44% regulation

Figure 2. Shows the correlation between the number of citations and average citations per year

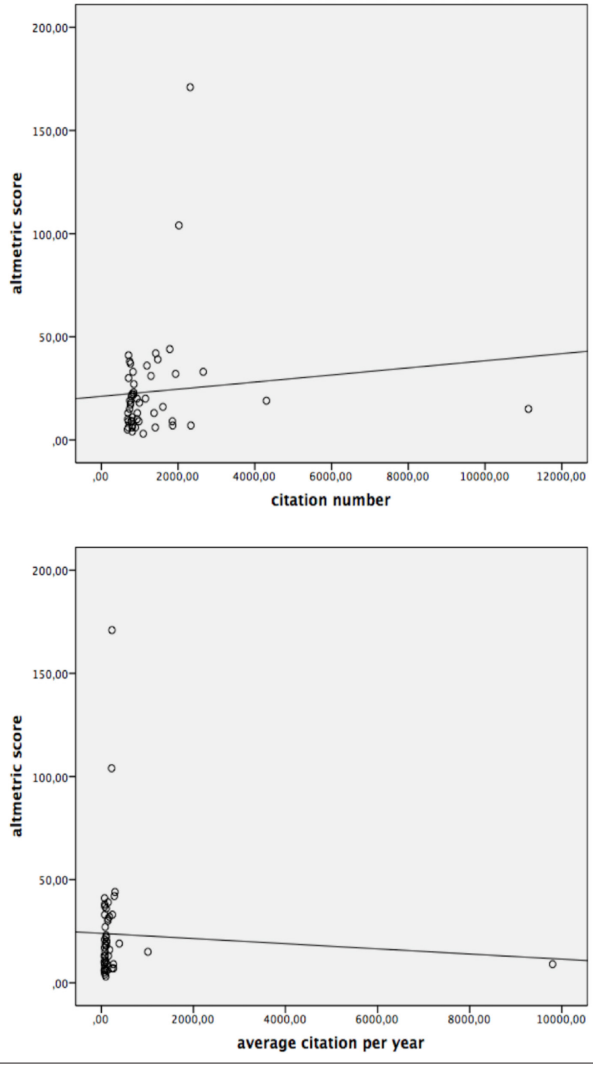


Table 3. Top-50 cited articles were classified according to research categories and subgroup

Rank	Main Subject	Subgroup
1.	Gene Expression Regulation	miRNA target recognition
2.	Gene Expression Regulation	miRNA target recognition
3.	Cancer and Disease	siRNA and mRNA biogenesis pathway
4.	Gene Expression Regulation	mRNA of protein-coding genes repression
5.	Gene Expression Regulation	protein-miRNA interactions
6.	Cancer and Disease	miRNAs and ncRNAs role of in cancer
7.	Gene Expression Regulation	miRNA-based therapies
8.	Gene Expression Regulation	CDR1 functions to bind miR-7
9.	Gene Expression Regulation	Circular RNA sponge for miR-7
10.	Gene Expression Regulation	Regulation of microRNA biogenesis
11.	Cancer and Disease	Ago2-miRNA complexes
12.	Gene Expression Regulation	MicroRNAs RNA- BindingProteins
13.	Gene Expression Regulation	miRNA target recognition
14.	Cancer and Disease	miRNA cancer biogenesis
15.	Diagnostic Biomarkers	HDL-miRNAs transports complexes
16.	Gene Expression Regulation	Myogenic Regulatory Factor miR-133
17.	Cancer and Disease	Expression of miR-26a by HCC cells
18.	Biomarkers	miRNAs in body fluids as biomarkers
19.	Gene Expression Regulation	Ago HITS-CLIP and miR-124 complexes
20.	Gene Expression Regulation	miRNA binding sites-miRWalk
21.	Cancer and Disease	Communication pathway by secretory miRNAs
22.	Gene Expression Regulation	miR-145 and miR-143 regulate smooth muscle cell
23.	Cancer and Disease	Stress signaling pathways
24.	Gene Expression Regulation	Extracellular circulating miRNA
25.	Cancer and Disease	miRNA target recognition
26.	Cancer and Disease	miR-9 metastasis
27.	Gene Expression Regulation	miRNA target gene recognition
28.	Cancer and Disease	miRNA diagnostics, monitoring and therapeutics
29.	Cancer and Disease	Diagnostic biomarker
30.	Gene Expression Regulation	microRNA-target interactions
31.	Cancer and Disease	miR-126 and other microRNAs in type 2 diabetes
32.	Cancer and Disease	Immunology/microRNA T cells to antigen-presenting
33.	Cancer and Disease	miRNA intercellular transfer
34.	Gene Expression Regulation	miRDeep2 Algorithm
35.	Biomarkers	miR122-miR192 circulating- drug-induced liver injury
36.	Gene Expression Regulation	Tumor Suppressor Protein p53
37.	Gene Expression Regulation	Protein-RNA interaction networks
38.	Gene Expression Regulation	miRNA profiling
39.	Cancer and Disease	Diagnostic biomarker
40.	Cancer and Disease	miRNA-200c links diseases
41.	Cancer and Disease	Diagnostic biomarker
42.	Gene Expression Regulation	Signal Transduction Network
43.	Cellular Reprogramming	miR302/367-mediated reprogramming
44.	Cancer and Disease	miRNA biogenesis pathway
45.	Cancer and Disease	miR-33 links liver and cellular cholesterol
46.	Cancer and Disease	Diagnostic marker for lung cancer
47.	Cancer and Disease	Diagnostic biomarker
48.	Gene Expression	IPSC and ESC are distinguished
49.	Cancer and Disease	Immune system regulatory
50.	Cancer and Disease	miR-1, miR-133a, miR-499 and miR-208a diagnostic biomarker for AMI

