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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.

European Journal of Therapeutics aims to contribute to the international literature by publishing original clinical and experimental research articles, short communication, review articles, technical notes, and letters to the editor in the fields of medical sciences. The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in in all medical disciplines.

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Editor in Chief: Prof. Murat Sucu
Address: Gaziantep Üniversitesi Tıp Fakültesi, 27310 Şehitkamil, Gaziantep, Turkey
Phone: +90 342 360 60 60 / 77751
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E-mail: info@eurjther.com

Publisher: Gaziantep University School of Medicine
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Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	250 (Structured)	30	6	7 or total of 15 images
Review Article	5000	250	50	6	10 or total of 20 images
Short Communication	1500	200	20	5	1 or total of 5 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

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.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. DOI: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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Address: Gaziantep Üniversitesi Tıp Fakültesi, 27310 Şehitkamil, Gaziantep, Turkey
Phone: +90 342 360 60 60 / 77751
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ORIGINAL RESEARCH ARTICLES

LETTER TO THE EDITOR

SHORT COMMUNICATION

European Journal of Therapeutics

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Original Research

Comparison of Human Papilloma Virus Results in Women with and without Atopic Disease

Gülsüm Uysal[1], Güzin Özden[2], Şevki Göksun Gökulu[3]

1 Adana City Training and Research Hospital, Department of Obstetrics and Gynecology, Adana, Turkey
2 Adana City Training and Research Hospital, Division of Allergy and Immunology, Adana,Turkey
3 Mersin University, Division of Gynecologic Oncology, Mersin, Turkey

ABSTRACT

Objective: Virus infected cells are destroyed by the natural immune system. However, HPV genotype and concomitant diseases are effective in HPV persistence. The aim of the study is to compare HPV screening results between patients with and without atopic disease.

Methods: This cross-sectional controlled study was conducted between August 2019 February 2020 in a tertiary research hospital. Patients between the ages of 30-65 with allergic complaints were diagnosed "atopic disease" by same clinician. HPV test results were compared to healthy controls. Subgroup atopic disease diagnoses, duration of illness, treatments regimes and treatment time of the patients in the study group were also questioned and recorded.

Results: A total of 239 women were recruited (121 study and 118 controls). Of recruited 121 atopia diagnosed women, 65 had rhinitis,9 had urticaria, 20 had asthma and remaning 27 had both rhinitis and asthma. 9 patients in control group and 7 patients in study group were HPV positive. No cervical carcinoma was reported. The mean duration of atopia was 50 months (12-360). 78 patients were treated medicaly while 43 were vaccinated during medical therapy

Conclusion: HPV test results of groups were similar. No extra screening schedules are needed for patients with atopic diseases such as asthma, rhinitis or urticaria.

Keywords: Human papilloma virus, screening test, atopic diseases, asthma, rhinitis, urticaria

INTRODUCTION

Cervical cancer is an important health problem in women. Human papillomavirus (HPV) is considered the primary etiological agent of cervical cancer worldwide (1). Cervical cancer screening methods are proved one of the few screening methods that are thought to decrease invasive cancer incidence and mortality (1-3). In our country, HPV screening is carried out free of charge by public health to women between the ages of 30-65 (4,5). Abnormal results, in cases where the HPV test is positive or abnormal cells are seen in the Pap-smear, the individual is directed to obstetricians at advanced centers (5). HPV screening is repeated every five years.

As with all virus infections, the cellular and humoral response of the immune system is very important in defense against the virus (6). Usually virus and virus infected cells are destroyed by the natural immune system (6). HPV genotype and concomitant diseases are effective in HPV persistence (7). Urticaria, Hepatitis B or C infection, chronic obstructive pulmonary disease and asthma are some examples for concomitant diseases (8). In some studies, systemic lupus disease and rheumatoid arthritis have also been shown to be associated with HPV infection (7,8).

Atopia causes tendency to develop allergic asthma, allergic rhinoconjunctivitis and atopic dermatitis due to creating type I hypersensitivity to certain antigens under the influence of environmental factors in individuals with genetic predisposition (9). The best known of atopic or allergic diseases are asthma, hay fever and eczema (9,10). T helper-1(Th-1) response decreased and T-helper-2 (Th-2) response increased in atopic patients (10,11). There are studies investigating the relationship between atopic dermatitis and HPV in the literature [10-11]. Moreover, it has been shown that a history of childhood atopic dermatitis may play a role in an increased risk of cervical cancer (12). Although mechanisms are not clarified (probably changes in T cell responses), patients with atopic disease may have tend to persistence of HPV or late cleansing. Our aim is to compare HPV screening results between women diagnosed with atopic disease and healthy women without a history of atopy. To our knowledge, it is the first study investigating HPV relationship under the title of atopic disease (allergic dermatitis, urticaria, atopic asthma, allergic rhinitis). If HPV is detected more frequently in patients with atopy, it may be recommended to follow more closely in vaccination and screening programs. Therefore, our study was designed to evaluate whether HPV positivity is common in atopic women.

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Corresponding Author: Gulsum Uysal **E-mail:** gulsumaykut@yahoo.com

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METHODS

This cross-sectional controlled study was conducted between August 2019 February 2020 at a tertiary referral research and education hospital. Informed written consent was received from all participants and ethics committee of City Education and Research Hospital approved the study with the number 494/2019.

Patients between the ages of 30-65 with allergic complaints (sneezing, itching, runy nose, respiratory distress) were diagnosed "atopic disease" by same clinician in Immunology and Allergy Department. The HPV results of the screened patients were noted retrospectively through the result reports of the patients, and those who were not screened were directed to Gynecology Department and HPV results were followed. Healthy women without allergic complaints applied for routine gynecologic examination occurred control group. Similary, the HPV results of the control group were recorded. Pregnancy, immunosuppressive therapy history (including chemotherapy and chronic corticosteroid therapy), HIV positivity, cancer and hysterectomized patients were excluded.

Patients' height, weight, demographic features (age, marital status, income level), obstetric histories (gravida, parity, mode of delivery), contraception methods and smoking status were noted. Subgroup atopic disease diagnoses, duration of illness, treatments regimes and treatment time of the patients in the study group were also questioned and recorded. Leukotriene receptor antagonists and histamine receptor antagonists were the main treatments for study group and all therapies were designed by the same clinician. Routine allergic skin tests were performed to all atopic patients during diagnosis.

Statistical Package for Social Sciences (SPSS) for Windows, version 18.0 (SPSS Inc. IL, USA) was used to compare the clinical features of groups. Normality of data distribution was tested with Kolmogorov-Smirnov test. Data was shown as means ± SD for continuous variables and the independent t test was used to assess the differences in variables between groups. Kruskal–Wallis, Mann–Whitney U-test and Bonferroni correction were used for values with non-normal distribution. P Values below 0.05 ($p<0.05$) was accepted as statistically significant.

RESULTS

A total of 239 women were recruited. Among patients, 121 were diagnosed atopia and 118 healty women occured control group. The baseline characteristics of the all patients were shown in Table 1. There was no significant difference between in mean scores of age, body mass index (BMI), age at first marriage. No other significant differences in frequency of smoking status was reported. The two groups were comparable in terms of gravidity, parity and abortion. Regarding delivery mode among groups; cesarean section was the highest in the study group, while normal delivery was the highest in the control group. Nulliparity rate was similar between groups. Income per month was found statistically higher in study group ($p=0.002$). The most common contraception method was condom in both groups (57.9 %, 38.1%, study and control group respectively.) while patients with no contraception was the highest number (30.6 %, 49.2%, study and control group respectively). 9 patients in control group and 7 patients in atopic group were HPV positive. One patient had HPV type 16 while remaining 8 patients had other types of HPV in the control group. No cervical carcinoma was reported.

Clinical diagnosis and treatment history of study group were shown in Table 2. Of recruited 121 atopia diagnosed women, 65 had rhinitis, 9 had urticaria, 20 had asthma and remaning 27 had both rhinitis and asthma. The mean duration of atopia was 50 months (12-360). 78 patients were treated medicaly while 43 were vaccinated during medical therapy. The median treatment time after diagnosis was 28 months. There was no patients with atopic dermatitis.

Main Points:

- Cervical cancer screening methods are proved one of the few screening methods that are thought to decrease invasive cancer incidence and mortality.
- It is the first study investigating HPV relationship under the title of atopic disease (allergic dermatitis, urticaria, atopic asthma, allergic rhinitis).
- There is no need to different and extra screening schedules for patients with atopic diseases such as asthma, rhinitis or urticaria.

DISCUSSION

This study reports that there was no significant difference in terms of HPV positivity. Between groups (controls and atopic diseases such as asthma, allergic rhinitis and urticaria).

In a recent retrospective study, Garritsen et al. (10) searched Pap smear results of 189 atopic dermatitis diagnosed patients medicating oral immunosuppressive drugs such as azatioprine, methotrexate and other similar drugs for more than 2 months between 1989 and 2014. They found no cervical carcinoma and suggested that extra screening for patients using oral immunosupressive drugs was unneccessary for patients with atopic dermatitis (10). Additionaly, although transplant patients have used more than one immunsupressive drug for a long period of time, some studies revealed low prevelance of cervical HPV infection in renal transplant recipients (13,14). This might be explained by being monogamous or sexual inactivity among that patient population. Moreover, HPV vaccine may have an impact on decreased incidence in the future, although it is not yet widely available.

Orrigoni et al. (15), enrolled solid organ transplanted 48 patients prospectively for 10 years and they checked Pap smear and HPV tests each year during follow up time. Compared to control group there was no statistically significant higher incidence of HPV infection or high-grade cervical dysplasia in transplant received patients (15). On the other hand, Meeuwis et al (16), reported high number of anogenital tract malignancies and detected high-risk HPV (especially type 16) in 91.7% of investigated lesions among 1023 patients who were performed renal transplantation between 1968 and 2008. Also, they suggested periodically screening before and after the transplantation (16). There has been no consensus in literature on this issue.

Table 1. Basic Characteristics Of Groups

	Control group n= 118	Study group n= 121	P value
Age (y)	42.9±7.5	41. 3±7.3	0.1
BMI (kg/m2)	27.0±4.7	26.8±4.5	0.2
Age of first marriage (y)	21.3±4.9	21.8±3.7	0.3
Smoking (yes) N, (%)	30	26	0.47
Gravidy	2.9±1.9	3.4±2.0	0.06
Parity	2.3±1.2	2.6±1.5	0.07
Abortion *	0 (0-7)	0 (0-13)	0.4
Type of delivery, N, %			
No delivery	7 (59)	6 (5)	
Vaginal birth	66 (55.9)	45 (37.2)	0.009
Cesarean section	45 (38.1)	70 (57.9)	
Income (tl)*	2000 (1000-10.000)	2600 (700-24000)	0.002
HPV positivity	9	7	0.56

BMI: body mass index, y: year, n:number, OCT: oral contraceptives, IUD: Intrauterine device,

*: median (min-max)

Table 2. Clinical Diagnosis And Treatment History Of Study Group

Study Group N=121	
Type of Atopia	
Rhinitis	65
Asthma	20
Rhinitis+ Asthma	27
Urticaria	9
Time of Atopia (month)*	50 (12-360)
Type of treatment	
Medical	78
Medical+ vaccine	43
Time of treatment (month)*	28 (2-360)

*: median (min-max)

In another study, they investigated the risk of cervical dysplasia and cervical cancer in women with systemic inflammatory diseases such as inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE), psoriasis and rheumatoid arthritis (RA) (17). They concluded that SLE and RA patients had 1.5 times higher cervical dysplasia and cervical cancer compared to patients with non-systemic diseases (17). This result was thought to be related with different severity of inflammation and doses of systemic immunosuppressive drugs. It was also reported that asthma, chronic rhino-sinusitis, atopic dermatitis, eosinophilic esophagitis, and IBD had similar tight junctions leading pathogenic mechanisms of these diseases (18). T-helper 2 (Th2) cells play one of the major role in the pathogenesis of the allergic asthma and allergic dermatitis through pro-inflammatory cytokines (9,19). Moreover, HPV infection was well known to promote Th2 cells while reducing Th1 cells (20). Therefore, we chose to screen HPV positivity in atopic diseases including asthma, allergic rhinitis, atopic dermatitis or urticaria. Morgan et al. (11) compared the characteristics of patients with HPV infection to negative controls. As a result, they found that atopic dermatitis was statistically significantly high in HPV positive ones. However, allergic rhinitis or patients who had both allergic dermatitis and rhinitis were similar between groups. It was thought that difference of Th1 and Th2 balance may be related to results of the study. Cellular immunity declines due to reduced Th1 response (21). In our study, allergic patients were selected first and then

HPV results were checked. We preferred to compare the atopic and non-atopic patients results regarding HPV tests. The main significant difference was monthly income between groups. Allergic patients had higher income. It was well known that patients who had more income pay more attention to their allergic or cosmetic problems. The rates of contraception methods and delivery modes were also different between groups. Study group had higher cesarean rates and higher condom usage which may be in parallel with high income and may affect the HPV results. On the other hand, we had no patients with atopic dermatitis. It was thought to be related with preference of those patients to dermatology department mainly in our country.

In another different relationship between asthma and HPV was genetic control mechanism via serine/arginine rich splicing factors (SRSFs) (22,23). Those studies showed that SRSF2 and SRFS3 were important modulators of HPV16 for protein expression and maintenance of cervical tumor (22,23). Interestingly linked, SRFS6 was reported a trigger of asthma in horses and they also claimed that new therapy methods could be tried due splicing factors in asthma (24). The relationship between HPV and asthma seems complicated and larger, future studies are needed.

In a recent study, they analyzed the expression of HPV capsid protein by immunohistochemistry, for presence of HPV DNA via polymerase chain reaction in placenta specimens with villitis of unknown etiology (VUE) and chronic deciduitis with plasma cells (CD) (25). VUE and CD in miscarriage, preterm delivery, and adverse pregnancy outcome are supposed to be non infectious placental lesions caused by a pathologic immune reaction similar to a host versus graft mechanism and the frequent detection of autoimmune diseases in the VUE was 21% with atopic disease, 15.5% with other autoimmune disease, 10.5%with thrombophilia (25). Finally, the results show that a causal role for HPV in the development of VUE and CD is unlikely. A pathologic immune reaction is more probable since clinical signs of infection are usually not seen in association with the mentioned chronic inflammatory lesions (26). Therefore, the inflammation caused by autoimmune disease are not correlated with HPV in line with our smear test results.

As for the limitations, it was not a long follow-up study. The mean ages of the all partipicants were between 41 and 42. Therefore, HPV reports may change in years due to immune system and treatments. Second, patient population was small compared to retrospective studies.

A major strength of the present study was being the first study evaluating HPV results in women regarding "atopia" title that involves allergic asthma, rhinitis or both.

CONCLUSION

The significance of human papilloma virus as an etiologic factor in cancers among immun suppresive treated patients is known but for atopic disease it is unclear and still subject of debate. Our results showed that HPV positivity in smear test was not associated with atopic disease. Although HPV vaccination should still be encouraged to prevent carcinoma of the cervix, there is no need to different and extra screening schedules for patients with atopic diseases such as asthma, rhinitis or urticaria.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Trials Ethics Committee of City Education and Research Hospital (decree number: 494/2019).

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Original Research

Investigation of Antibiotic Resistance Profiles and Carbapenemase Resistance Genes in Acinetobacter Baumannii Strains Isolated From Clinical Samples

Osman Karabay[1], Fahriye Ekşi[1], Mehmet Sait Yıldırım[2], Deniz Gazel[1]

[1] Gaziantep University, Faculty of Medicine, Department of Medical Microbiology, Gaziantep, Turkey
[2] Dr. Ersin Arslan Training and Research Hospital, Microbiology Laboratory, Gaziantep, Turkey

ABSTRACT

Objective: Acinetobacter baumannii is an important pathogen that can develop multiple drug resistance. Here, we aimed to investigate the antibiotic resistance profiles of A. baumannii strains isolated from the various clinics of our hospital and determine the class D beta-lactamase resistance genes causing carbapenem resistance.

Methods: Between June-2016 and June-2017, 157 A. baumannii strains isolated from clinical specimens of our hospital were identified with automatic bacterial identification system and antibiograms were determined by the same system. Among the carbapenem resistant strains, bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-23} and bla_{OXA-24} genes were also investigated by PCR method.

Results: When we analyze the resistance profiles of the strains, we observed that the lowest resistance rate was against colistin with 5 (3.2%) strains. OXA-51 and OXA-23 genes were found positive in all isolates, while OXA-24 was found positive in 16 (32%) strains; OXA-58 was not detected in any of the strains.

Conclusion: The most effective antibiotics for carbapenem resistant A. baumannii isolates were colistin, tigecycline and amikacin. Prevelance of OXA-24 enzyme gene was found higher than other similar studies. Monitoring antibiogram profiles and conducting molecular epidemiological studies may help us detect resistant bacteria at the source and reduce the development of resistance.

Keywords: Acinetobacter baumannii, OXA-23, OXA-24, OXA-51, OXA-58

INTRODUCTION

Acinetobacter baumannii is one of the the most important species in the Acinetobacter genus and has become one of the most important pathogens in hospital settings globally. In the last 15 years, its clinical importance has increased along with antibiotic resistance rates. These features have made it one of the main organisms threatening the current antibiotic use (1). A. baumannii generally targets patients who are most susceptible and have suppressed immune systems. Health-care associated pneumonia is accepted as the most common infection caused by A. baumannii; however, in recent times, infections involving the central nervous system, skin and soft tissue and bone have become very problematic for hospitals and health organizations (1). In A. baumannii strains, resistance to beta-lactam antibiotics involves production of beta-lactamase coded by chromosomes or plasmids. The reason for resistance against carbapenem group antibiotics is changes in proteins binding to porin and penicillin associated with acquiring genes coding class B or class D beta-lactamases. The most common beta-lactamase type acquired and causing carbapenem resistance in A. baumannii are some OXA-23, OXA-24, OXA-40, OXA-58 and OXA-143 enzymes from oxacillinases. Additionally, overproduction of OXA-51 type natural oxacillinase in conjunction with other OXA enzymes causes high levels of carbapenem resistance. Phenotypic tests are inadequate for identification of OXA enzymes and with the lack of reliability; these enzymes can be definitely identified using molecular methods (2, 3).

In this study, the aim was to research A. baumannii strains isolated during a one-year period from patient samples in a variety of clinics in Dr. Ersin Arslan Training and Research Hospital to determine antibiotic resistance status, and to determine the class D beta-lactamase (OXA-23, OXA-24, OXA-51, OXA-58) resistant genes causing carbapenem resistance in strains resistant to imipenem and meropenem with the real-time polymerase chain reaction (PCR) method.

METHODS

Sample Selection

The study included 157 A. baumannii strains isolated from patient samples sent under appropriate conditions to the Medical Microbiology laboratory of Dr. Ersin Arslan Training and Research

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Corresponding Author: Deniz Gazel **E-mail:** denizgazel@yahoo.com

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Hospital from June 2016 to June 2017. From these 157 strains, 50 A. baumannii strains resistant to imipenem and meropenem chosen randomly were researched for OXA-23 group, OXA-51 group and OXA-58 group genes with real-time PCR, while OXA-24 group resistant genes were researched with the optimized PCR method. Only one A. baumannii strain isolated from the same patient was included in the study.

Detection of Isolates and Antibiogram

Urine samples from patients were seeded on 5% sheep blood agar (RTA, Turkey) and EMB agar (GBL, Turkey) while other samples were seeded on 5% sheep blood agar, EMB agar and chocolate agar (RTA, Turkey) and left for 18-24 hours incubation at 37 °C. The blood culture samples were incubated in an automated culture system (BD BACTEC™ FX blood culture system, USA). Samples with proliferation in the automated blood culture system had seeding performed on 5% sheep blood agar and EMB agar after the device warning and were left for 18-24 hours incubation at 37 °C. Proliferating bacteria had smear preparate created from oxidase negative, non-hemolytic, catalase test positive colonies. Samples investigated with the microscope with gram negative coccobacillary appearance were defined at species level with a BD Phoenix™ (BD Phoenix™ ID & AST System, USA) automatic identification system and antibiotic susceptibility profiles were determined. The results of the susceptibility tests categorized samples as susceptible, moderately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) criteria (4). The A. baumannii strains obtained from pure culture were stored at -20 °C in beaded storage media for use in advanced studies.

Determination of Carbapenem-Resistant Isolates

The randomized 50 A. baumannii strains from isolates which were resistant to imipenem and meropenem in the automated system were also susceptible to meropenem and imipenem via Kirby-Bauer disk diffusion method according to the CLSI recommendations. Additionally, the minimum inhibitory concentration (MIC) values for these two antibiotics were determined with the gradient strip test (Bioanalyse, Turkey). The Pseudomonas aeruginosa ATCC 27853 standard strain was used as quality control strain.