HCC: Hepato Cellular Carcinoma, AMI: Acute Myocardial Infarction, IPSC: Induced Pluripotent Stem Cells, and ESC: Embryonic Stem Cells

Table 4. Journals with top–50 articles, ranked according to the number of articles, Impact Factory, Quartile Category and H Index

Journal name	Number of articles	Impact Factory	Quartile Category	H Index
Cell	7	36,216	Q1	705
Nature	6	43,070	Q1	1096
Nature Reviews Genetics	5	43,704	Q1	320
Nucleic Acids Research	4	11,147	Q1	452
Proceedings of the National Academy of Sciences of The United States of America	3	9,580	Q1	699
Nature Reviews Molecular Cell Biology	2	43,351	Q1	386
Cell Stem Cell	2	21,464	Q1	212
Nature Cell Biology	2	17,728	Q1	337
Annual Review of Biochemistry	1	26,922	Q1	268
Elife	1	7,551	Q1	93
Annual Review of Medicine	1	10,091	Q1	148
Clinical Chemistry	1	6,891	Q1	201
Journal of Biomedical Informatics	1	2,950	Q1	83
Journal of Biological Chemistry	1	4,106	Q1	477
Nature Reviews Drug Discovery	1	57,618	Q1	289
Genome Research	1	9,944	Q1	269
Embo Molecular Medicine	1	10,624	Q1	90
Gut	1	17,943	Q1	262
Circulation Research	1	15,862	Q1	306
Nature Communications	1	11.878	Q1	248
Cancer Science	1	4.751	Q1	129
Nature Reviews Clinical Oncology	1	34.106	Q1	127
Nature Reviews Cancer	1	51.848	Q1	396
Science	1	41.037	Q1	1058
Clinical Lung Cancer	1	4.117	Q1	52
Methods	1	3.782	Q1	132
European Heart Journal	1	23.239	Q1	265

gene expression (22 publications), 6% biomarkers (3 publications), and 2% cellular reprogramming (1 publication) (Table 3).

Journal

MiRNA articles were classified according to the number of articles published in various journals. We saw that 7 articles were published in Cell journal, 19 articles in Nature and Nature Review Genetics, 4 articles in Nucleic Acid Research, and 3 articles in the United States National Academy of Sciences Papers. The remaining 17 articles were published in various declaration journals. The IF values of the journals varied between 2.9 and 57.6. Journal of Biomedical Informatics had the lowest IF, whereas Nature Reviews Drugs Discovery had the highest IF. All miRNA publications were published in the Quartile (Q) 1 category. It was observed that the journal with the highest H index was Nature (1.096), and the journal with the lowest H index was Clinical Lung Cancer (52). The journal name, number of articles, IF, Q category, and H index are presented in Table 4.

Correlation Analyses

Correlation analyses revealed a weak correlation both between AAS and the number of citations ($r = 0.207$, $p < .15$) and between

AAS and ACPY (r =.241, p <.09). In addition, a strong correlation was observed between the number of citations and ACPY (r = 0.866, p <.01). Correlation analysis is shown in Figure 2.

DISCUSSION

With the increase in the number of social network users worldwide, social media has an extremely important place in the dissemination of scientific and interdisciplinary information (31). Healthcare professionals use social media to share medical information about patients and to connect with colleagues around the world (32). To our knowledge, this is the first review to evaluate the online attention received by articles published in the miRNA field.

To understand the functions of miRNAs in physiological and pathological processes, miRNAs biogenesis must be known. The biogenesis and the functions of miRNAs are mentioned in 8 articles. In addition, miRNAs perform their functions through their target genes. The identification of target genes of miRNAs was reported in 9 articles. Various databases are used to determine target genes. One of these databases, mirWalk, determines the binding sites of miRNAs using information about genes known in humans, mice, and rats (33) and identifies not only the matches present that are complementary to 3′ UTRs but also other known regions of the gene.

MiRNAs whose target genes have been identified can be used for therapeutic purposes (34). Identification of miRNAs involved in the regulation of cellular processes will enable the functional significance of miRNAs to be determined. It has been reported that miR-26a acts as a proliferation inhibitor in hepatocellular carcinoma (35). A total of two miRNAs—miR-143 and miR-145—have been shown to play a role in the differentiation of smooth muscle cells and the regulation of plasticity (36). Moreover, it is stated that miRNAs play a balancing role in the regulation of cholesterol homeostasis. It has been reported that miR-33 is involved in both high-density lipoprotein biogenesis and the regulation of cellular cholesterol efflux in the liver (37). It has been shown in one study that miRNAs are involved in the formation of the immune response against autoimmune diseases and cancer (28). The functions of miRNAs in pluripotency have been described in two articles. In these studies, embryonic and pluripotent stem cells can be distinguished from each other owing to differences in gene expressions (38).

miRNAs can be used as biomarkers for a variety of diseases. The presence of miRNAs in body fluids has been reported in 12 separate articles. It is estimated that miRNAs found in body fluids can be used to evaluate and monitor various pathophysiological conditions (39). It has further been stated that miRNAs especially found in the blood of patients with cancer can be used as a new diagnostic criterion (40). The miRNAs whose expression changes in cancer disease have been mentioned in seven articles. It has been stated that the upregulation of miR-92 can be used as a biomarker in the plasma of patients with colorectal cancer (41). miRNAs may also play a role in preventing cancer metastasis. miR-9 has been shown to inhibit breast cancer metastasis (42). In addition, it has been stated that the upregulation of miR-208a in plasma can be used as a biomarker for early detection of myocardial damage (27).

It is extremely important to identify new miRNAs responsible for the emergence of human diseases. Various algorithms are used to identify new miRNAs. miRDeep2 is an algorithm used to identify canonical and noncanonical miRNAs (43). In determining the functions of miRNAs, their relationships with other noncoding RNAs and proteins need to be evaluated. One of the noncoding RNAs, competing endogenous RNAs, regulate the distribution of miRNA molecules on their targets (44). The other is small nucleolar RNAs that provide cellular homeostasis (45). It has been reported in two articles that noncoding RNAs are responsible for the occurrence of human diseases. For this purpose, a database called starBase is used to show the interaction of noncoding RNAs with miRNA and other proteins. Using this database, the functions of noncoding RNAs and the coordination of the networks they organize can be elucidated (46).

Correlation analyses revealed a weak correlation both between AAS and the number of citations (r = 0.207, p <.15) and between AAS and ACPY (r = 0.241, p <.09). These results show that the authors do not prefer to share their articles on social media. Although some articles received enormous citations, it was found that they were not common enough on social media. Although Bartel DP's publication had 11.131 citations, the AAS of this article was found to be 41. If these articles are shared on social platforms, they can be more enlightening or can attract the attention of different researchers. Because miRNA studies have been evaluated by a limited number of experts working in this field, it can be expected that the AASs of these studies are low. It has been stated that altmetric citations do not always reflect the impact value of highly cited articles (47). In a cross-sectional study conducted in the general medical journal, high-impact original research articles published in the full text were analyzed. In this study, it was shown that there is a weak correlation between AAS and the number of citations (48). It has also been reported that there is a moderate correlation between articles published in the cardiovascular field (49). It has been shown that there is a weak correlation between studies conducted in the field of radiology (50). Our results appear to be consistent with the literature. In addition, in our study, we observed a strong correlation between the number of citations and ACPY (r = 0.866, p <.01). These results give clues that the articles reviewed do not lose their validity. In addition, it has been observed that these publications are regularly cited every year. This shows that the subject of miRNA is still up to date and popular on the academic platform.