DNA isolation and molecular study:

A. baumannii DNA isolation was performed using a QIAamp DNA Mini Kit (Qiagen, Germany). The procedure was repeated according to the manufacturer's advice. Later the OXA-23 group, OXA-51 group and OXA-58 group genes were researched with real time PCR. The 50 A. baumannii strains determined to be resistant to meropenem and imipenem with the disc diffusion and gradient strip method and chosen randomly were researched for the presence of OXA-type carbapenemase groups (OXA-23 group, OXA-51 group and OXA-58 group). Four strains obtained from Gaziantep University were used as control strains. The first control strain was used as positive control for OXA-51 and OXA-58, the second control stain was used as positive control for OXA-51 and OXA-24 and the third control strain was used as positive control for the OXA-51 and OXA-23 gene regions. Additionally, a negative control strain was used without detection of these gene regions. To determine primers and probes, firstly some of the variant arrays for the OXA-51, OXA-23 and OXA-58 groups observed in A. baumannii were downloaded from the NCBI database. Alignment files were created using the ClustalW program. This file was opened with the jalview program and unchanging regions within the groups and regions displaying differences from other groups were observed and the primer and probe alternatives were determined with the aid of the Vector NTI program. As the multiplex PCR in the study can be optimized, the interactions of the relevant primers and probes were researched with the Vector NTI program and appropriate arrays were synthesized (care was taken that Tm degrees of primers were kept close to each other and Tm degrees of probes were at least 6-7 degrees more distant from primers). Differentiation of the proliferating regions was performed with the aid of probes synthesized with OXA 51-FAM, OXA 58-Cy5, and OXA 23-HEX stains. To check the synthesized primers and probes in the study, the experiments were designed with the same reaction program though samples were run independently for OXA-23, OXA-51 and OXA-58. All samples were trialed with a triple mix created in the same way with the Fluorion detection system (İontek, Turkey) and expected results were obtained. Then the multiplex reaction was optimized. Separate from these, PCR optimized with the primers designed with the method above for OXA-24 was used to study positive controls and other samples. Positive bands were checked with a gel imaging system.

Main Points:

- All isolates were positive for chromosomal-derived OXA-23 and OXA-51 enzymes.
- The OXA-24 enzyme had the rate of 32% which was higher when compared to other studies
- Monitoring antibiotic susceptibility patterns and performing molecular epidemiological studies may help reducing the infections by detecting the source of the bacterial isolate.

RESULTS

The samples containing the 157 A. baumannii strains included in the study comprised 64 samples from women (41%) and 93 from men (59%). The mean age of these patients was 53 years, with age interval from 1 to 91 years. Isolates produced from the samples were obtained from tracheal aspirate culture for 65 samples (41.4%), wound culture for 35 (22.3%), blood culture for 30 (19.1%), sputum culture for 17 (10.8%), urine culture for 5 (3.2%), CSF culture for 3 (1.9%) and catheter culture for 2 (1.3%). Among the patients with isolates obtained, 121 (77.2%) were intensive care patients, and 36 (32.8%) were ward patients. According to the distribution of units, A. baumannii strains were isolated from patient samples with 102 from the general ICU (65.1%), 15 from the wound care ward (6.4%), 10 from the chest diseases ward (6.4%), 7 from the additional building ICU (4.5%), 6 from the internal medicine ICU (3.8%), 4 from the cardiovascular surgery intensive care (2.5%), 4 from the orthopedics and traumatology ward (2.5%), 3 from the infectious diseases ward (1.3%), 2 from the neurology ICU (1.3%), and 1 each from the burns, nephrolo-

gy, brain surgery, oncology and rheumatology wards (0.6%). The distribution of the antimicrobial susceptibility profiles of 157 isolates are given in Table 1.

The susceptibility pattern of 50 A. baumannii isolates that were 100% resistant to imipenem and meropenem with genotyping performed found 37 strains (74%) were resistant, 1 strain (2%) was moderately susceptible and 12 strains (24%) were susceptible to amikacin. For cefepime 50 strains (100%) were resistant, for ceftazidime 50 strains (100%) were resistan, for ceftriaxone 50 strains (100%) were resistant and for ciprofloxacin 50 strains (100%) were resistant. For gentamicin, 47 strains (94%) were resistant and 3 strains (6%) were susceptible. For colistin, 3 strains (6%) were resistant, and 47 strains (94%) were susceptible. For netilmicin, 48 strains (96%) were resistant and 2 strains (4%) were susceptible. For piperacillin, 50 strains (100%) were resistant, while 50 strains (100%) were also resistant to piperacillin tazobactam. For tigecycline, 32 strains (64%) were resistant and 18 strains (36%) were susceptible. For trimethoprim sulfamethaxasole, 44 strains (88%) were resistant and 6 strains (12%) were susceptible. Results are summarized in Table 2.

In the chosen 50 A. baumannii isolates, the A. baumannii specific structural gene group of bla_{OXA-51} and the gene groups stated to be responsible for carbapenem resistance in the literature of bla_{OXA-23}, bla_{OXA-58} and bla_{OXA-24} gene groups were researched with real-time PCR. The structural gene group for A. baumannii of bla_{OXA-51} and the bla_{OXA-23} gene group were identified in 50 of the isolates (100%). None of the isolates had the bla_{OXA-58} gene group encountered, while 16 (32%) had bla_{OXA-24} gene group positivity identified. The bla_{OXA} gene distribution for the isolates identified with PCR is shown in Table 3 (Figure 1, 2 and 3). In terms of location, 10 isolates from the general ICU had bla_{OXA-24} gene identified, while 1 isolate each from the CVS ICU, wound service, internal medicine ICU, neurology ICU, infection and chest diseases wards had bla_{OXA-24} gene identified The other clinics did not have bla_{OXA-24} gene identified. The distribution of OXA beta lactamase genes according to clinic is shown in Table 4.

Table 1. Antibiotic susceptibilities of A. baumannii isolates with automated system

Antibiotic	Resistant	Intermediate	Susceptible
	n (%)	n (%)	n (%)
AK	113 (72)	2 (1.3)	42 (26.8)
FEP	148 (94.3)	0	9 (5.7)
CAZ	148 (94.3)	0	9 (5.7)
CRO	155 (98.7)	0	2 (1.3)
CIP	145 (92.4)	0	12 (7.6)
CN	139 (88.5)	0	18 (11.5)
CT	5 (3.2)	0	152 (96.8)
IPM	145 (9,4)	0	12 (7.6)
MEM	142 (9.4)	1 (0.6)	14 (8.9)
NET	153 (97.5)	0	4 (2.5)
PIP	149 (94.9)	0	8 (5.1)
TZP	149 (94.9)	0	8 (5.1)
TGC	89 (56.1)	0	68 (43.9)
SXT	126 (8.3)	0	31 (19.7)

AK: Amikacin, FEP: Cefepime, CAZ: Ceftazidime, CRO: Ceftriaxone, CIP: Ciprofloxacin, CN: Gentamicin, CT: Colistin, IPM: Imipenem, MEM: Meropenem, NET: Netilmicin, PIP: Piperacillin, TZP: Piperacillin tazobactam, TGC: Tigecycline, SXT: Trimethoprim–Sulfamethoxazole.

Table 2. Antibiotic Susceptibility Rates Of 50 Genotyped Isolates

Antibiotic	Resistant	Intermediate	Susceptible
	n (%)	n (%)	n (%)
AK	37 (74)	1 (2)	12 (24)
FEP	50 (100)	0	0
CAZ	50 (100)	0	0
CRO	50 (100)	0	0
CIP	50 (100)	0	0
CN	47 (94)	0	3 (6)
CT	3 (6)	0	47 (94)
IPM	50 (100)	0	0)
MEM	50 (100)	0	0
NET	48 (96)	0	2 (4)
PIP	50 (100)	0	8 (5.1)
TZP	50 (100)	0	8 (5.1)
TGC	32 (64)	0	18 (36)
SXT	44 (88)	0	6 (12)

AK: Amikacin, FEP: Cefepime, CAZ: Ceftazidime, CRO: Ceftriaxone, CIP: Ciprofloxacin, CN: Gentamicin, CT: Colistin, IPM: Imipenem, MEM: Meropenem, NET: Netilmicin, PIP: Piperacillin, TZP: Piperacillin tazobactam, TGC: Tigecycline, SXT: Trimethoprim-Sulfamethoxazole.

Table 3. blaOXA Gene Distribution In A. Baumannii Isolates

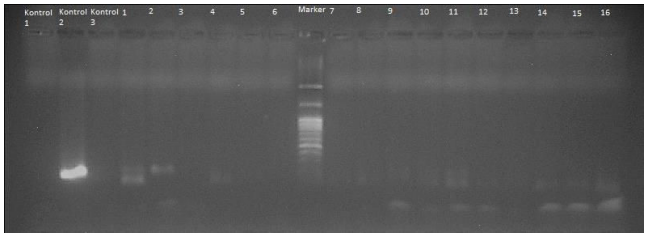
Isolate No	bla_{OXA-23}	bla_{OXA-51}	bla_{OXA-58}	bla_{OXA-24}
1	+	+	-	+
2	+	+	-	+
3	+	+	-	-
4	+	+	-	+
5	+	+	-	-
6	+	+	-	-
7	+	+	-	-
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-
11	+	+	-	+
12	+	+	-	-
13	+	+	-	-
14	+	+	-	+
15	+	+	-	+
16	+	+	-	+
17	+	+	-	-
18	+	+	-	-
19	+	+	-	-
20	+	+	-	-
21	+	+	-	-
22	+	+	-	-

Isolate No	bla_{OXA-23}	bla_{OXA-51}	bla_{OXA-58}	bla_{OXA-24}
23	+	+	-	-
24	+	+	-	-
25	+	+	-	-
26	+	+	-	+
27	+	+	-	-
28	+	+	-	+
29	+	+	-	+
30	+	+	-	+
31	+	+	-	+
32	+	+	-	+
33	+	+	-	-
34	+	+	-	-
35	+	+	-	+
36	+	+	-	+
37	+	+	-	-
38	+	+	-	-
39	+	+	-	+
40	+	+	-	-
41	+	+	-	-
42	+	+	-	-
43	+	+	-	-
44	+	+	-	-
45	+	+	-	-
46	+	+	-	-
47	+	+	-	-
48	+	+	-	-
49	+	+	-	-
50	+	+	-	-

Table 4. Distribution of OXA beta-lactamase genes detected in A. baumanni isolates according to clinics

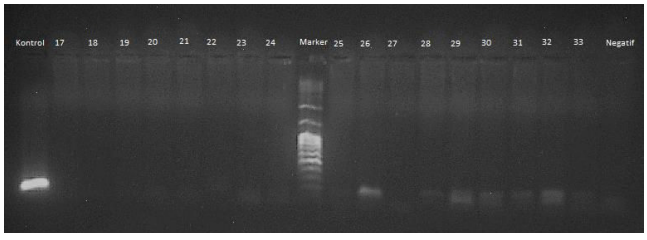
Departments	bla_{OXA-23}	bla_{OXA-51}	bla_{OXA-58}	bla_{OXA-24}
	n (%)	n (%)	n (%)	n (%)
General ICU	34 (68)	34 (68)	0	10 (67.5)
Wound Service	3 (6)	3 (6)	0	1 (6.25)
Pulmonology Unit	3 (6)	3 (6)	0	1 (6.25)
Internal Medicine ICU	3 (6)	3 (6)	0	1 (6.25)
Cardiovascular ICU	2 (4)	2 (4)	0	1 (6.25)
Neurology ICU	1 (2)	1 (2)	0	1 (6.25)
Infectious Diseases Unit	1 (2)	1 (2)	0	1 (6.25)
Burn Unit	1 (2)	1 (2)	0	0
Nephrology Unit	1 (2)	1 (2)	0	0
Neurosurgery Unit	1 (2)	1 (2)	0	0
Total	50 (100)	50 (100)	0	16 (100)

Figure 1. OXA-24 PCR gel image (From our study)



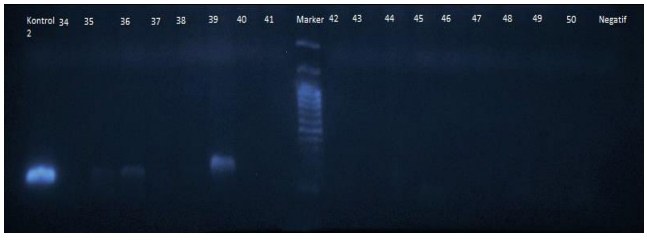
*Positive samples: 1, 2, 4, 11, 14, 15, 16.

Figure 2. OXA-24 PCR gel image (Continued)



*Positive samples: 26, 28, 29, 30,31, 32.

Figure 3. OXA-24 PCR gel image (Continued)



*Positive samples: 35, 36, 39.

DISCUSSION

In our study, A. baumannii strains were most frequently isolated from tracheal aspirate samples (n: 65/157, 41.4%). Previously, Özdem et al. (5) isolated 465 Acinetobacter isolates from 2007-2010 with 39.5% in tracheal aspirate culture, 19.8% in wound culture and 15.3% in blood culture. A study by Salih et al. (6) in a variety of provinces obtained most strains from tracheal aspirate culture at 42.5%. Our study generally appears to be consistent with these studies in terms of the units where strains were isolated.

In spite of identification of resistance rates from 72-88.5% in our study, amikacin was the most effective antibiotic after colistin and tigecycline. Altunok et al. (7) reported that among Acinetobacter isolates, amikacin resistance had a reducing trend through the years, while gentamicin resistance increased. A total of 124 Acinetobacter strains isolated from a variety of clinical samples in Hacettepe University detected 65.3% resistance to imipenem (8). A study in İzmir reported this rate was 86% for A. baumannii strains isolated from intensive care patients (9). Studies performed in 2014 observed that carbapenem resistance rate rose above 90% (7). A study in İstanbul by Barış et al. (10) reported the carbapenem resistance among the isolates was 96.3%. In our study, imipenem resistance was detected as 92.4% and was consistent with other studies showing an increase in resistance rates developing against carbapenem in recent years. The increase in carbapenem resistance in recent years may be explained by clonal association of isolates from A. baumannii strains obtained in excessive amounts from intensive care units due to the frequent use of carbapenem group antibiotics for empirical treatment.

Analyzing Acinetobacter baumannii isolates collected from different regions in Turkey (12 provinces), Beriş et al. (11) found 0.6% colistin resistance in a multicenter study. Research in 2019 by Çağlan et al. (12) with the broth microdilution method identified colistin resistance rate was 28% in Acinetobacter baumannii isolates. In recent years, colistin monotherapy was reported to cause problems like heteroresistance and resistance development; however, commonly used routine antimicrobial susceptibility tests cannot easily identify heterogeneous resistance against colistin. Two studies performed in Turkey in 2019 identified colistin heteroresistance at 34% and 21.4% rates in Acinetobacter baumannii isolates with carbapenem resistance (12, 13). In our study, colistin was identified as the most effective in vitro antimicrobial agent with 96.8% susceptibility, while tigecycline was in second place with 43.9% susceptibility. To prevent increasing resistance profiles and the spread of resistant strains, infection control precautions and smart antibiotic use policies should be applied. Additionally, the combined use of colistin for infections is important to prevent resistance development. Among the oxacillinases which can hydrolyze carbapenem acquired by Acinetobacter species, the bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48} and bla_{OXA-58} type enzymes were identified at various rates in different regions of the world. Al-Sultan et al. (14) reported 58% bla_{OXA-23}, 13% bla_{OXA-40}, and 0% bla_{OXA-58} in Saudi Arabia, while Mohajeri et al. (15) reported 77.9% bla_{OXA-23} and 19.2% bla_{OXA-24} positivity in Iran. When studies in our country are analysed, a study in Istanbul and Ankara by Gür et al. (16) investigated 321 A. baumannii strains and detected carbapenem resistance 44 out of 75 isolates (58.6%) in 2008. They reported that 26 of these isolates (59.1%) carried genes coding OXA-23 and 18 (40.9%) isolates carried genes coding OXA-58. Of 18 strains isolated in Ankara, 17 had OXA-23 and all had OXA-58, while all of the 26 strains isolated in Istanbul, bar one, had OXA-23 and one was identified to carry OXA-58 type genes (17). Again, in Turkey, the presence of bla_{OXA-58} genes from 0-23%, bla_{OXA-23} from 31-78% and low rates of bla_{OXA-24} gene were reported (18). Just as with different studies in our country, in our study the bla_{OXA-51} gene was identified in all isolates. Additionally, studies by Keskin et al. (19) reported 91.5% bla_{OXA-23}, 7% bla_{OXA-58} and 2% bla_{OXA-24}; Keyik et al. (17) reported 46.7% bla_{OXA-23} and 53.3% bla_{OXA-58}; Ertürk et al. (18) 94.5% bla_{OXA-23}; and Çiçek et al. (20) reported 78% bla_{OXA-23} gene presence. In our study, bla_{OXA-23} gene positivity rate was determined as 100%. All these results emphasize that the bla_{OXA-51} and bla_{OXA-23} gene regions comprise the dominant mechanism for imipenem resistance in A. baumannii isolates. Additionally, in our study, there was 32% positivity for the bla_{OXA-24} gene region. When other studies are examined, this rate was observed to be high. Additionally, the bla_{OXA-58} gene region was not identified in any sample. A multicenter study by Çiftçi et al. (21) reported that all isolates carried the bla_{OXA-51} gene, 74.4% of carbapenem-resistant isolates carried bla_{OXA-23}, and 17.3% carried the bla_{OXA-58} gene.

When 2008 isolates are compared with 2011 isolates, the bla_{OXA-23} gene was identified at 3 times higher rates (21).

In a research about molecular typing and carbapenemase in carbapenem-resistant A. baumannii, Özbey et al. (2) investigated the oxacillinase enzyme genes of bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, and bla_{OXA-58} gene regions and the metallo-beta-lactamase enzymes of IMP, VIM, SIM and SPM enzyme genes with multiplex PCR. While bla_{OXA-23} and bla_{OXA-51} positivity was identified in all isolates, it was stated that carbapenem resistance was due to excessive production of bla_{OXA-51} type natural oxacillinases and bla_{OXA-23} enzyme gene as samples were negative for bla_{OXA-24}, bla_{OXA-58}, IMP, VIM, SIM and SPM enzymes (2). Additionally, they reported variability in bla_{OXA} gene diversity in A. baumannii isolates between geographic regions with gene variations observed over time in the same geographic region (16). As a limitation of our study, the research isolates which were included in the study were obtained from only one center in Gaziantep. As another limitation, the MIC values for colistin drug were determined and screened by BD Phoenix™ (BD Phoenix™ ID & AST System, USA) automatic identification system which is not gold standard for antibiotic susceptibility testing. Further multi-center studies are necessary to obtain molecular epidemiology data from Acinetobacter baumannii clinical isolates.

CONCLUSION

In our study, 96.8% rate of susceptibility to colistin was reported, with colistin being the most effective antibiotic. This was followed by 43% susceptibility rates for tigecycline and 26% susceptibility rates for amikacin. To prevent the development of resistance or heteroresistance against these antibiotics, the combined use of these last-resort medications will be appropriate. According to real-time PCR results used to research oxacillinase enzymes causing carbapenem resistance, all strains were positive for chromosomal-derived OXA-23 and OXA-51 enzymes, with no isolate containing OXA-58 enzyme gene. The OXA-24 enzyme gene investigated with the optimized PCR method had the rate of 32% which was higher compared to other similar studies. We think that monitoring antibiotic susceptibility patterns and performing molecular epidemiological studies will detect the source of resistant bacteria and reduce the infections and resistance development.

Ethics Committee Approval: Ethics committee approval was received for this study from the Gaziantep University Clinical Researches Ethical Committee (Decision number: 2016/188, Date: 20.06.2016).

Informed Consent: All participants signed informed consent forms before study inclusion.

Peer-review: Externally peer-reviewed.

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

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

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Original Research

Stereological Estimation of Bone Cyst Volume Using Computed Tomography Images: A Comparison with the Planimetry Technique

Murat Gölpınar[1], Ferhat Say[2], Nükhet Göçmen Karabekir[3], Bünyamin Şahin[4]

[1] Hitit University, Faculty of Medicine, Department of Anatomy, Çorum, Turkey
[2] Ondokuz Mayis University, Faculty of Medicine, Department of Orthopedics and Traumatology, Samsun, Turkey
[3] Dokuz Eylül University, Faculty of Medicine, Department of Anatomy, İzmir, Turkey
[4] Gazi Osmanpaşa University, Faculty of Medicine, Department of Anatomy, Tokat, Turkey

ABSTRACT

Objective: The exact volume of the bone cyst is fundamental for evaluation, treatment, and surgical management of the pathology related to any bone. The aim of this study was to introduce the stereological point-counting technique in bone cyst volume measurements on computed tomography images and to compare it with the planimetry technique.

Methods: A total of 30 bone cyst volumes were estimated on CT images using stereological point-counting and the planimetry technique, which is based on the Cavalieri principle. The planimetric measurements were regarded as reference values. The stereological and planimetric volume estimations were performed by two independent observers twice. The estimation results of the two volumetric techniques were compared with the Wilcoxon signed-rank test. Intra-observer and inter-observer reliability of each volumetric technique was assessed.

Results: For each bone cyst, 7-11 systematically sampled CT slices enabled reliable cyst volume estimations with a low coefficient of error (0.39%-3.12%). There was no significant difference between point counting and planimetry methods regarding volume measurements in both sessions ($p>0.05$), and these methods correlated well with each other. There was a significant inter-and intra-observer agreement for each volumetric method (ICC=0.9984 to 0.9988). The stereological approach was observed to take less time than the planimetric approach (mean 01:43±0.44 vs. 03.33±1:47 minutes)

Conclusion: The stereological point-counting method can be well pertained to CT images for the reliable and reproducible assessment of bone cyst volume. Application of the point-counting method for volume estimation of bone cysts with different morphological features provides a great advantage in terms of both time-saving, applicability, and practicality in comparison with the planimetry technique.