This study shows the impact of social media on the 50 most-cited miRNA articles. It has shown that miRNAs in circulation can be used, especially in the diagnosis, prognosis, and treatment of cancer and cardiovascular diseases.

The limitations of this study are that the altmetric analysis performed covers a certain period. Because altmetric analyses are constantly updated, fluctuations may be seen in the results of the analysis over time. In addition, it is necessary to reach the full text of the articles in order to make a few metric calculations. The

full text of only 57% of the miRNA articles (26.055 publications) selected in our study (45.911 publications) can be accessed. We think that this situation may change with the increase in open access opportunities.

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Original Research

High Endometrial Thickness Does not Affect IVF/ICSI Outcomes

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ABSTRACT

Objective: We examined the impact of thicker endometrium (>15 mm) on in vitro fertilization (IVF) outcomes by evaluating the rates of clinical pregnancy, ongoing pregnancy, and live birth.

Methods: Intracytoplasmic sperm injection procedures performed at a single IVF center were retrospectively examined. A total of 380 cases from patients aged between 19 and 39 years were included. The patients were divided into 2 groups according to their endometrial thickness (EMT) value determined using ultrasonography on the day of human chorionic gonadotropin administration.

Results: Embryo day was 5 in 78.4% of cases with EMT <15 mm and 3 in 89.8% of cases with EMT ≥ 15 mm (p<.001). In the group with EMT <15 mm, IVF outcomes were 61.5% clinical pregnancy, 54.7% ongoing pregnancy, and 49.0% live birth. In the group with EMT > 15 mm, IVF outcomes were 64.3% clinical pregnancy, 52.4% ongoing pregnancy, and 41.7% live birth. There was no significant difference between the groups in terms of clinical pregnancy, ongoing pregnancy, and live birth rates (p>.05). Determining the EMT cut-off value as 14 mm also did not yield significant results. Live birth was present in 47.4% of the cases. There were no statistically significant differences between the groups with and without live birth in terms of the variables examined.

Conclusion: There was no significant relationship between EMT and achieving a live birth through IVF. Nevertheless, conducting prospective and comprehensive studies on thicker endometrium may yield data that could be beneficial for IVF practitioners.

Keywords: Endometrium, IVF/ICSI, live birth rate, pregnancy

INTRODUCTION

Identifying the most suitable endometrium for a good embryo transfer has always been very important for in vitro fertilization (IVF) practitioners. The endometrium thickness in the proliferative phase of the menstrual cycle and provides the necessary environment for implantation of the embryo. Progesterone secreted after ovulation makes the endometrium suitable for embryo implantation. If pregnancy occurs after fertilization, progesterone continues to be secreted from the ovaries, and the endometrium continues to facilitate the development of embryo until the formation of placenta (1-3). Therefore, morphologically normal and receptive endometrium is essential for successful implantation in IVF treatment.

Detailed assessment of endometrial thickness (EMT) and endometrium patterns became possible with the use of ultrasonography (USG). Different views about the clinical importance of EMT are provided to patients who undergo IVF treatment owing to infertility. It has been suggested that hypoechoic endometrium is better than isoechoic endometrium or hyperechoic endometrium for embryo implantation.[4] However, the ideal EMT is still controversial. In most of the previous studies evaluating the effect of EMT on IVF outcomes, it was reported that a cut-off value of 6-8 mm could be used (1-3). When the clinical results after IVF and intracytoplasmic sperm injection (ICSI) were examined according to these cut-off values, it was found that the results were significantly more negative in cases with thinner EMT values in almost all the studies (5-16). However, in contrast to the unanimous opinion against thin endometrium, the number of studies investigating the role of thicker endometrium (>14 mm) is limited, and it remains controversial whether a thick endometrium can affect endometrial receptivity (17-22). Although there are studies showing that thicker endometrium positively (14,18) or negatively (17,19) impacts IVF outcomes, there are also various studies showing that thicker endometrium has no impact on IVF outcomes (23-26).

The impact of EMT on the success of IVF procedure is still unclear. When the endometrium is thinner than the previously defined cut-off values, transfers may be canceled confidently. However, when the endometrium is thicker (often taken as being >14 mm or >15 mm thick), there is still no consensus on whether the

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Table 1. Individual characteristics of participants

Characteristics	Endometrial thickness			*p*
	<15 mm (n=296)	≥15 mm (n=84)	Total (n=380)	
Age (year)	28.86±4.77	28.56±5.06	28.79±4.83	.618
Duration of infertility (year)	5 (1–24)	6 (1–22)	5 (1–24)	.276
Body mass index (kg/m^2)	26.24±2.68	26.64±1.90	26.33±2.53	.125
PCOS	278 (93.92%)	83 (98.81%)	361 (95.00%)	.088
Tubal factor	4 (1.35%)	0 (0.00%)	4 (1.05%)	.580
Unexplained	20 (6.76%)	1 (1.19%)	21 (5.53%)	.057
Other	2 (0.68%)	0 (0.00%)	2 (0.53%)	1.000
Endometrial thickness (mm)	10 (6–14)	16 (15–21)	11 (6–21)	<.001
E2 on hCG day (pg/mL)	2923 (616.8–8,320)	2995 (1023–9300)	2948 (616.8–9300)	.840
P on hCG day (ng/mL)	0.9 (0.1–4)	0.9 (0.09–3.1)	0.9 (0.09–4)	.553
M2	14.37±4.00	14.26±3.14	14.35±3.82	.785
Embryo transfer day				
2	1 (0.34%)	0 (0.00%)	1 (0.26%)	<.001
3	62 (20.95%)	67 (79.76%)	129 (33.95%)	
5	232 (78.38%)	17 (20.24%)	249 (65.53%)	
6	1 (0.34%)	0 (0.00%)	1 (0.26%)	
Freshcycles	296 (100.00%)	84 (100.00%)	380 (100.00%)	N/A
Clinical pregnancy	182 (61.49%)	54 (64.29%)	236 (62.11%)	.641
Ongoing pregnancy	162 (54.73%)	44 (52.38%)	206 (54.21%)	.703
Live birth	145 (48.99%)	35 (41.67%)	180 (47.37%)	.236

Data are presented as mean ± standard deviation or median (minimum–maximum) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables.
E2, estradiol; hCG, human chorionic gonadotropin; M2, mature oocyte; N/A, not available; P, progesterone; PCOS, polycystic ovary syndrome.

transfer should be canceled. In this study, we aimed to examine the impact of thicker endometrium (>14 mm and >15 mm) on IVF outcomes based on the rates of clinical pregnancy, ongoing pregnancy, and live birth to help clinicians who encounter thicker endometrium in practice.

METHODS

ICSI procedures performed at the Kayseri Memorial IVF Center between 2019 and 2020 were retrospectively examined. Patients aged between 19 and 39 years were included in the study. Ethics approval was obtained from the institutional review board (approval number: 3, date: 16/01/2020). Verbal informed consent was obtained from all participants included in the study. Controlled ovarian hyperstimulation of the patients was performed using an antagonist protocol with recombinant follicle-stimulating hormone. When at least 3 follicles were ≥17 mm in size, maturation was induced using standard recombinant 250 µg dose of human chorionic gonadotropin (hCG) (Ovitrelle; Merck Group, Darmstadt, Germany). Oocyte pickup (OPU) was performed via transvaginal route under anesthesia at 36 h after hCG. Embryo transfer was performed on the third or fifth day after OPU. The patients were divided into 2 groups according to the EMT value determined by USG on the day of hCG administration (group A had EMT <15 mm and group B had EMT≥15 mm). The age of the patients, cause and duration of the patients' infertility, hormonal characteristics of the patients, other characteristics, and clinical outcomes of the participants were recorded.

Main Points:

- Ideal endometrial thickness (EMT) is important for implantation in IVF procedure.
- Previous studies have shown that 7-15 mm EMT is the most suitable range, but there are not enough studies showing the impact of EMT > 15 mm on pregnancy outcomes.
- In this study, it was shown that EMT > 15 mm does not have a negative impact on pregnancy outcomes.