Keywords: Bone cyst, computed tomography, planimetry, stereology, volume measurement

INTRODUCTION

Bone cysts are clear fluid-filled cystic lesions that tend to expand and weaken the bone. This benign lesion represents one of the most frequent osseous lesions. The cysts have a predilection to occur in males more frequently than in females. Bone cysts are one of the most frequently seen osseous lesions, and most commonly observed in the period from birth to 20 years of age (1,2). Common locations include the proximal humerus and femur, although any section of any long bone may be involved (3). Most bone cysts are asymptomatic and detected incidentally on imaging. However, cysts may also be diagnosed because of pain, which may reflect a microscopic pathological fracture as a result of a minor trauma (4). Treatment methods include intralesional injections, decompression, and combined surgical techniques (5).

The volume of benign bone lesions such as a bone cyst or enchondroma is fundamental for evaluation, treatment, and surgical management of the pathology related to any bone (6). As most benign lesions respond well to surgical removal, surgical treatment is one of the preferred options for the management of benign lesions. Precise information of cyst volume is often required to able to establish the healing process of the cyst as cyst volume is closely pertaining to pathological fracture risk (7). The exact volume of the cyst is also necessary for the planning of surgical treatment, especially to estimate the amount of graft required to fill the defect. Therefore, a technique for accurate and reliable estimation of the cyst volume has to be clinically oriented (6,7).

According to the Cavalieri principle, the two main volumetric methods for estimating the volume of anatomical structures are point-counting and planimetry techniques. In the Cavalieri principle, the volume of any structure can be estimated using 2-dimensional parallel sections separated by a definite distance (8).

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Corresponding Author: Murat Gölpınar **E-mail:** muratgolpinar@hitit.edu.tr

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The point-counting technique of modern design stereology is based on surface area estimation of a region of interest on cross-sectional images via the simple method of point counting. The stereological technique is an accurate and effective approach, which can be utilized to safely assessing the volume of biological structures (9). Planimetry is an approach for estimating the sectional area particularly by tracing the boundaries of the object of interest and is regarded as a valid method for volume estimation (10).

The goals of this study were to introduce combining stereological point-counting technique with computed tomography (CT) image sections for bone cyst volume estimation, and compare the stereological measurements with those obtained with the planimetry technique.

METHODS

CT Scans

Before the study commenced, approval was obtained from the Institutional Ethics Review Board of our institution (Decision No: 611-2019). After obtaining Institutional Ethics Review Board approval for this study, we conducted a retrospective analysis of preoperative CT scans of 30 patients with varying sizes and shapes of the simple bone cysts, which were selected from the archives of the Department of Orthopedics and Traumatology, Faculty of Medicine, 19 Mayıs University, Samsun, Turkey. The 30 patients comprised 12 males and 18 females, with a mean age of 24.47±13.30 years. Axial plane CT scans of the bone cysts were obtained using a helical CT scanner (Toshiba Aquilion, Canon Medical Systems Corporation Toshiba, Dalian) with the following parameters; thickness: 1 mm, 120 kVp, 150-220 mAs. The axial image series were transferred to Digital Imaging and Communication in Medicine (DICOM) viewer software (Horos v.1.1.7, Purview, Annapolis, MD 21401, USA). In order to determine the superior and inferior margins of the cysts, CT scans were reconstructed in the coronal plane. Afterward, CT scans were divided into 1 mm sections without the slice gap in the coronal plane and recorded as separate DICOM files. Simple bone cysts with pathological fractures were not included in this study because the determination of the boundaries of the cysts was not possible on CT images. The localization of the cysts was 11 in the femur, 7 in the tibia, 4 in the radius, 3 in the humerus and fibula, and 1 in the metacarpal bone and calcaneus. Stereological and planimetric volume measurements were performed by utilizing ImageJ (ImageJ,1.37v: http://rsb.info.nih.gov/ij/) software, which is distributed free of charge by the National Institutes of Health, USA.

Main Points:

- Stereological point-counting technique is an efficient alternative approach for estimating bone cyst volume.
- Stereological bone cyst volume estimations from computed tomography images are practical, rapid, reproducible and accurate.
- The application of point-counting technique to measuring cyst volume reduces processing time.

Volume Estimation Techniques

Planimetry Technique

The cyst boundaries were manually delineated on each CT image using the polygon selection tool of the software (Figure 1). The sectional surface area of the cyst was automatically calculated by the program for each slice. The total volume of the bone cyst was calculated by multiplying the obtained sectional surface areas by the slice thickness. The total volume of the bone cyst can be estimated using the following formula (10). Where T is the slice thickness of consecutive sections, a_i is the area of the cyst outlined in section i, and m is the total number of slices containing the region of interest.

In the planimetry technique, the coefficient of error (CE) was estimated with the formula described in previous studies (11). All calculations were recorded in a Microsoft Excel worksheet where the final data were automatically obtained by entering the section thickness and surface area of the structure into the worksheet. The mean time required to complete planimetric volume

Figure 1A. A coronal CT image showing a bone cyst in the proximal femur of patient no 28.

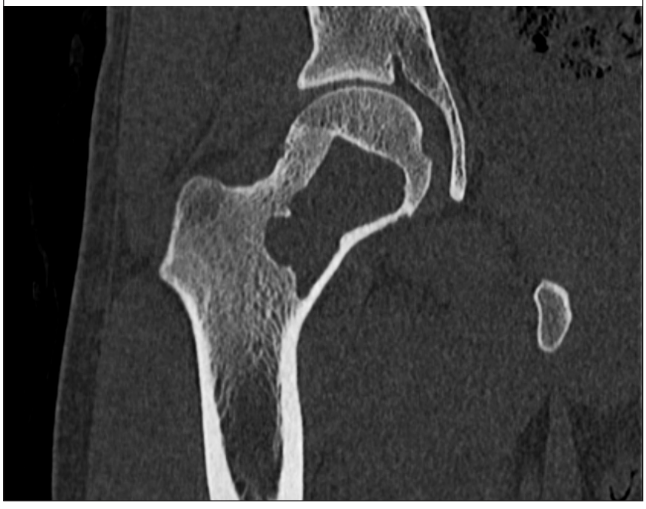
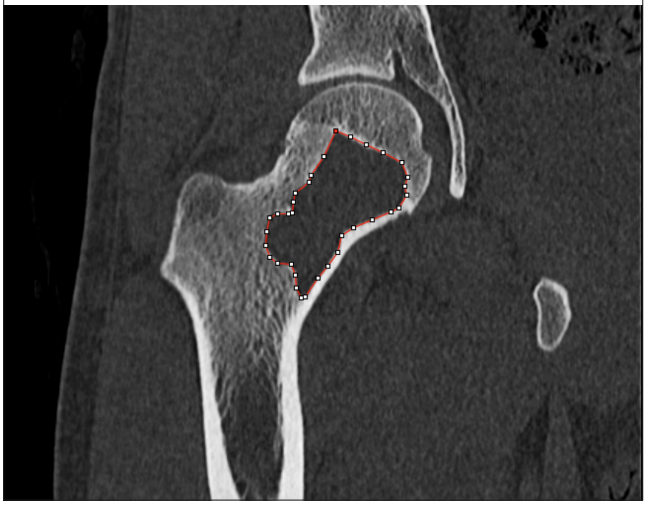


Figure 1B. Delineation of the borders of the bone cyst for planimetric volume measurement.



estimations was recorded. In the present study, planimetric volume estimates were regarded as reference values.

Stereological Technique

In the point-counting technique, instead of tracing the boundary of the region, area estimation was performed by superimposing a point-counting grid on the sections. By systematically sampling CT slices and regulating an optimum distance between test points of the grid, the point-counting technique may be optimized (12). This systematic slice sampling procedure is dependent on using a minimum number of systematically sampled slices to ensure precise and reproducible volume measurements with minimal user intervention (10). In the current study, the bone cyst was depicted on mean of 17.70±6.9 images (range 7-32). The sample types 1/2 and 1/3 were selected for stereological volume estimation. Finally, 7-11 CT sections including the cyst were obtained. Prior to the study, the point-counting grid with d= 0.7, 0.8, 0.9, and 1.0 cm between the test points, i.e. 0.49, 0.64, 0.81, and 1.00 cm^2 symbolizing area per point was tested on the first ten patients to determine proper point spacing. The point-counting grid with d=0.9 cm between the test points was selected as it enabled the determination of the bone cyst volume with CE<2%.

The grid was randomly overlaid on each CT image by the software and the number of test points accommodating within the cyst area was counted by the examiner on each section (Figure 2). According to the stereological technique, the bone cyst volume was assessed with the following formula (Figure 3) (13).

Where T is the section thickness (including interval) of consecutive sections, A represents the area associated with each test point, m is the number of sections depicting the cyst and Pi is the number of points lying within the cyst on section i.

The accuracy of volume assessment of any object using the stereological approach may be defined to obtain a CE<5% as described in previous studies (14). Calculation of the cyst volume, estimation of the CE value, and other associated data were implemented on MS Excel worksheets, where the results were automatically obtained by entering the point count, section thickness, and other related data into the worksheet. The mean time required to perform the stereological volume estimations was recorded.

The two observers independently estimated the bone cyst volume using both volumetric methods twice at an interval of one month to reduce recall bias. Each observer was blinded to the results of the other and to their own previous measurements of the same images for each measurement method. Prior to the study, 30 bone cysts were morphologically evaluated by an orthopedist (FS) in order to evaluate the efficacy and applicability of the volumetric techniques on cyst volume measurements.

Statistical Analysis

Version 22 of Statistical Package for Social Sciences for Windows software (SPSS, Chicago, IL, USA) was used for statistical analysis. Continuous variables were expressed as mean±standard deviation (SD), and min-max where appropriate. Conformity of the volumetric data to normal distribution was tested using the Shapiro-Wilk test.

Figure 2A. The point counting grid was superimposed to cover the entire cyst projection area randomly.

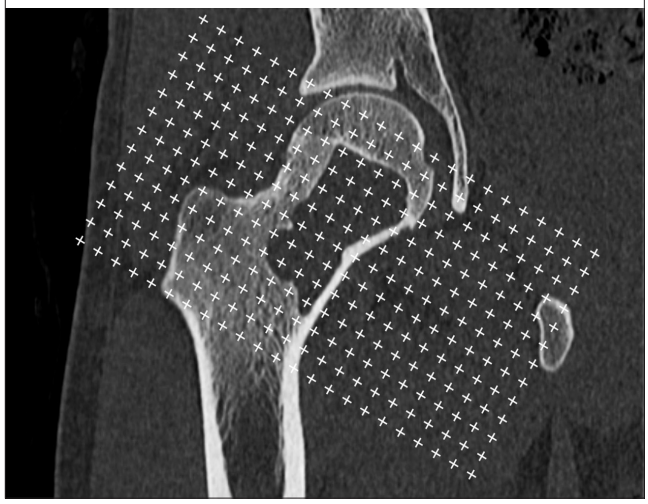


Figure 2B. The number of points within the area of the cyst were selected and counted for stereological volume estimation.

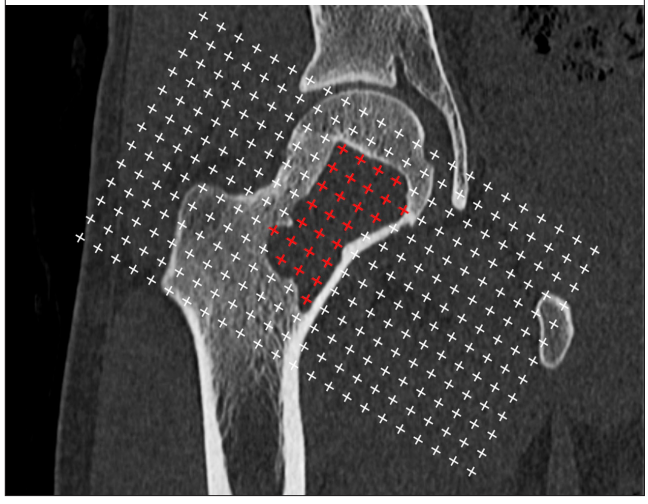
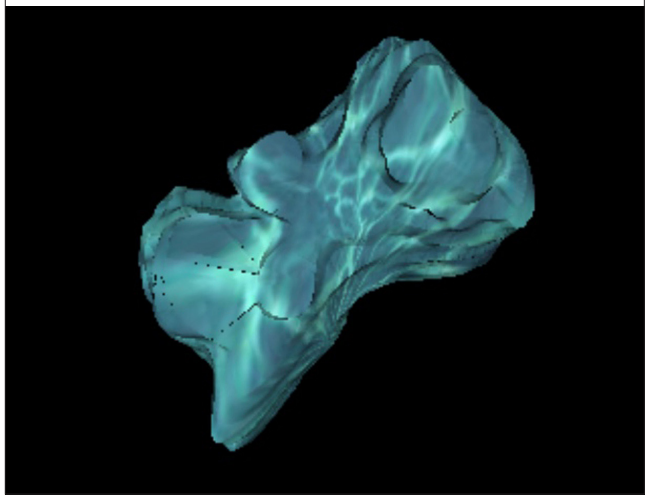


Figure 3. 3D-model of the segmented bone cyst for estimation of the total volume.



The Wilcoxon signed-rank test was conducted to detect statistical differences between the stereological and planimetric volume estimations obtained by the two observers. The intraclass correlation coefficient (ICC) (two-way mixed model) was calculated to define the intra- and interobserver reliability of each volumetric technique. The relationship between the estimation results of the two methods was examined using the Spearman correlation coefficient. Bland-Altman plots were generated to define 95% limits of agreement between the point-counting and planimetry methods. In addition, consistency between the two methods of measurement was assessed with Passing-Bablok regression analysis. A P value<0.05 was considered to be statistically significant for all tests.

RESULTS

There was no significant difference in age between male and female patients (P=0.638). The overall mean bone cyst volume estimated by the two observers using the optimized point-counting and planimetry methods was 20.71±19.60 cm^3 and 20.65±19.60 cm^3, respectively.

Evaluation of Stereological Volume Measurements

The mean cyst volume obtained using the stereological technique by the two observers in the first and second sessions was 20.67±19.62 cm^3 and 20.75±19.74 cm^3, respectively. According to the results of the Wilcoxon signed-rank test, there were no significant differences between each observer's estimation results in both sessions (p>0.05) (Table 1). There were no significant differences between the estimation results of the two observers in both the first and second sessions (p=0.651, p=0.829, respectively).

The ICC showed a high degree of intra-observer agreement in the stereological estimation for both the first and second observer (ICC=0.9986, p<0.001; ICC=0.9984, p<0.001, respectively). There was high inter-observer agreement in the stereological estimation for the first and second sessions (ICC=0.9988, p<0.001; ICC=0.9988, p<0.001 respectively). The mean CE of the volume estimations obtained using stereology was 1.43±0.61%.

Evaluation of Planimetric Volume Measurements

The mean cyst volume obtained using the planimetry technique by the two observers in the first and second sessions was 20.67±19.70 cm^3 and 20.62±19.67 cm^3, respectively. Based on the results of the Wilcoxon signed-rank test, no statistical difference was found between each observer's estimation results in both sessions (p>0.05) (Table 2). No statistically significant differences were determined between the planimetric volume estimates of the two observers in the first and second sessions (p=0.341, p=0.382, respectively).

The ICC showed a high degree of intra-observer agreement in the planimetric estimation for the first and second observer (ICC=0.9988, p=0.001; ICC=0.9988, p<0.001, respectively). Inter-observer agreement of the planimetric volume measurements was found to be almost perfect for the first and second sessions (ICC=0.9992, p<0.001; ICC=0.9991, p<0.001, respectively). The mean CE of the volume measurements estimated using the planimetry method was 1.36±0.66 %.

Comparison of Two Volumetric Techniques

There was no statistical difference between the stereological and planimetric volume measurements obtained by each observer in the first and second sessions (p=0.711, p=0.658; p=0.339, p=0.666, respectively). Total cyst volume obtained using stereology by the first observer in both sessions was highly correlated with those obtained with the planimetry technique (r=0.997, p=0.001; r=0.996, p=0.001, respectively). The stereological estimation results of the second observer in both sessions showed a high correlation with those obtained from the planimetry method (r=0.995, p=0.001; r=0.996, p=0.001, respectively).

Bland-Altman Analysis revealed that both volumetric techniques were in good agreement for each observer and each session. The mean difference of cyst volume obtained using stereology and planimetry by the first observer in the first session was -0.07 cm^3, and the limits of agreement were -1.60 and 1.47 cm^3. The mean difference of cyst volume estimated using stereology and planimetry by the second observer in the first session was 0.16 cm^3. The limits of agreement were -1.31 and 1.62 cm^3. The mean difference of cyst volume evaluated with stereology and planimetry by the first observer in the second session was 0.07 cm^3 and limits of agreement were found to be 1.41 and -1.55 cm^3. The mean difference of the cyst volume estimated using stereology and planimetry by the second observer in the second session was 0.09 cm^3 and limits of agreement were found to be -1.45 and 1.63 cm^3 (Figure 4).

Table 1. The results of the bone cyst volume (cm3) estimations obtained using stereology in two sessions

Observer	Mean ± SD	Min-max	Mean ± SD	Min - max	P value
	First Session		Second Session		
Observer 1	20.66 ± 19.83	1.94 - 82.82	20.74 ± 19.90	2.08 - 83.60	0.551
Observer 2	20.68 ± 19.74	2.18 - 82.62	20.76 ± 19.93	2.04 - 84.62	0.572

Table 2. The results of the bone cyst volume (cm3) estimations obtained using planimetry in two sessions

Observer	Mean ± SD	Min-max	Mean ± SD	Min - max	P value
	First Session		Second Session		
Observer 1	20.72± 19.88	2.22 - 84.23	20.58 ± 19.74	1.79 - 82.60	0.162
Observer 2	20.61 ± 19.86	2.02 - 83.58	20.67 ± 19.94	1.88 - 83.32	0.517

Table 3. Passing-Bablok regression analysis results for measurement agreement between the stereology and planimetry techniques in the first and second sessions

Session	Observer	Intercept	95% CI	Slope	95% CI	Linearity
Session 1	**Observer 1**	- 0.31	- 0.51 to - 0.05	0.99	0.98 to 1.02	Yes
	Observer 2	0.37	- 0.17 to 0.61	1.00	0.99 to 1.02	Yes
Session 2	**Observer 1**	0.24	- 0.21 to 0.45	0.99	0.97 to 1.01	Yes
	Observer 2	0.23	0.08 to 0.47	0.99	0.97 to 1.01	Yes

Figure 4A. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the first observer in the first session. The dashed line represents the 95% limit of agreement.

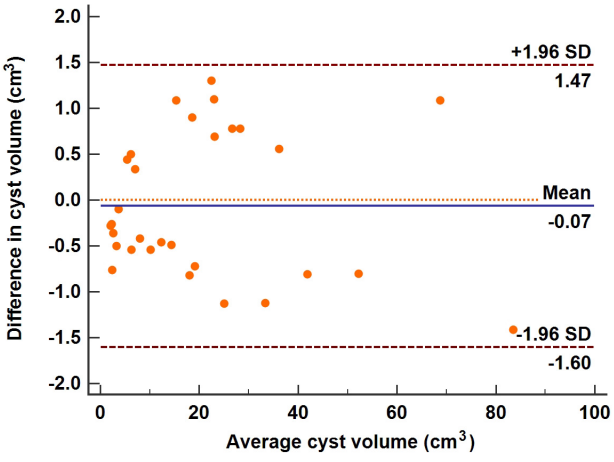


Figure 4B. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the second observer in the first session. The dashed line represents the 95% limit of agreement.

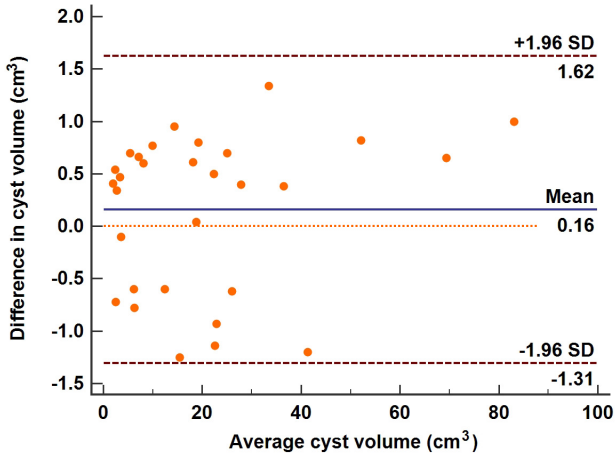


Figure 4C. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the first observer in the second session. The dashed line represents the 95% limit of agreement.

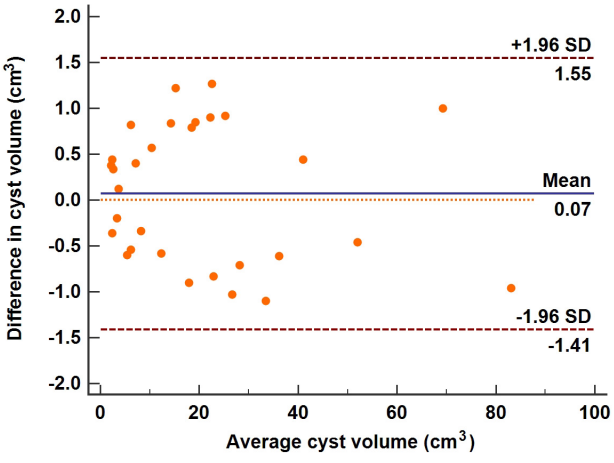
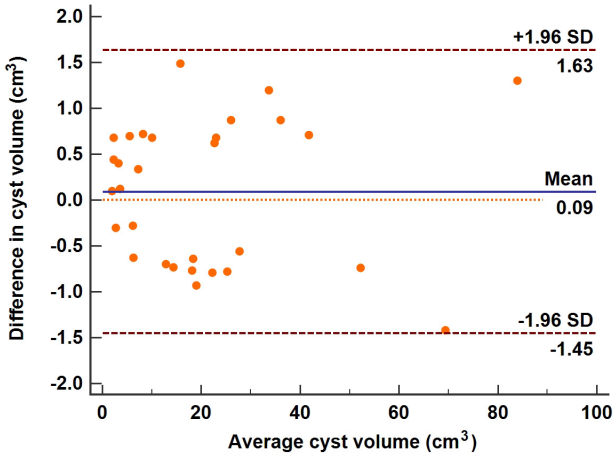


Figure 4D. The Bland–Altman plot showing the differences between the mean cyst volumes obtained using stereology and planimetry by the second observer in the second session. The dashed line represents the 95% limit of agreement.