Statistical Analysis

All analyses were performed on the Statistical Package for the Social Sciences, version 21 (IBM SPSS Corp.; Armonk, NY, USA). Q-Q and histogram plots were used to determine whether

Table 2. Summary of individual characteristics with regard to the presence of live birth

Characteristics	Live birth		*P*
	Absent (n=200)	Present (n=180)	
Age (year)	28.92±5.10	28.65 ± 4.52	.587
Duration of infertility (year)	5 (1–24)	5 (1 – 20)	.652
Body mass index (kg/m^2)	26.47±2.37	26.18 ± 2.70	.262
PCOS	192 (96.00%)	169 (93.89%)	.480
Tubal factor	2 (1.00%)	2 (1.11%)	1.000
Unexplained	8 (4.00%)	13 (7.22%)	.251
Other	1 (0.50%)	1 (0.56%)	1.000
Endometrial thickness (mm)	12 (6–21)	11 (6 – 19)	.095
E2 on hCG day (pg/mL)	3000 (616.8–8697)	2892.5 (919–9300)	.307
P on hCG day (ng/mL)	0.9 (0.09–4)	0.9 (0.1–3.1)	.820
M2	14.43±3.79	14.26±3.87	.648
Embryo transfer day			
2	1 (0.50%)	0 (0.00%)	.223
3	75 (37.50%)	54 (30.00%)	
5	124 (62.00%)	125 (69.44%)	
6	0 (0.00%)	1 (0.56%)	

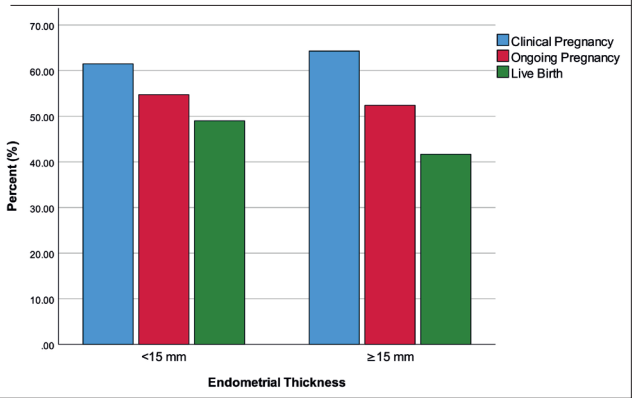
Data are presented as mean±standard deviation or median (minimum–maximum) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables.

the variables were normally distributed. Data are presented as mean±standard deviation or median (minimum-maximum) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables. Normally distributed variables were analyzed with independent samples *t*-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Categorical variables were analyzed using the chi-square test or Fisher's exact test. P =.04 was considered statistically significant.

RESULTS

A total of 380 women aged 19-39 years with fresh embryo transfer were included in the study. The EMT on the day of hCG administration ranged from 6 to 21 mm. Embryo day was 5 in 78.4% of cases with EMT <15 mm and 3 in 89.8% of cases with EMT≥15 mm. There was a significant difference between the groups in terms of embryo day (p<.001). In the group with EMT <15 mm, IVF treatment results were as follows: 61.5% clinical pregnancy, 54.7% ongoing pregnancy, and 49.0% live births. In the group with EMT>15 mm, IVF outcomes were as follows: 64.3% clinical pregnancy, 52.4% ongoing pregnancy, and 41.7% live births (Figure 1). There was no significant difference between the groups in terms of clinical pregnancy, ongoing pregnancy, and live birth rates (p>.05) (Table 1). When the EMT threshold was accepted as 14 mm, we did not find any differences between the groups.Live birth was present in 47.4% of the cases. There was no statistically significant difference between patients who had live births and those who did not in terms of the variables examined (Table 2).

Figure 1. Pregnancy and live birth percentages with regard to endometrial thickness



DISCUSSION

Studies investigating whether EMT is a determinant for IVF-achieved pregnancy in the literature have reported controversial results. In this study, which was carried out to evaluate the effect of EMT on the clinical outcomes of IVF, it was found that the frequency of clinical pregnancy and live birth is not associated with whether the EMT values are <15 mm or >15 mm. There was also no relationship between EMT and IVF outcomes in terms of live birth.