The Passing-Bablok regression analysis indicated close agreement between the stereological and planimetric volume estimations for each observer in both sessions (Figure 5). The Cusum linearity test indicated a linear relationship between the volume estimates of the two measurement methods in all comparisons ($p>0.05$). Details of the Passing-Bablok regression analysis in the comparisons of the two techniques are given in Table 3. The mean time required to perform stereological volume analysis was 01:43±0:44 minutes (range, 0:41-03:10). The mean time needed to complete volumetric analysis of the cyst using planimetry was 03:33±1:47 minutes (range, 1:28-06:43).

Evaluation of the Bone Cysts in Respect of Morphological Characteristics

The 30 bone cysts were separated into 4 main groups according to the morphological characteristics. Group 1 included complete bone cysts with regular borders (example patient no 28-Figure 1). Group 2 included bone cysts that were complete but the borders were irregular (example patient no 18-Figure 6). Group 3 included bone cysts separated into two or more sections (example patient no 16-Figure 7). Group 4 included bone cysts which appeared as many separate independent cystic islets (example patient no 24-Figure 8).

Figure 5A. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the first observer in the first session. The solid blue line represents the regression line. Dashed brown lines indicate the confidence interval for the regression line. The dotted line shows the identity line. Orange dots represent measured samples.

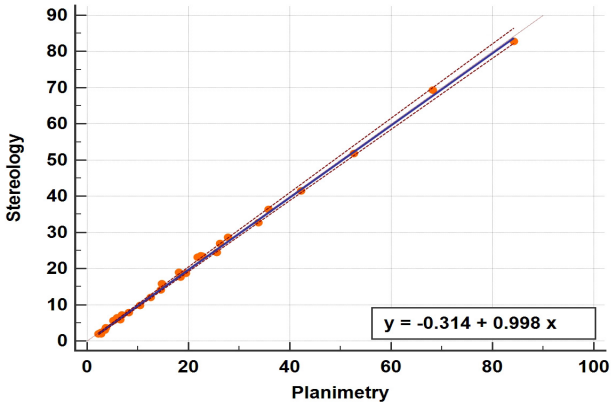


Figure 5B. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the second observer in the first session.

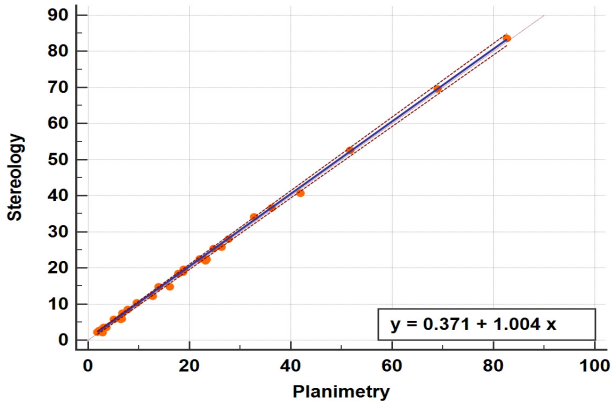


Figure 5C. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the first observer in the second session.

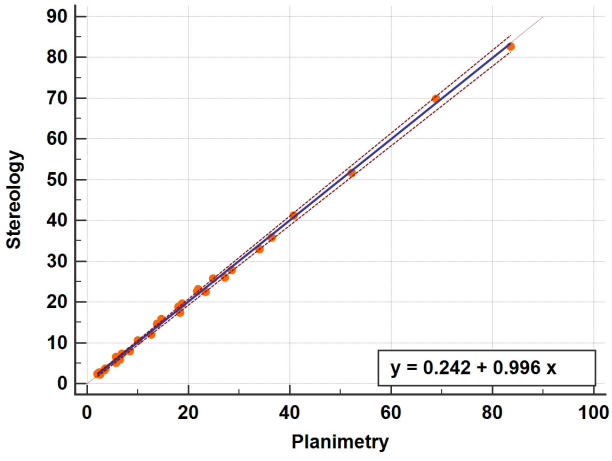


Figure 5D. Passing–Bablok regression analysis for comparison of the stereological and planimetric volume measurements for the second observer in the second session.

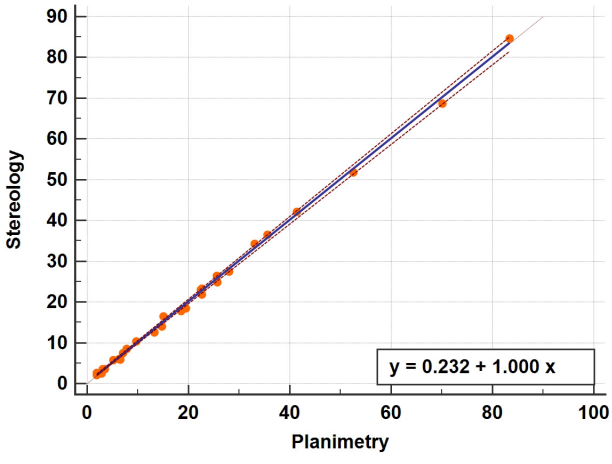


Figure 6A. Application of the planimetry technique in volume estimation of the bone cyst, which has an irregular shape located in the distal femur of patient no 18.

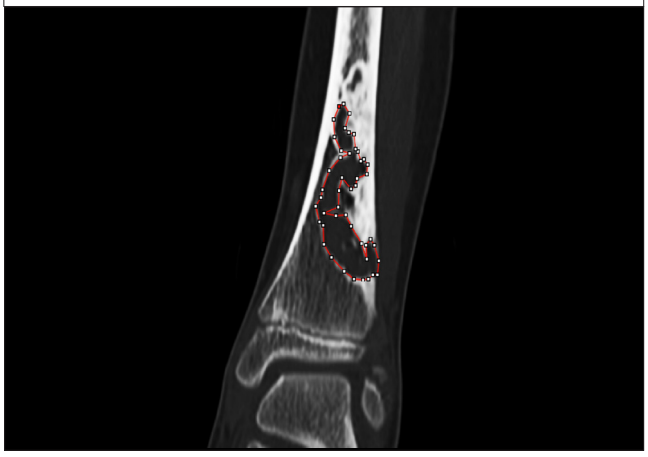


Figure 6B. Application of the point-counting technique in volume estimation of the bone cyst, which has an irregular shape located in the distal femur of patient no 18.

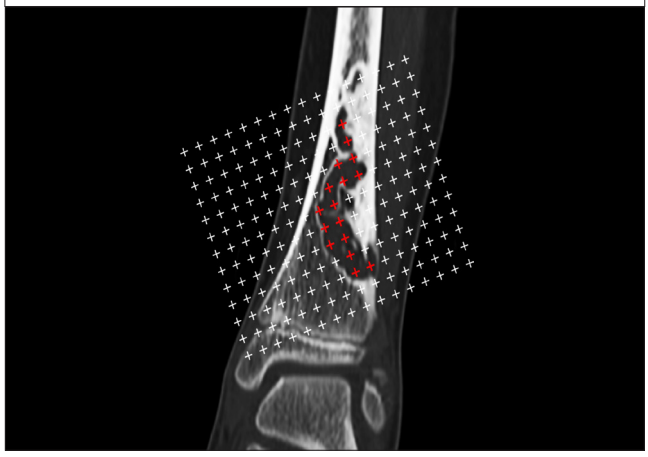


Figure 7A. Application of the planimetry technique in the volume measurement of the bone cyst, which is divided into sections by two septum located in the distal femur in patient no 16.

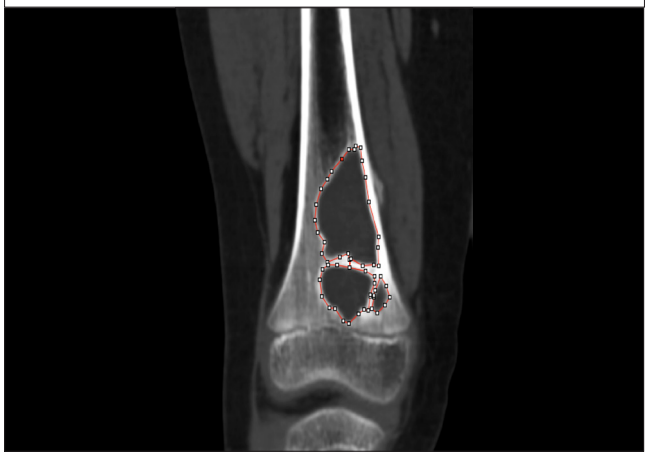


Figure 7B. Application of the point-counting technique in the volume measurement of the bone cyst, which is divided into sections by two septum located in the distal femur in patient no 16.

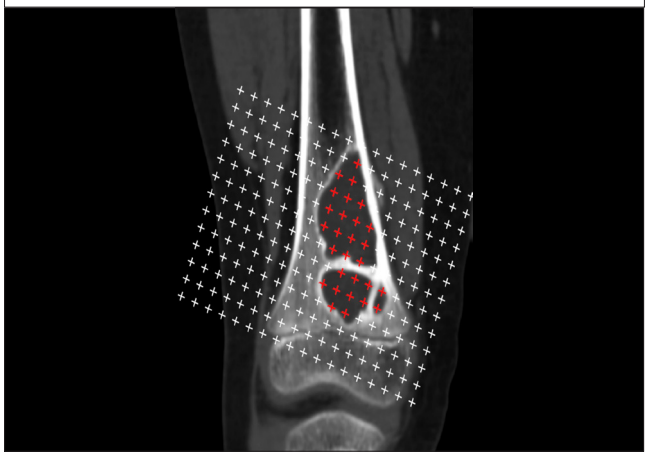


Figure 8A. Application of the planimetry technique in the volume measurement of the bone cyst which consists of a large number of cystic islets in the distal fibula in patient no 24.

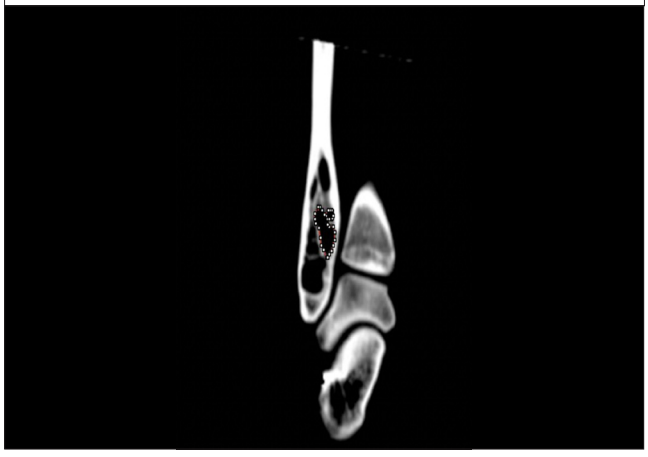
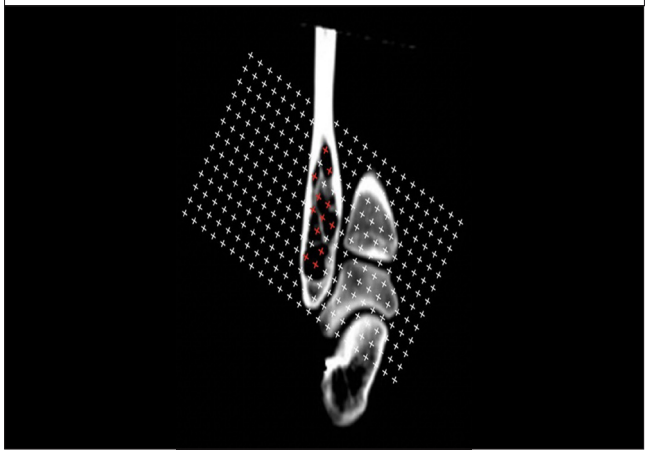


Figure 8B. Application of the point-counting technique in the volume measurement of the bone cyst which consists of a large number of cystic islets in the distal fibula in patient no 24.



DISCUSSION

The natural history of bone cysts has not been fully clarified. Active cysts are those which are closely adjacent to a growth plate and when localized at a distance from the growth plate, those are known as latent cysts. An active cyst in a child can be seen to grow in volume throughout the period of natural growth and development of the child. In the evaluation of radiographic healing following an intervention, knowledge of the cyst volume is crucial. Preoperative evaluation of cyst volume allows the preparation of a sufficient amount of therapeutic agent to fill the defect. Thus, in clinical practice, accurate and reliable estimation of the cyst volume is important (7).

Several studies have been conducted for volume estimation of similar pathological lesions using different imaging techniques. Gobel et al. (15) have calculated tumor volume in patients with Ewing sarcoma by assuming an ellipsoidal or cylindrical configuration on X-rays and CT scans. Similarly, Glowacki et al. (16) also utilized the above technique for the evaluation of solitary cyst volume using X-ray images. In another study, Docquier et al. (17) described a semi-automatic segmentation method for the measurement of bone cyst volume from magnetic resonance images. As the reliability and clinical applicability of the suggested methods have been shown to be limited, none of those approaches assessing bone cyst or lesion volume has been absolutely acknowledged.

The point-counting technique has been reported in the literature as a good alternative to the conventional planimetric technique for estimating the volume of various organs or structures (11,18,19). In those studies, the stereological technique has provided very similar results to those of the planimetry technique and it has shown a strong correlation with the planimetric approach.

In the current study, the stereological point-counting technique is introduced for the assessment of bone cyst volume on CT images. The study results showed that total cyst volume estimated by stereology in both sessions was not statistically different from the values obtained with planimetry ($p>0.05$) and stereological volume estimates displayed a high correlation with the planimetry values (ranging between 0.995 and 0.997). Bland-Altman plots indicated that the 95 % limits of agreement between the two volumetric methods were quite narrow and clinically acceptable. In addition, Passing-Bablok regression analysis showed close agreement between the stereological and planimetric volume estimations for each observer in both sessions. No significant deviation from linearity was observed ($p>0.05$). With respect to these results, the point-counting and planimetry techniques seemed to show sufficient agreement to be able to be used interchangeably for volume estimation of bone cysts.

Previous studies that have compared the two techniques have generally measured the volumes of structures and organs that have a holistic structure and specific shapes, such as the eye, brainstem, spleen, and liver (18,20,21). Unlike previous studies, in this study, the volume was measured of 30 bone cysts different from each other in size and structure. For example, as in the bone cyst situated at the proximal end of the femur of patient no 28, some of the bone cysts were whole and the borders were very regular. In such a case, both methods may be suitable for measuring cyst volume in respect of applicability and efficacy.

In other cases, although the bone cyst was complete, it may have quite irregular, indented, and protruding edges, as in patient no 18. Or the borders of the cyst may not be very obvious. In these cases, manually drawing the complex boundaries of the cyst on all CT sections using planimetry, is time-consuming and requires skilled users, while the stereological method has a great advantage in volume measurements as it uses the simple and fast process of point counting.

Some bone cysts may be divided into two or more sections by a septum located between the cysts as shown in patient no 16, or a cyst may consist of a large number of islets that appear to be independent of each other, as in patient no 24. If a cyst is divided into two or more cystic areas by a septum, the planimetric approach is more time-consuming as compared to the point-counting approach since the process of tracing the boundaries of the cystic areas is longer and laborious. If the bone cyst consists of a large number of lesions that seem to be independent of each other, it is necessary to trace the boundary of each cyst area individually. Application of the planimetry is both time-consuming and difficult in this condition. In both cases, the point-counting method offers a very practical, fast, and effective measurement opportunity as compared to the planimetry method.

Since the point-counting method relies on the simple and fast process of counting, the short processing time for volume estimation is its main benefit. Sahin and Ergur (21) reported that the point-counting method takes less time as compared to the planimetric method (mean 5:37 vs. 7:22 minutes) for the assessment of liver volume on MR images. Acer et al. (22) showed that the stereological technique is 30% faster than the planimetric technique for the assessment of total intracranial volume. In another study by Acer et al. (23), it was stated that the application of stereology to estimate cerebellum volume reduces processing time in comparison with the planimetry method (mean 8±3:6 vs. 15±5:5 minutes). In the current study, the time taken for stereological volume estimations was 48% shorter than planimetric volume estimations.

The stereological technique offers researchers the opportunity to make suitable changes in their sampling strategies and point density of the grids by assessment of the CE. A CE <5 % is in an acceptable range. Determination of the suitable grid size and the number of slices necessary for volume assessment of an object is essential at the beginning of the procedure as there is no need to calculate CE for repeated measurements. Mazonakis et al. (24) stated that bladder and rectum volume calculations can be performed on 5-7 CT image slices obtained by the adoption of the 1/3 systematic sampling procedure. In another study, Mazonakis et al. (18) also reported that 5-8 systematically sampled slices provided enlarged splenic volume measurements while 4-7 systematically selected slices enabled a precise normal splenic volume measurement. Manious et al. (10) suggested that abdominal fat volume

may be estimated on only 6 CT slices by means of a systematic sampling process. In the present study, the bone cysts of patients were depicted on a varying number of CT images ranging from 7 to 32. Therefore, a sample type of 1/2 and 1/3 was performed to obtain the minimum number of slices containing the bone cyst. The results showed that 7-11 systematically sampled CT slices per bone cyst provided reliable cyst volume estimations with a low coefficient of error (0.39%-3.12%). In the point-counting technique, the slice sampling procedure allows the minimization of user interaction by providing a minimum number of systematically sampled CT sections for volume measurements. Stereological volume measurements are independent of cyst shape, which may vary widely among individuals and do not require user experience in outlining cyst contours.

This study was also designed to evaluate intra- and interobserver reliability and accuracy of the point-counting and planimetry methods for bone cyst volume measurement. There was high agreement in the intra- and interobserver results both in the planimetry and point-counting techniques (with all ICC values>0.998). Therefore, these techniques can be considered reliable techniques with low intra- and interobserver variation in cyst volume estimations.

Relatively higher doses of radiation exposure of the patients with CT imaging is a limitation of this technique. However, particularly for the bone cysts localized in the spine or pelvis as they cannot be easily identified on plain X-Ray images, CT scanning is recommended for initial evaluation. Another indication for CT is when there is a high index of suspicion regarding the structural integrity of a weight-bearing area (25,26). Nevertheless, it must be kept in mind that for pediatric patients, repeated CT evaluations are less desirable. Measurement and evaluation of only 30 CT images and differences between male and female patients in terms of the number are other limitations of the present study.

CONCLUSION

In conclusion, the stereological approach, based on point-counting and systematic sampling, may be efficaciously applied to CT images for identifying bone cyst volume. The estimated volumes of the point-counting technique were compatible with the results of the planimetry technique, which is a robust indicator of the accuracy of this technique. In addition to providing trustworthy and accurate estimations of bone cyst volume, the stereological point-counting technique provides a great advantage in terms of both time savings, applicability, and practicality for the volume estimation of bone cysts showing different morphological features.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University of School of Medicine (2019/611).

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

Peer-review: Externally peer-reviewed.

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

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

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Original Research

The Localizations of Osteoarthritis in the Knee, Ankle and Foot Joints of Cadaver: Comparison in Radiological, Morphological and Histopathological Aspects

Menekse Cengiz[1], Serra Ozturk[1], Ramazan Yavuz Arican[2], Ceren Can Bacanli[3], Inanc Elif Gurer[4], Gulcan Gurer[5], Tiraje Tuncer[6], Timur Sindel[7], Muzaffer Sindel[1]

1 Akdeniz University, Faculty of Medicine, Departments of Anatomy, Antalya, Turkey
2 Balıkesir University, Faculty of Health Sciences, Balıkesir, Turkey
3 Private Akdeniz Sifa Hospital, Departments of Physical Medicine and Rehabilitation, Antalya, Turkey
4 Akdeniz University Faculty of Medicine, Department of Pathology, Antalya, Turkey
5 Adnan Menderes University, Departments of Physical Medicine And Rehabilitation, Aydın, Turkey
6 Akdeniz University Faculty of Medicine, Departments of Physical Medicine and Rehabilitation, Antalya,Turkey
7 Akdeniz University Faculty of Medicine, Departments of Radiology, Antalya, Turkey

ABSTRACT

Objective: Osteoarthritis (OA) is the most common joint disease. In this study it was aimed to compare the general features of OA such as location, placement, severity and shape of the lesions in terms of radiological and morphological aspects and to determine their relationship with each other.

Methods: In our study, the antero-posterior and lateral radiographies of knee talocrural and transverse tarsal joints of 20 cadavers by age between 30 and 50 years were taken. The results obtained from the radiological examination were graded according to the Kellgren and Lawrence scale. For each of the identified regions, the presence of degenerative changes was noted. Then samples were taken from these regions were examined by microscopic methods. The cartilage degeneration changes, presence of fibrillations, density, depth, chondrocyte aggregation, and necrotic changes were evaluated.

Results: In the radiological examination OA was found in 35% in knee joint, 25% in the talocrural joint, 15% in the transverse tarsal joint. In the morphological examination OA was found in 31.5% knee joint, 25% ankle joint and 5% transverse tarsal joint. In the microscopic examination OA was found in 94.7% knee joint, in 94.7% ankle joint and in 100% transverse tarsal joint.

Conclusion: Although radiological and macroscopic OA was detected in approximately 1/3 of cadavers aged between 30 and 50 years, degeneration of varying degrees was detected in all joints examined in microscopic examination. This shows that an advanced age disease OA, starts at a very early age.

Key words: Osteoarthritis, Knee joint, Talocrural joint, Transverse tarsal joint

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease in the world, and pain, deformity and loss of function arises by the progresive damage of the joint cartilage (1). Radiological images are important in defining the OA. Narrowing in the joint space, osteophyte, subchondral sclerosis, cyst formation, bone contour abnormalities are common radiological findings. OA frequency increases with age and pain is the most important symptom. However, the relationship between radiological image and joint pain is not always correlated (2).

Knee joint is a large joint of medial, lateral and patellofemoral components. These regions may be affected seperetaly or in different combinations in OA. Knee OA development is slow and usually takes years. The relationship between the clinic and radiology of knee OA is not strong (3).

The foot consists of many bones and small joints. All or a few of these small joints may be affected by the OA. The ankle OA is more rare, although it is a traumatic zone and is a load-bearing joint. OA may be less common due to the complicated geometry

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Corresponding Author: Muzaffer Sindel **E-mail:** sindelm@akdeniz.edu.tr

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and limited known biomechanics of the joints in the foot area. OA is more frequent in subtalar joint, but it rarely causes clinical symptoms.

Large osteophytes can often be seen in the talonavicular, calcaneocuboid joints. In the anterior part of the foot, OA is most common in the first metatarsophalangeal joint (4).

OA is mainly a disease in which articular cartilage destruction is accompanied by subchondral bone changes. Although the general approach is to prioritize the destruction of the articular cartilage, it has also been reported that changes in the subchondral bone initiate the destruction of the articular cartilage in some cases. However, when symptoms occur, as both articular cartilage and subchondral bone are usually affected, it is not known which of them started first (5) .

Cadaver studies can provide more accurate information on this issue. In addition, the examination of the bone also provides important data about the relationship of degenerative changes in the joints (6).

In this study, to determine the frequency of OA in the knee, ankle and foot joints in cadavers aged between 30-50 years, radiologically, macroscopically and microscopically, to compare the general features of OA such as location, location, severity and the shape of the lesions in terms of radiological and morphology and to reveal their relationship with each other was aimed.

METHODS

Cases

In our study, 20 amputee lower extremity materials, which were have data usage permission with the decision numbered 2008.01.02.0016 and were fixed with 10% formaldehyde solution in Akdeniz University Faculty of Medicine, Department of Anatomy and with data usage permission, were used. The ages of the cases were between 30 and 50 years.

Radiological Examination

The results obtained from radiographs of the knee joint, talocrural joint and transverse tarsal joint were graded for each joint according to the Kellgren and Lawrence scales. According to this system, joints with different OA were evaluated in 5 degrees between 0-4 (7, 8).

According to this rating; each case rated as;
0 - Normal (OA table absent),
1 - Suspicious (Suspicious appearance for small osteophytes),
2 - Minimal (There are osteophytes. Joint space is intact),
3 - Moderate (Moderate narrowing at the joint space is observed),
4 - Severe (The joint space is greatly impaired and there is sclerosis increase of the subchondral bone.).

Main Points:

- OA is a disorder characterized by pain, loss of function and joint stiffness in advanced ages.
- OA frequency increases with age and pain is the most important symptom.
- According to the data obtained in our study, it was concluded that OA, an advanced age disease, actually begins at a very early age.

Macroscopic Examination

The knee joint, talocrural joint, and transverse tarsal joint opened with proper dissection. Each joint surface divided in to five areas as anterior, posterior, medial, lateral and central (Figure 1, Figure 2 and Figure 3). Articular surfaces were examined morphologically. The joint degeneration was evaluated in 5 grades from 0 to 4 (9, 10).

According to this score, each examined joint surface were graded as;
0 - Normal (No degeneration)
1 - Suspicious (slight deterioration of the cartilage)
2 - Minimal (slight defect at the cartilage, fissure)
3 - Moderate (Obvious erosion bigger than 1.5 cm cartilage defect)
4 - Severe (a defect that has reached the bone)

Microscopic Examination

The samples taken from the knee joint, talocrural joint, and transverse tarsal joint stained by toluidin-blue and hematoxylin-eosin by using routine examination methods. The slides that we prepared were examined and the degeneration changes in cartilage, presence of fibrillation, density, depth, clustering of cartilage cells, necrotic changes were evaluated according to the literature in 5 degrees from 0 to 4 (5, 11).

Figure 1. The division of the articular surfaces into regions is schematized on the distal articular surface of the femur in cadaver images.

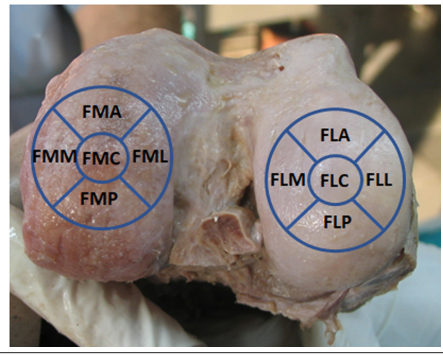
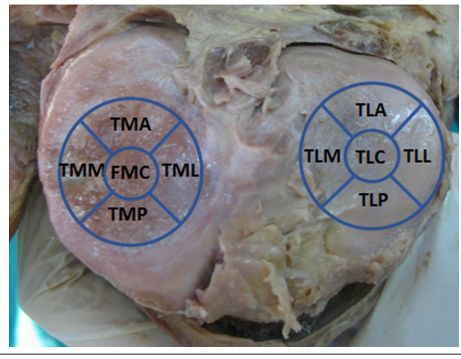
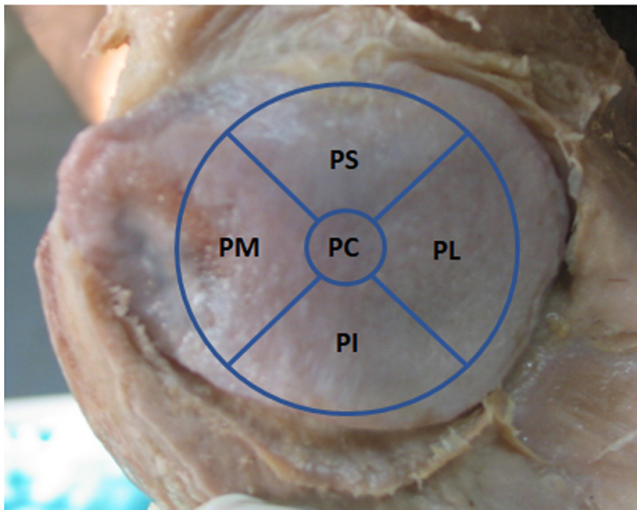


Figure 2. The division of articular surfaces into regions in cadaver images is schematized on the proximal articular surface of the tibia.



According to this classification, each case graded as;
0 - No pathology
1 - Nearly normal structure
2 - Fibrillation (for predegenerative changes)
3 - Cleft or significant erosion (more intense and deeper fibrillations)
4 - Cartilage degeneration, severe changes, intense fibrillation, aggregation of cartilage cells, and necrotic changes.

Figure 3. The division of articular surfaces into regions in cadaver images is schematized on the joint surface of the patella.



Statistical Analyses

All analyzes were performed with the IBM SPSS 23.0 package program (IBM Corp., Armonk, NY). Categorical variables were shown by frequency and percentage. Kappa coefficient was calculated in order to evaluate the agreement of microscopic and macroscopic measurements. The degree of agreement is "insignificant" if the kappa coefficient is equal to or less than 0.20, "weak" if it is between 0.21-0.40, "moderate" if it is between 0.41-0.60, "significant" if it is between 0.61-0.80 and "significant" if it is between 0.81-0.00 defined as "very good" fit. Spearman correlation test was used to determine the relationship between the measurement values obtained from the two methods. P values less than 0.05 were considered statistically significant.

RESULTS

Radiological Results

In the morphological examination, the joint surfaces were divided into five zones, while in the radiological examination, only each joint surface was scored without dividing the joint surfaces into five zones due to the visual limitation of the joint surfaces from all directions. The Kellgren and Lawrence classification is graded 0 to 4. In order to compare the radiological and morphological results more easily, we accepted this rating as 0 normal, 1 and 2 points as mild signs of OA, and generalized it by giving 1 point. We accepted the 3 and 4 scoring groups as severe OA signs and generalized by giving 2 points.

As a result of radiological examination, OA was found in 7 (35%) of the 20 patients, of which 5 were severe and 2 were mild in knee joint. Ankylosis was detected in one case. In the examination of talocrural joint, OA was found in 5 (25%) of the cases, of which 3 were severe and 2 were mild. Severe OA was found in 3 cases (15%) in transverse tarsal joint. In summary, the involvement of the knee joint is observed in the radiological examination most commonly (Figure 4, Figure 5 and Figure 6).

Figure 4. AP radiographs of both knees, bilateral osteoarthritis in the knee joint. Lateral narrowing in both two joints, tapering in eminentia and joint corners.

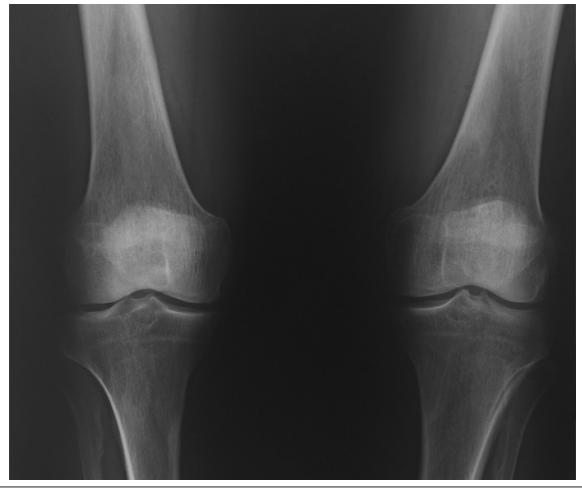


Figure 5.Lateral knee joint radiograph, retropatellar tapering and distance narrowing.

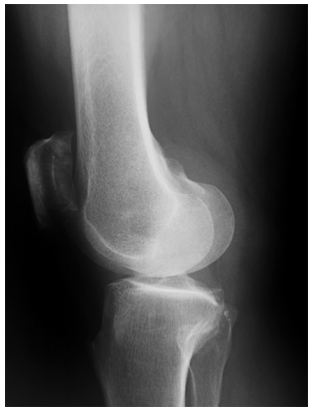
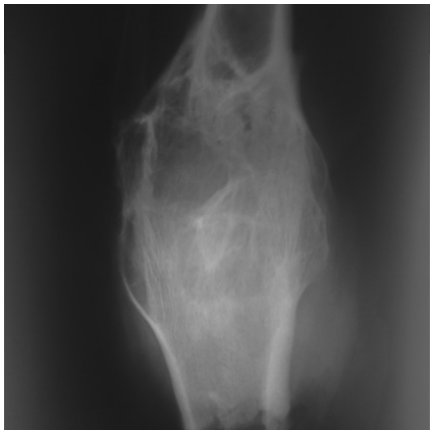


Figure 6. AP knee joint radiograph, ankylosis in the knee joint.



Macroscopic Results

In the macroscopic examination, 67 different areas of 20 cadavers were examined. The knee joint of only 1 case could not be examined because of ankylosis. As a result of the macroscopic examination, the most frequently affected areas in the knee joint of 20 cases were the anterior part of the medial condyle of the femur (FMA), the inner lateral part of the patella (PM), and the least affected areas were the inner lateral part of the lateral condyle of the femur (FLM) and the outer lateral part (FLL) of the lateral condyle of the femur. On the tibial surface, the most affected area was the center of the medial condyle of the tibia (TMC), and the least affected area was the outer lateral part of the lateral condyle of the tibia (TLL).

In the talocrural joint examination, the most affected areas were the inner lateral part of the distal articular surface of the talus (TAM) and the posterior part of the distal articular surface of the talus (TAP), the least affected areas were the center of the distal articular surface of the talus (TAC), the distal center of the articular surface of tibia (TIC), the inner lateral part of the distal articular surface of the tibia (TIM), and the outer lateral part of the distal articular surface of the tibia (TIL) were found. According to the transverse tarsal joint examination, it was observed that this joint was not affected except the anterior part of the distal articular surface (TAA) of the talus. In summary, it was revealed that the medial and patellofemoral surfaces are mostly affected in the knee joint, while the anteroposterior and medial aspects of the talus are mostly affected in the talocrural joint. In general morphological examination, in 6 of 20 cases (4 definite, 2 suspicious, 1 ankylosis) (31.5%) knee OA, in 5 cases (1 definite, 4 suspected) (25%) ankle OA and 1 case (suspected) (5%) transverse tarsal joint OA was determined.

Microscopic Results

As a result of microscopic examination, the most frequently affected areas in the knee joint of 20 cases were found as FMA in the femur (Figure 7), the center of the medial condyle of the femur (FMC), the upper part of the os patella (PS), the PM, and the outer lateral part of the os patella (PL) (Figure 8). It was observed that the lateral part was least affected. On the tibial surface, the most affected area was the outer lateral part of the medial condyle of the tibia (TML) (Figure 9), and the least affected area was the posterior part of the medial condyle of the tibia (TMP).

In the talocrural joint examination, the most affected area in the talus was the posterior part of the proximal articular surface of the talus (TP), the least affected areas were the center of the proximal articular surface of the talus (TC), the most frequently affected area in the tibia was the distal center of the articular surface of tibia (TIC), the least affected area was the anterior part of the distal articular surface (TIA) of the os tibia.

In the transverse tarsal joint, in the talonavicular joint, the most frequently affected area was center of the distal articular surface of the talus (TAC) and the posterior part of the navicula (NAP), while the least affected areas were the outer lateral part of the distal articular surface of the talus (TAL), the anterior part of the navicula (NAA), and the anterior part of the distal articular surface of talus (TAA) and in calcaneocuboid joint it was found that the center of the cuboideum (CUC), the posterior part of the cuboideum (CUP) and the outer lateral part of the cuboideum (CUL) were most common.

In general microscopic examination, the degenerative changes were found in 18 of 20 cases (9 definite, 9 mild) (94.7%) knee joint, in 18 cases (94.7%) (8 definite 10 mild) ankle joint and 20 cases (100%) (9 definite 11 mild) transverse tarsal joint. we found that the knee joint had ankylosis in one case. In another case we observed osteophytic tissue (Figure 10).

Figure 7. Images of microscopic findings in the anterior region of the medial condyle of the femur (FMA).

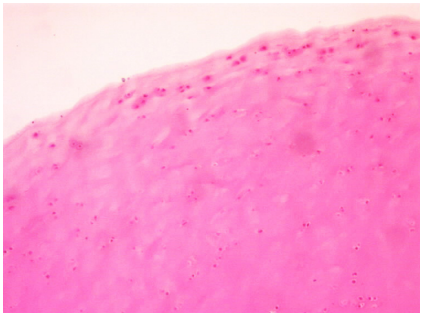


Figure 8. Images of microscopic findings in the lateral region (PL) of the patella and fibrillation in the cartilage tissue

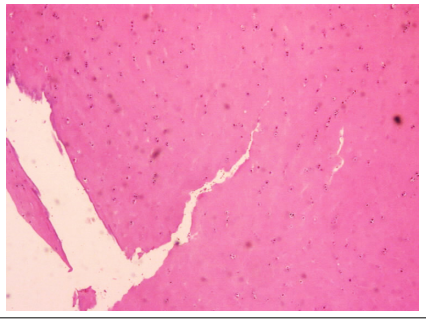


Figure 9. Fibrillation in the cartilage tissue of the lateral region of the outer condyle of the tibia (TML).

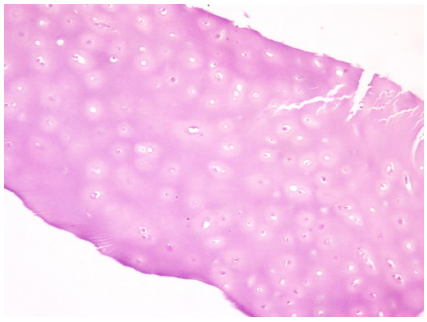
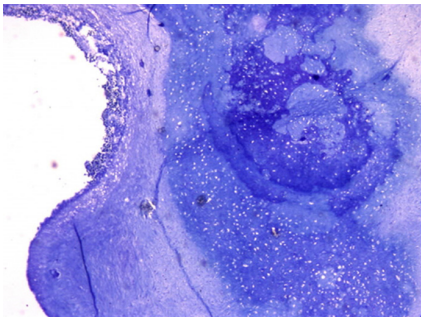


Figure 10. Osteophytic tissue (image taken at 50x magnification).



Radiological, microscopic and macroscopic results are not statistically compatible with each other. Kappa rate is less than 0.800 (Table 1).

When macroscopic and microscopic findings of osteoarthritis were compared in the knee joint, statistically significant difference was observed in PI, PM, PL, FLP, FLM, FMA, FMP, TMM values. In the ankle; Statistically significant difference was observed in TM and TIA values. In foot; statistically significant difference was observed in TAC and CUA values. There was no significant difference in the findings of other parametersin both knee joint, ankle joint and foot (Table 1). The macroscopic and microscopic evaluation of knee joint, talocrural joint and transverse tarsal joint were shown in Table 2.

Table 1. Macroscopic and microscopic evaluation of osteoartrit

	Macroscopic		Microscopic			
Variables, n (%)	**No**	**Yes**	**No**	**Yes**	**Kappa**	**p**
Knee						
FS	16(84.2)	3(15.8)	14(73.7)	5(26.3)	0.066	0.764
FI	15(78.9)	4(21.1)	15(78.9)	4(21.1)	0.050	0.827
PS	16(84.2)	3(15.8)	12(63.2)	7(36.8)	–0.027	0.891
PC	14(73.7)	5(26.3)	13(68.4)	6(31.6)	0.362	0.111
PI	17(89.5)	2(10.5)	13(68.4)	6(31.6)	0.406	**0.028**
PM	13(68.4)	6(31.6)	12(63.2)	7(36.8)	0.650	**0.004**
PL	17(89.5)	2(10.5)	13(68.4)	6(31.6)	0.406	**0.028**
FLA	16(84.2)	3(15.8)	16(84.2)	3(15.8)	0.208	0.364
FLC	17(89.5)	2(10.5)	17(89.5)	2(10.5)	–0.118	0.608
FLP	18(94.7)	1(5.3)	16(84.2)	3(15.8)	0.457	**0.018**
FLM	16(84.2)	3(15.8)	12(63.2)	7(36.8)	0.486	**0.013**
FLL	19(100)	0(0)	19(100)	0(0)	–	–
FMA	11(57.9)	8(42.1)	14(73.7)	5(26.3)	0.431	**0.046**
FMC	13(68.4)	6(31.6)	13(68.4)	6(31.6)	0.269	0.241
FMP	16(84.2)	3(15.8)	14(73.7)	5(26.3)	0.689	**0.002**
FMM	16(84.2)	3(15.8)	17(89.5)	2(10.5)	0.313	0.161
FML	15(78.9)	4(21.1)	14(73.7)	5(26.3)	0.275	0.226
TLA	19(100)	0(0)	16(84.2)	3(15.8)	–	–
TLC	18(94.7)	1(5.3)	11(57.9)	8(42.1)	0.142	0.228
TLP	18(94.7)	1(5.3)	12(63.2)	7(36.8)	–0.101	0.433
TLM	15(78.9)	4(21.1)	11(57.9)	8(42.1)	0.073	0.719
TLL	18(94.7)	1(5.3)	16(84.2)	3(15.8)	–0.086	0.656
TMA	16(84.2)	3(15.8)	15(78.9)	4(21.1)	0.128	0.570
TMC	18(94.7)	1(5.3)	9(47.4)	10(52.6)	–0.106	0.279
TMP	18(94.7)	1(5.3)	18(94.7)	1(5.3)	–0.056	0.809
TMM	16(84.2)	3(15.8)	16(84.2)	3(15.8)	0.999	**<0.001**
TML	17(89.5)	2(10.5)	12(63.2)	7(36.8)	0.070	0.683
Ankle						
TA	17(85)	3(15)	16(80)	4(20)	–0.207	0.348
TC	20(100)	0(0)	19(95)	1(5)	–	–
TP	18(90)	2(10)	16(80)	4(20)	0.231	0.264
TM	17(85)	3(15)	19(95)	1(5)	0.459	**0.015**
TL	19(95)	1(5)	20(100)	0(0)	–	–
TIA	19(95)	1(5)	17(85)	3(15)	0.459	**0.015**
TIC	20(100)	0(0)	17(85)	3(15)	–	–
TIP	20(100)	0(0)	16(80)	4(20)	–	–
TIM	19(95)	1(5)	17(85)	3(15)	–0.081	0.666
TIL	19(95)	1(5)	16(80)	4(20)	–0.087	0.608
Foot						
TAA	20(100)	0(0)	16(80)	4(20)	–	–
TAC	19(95)	1(5)	17(85)	3(15)	0.459	**0.015**