Many studies have examined the relationship between EMT and IVF outcomes, and there are systematic reviews and meta-analyses evaluating the findings of these studies. In a recent and comprehensive meta-analysis examining the impact of EMT on IVF cycle outcomes, Gao et al.[1] showed that decreased EMT was significantly associated with decreased rates of implantation, pregnancy, ongoing pregnancy, and live birth. In another systematic review and meta-analysis examining the effect of EMT on pregnancy rate with IVF, Kasius et al. (2) reported that EMT was not tenable as a parameter to predict pregnancy with IVF. In addition, they showed that the possibility of pregnancy and live birth rate decreased in cases with EMT≤7 mm (2). In a similar meta-analysis by Momeni et al. (3), it was reported that the EMT of women who became pregnant with IVF was higher than that of women who did not become pregnant. In addition, they found that the mean EMT difference between the groups was <1 mm.[3] In many cohorts, randomized controlled trials, and cross-sectional studies, the suitability of EMT as a tool to predict IVF outcomes at certain cut-off points was studied, and the results were significant (5-22). In our study, no significant difference was found in

EMT values when cases grouped according to the presence or absence of live births were compared. Moreover, no significant difference was found between the 2 groups (i.e., cases with EMT <15 mm vs cases with EMT≥15 mm) in terms of IVF outcomes.

In previous studies, IVF outcomes were compared according to different EMT cut-off values. Our literature review found that a cut-off value between 6 and 8 mm was determined for EMT in most of these studies (1-3) Although few studies focused on the impact of higher EMT values (e.g., EMT≥15 mm) on IVF outcomes, there is no study showing the relationship between high EMT values and pregnancy rates (11, 17-22). Some studies reported that an EMT ≥15 mm decreases the frequency of clinical pregnancy (17). In contrast, in some other studies, it was reported that the frequency of clinical pregnancy increased significantly with higher EMT values (14, 18). Another study reported that the frequency of miscarriage was higher in cases with EMT≥15 mm (19). It was thought that the negative results for thicker EMT may be caused by mechanical trauma in thicker endometrium during embryo transfer. In a study examining the effect of EMT on the day of hCG administration, Bu and Sun (20) reported that in all 3 groups (poor, medium, and high responders), the prevalence of pregnancy was significantly higher in cases with an EMT >14 mm than in cases with lower EMT value. In a study conducted in Turkey, Bozdag et al. (21) found that an EMT >14 mm had a positive impact on IVF outcomes. In some studies, different EMT cut-off values, such as 12 mm (11, 27, 28) and 16 mm (14, 29), were determined, and positive or negative IVF outcomes were reported. In some other studies, patients were classified into 3 groups according to EMT values (2 cut-off values). The results showed a significant difference between the group with the highest EMT values and that with the lowest value in terms of pregnancy and live birth rate (30, 31). The results of our study, in contrast to those of the aforementioned studies, show that IVF outcome is not associated with EMT, indicating that 14 or 15 mm values for EMT cannot be used as cut-off thresholds to predict IVF outcomes. Singh et al. (11) suggested that the minimum value for EMT should be 5.8 mm for clinical pregnancy, and an EMT value between 8 and 10 mm was ideal. They stated that there may be an increasing and then decreasing relationship between a higher value of EMT and success of IVF, suggesting that the success of IVF could decrease at extreme values of EMT (11).The similarities in IVF outcomes between the studies that grouped patients according to the primary cut-off value of EMT (15 mm) and our study in which a secondary analysis for EMT cut-off value of 14 mm contradict most of the previous findings. With more comprehensive and standardized future studies on this subject, our results can be examined in more detail.

Although the retrospective design of our study, an important limitation, can be considered a factor that could skew results, other studies including meta-analysis, prospective studies or observational studies have obtained similar conclusions (2, 23-26). In the meta-analysis by Kasius et al. (2) it was concluded that pregnancy with IVF could not be predicted with EMT. Rashidi et al. (26) reported that there was no significant difference between the EMT values of pregnant women and those of non pregnant women who underwent IVF. Similarly, Dietterich et al. (22) found that there was no significant relationship between EMT and IVF outcomes. In a study conducted in Turkey, Kınay et al. (13) reported that EMT was not a determinant for the development of clinical pregnancy with IVF.

CONCLUSION

Although there are conflicting views in the literature on whether EMT has a positive or negative impact on IVF outcomes (given that it is often accepted when it is above a certain threshold, such as 6 mm), it was generally suggested that lower EMT values would negatively impact IVF outcomes. In our study, we determined that there is no significant relationship between EMT and achieving a live birth with IVF when the patients were divided into two groups based on a 15 mm cut-off value. Determining the EMT cut-off value as 14 mm also did not yield any significant role in the prediction of IVF outcomes. Further prospective and comprehensive studies on thicker endometrium should be conducted in future to guide IVF practitioners on this matter.

Ethics Committee Approval: Ethics committee approval was received for this study from the institutional review board of Memorial Hospital (Approval date: 16.01.2020, No: 3).

Informed Consent: Verbal informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

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