Table 1. Macroscopic and microscopic evaluation of osteoartrit

	Macroscopic		Microscopic			
Variables, n (%)	No	Yes	No	Yes	Kappa	p
Foot						
TAP	20(100)	0(0)	17(85)	3(15)	-	-
TAM	20(100)	0(0)	18(90)	2(10)	-	-
TAL	20(100)	0(0)	18(90)	2(10)	-	-
NAA	20(100)	0(0)	17(85)	3(15)	-	-
NAC	20(100)	0(0)	17(85)	3(15)	-	-
NAP	20(100)	0(0)	14(70)	6(30)	-	-
NAM	20(100)	0(0)	15(75)	5(25)	-	-
NAL	20(100)	0(0)	20(100)	0(0)	-	-
CAA	19(95)	1(5)	14(70)	6(30)	0.219	0.117
CAC	19(95)	1(5)	15(75)	5(25)	-0.091	0.554
CAP	20(100)	0(0)	18(90)	2(10)	-	-
CAM	20(100)	0(0)	17(85)	3(15)	-	-
CAL	20(100)	0(0)	19(95)	1(5)	-	-
CUA	19(95)	1(5)	18(90)	2(10)	0.643	**0.002**
CUC	20(100)	0(0)	15(75)	5(25)	-	-
CUP	20(100)	0(0)	15(75)	5(25)	-	-
CUM	20(100)	0(0)	19(95)	1(5)	-	-
CUL	20(100)	0(0)	18(90)	2(10)	-	-

FS: Upper part of the intercondylar space of the femur, FI: The lower part of the intercondylar space of the femur, PS: Upper part of patella, PC: Center of patella, PI: Lower part of patella, PM: Inner lateral part of os patella, PL: Outer lateral part of patella, FLA: Anterior part of the lateral condyle of the femur, FLC: Center of the lateral condyle of the femur, FLP: Posterior part of the lateral condyle of the femur, FLM: Medial lateral portion of the lateral condyle of the femur, FLL: Lateral part of the lateral condyle of the femur, FMA: Anterior part of the medial condyle of the femur, FMC: Center of the medial condyle of the femur, FMP: Posterior part of medial condyle of femur, FMM: Medial condyle of femur medial lateral part, FML: Lateral part of medial condyle of femur, TLA: Anterior part of lateral condyle of tibia, TLC: Center of lateral condyle of tibia, TLP: Posterior part of lateral condyle of tibia, TLM: Inner lateral portion of the lateral condyle of the tibia, TLL: Outer lateral portion of the lateral condyle of the tibia, TMA: Anterior part of medial condyle of tibia, TMC: Center of the medial condyle of the tibia, TMP: Posterior part of medial condyle of tibia, TMM: Inner lateral part of the medial condyle of the tibia, TML: Lateral part of medial condyle of tibia, TA: Anterior portion of the proximal articular surface of the talus, TC: Center of the proximal articular surface of the talus, TP: Posterior part of the proximal articular surface of the talus, TM: Inner lateral portion of the proximal articular surface of the talus, TL: Lateral part of the proximal articular surface of the talus, TIA: the least affected area was the anterior part of the distal articular surface, TIC: the distal center of the articular surface of tibia, TIP: Posterior part of the distal articular surface of the tibia, TIM: Inner lateral part of the distal articular surface of the tibia, TIL: Lateral part of the distal articular surface of the tibia, TAA: Anterior portion of the distal articular surface of the talus, TAC: Center of the distal articular surface of the talus, TAP: Posterior part of the distal articular surface of the talus, TAM: Inner lateral part of the distal articular surface of the talus, TAL: Lateral part of the distal articular surface of the talus, NAA: Anterior part of the navicular, NAC: Center of the navicular, NAP: Posterior part of navicular, NAM: Inner side of navicular, NAL: Outer part of navicular, CAA: Anterior part of calcaneus, CAC: Center of calcaneus, CAP: Posterior part of calcaneus, CAM: Inner side of calcaneus, CAL: Lateral part of calcaneus, CUA: Anterior part of cuboideum, CUC: Center of the cuboideum, CUP: Posterior part of cuboideum, CUM: Inner side of cuboideum, CUL: outer part of cuboideum.

Table 2. Correlation between macroscopic and microscopic evaluation of knee joint, talocrural joint and transverse tarsal joint

Variables	r	p
Knee		
FS	0.176	0.470
FI	0.254	0.294
PS	0.030	0.903
PC	0.541	0.017
PI	0.679	0.001
PM	0.583	0.009
PL	0.651	0.003
FLA	0.387	0.101
FLC	0.284	0.238

FLP	0.529	0.020
FLM	0.320	0.181
FLL	0.255	0.292
FMA	0.346	0.147
FMC	0.677	0.001
FMP	0.607	0.006
FMM	0.562	0.012
FML	0.585	0.009
TLA	-0.045	0.853
TLC	0.158	0.518
TLM	-0.036	0.884
TLL	0.330	0.168
TMA	0.463	0.046
TMC	0.098	0.690
TMP	0.111	0.650
TMM	0.490	0.033
TML	0.350	0.141
Ankle		
TA	-0.261	0.267
TC	0.085	0.722
TP	0.325	0.162
TM	0.550	0.012
TL	-0.088	0.712
TIA	0.366	0.113
TIC	0.404	0.078
TIP	0.365	0.113
TIM	0.091	0.704
TIL	0.370	0.108
Foot		
TAA	0.278	0.235
TAC	0.069	0.772
TAP	0.216	0.360
TAM	0.432	0.057
TAL	-0.070	0.768
NAA	0.548	0.012
NAC	0.218	0.355
NAP	-0.063	0.792
NAM	0.509	0.022
NAL	0.242	0.304
CAA	0.340	0.143
CAC	0.182	0.441
CAP	0.385	0.094
CAM	0.430	0.058
CAL	0.380	0.098
CUA	-0.120	0.615
CUC	0.113	0.634
CUP	0.253	0.281
CUM	-0.033	0.889
CUL	0.349	0.131

FS: Upper part of the intercondylar space of the femur, FI: The lower part of the intercondylar space of the femur, PS: Upper part of patella, PC: Center of patella, PI: Lower part of patella, PM: Inner lateral part of os patella, PL: Outer lateral part of patella, FLA: Anterior part of the lateral condyle of the femur, FLC: Center of the lateral condyle of the femur, FLP: Posterior part of the lateral condyle of the femur, FLM: Medial lateral portion of the lateral condyle of the femur, FLL: Lateral part of the lateral condyle of the femur, FMA: Anterior part of the medial condyle of the femur, FMC: Center of the medial condyle of the femur, FMP: Posterior part of medial condyle of femur, FMM: Medial condyle of femur medial lateral part, FML: Lateral part of medial condyle of femur, TLA: Anterior part of lateral condyle of tibia, TLC: Center of lateral condyle of tibia, TLP: Posterior part of lateral condyle of tibia, TLM: Inner lateral portion of the lateral condyle of the tibia, TLL: Outer lateral portion of the lateral condyle of the tibia, TMA: Anterior part of medial condyle of tibia, TMC: Center of the medial condyle of the tibia, TMP: Posterior part of medial condyle of tibia, TMM: Inner lateral part of the medial condyle of the tibia, TML: Lateral part of medial condyle of tibia, TA: Anterior portion of the proximal articular surface of the talus, TC: Center of the proximal articular surface of the talus, TP: Posterior part of the

proximal articular surface of the talus, TM: Inner lateral portion of the proximal articular surface of the talus, TL: Lateral part of the proximal articular surface of the talus, TIA: the least affected area was the anterior part of the distal articular surface, TIC: the distal center of the articular surface of tibia, TIP: Posterior part of the distal articular surface of the tibia, TIM: Inner lateral part of the distal articular surface of the tibia, TIL: Lateral part of the distal articular surface of the tibia, TAA: Anterior portion of the distal articular surface of the talus, TAC: Center of the distal articular surface of the talus, TAP: Posterior part of the distal articular surface of the talus, TAM: Inner lateral part of the distal articular surface of the talus, TAL: Lateral part of the distal articular surface of the talus, NAA: Anterior part of the navicular, NAC: Center of the navicular, NAP: Posterior part of navicular, NAM: Inner side of navicular, NAL: Outer part of navicular, CAA: Anterior part of calcaneus, CAC: Center of calcaneus, CAP: Posterior part of calcaneus, CAM: Inner side of calcaneus, CAL: Lateral part of calcaneus, CUA: Anterior part of cuboideum, CUC: Center of the cuboideum, CUP: Posterior part of cuboideum, CUM: Inner side of cuboideum, CUL: outer part of cuboideum.

DISCUSSION

OA is one of the most common rheumatic diseases and its frequency increases with age. It is rare before the age of 50. When it gives symptoms, it is usually present in radiological findings.

Therefore, it is accepted that joint degeneration begins long before it causes symptoms or radiological signs and progresses over the years and becomes symptomatic (1, 2). In this study, the knee, ankle and foot joints of cadavers aged 30-50 years were examined. Therefore, we think that this study, which examines the degeneration in the joints before the age of 50 from a macroscopic, microscopic and radiological point of view, may shed light on the concept of early OA diagnosis. In the literature, cadaveric materials have not been used in studies on OA so far. Takahama (11) performed histological examination of OA on mice. There are studies about the incidence of OA in the intervertebral disc, ankle, hand fingers, carpometacarpal joint, trapezio-metecarpal joint, and trapezium bone (12-16). Especially in the Turkish population, we could not come across such a study in decapitated material between the ages of 30 and 50, and any studies comparing all three methods with each other. Radiological assessment is an important diagnostic criteria for OA determination. Especially in the complaints of the human population accompanied by joint pain, simple and inexpensive as radiography method is preferred. In the study of Claessens et al. (17), it was stated that radiological findings are the most important clinical finding in the definition of OA. In our study, reading was made by a single observer to minimize the reading errors of the radiological graphies. Kellgren and Lawrence performed the radiological evaluation of OA and graded osteoarthritis in five grades (8). We have graded osteoarthritis based on this scale in all of our reviews. The incidence of OA in the knee joint is higher than in other joints (9). In this study, which is consistent with the literature, radiologically detected degeneration is more common in the knee joint than in the talocrural and tarsi transversa joints. Macroscopic examination is an invasive method used by researchers to observe degenerative changes. It cannot be used routinely for diagnostic purposes. In the study of Hirose et al. (18), cartilage degeneration in the talocrural and talocalcaneal joints of cadaver donors was examined macroscopically and it was reported that degenerative changes were seen more frequently in the talocrural joint. In the study of Nakamura et al. (19), degenerative changes in the hand finger joints of elderly cadavers were examined macroscopically and degenerative changes were detected more intensely in the thumb and middle finger. In the study of Koepp et al. (20) in the examination of degenerative changes in the ankle and knee joints in human donors, degeneration was observed increasing with age in men compared to women. Waldron examined the prevalence and distribution of OA in the former Londoner and German population and reported that the shoulder joint was the most affected joint macroscopically (21). In the study of Hirose et al. (18) in which they examined cartilage degeneration in the talocural and talocalcaneal joints from a macroscopic point of view, they observed degenerative changes more frequently in the anterior joint surfaces of the talocalcaneal joint and the medial joint surfaces of the talocrural joint. Similarly, in our study, TAM and TAP were the most degenerated regions in the talocrural joint.

In our study, degenerative changes were found in 31.5% of the knee joints, 25% of the talocrural joints and 5% of the tarsi transversa joints. As can be seen, the most affected joint is the knee joint. In the knee joint, it was determined that the medial and patellofemoral surfaces were mostly involved. This is the part that is most clinically affected. Waldron et al. (21) did not make a detailed evaluation and investigated the rate of involvement of the knee joint with other joints. Binks et al. (22) performed magnetic resonance imaging and histological evaluation in cadaver tissue for the potential role of the synovium of the posterior cruciate ligament in the joint effusion complex in early osteoarthritis. They detected prominent findings associated with microscopic OA changes of the posterior cruciate ligament and showed common pathological features in knee OA detected by magnetic resonance imaging (22). Iriuchishima et al. (23) evaluated OA changes in the patella-femoral joint on 203 cadaver knees and found that the medial area of the patella was the most affected area. In our study, various degrees of degeneration were detected in almost all cases in microscopic examination. The fact that the cases are in the 30-50 age group and there are mild or severe signs of degeneration in all examined joints indicate that OA begins at a very early age. Therefore, we can say that this is a very important finding for OA. Undoubtedly, mild microscopic findings cannot be detected macroscopically and radiologically. In our study, we also could not found relationship between the methods. However, it seems fit in terms of macroscopic and microscopic as can be seen from the table 1 as viewed by the severity. Irlenbuch et al. (23) reported that OA begins with subchondral ossification and progresses in a similar pathogenic mechanism to large joints. In our study, only cartilage was examined. Subchondral bone was not examined. In the study of Takahama (11) on the knees of C 57 mice, OA was detected in all 18-24 month-old mice, no fibrillation in the cartilage and no changes in the synovium were observed, and they evaluated the reason as the mice were not exposed to repetitive microtraumas (11). The presence of OA is observed more clearly in microscopic examination. However, the fact that it is an invasive method makes it impossible to use in healthy individuals. For this reason, it would be beneficial to develop other methods in order to detect degeneration earlier and taking necessary precautions.

CONCLUSION

OA is a disorder characterized by pain, loss of function and joint stiffness in advanced ages. In our study, we preferred age-determined cadaver material. We determined that OA most commonly affects the knee joint in the lower extremity and the medial and patellofemoral parts of this joint. There was no statistical relationship between the three methods we used. Although radiological and macroscopic OA was detected in approximately 1/3 of the cadavers, degeneration of varying degrees was detected in all joints examined in microscopic examination. According to the data obtained in our study, it was concluded that OA, an advanced age disease, actually begins at a very early age.

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Original Research

Examining the Relationship Between Preventable Psychiatric Problems and Child Extremity Fractures

Seda Aybuke Sari[1], Ozhan Pazarci[2], Seyran Kilinc[2], Ayla Uzun Cicek[1]

1 Cumhuriyet University School of Medicine, Department of Child and Adolescent Psychiatry, Sivas/Turkey

2 Cumhuriyet University School of Medicine, Department of Orthopaedics and Traumatology, Sivas/Turkey

ABSTRACT

Objective: Extremity fractures (EF) are among the most common causes of admission to hospitals in children. We aimed to evaluate children treated for EFs by comparing them with the control group from a psychiatric perspective.

Method: Thirty-six children aged between 3 and 17 years who administered to the Orthopedics and Traumatology clinic due to EF were included in the study. 36 children of similar age and gender with the study group were included as the control group. A child psychiatrist evaluated all children included in the study. A psychiatric diagnosis interview was conducted. The parents filled out the Conner's Parent Rating Scale-Revised Short Form (CPRS-R:S).

Results: Of the cases in the patient group, 66.7% were male. The ratio of rural residents in the patient group was higher compared to the control group. The most common fracture location was lower extremity (55.6%). The most common cause of the fracture was falling (52.8%). In the patient group, the ratio of the children who had previously experienced fracture was 36.1%. Psychopathology was detected to be at a higher level in the patient group. The most common was Attention Deficiency and Hyperactivity Disorder (ADHD). Children in the patient group scored higher on the CPRS-R:S than the control group.

Conclusion: Children with EF exhibited more impulsive and hyperactive behaviours than controls and had more psychopathology. For this reason, it is essential to evaluate children who apply due to fracture in terms of psychopathology.

Keywords: Extremity fracture, psychopathology, children

INTRODUCTION

Extremity fractures are among the most common causes of admission to emergency services and hospitalization in children (1). Although there are differences in the conducted studies, the risk of the occurrence of at least one fracture in childhood was found to be approximately 42-64% in boys and 27-40% in girls (2). Upon examining these fractures, it is observed that the age, at which the fracture occurs, displays a bimodal distribution. Accordingly, the first peak is observed at 6-7 years of age, and the second peak is observed at 13-14 years of age (3). Examining the etiology of extremity fractures observed in children, there are three leading causes, including trauma after an accident, non-accident trauma, and pathological conditions (4). The causes of trauma are usually falling, motor vehicle accidents, sports or bicycle injuries, and are mostly observed in the upper extremity (5).

Psychosocial characteristics of children applying with fractures that occurred due to traumatic injuries have been examined in various studies (6, 7). Childhood behavior disorders have been reported to increase the risk of injury in children 1.5 times (8). Among these disorders, Attention Deficiency and Hyperactivity Disorder (ADHD), which is characterized by intense inattention, mobility, and impulsivity, is the most common neurodevelopmental disorder of childhood (9). It was reported that ADHD adversely affects a person's daily life, and patients diagnosed with ADHD are more prone to accidents (10). The risk of fracture increases in patients with ADHD due to behavioral characteristics such as negligent behavior, clumsiness, disregard of rules during activities, and neglect of safety measures (11). It was suggested that children treated for extremity fractures had higher levels of impulsivity and hyperactivity than children treated for non-traumatic reasons (12).

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Corresponding Author: Seda Aybuke Sari **E-mail:** aybuke_sari@hotmail.com

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In our study, the presence of psychiatric disorders in pediatric patients who applied to the orthopedic clinic due to extremity fractures was compared with the control group. In this prospective controlled study, we investigated whether there were any psychiatric disorders, especially Attention Deficiency and Hyperactivity Disorder (ADHD), Conduct Disorder (CD), and Oppositional Defiant Disorder (ODD).

MATERIAL AND METHODS

Children between the ages of 3-17 who administered to orthopedics and traumatology clinic of our hospital due to extremity fracture between 1 January and 1 May 2019 were included in the study. Children with fractures due to intra-vehicular traffic accidents and due to suicidal attempt were excluded from the study. Also, children who were prone to accidents due to neuropsychiatric disorders, including mental retardation, autism spectrum disorder or epilepsy, and who had bone metabolism disorders such as osteogenesis imperfecta, and children with auditory or visual impairments were not included in this study. Finally, thirty-six children with fractures who met these criterias were included in this study as a patient group. The control group consisted of 36 children of similar age and gender with the patient group, who did not receive psychiatric treatment, and did not have a history of fracture according to the information obtained from the parents. After the treatment of the children was completed, they were referred to the child and adolescent psychiatry outpatient clinic for psychiatric evaluation. All children included in the study were evaluated by a semi-structured psychiatric interview is a detailed interview conducted by a child psychiatrist with the child himself/herself and his/her parents to determine whether there is any mental disorder in the child. We used the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Aged Children-Present and Lifetime Version (KSADS-PL) for this interview (13). The information form prepared by the clinician was completed by asking the questions to the parents. Also, we asked to fill out the Conner's Parent Rating Scale-Revised Short Form by all parents (14). Ethical committee approval was obtained on 02.01.2019 with the decision number 2019-01/02. Written informed consent was obtained from all children and parents included in this study.

DATA COLLECTION TOOLS

Information Form

This form was prepared by the researcher. The socio-demographic information of both groups was obtained by these forms (Name-surname, age, the place of living, class, parents' profession, parents' educational level, and monthly income of the family). Furthermore, fracture information was collected in the patient group (location of the fracture, aetiology, treatment modality, the presence of a previous fracture, etc.).

Main Points:

- Cervical cancer screening methods are proved one of the few screening methods that are thought to decrease invasive cancer incidence and mortality.
- It is the first study investigating HPV relationship under the title of atopic disease (allergic dermatitis, urticaria, atopic asthma, allergic rhinitis).
- There is no need to different and extra screening schedules for patients with atopic diseases such as asthma, rhinitis or urticaria.

Conner's Parent Rating Scale-Revised Short Form (CPRS-R:S)

It consists of 27 items. The items were collected in three subscales (Oppositional Disorder-OD, Cognitive Problems-Inattention-CP/I, Hyperactivity-H) and one assistant scale (ADHD Index-ADHD). There are four answer options for each item with a score value ranging from 0 to 3: never correct (never, very rarely), 0 points; slightly correct (sometimes), 1 point; quite accurate (often, quite a lot), 2 points; very accurate (very often), 3 points. A high score indicates that the child has problems identified in the CPRS-R:S. The validity and reliability of the Turkish version of the scale were conducted by Kaner et al [15].

Statistical Analysis

Data were analyzed using the SPSS 22.0 program (Statistical Package for Social Sciences for Windows 22, SPSS Inc., Chicago, USA). The Shapiro-Wilk test was used to determine whether the data were normally distributed. For the data with non-normal distribution, the Mann-Whitney U test was used to compare two independent groups. The chi-square test was used to analyze categorical data. A p-value less than 0.05 was considered to be statistically significant with a 95% confidence interval (CI).

RESULTS

The sociodemographic data of the patient and control groups are presented in Table 1. There was no significant difference in terms of sociodemographic characteristics between the two groups matched by age and gender.

The fracture information of the patient group is presented in Table 2. We compared the localization of the fracture and the CPRS-R:S scores according to Mann Whitney-U test No statistically significant difference was found between these parameters (p: 0.924). Similarly, no significant difference was found between the treatment modality of the fracture and CPRS-R:S scores (p: 0.502).

Data on the comorbid psychiatric disorders of the patient and control groups are given in Table 3. According to our findings, half of the children in the patient group had at least one comorbid psychiatric disorder. ADHD was the most detected psychiatric disorder. In other words, the rate of comorbid psychiatric disease in the patient group was significantly higher than in the control group (p<0,001). Five patients in the patient group had two comorbid psychiatric disorders. 3 of them had ADHD+ODD, 2 of them had ADHD and CD. On the other hand, there was only one children had ADHD+ODD in the control group. There was no significant difference between the two groups in this respect (p=0,199).

In 61.5% (n: 8) of the comorbid ADHD patients, fracture occurred due to falling, in 23.1% (n: 3) due to pedestrian injuries, in 7.7% (n: 1) due to a bicycle injury, and in 7.7% (n: 1) due to a playground injury. No significant difference was detected between ADHD comorbidity and the causes of fracture formation (p = 0.520).

Table 1. Sociodemographic Data of The Patient and Control group

		Patient	Control	P
Sex	Female	12 (33,3%)	10 (27,8%)	0,609
	Male	24 (66,7%)	26 (72,2%)	
Age (Year)		10,7 ± 3,6	10,0 ± 3,2	0,349
Place Of Residence	Province	20 (55,6%)	22 (61,1%)	0,841
	District	7 (19,4%)	7 (19,4%)	
	Town/Vİllage	9 (25,0%)	7 (19,4%)	
Mother's Educational Status	Illiterate	5 (13,9%)	1 (2,8%)	0,053
	Primary School	20 (55,6%)	12 (11,1%)	
	Secondary School	3 (5,6%)	6 (16,7%)	
	High School	4 (13,9%)	11 (30,5%)	
	University	4 (13,9%)	6 (16,7%)	
Father's Educational Status	Primary School	13 (36,1%)	12 (33,3%)	0,716
	Secondary School	12 (33,3%)	9 (25,0%)	
	High School	7 (19,4%)	8 (22,2%)	
	University	4 (11,1%)	7 (19,4%)	
Family Structure	Nuclear Family	27 (75,0%)	29 (80,6%)	0,571
	Extended Family	9 (25,0%)	7 (19,4%)	
Family's Monthly Income*	< 2.000	17 (47,2%)	15 (41,7%)	0,422
	2.000 - 4.000	10 (27,8%)	15 (41,7%)	
	> 4.000	9 (25,0%)	6 (16,7%)	

*Turkish Liras

Table 2. Fracture information of patient group

		n	%
Fracture Localization	Lower Extremity	20	55,6
	Upper Extremity	16	44,4
Causes Of Fractures	Falling	19	52,8
	Fighting	2	5,6
	Motor Vehicle Accidents (Pedestrian İnjuries)	8	22,2
	Sport Injury	0	0
	Bicycle Injury	2	5,6
	Playground İnjury	5	13,9
Treatment of fractures	Surgical	24	66,7
	Conservative	12	33,3
Previous fracture history	Yes	13	36,1
	No	23	63,8

Table 3. Comorbid Psychiatric Disorders In The Patient And Control Group

	Patient* n:36	Control n:36	P
Ratio of comorbid psychiatric disorders in the whole group	18 (50,0%)	4 (11,1%)	**< 0,001**
ODD**	8 (22,2%)	2 (5,6%)	**0,042**
CD***	2 (5,6%)	0 (0,0%)	0,246
ADHD****	13 (36,1%)	3 (8,3%)	**0,005**

* Five patients in the patient group had two comorbid psychiatric disorders.
ODD: Oppositional Defiant Disorder, **CD***: Conduct Disorder, **ADHD****: Attention Deficiency and Hiperactivity Disorder

Table 4. Conners' Parent Rating Scale-Revised Short Form Sub-Scale Scores of patient and control group			
Sub-scales	**Patient (mean ± sd)**	**Control (mean ± sd)**	**p**
Oppositional-O	7,7 ± 5,8	3,2 ± 3,7	< **0,001**
Cognitive Problems-Inattention-CP/I	12,5 ± 10,0	4,5 ± 4,5	< **0,001**
Impulsivity/Hyperactivity-I/H	6,4 ± 4,8	1,9 ± 2,7	< **0,001**
Total Score	26,8 ± 17,7	9,7 ± 9,6	< **0,001**

In 50.0% (n: 4) of the Oppositional Defiant Disorder (ODD) patients, fracture occurred due to falling, in 12.5% (n: 1) due to fighting, in 25.0% (n: 2) due to a pedestrian injury, and in 12.5% (n: 1) due to a playground injury. No significant difference was detected between ODD comorbidity and the causes of fracture formation (p = 0.772).

In the patient group, children with a history of a previous fracture (n: 13) and children who had a fracture (n: 23) for the first time were compared. When a comparison was performed in terms of comorbid psychiatric diagnoses, a statistically significant difference was found (p: 0.02). According to this, 69.2% (n: 9) of children with a history of a previous fracture had a comorbid psychiatric disorder, and this rate was higher compared to those who had a fracture for the first time. A significant difference was found when the children with a history of fracture and the children who had a fracture for the first time were compared in terms of the scores obtained from the CPRS-R:S scale (p<0.01).

Table 4 displays the CPRS-R:S scores of the children in the patient and control groups. Accordingly, the children in the patient group scored significantly higher in all three subscales than their peers in the control group.

DISCUSSION

In the present study, children with extremity fractures were examined by being compared with the control group in terms of comorbid psychiatric disorders. Psychiatric disorders, especially ADHD, CD, and ODD, were evaluated. The most important finding we achieved as a result of our study is that children with a fracture are more likely to have comorbid psychiatric disorders than the control group. It has been reported that children with a fracture display more hyperactive-impulsive behaviours, psychosomatic complaints, and behaviour disorders (7).

It has also been indicated in many studies to date that children with behaviour disorders have a 1.5-times higher risk for injuries and that from these behaviour disorders especially ADHD constitutes a risk factor for injuries (8,15-18). However, our study stands out in terms of conducting face-to-face interviews by a child psychiatrist.

ADHD makes children vulnerable to accidents and injuries due to many reasons, especially inattention and impulsivity (19). Injuries in these children have been reported to be caused by reasons such as inattentive behaviours, clumsiness, disobedience to the rules during activities like a game, and difficulties in peer relationships (16,20). It has also been reported that children diagnosed with ADHD are inadequate in predicting the possible severe consequences of risky behaviours and in taking precautions against injuries, thus are more prone to accidents (18). In a study comparing children with and without ADHD, individuals in the ADHD group were found to apply to emergency services and to experience recurrent injuries at higher rates than those without ADHD (21). In a recent study conducted in our clinic in which 212 ADHD children and 215 healthy controls were compared, both one-time fracture (35.8%, 18.1% respectively; p<0.001) and recurrent fracture (12.7%, 6.0% respectively; p=0.018) rates were found to be significantly higher in the ADHD group than in the controls (22). In the present study, the most common comorbid psychiatric disorder was observed to be ADHD in the patient group.

In our study, the patient group had higher scores than the control group in all three subscales of the CPRS-R:S (Table 4). In other words, children who apply due to a fracture display more impulsive/hyperactive behaviours than the control group. Detecting the higher rate of psychopathology in the fracture group is similar to the results obtained by Uslu et al (11). Similarly to our study, a study reported that from the cases who applied to the hospital due to a fracture, those displaying impulsive/hyperactive behaviours had fractures mostly in the lower extremity and these fractures were more severe and required open reduction (23). In our study, we encountered mostly lower extremity fractures requiring surgical treatment in the patient group (Table 2). In addition to this, in terms of the general distribution, it is known that childhood extremity fractures are mostly observed in the upper extremity, unlike adults (4,5). It has been reported that children with ADHD have slower reaction times, and this situation may be associated with lower extremity fractures (24). Likewise, Clancy et al. (20) suggested that children with ADHD exhibit defective conservative reactions under experimental conditions and that this situation may explain why upper extremity fractures are less common in these children.

Several studies in the literature reported that the majority of the patients with fracture were male (3,5). In our study, the majority of the patients with extremity fracture were also male (Table 1). It is emphasized that boys are exposed to more severe injuries than girls due to a higher number of risky behaviours (25).

Some studies investigating the difference between urban and rural life in terms of fracture occurrence demonstrated that the rate of accidents and fractures was higher in rural areas (26,27). In our study, the majority of the children in the patient group were also children of families with low socioeconomic status.

In our study, the most common cause of fracture occurrence was found to be falling with a ratio of 52.8%, and this result was similar to the study, conducted by Rennie et al (3). Again in a study,

the most common cause of injury was found to be falling, followed by motor vehicle accidents and burns (28).

Of the fracture group, 36.1% had a history of a previous fracture. A higher rate of comorbid psychiatric diagnosis, especially ADHD, was detected in children with a history of fracture than children who had a fracture for the first time. The presence of comorbid psychiatric disorders in children with more than one fracture has also been emphasized in previous studies (29).

Our study has some limitations. These are the facts that the sample number was small, the duration of the study was short, and the study was single-centered. On the other hand, the prospective nature of our study, the fact that the data were not based solely on scale information, and in addition to the information received from the parent, the children were evaluated from a psychiatric perspective by conducting a diagnostic interview by the child psychiatrist are the superior aspects of our study.

CONCLUSION

Consequently, we found that the fracture group was determined to have more psychiatric disorders, especially ADHD, than the control group. Psychopathology was more common in children with more than one fracture history. Furthermore, these children were determined to exhibit more hyperactive/impulsive behaviours by both clinical examination and the CPRS: R-S.

Based on all these results, we recommend that children who present to the emergency department and/or orthopedics outpatient clinic for fractures and exhibit risky behaviors and/or have a history of multiple fractures should be evaluated by a child and adolescent psychiatrist. This situation will also reduce the mortality and morbidity associated with future fractures in these children.

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

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Original Research

The Reliability of the Projection Area Per Length Squared for Measuring Lumbar Lordosis on Lateral Radiographs: A Comparison with Cobb Method

Murat Golpinar[1], Erdal Komut[2]

[1]Hitit University, Faculty of Medicine, Department of Anatomy, Çorum, Turkey
[2]Hitit University, Faculty of Medicine, Department of Radiology, Çorum, Turkey

ABSTRACT

Objective: The assessment of the degree of lumbar lordosis in patients with spinal disorders is essential to determine disease progression and the effectiveness of treatment. The aim of this study was to examine the reliability of the projection area per length squared (PAL) for measuring lumbar lordosis on lateral radiographs and to compare it with the Cobb method.

Methods: Two independent investigators measured lumbar lordosis twice on 100 lateral radiographs using PAL and Cobb methods. Intra- and interobserver agreements of each radiological method were evaluated using intraclass correlation coefficients (ICC) and Bland–Altman plots. Correlations between the PAL estimations and Cobb angle measurements were tested using the Spearman rank correlation coefficient.

Results: Intra- and interobserver agreements for PAL and Cobb methods were excellent with all ICC values>0.976. The Bland–Altman plots indicated strong intra-observer and interobserver concordance in the measurement of the lumbar lordosis using the PAL method. A strong correlation was determined between the PAL and Cobb angle values in the first and second measurements ($r=0.825$; $p<0.001$ and $r=0.815$; $p<0.001$, respectively).

Conclusion: The PAL technique is easy to apply on digital images and provides quantitative information independent of the vertebral surface pathologies of the end vertebrae. It could be used as an alternative and potent diagnostic criterion for evaluating lumbar lordosis.

Keywords: Cobb angle, length squared, lumbar lordosis, planimetry, radiography

INTRODUCTION

Lumbar lordosis is a crucial structural component of the human spine in maintaining sagittal spinal alignment (1, 2). Ideal sagittal alignment in the lumbar region of the spine or normal lumbar lordosis is the primary goal for clinicians in surgical, ergonomic, and physiotherapeutic interventions (3). Therefore, physicians routinely measure and evaluate the lumbar curvature in the management of spinal deformity. Measurements of lumbar lordosis can provide quantitative data for monitoring disease progression or evaluation of the surgical approaches designed to restore the lordosis (4, 5). Therefore, appropriate and reliable measurement of the lumbar lordosis is important for clinical decisions.

Various techniques have been developed over the years for the quantitative evaluation of lumbar lordosis (1). Most of the existing methods are based on angle measurements formed by drawing straight lines from different landmarks of the lumbar vertebrae (1,5). The Cobb method, one of the first methods, is regarded as the gold standard for measuring lordotic curvatures on two-dimensional images in clinical practice because it provides the practical and rapid measurement of the sagittal spinal curvatures (6, 7). However, the Cobb method has some limitations, which can increase the variability in Cobb angle measurements (1, 8). In consideration of the limitations of the Cobb angle measurements, several alternative methods have been described by investigators to overcome these limitations (9). Suggested methods have involved multiple steps, used non-standardized terminology, and different anatomic landmarks when examining lumbar lordosis, so these methods are not widely used in clinical practice (9, 10). A more objective and standardized method is required for accurate and reliable measurement of lumbar lordosis.

The aim of this study was to describe an alternative approach for quantifying the degree of lumbar lordosis on lateral radiographs and to compare it with the Cobb method.

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Corresponding Author: Murat Golpinar **E-mail:** muratgolpinar@hitit.edu.tr

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METHODS

Study Design

Before the present study that is designed as a retrospective study commenced, approval was obtained from the local ethics committee of our institution (Decision Date: 2021 Approval No:436). A total of 100 standing lateral lumbar radiographs from 50 males and 50 females with varying degrees of lumbar lordosis were randomly selected between 2010-2019 from the archives of the Radiology Department of Hitit University, Çorum, Turkey. Patients with spinal deformity, cauda equina syndrome, previous back surgery, and spinal tumors were not included in the present study. All X-rays had previously been assessed for eligibility.

Two investigators with different levels of measurement experience were involved in this study. Investigator 1 had six years of experience using the PAL technique and four years of experience using the Cobb method. Investigator 2 had no measurement experience with either the PAL or the Cobb methods. As investigator 2 was unfamiliar with both the PAL and Cobb methods training was given on twenty digital radiographs for each measurement method before the study.

Radiographic Measurements

PAL Method

The planimetry technique was used to estimate the PAL of lordotic curvature on the digital images. Planimetry, which is based on the manual delineation of the margins of objects of interest on image sections, is the most widely preferred method for surface area measurement of irregularly shaped structures (11). All digital images were stored in the "Digital Imaging and Communications in Medicine (DICOM)" format. All measurements were performed using ImageJ software (Version 1.48, National Institutes of Health, Bethesda, Maryland, USA). The PAL estimation of lumbar lordosis on digital images was applied as follows.

The superoposterior corner of the first lumbar vertebra and the inferoposterior corner of the lower end vertebra were marked as anatomic bony landmarks. These landmarks were then connected with a straight line (Figure. 1A). The posterior boundaries of five lumbar vertebrae between the superoposterior and inferoposterior corners were drawn along the curvature, and the upper and lower ends of the elliptical-shaped line were connected to the beginning and end of the straight line. Finally, a semilunar area was obtained on the posterior side of the curvature (Figure. 1B). Both the semilunar region projection area and the length of the straight line were calculated using the ImageJ program (National Institutes of Health, Bethesda, MD, USA). Finally, the PAL was calculated as a percentage using the following formula (Figure. 1C) (12):

Where (*A*) denotes the semilunar region area and (*l*) represents the estimated length of the straight line between the superior and inferior end vertebrae. The PAL of the curvature expresses the surface area proportion of the semilunar area within the projection area of the square, which is the virtual reference surface area obtained from the square of the length (Figure. 1C).

Main Points:

- Projection area per length squared approach could provide accurate and reliable data for measuring the degree of lumbar lordosis.
- Projection area per length squared approach provides quantitative data independent of the vertebral surface pathologies of the reference vertebrae.
- Projection area per length squared approach could be used as an alternative diagnostic criterion for evaluating lumbar lordosis.

Figure 1A. White arrows show the superoposterior corner of the first lumbar vertebra and the inferoposterior corner of the fifth lumbar vertebra.

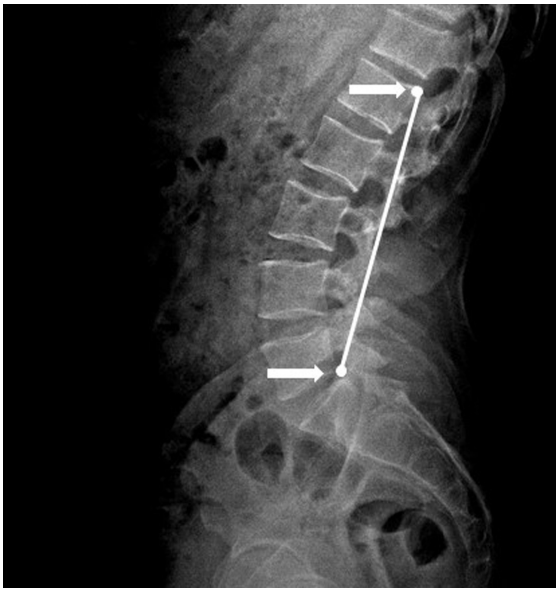


Figure 1B. Lateral digital radiograph showing the semilunar area drawn for the estimation of the projection area per length squared.

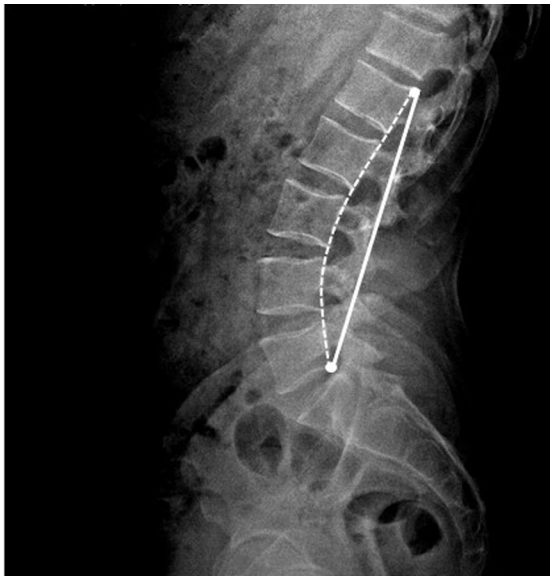


Figure 1C. The PAL of the curvature expresses the surface area proportion of the semilunar area within the projection area of the square, which is the virtual reference surface area obtained from the square of the length.

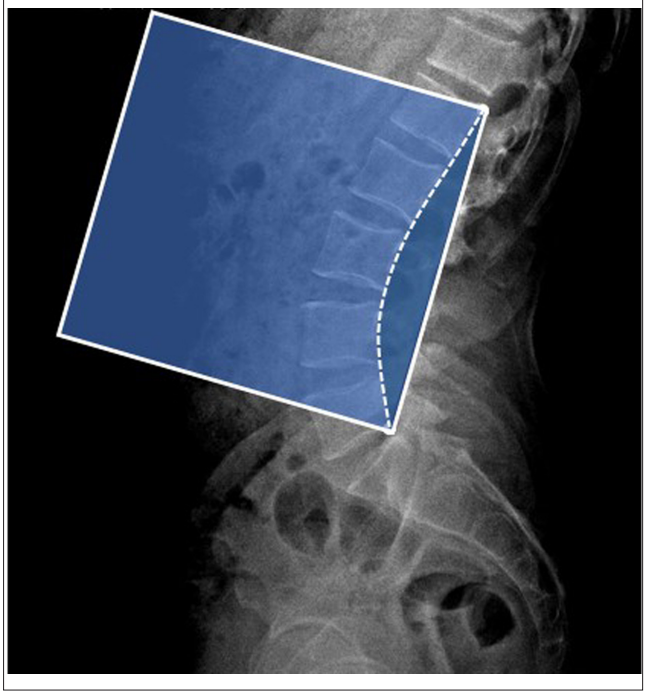
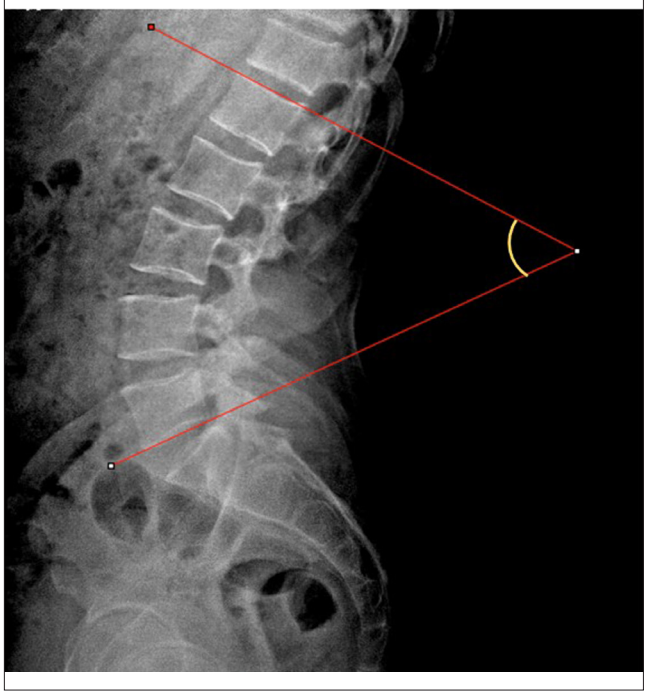


Figure 2. Computer-assisted Cobb angle measurement on lateral radiographs using OsiriX software. Cobb angle was formed by a lines drawn along the upper and lower surface of the first and fifth lumbar vertebrae.



Cobb Method

The Cobb angle measurements were performed using OsiriX software (OsiriX v.3.8.1 32 bit, Pixmeo SARL, Bernex, Switzerland). All digital images were transferred to OsiriX software. After opening the images, the investigators defined the superior and inferior endplates of the first and fifth lumbar vertebrae. Lines were drawn through and parallel to the superior and inferior endplate of the first and fifth lumbar vertebrae using the software tools. Finally, the program estimated the Cobb angle automatically (Figure. 2). The two investigators independently measured the lumbar lordosis on lateral radiographs using the PAL approach and the Cobb method twice at an interval of one month so as to reduce bias. Each investigator was blinded to the results of the other and to their own previous measurements of the same images for each measurement method.

Statistical Analysis

The data obtained were analyzed statistically using the Statistical Package for the Social Sciences for Windows, version 22 software (SPSS, Chicago, IL, USA). Conformity of the volumetric data to normal distribution was tested using the Shapiro-Wilk test. Estimation results obtained with each method were analyzed to detect statistical differences using the Wilcoxon signed-rank test. The intraclass correlation coefficient (ICC) (two-way mixed model) was calculated to define the intra- and interobserver reliability of each technique. The Bland–Altman method was used to examine the consistency between PAL measurements obtained by the two investigators in both sessions. The Spearman correlation test was used to analyze the degree of the relationship between the PAL approach and the Cobb method in both sessions.

RESULTS

The mean age of the subjects was 45.19±13.55 years (min-max, 22-77 years). The mean age of males and females were 47.62±9.44 and 42.76±10.25 years, respectively. There were no statistically significant differences between males and females in age (P=0.224). The overall mean PAL (±SD) obtained by both investigators was 6.23±2.15% (min-max, 2-11.90%). Three subjects with the minimum, medium, and maximum PAL values are shown in Figure 3.

According to the results of the Wilcoxon signed-rank test, there were no significant differences between each investigator's PAL estimation results in the first and second sessions (p=0.187, p=0.782, respectively). There were also no significant differences between the PAL estimation results of the two observers in the first and second sessions (p=0.432, p=0.853, respectively) The details of the PAL measurements of investigators in both sessions are given in Table 1. The ICC showed a high degree of intra-observer agreement in the PAL estimations for the first and second investigators (ICC=0.997, ICC=0.996, respectively). Interobserver agreement of the PAL estimations was found to be almost perfect for the first and second sessions (ICC=0.995, ICC=0.997, respectively).

Figure 3A. Subject with (A) minimum projection area per length squared (2.00%). The PAL value of the subject corresponded to Cobb angles of 11.34º.

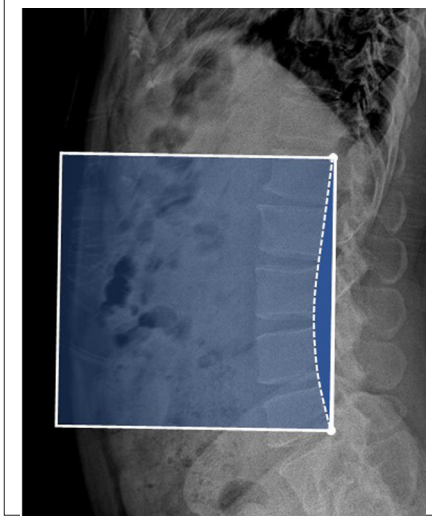


Figure 3B. Subject with (B) medium projection area per length squared (6.30%). The PAL value of the subject corresponded to Cobb angles of 48.45º

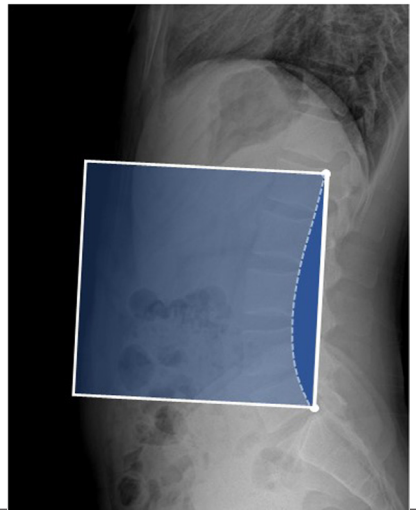
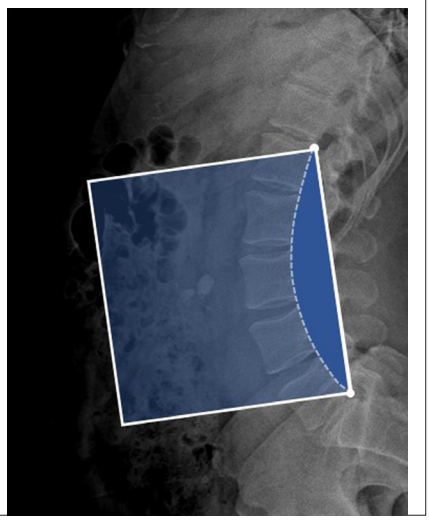


Figure 3C. Subject with (B) maximum projection area per length squared (11.90%). The PAL value of the subject corresponded to Cobb angles of 73.38º.



The Bland–Altman plots showed that the mean PAL estimated by the same investigator in two sessions differed between – 0.47 and 0.41%, and -0.51 and 0.51%, respectively (Figures 4 and 5). There was no significant difference between the repeated measurements of both investigators (p>0.145, p>0.817, respectively). The Bland–Altman blots indicated that the mean PAL estimations of the investigators in both session 1 and session 2 differed by –0.59% and 0.55%, and -0.43% and 0.45%, respectively (Figures 6 and 7). There was no significant difference between the PAL measurements of the investigators for the first and second sessions (p=0.585, p=0.660, respectively).

The average Cobb angle (±SD) on 100 digital radiographs was 45.27±13.35º (min-max, 11.34-73.38º). Based on the results of the Wilcoxon signed-rank test, no statistical difference was found between the repeated Cobb angle measurements of both investigators (p=0.503, p=0.152, respectively). No statistically significant differences were determined between the Cobb angle measurements of the two investigators in the first and second sessions (p=0.623, p=0.181, respectively). The details of the Cobb angle measurements in both sessions are given in Table 2. The ICC showed a high degree of intra-observer agreement in the Cobb angle measurements of the two investigators (ICC=0.987, ICC=0.989, respectively). Interobserver agreement of the Cobb angle measurements was found to be almost perfect for the first and second sessions (ICC=0.987, ICC=0.976, respectively).

There was a high correlation between the PAL estimations and Cobb angle measurements for the first and second sessions (r=0.825, p<0.001; r=0.815, p<0.001, respectively). The relationship between the PAL estimations and the Cobb angle measurements of both investigators in the first and second sessions are shown in Figures 8-11. According to the estimation results of the measurement methods in both sessions, the PAL estimates had high linear correlations with the Cobb angle measurements.

Figure 4. The Bland–Altman plot showing the differences between the mean PAL obtained by first investigator in the first and second sessions. The dashed line represents 95% limits of agreement.

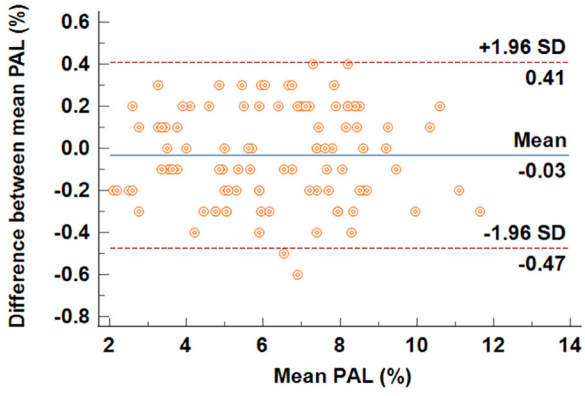


Figure 5. The Bland–Altman plot showing the differences between the mean PAL obtained by second investigator in the first and second sessions.

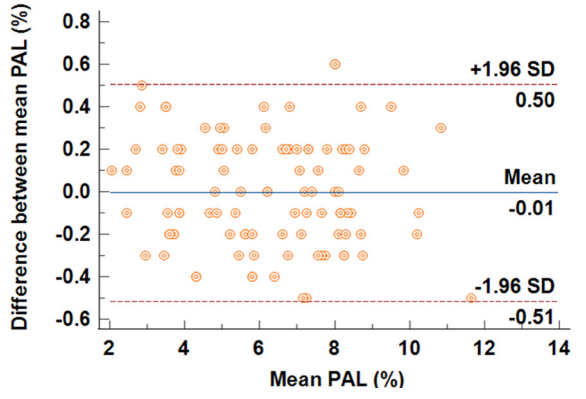


Figure 6. The Bland–Altman plot showing the differences between the mean PAL obtained by the two investigators in the first session.

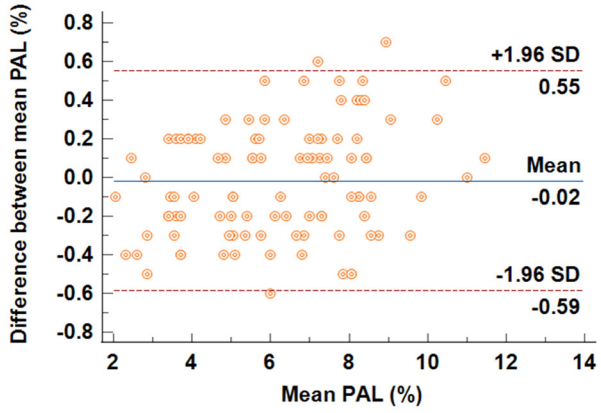


Figure 7. The Bland–Altman plot showing the differences between the mean PAL obtained by the two investigators in the second session.

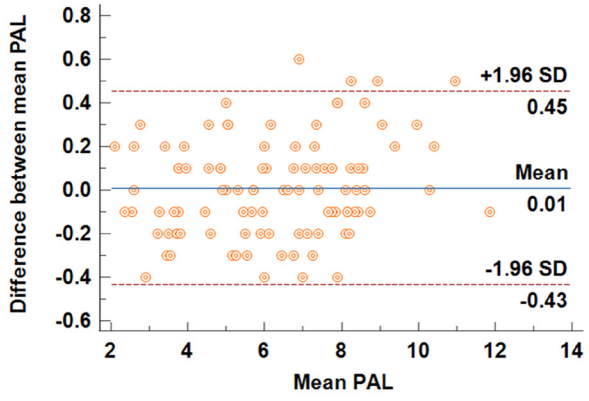


Figure 8. Correlation between the PAL estimations and Cobb angle measurements obtained by first investigator in the first session.

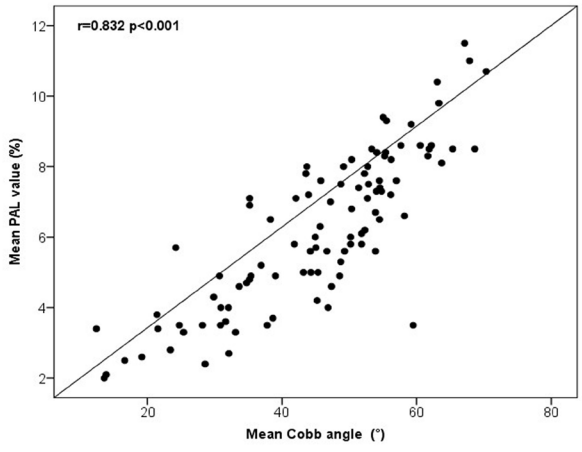


Figure 9. Correlation between the PAL estimations and Cobb angle measurements obtained by second investigator in the first session.

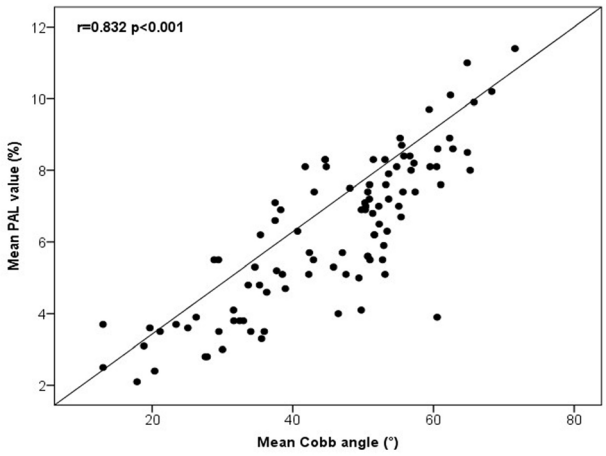


Figure 10. Correlation between the PAL estimations and Cobb angle measurements obtained by first investigator in the second session.

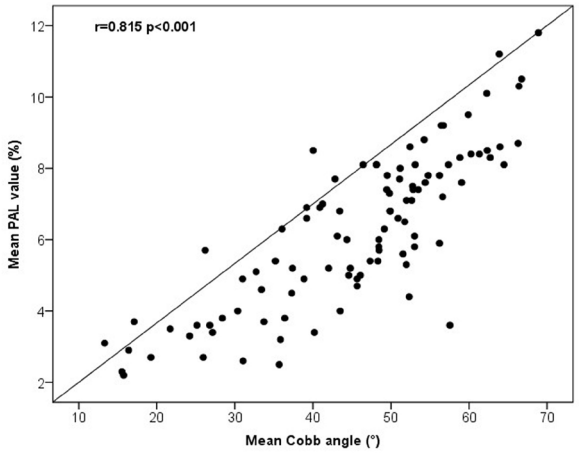
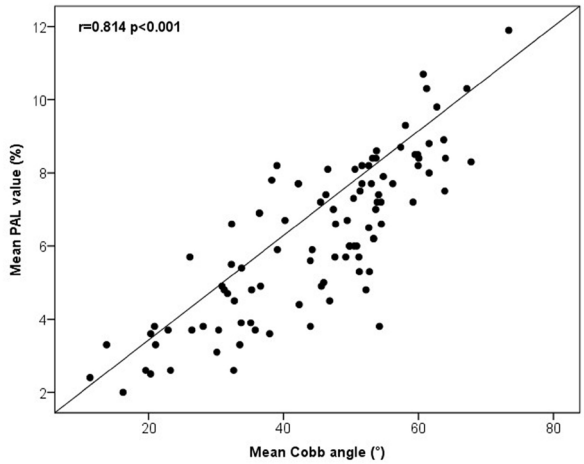


Figure 11. Correlation between the PAL estimations and Cobb angle measurements obtained Investigator 2 in the second session.



DISCUSSION

The Cobb method, originally proposed for the assessment of the severity of scoliosis, is a commonly accepted technique by clinicians for measuring the degree of lumbar lordosis (6, 7). However, it has several limitations, primarily that the Cobb angle predominantly reflects the endplate tilt of the superior and inferior end vertebrae (9). Therefore, two lumbar curvatures of different magnitudes may result in an identical Cobb angle (1, 13). Another limitation of the Cobb method is that Cobb angle measurement is influenced by irregularity in vertebral endplates (14). As the lateral projection of the vertebral end-plates is not suitable for drawing tangential lines, a variety of lines may be drawn parallel to vertebral endplates (14, 15). Thus, the value obtained in the Cobb method is affected by the pathology of the reference vertebral surface.

To date, various alternative methods have been developed for examining lumbar lordosis and these have been compared with the Cobb method (1,9). The most popular alternative methods are the Harrison Posterior Tangent Method (HTPM), the TRALL method, and the Vertebral Centroid method (15-17). In the HPTM, first described by Gore et al. (18), the angle of the lordotic curvature is defined between two straight lines, drawing tangentially to the posterior walls of the end vertebrae (16). Similar to the Cobb method, the HPTM provides a practical approach to segmental and global analysis of lumbar lordosis (5). However, like the Cobb method, the HPTM is sensitive to the irregular shape of the vertebral body. The concave-shaped posterior margin of the vertebral body affects how the straight line is drawn, resulting in different angles being measured (12). Harrison et al. (16) compared four different approaches for radiological analysis of lumbar lordosis on 30 lateral lumbar radiographs. It was reported that the HPTM results in different magnitudes of global lordosis from T12-S1 and L4-S1 than the centroid and Cobb method results (16). In the TRALL method, another tangential approach, the superior and inferior angles of the upper and lower end vertebrae are defined as points A and B, respectively. Point C is identified as the reference point with the maximal orthogonal distance from the spine to the straight lines AB. In this method, the TRALL angle is defined between straight lines AC and AB. Although the TRALL method is a reliable and reproducible method for the radiological assessment of lumbar lordosis, it is not suitable for the measurement of a substantial part of the sacrum (19). The TRALL method is not recommended as it does not allow the segmental analysis of lumbar lordosis (16). Another alternative technique based on vertebral centroid measurement of lumbar lordosis was proposed by Chen et al. (17). In the centroid method, the angle is measured between two straight lines that pass through the two vertebral centroids at both ends of the lumbar curvature. As the centroid method requires three or four vertebrae and the definition of more reference points on vertebral bodies, it is time-consuming and laborious in clinical applications (16). Centroid measurements of lumbar lordosis have been shown to be variable in specific conditions such as ankylosing spondylitis (20). Furthermore, the centroid method is not suitable for segmental analysis of L5-S1 in cases with spondylitis because the S1 vertebra is more sensitive to degenerative changes (7).

As can be seen from the literature, the suggested methods are influenced by irregularly shaped vertebral bodies and angular measurements are prone to error. The PAL approach was first described by Kuru et al. (12) in the measurement of lumbar lordosis on 24 plain radiographs. It was reported that the PAL approach could provide accurate data for evaluation of the degree of lumbar lordosis on plain radiographs. However, Kuru et al. (12) did not test the intra- and interobserver variability of the PAL method and did not compare it with the Cobb method, which is the gold standard method for measuring lumbar lordosis.

In the present study, the reliability of the PAL approach for quantifying lordotic curvature on lateral radiography was examined. The results of the study showed that the PAL approach had high intra- and interobserver reliability with all ICC values>0.929 for lumbar lordosis measurements on digital radiographs. Tangential radiographic evaluation of lumbar lordosis is influenced by irregularity in vertebral endplates and the posterior margin of the vertebral body (12, 14, 15). Degenerative changes make the vertebral endplates a less distinct landmark for the Cobb method (21). Therefore, the obtained value may be greatly influenced by the surface pathologies of the reference vertebrae. In the PAL technique, the observer can easily identify the anatomic bony landmarks on the first and fifth lumbar vertebral bodies. In contrast to tangential radiographic approaches, the estimated PAL value is not affected by the irregular shape of the vertebral body because the PAL technique is based on the manual delineation of the posterior margins of the vertebral bodies in the curvature (12, 14, 15). The PAL technique provides quantitative information independent of the vertebral endplate architecture or marginal convexity in the vertebra body (6). Thus, the PAL method may manage to assess an irregularly shaped curvature of the spine on lumbar lordosis measurement.

The main limitation of the Cobb method is that the Cobb angle is related to changes in the inclination of the end vertebrae rather than changes within the lordotic curvature. Therefore, it cannot reveal regional curvature changes (1,9). The PAL method reflects the curvature changes in the lumbar region because it is based on surface area measurements formed by the lordotic curvature. The values obtained are closely related to the magnitude of the curvature in the lumbar region. An increase in the severity of lumbar lordosis will be reflected in an increased PAL measurement. The method described in this study could overcome all the handicaps and limitations of the previous methods.

Cobb angle measurement still remains the gold standard in current clinical diagnosis when quantifying the magnitude of the lumbar curvature. Therefore, the PAL approach was compared with the Cobb method in this study. The PAL estimation results obtained by both investigators were seen to be highly correlated with the Cobb angle measurements for the assessment of the lumbar lordosis.

Limitations

A limitation of this study could be said to be that although Cobb angle cut-off values have been defined for normal or pathological degrees of measurement, cut-off values for the PAL method were not determined in this study. There is a need for further studies

with a wider range of examinations to be able to determine PAL cut-off values for the clinical diagnosis of lumbar lordosis.

CONCLUSION

In conclusion, the method described here is not only simple and fast but is also a reliable technique for measuring lumbar lordosis. The PAL method provides quantitative data independent of the vertebral surface pathologies of the reference vertebrae. This method could manage to evaluate the irregularly shaped curvature in the lumbar spine. Therefore, the PAL approach could be used as an alternative and potent diagnostic criterion to determine the degree of lumbar lordosis on lateral radiographs.

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Original Research

Morphometry of the Glenoid Cavity of Dry Scapulae of Human Adults

José Aderval Aragão[1], Iapunira Catarina Sant'Anna Aragão[2], Felipe Matheus Sant'Anna Aragão[2], Paola Cardoso[2], Pedro Henrique Adário Marassi[2], Bárbara Costa Lourenço[3],Francisco Prado Reis[4]

[1] Department of Morphology, Federal University of Sergipe (UFS), Aracaju, Sergipe, Brazil
[2] Medical Student, University Center of Volta Redonda (UNIFOA), Volta Redonda, Rio de Janeiro, Brazil
[3] Medical Student, Três Rios Faculty of Medical Sciences (FCM-TR), Três Rios, Rio de Janeiro, Brazil
[4] Titular Professor, Medical School of Tiradentes University (UNIT), Aracaju, Sergipe, Brazil

ABSTRACT

Objective: The shoulder joint is considered the most unstable in the human body and this is due to the measurement relationships between the bone surfaces of its components. This joint is subject to frequent dislocations, which can result in acute fracture or gradual bone loss, which can lead to recurrent instability, additional injury and pain. In this study, it was aimed to carry out a study of the maximum height and width measurements of the glenoid cavity of dry scapulae, correlating them with sex and dimidium.

Methods: Measurements of the maximum heights and widths of 90 dry scapulae glenoid cavities were performed using a 0.01 mm precision digital caliper, 54 were males and 36 were females, with a mean age of 51.9 years. Values of $p<0.05$ were considered statistically significant.

Results: In general, the height and width measurements of the glenoid cavity, as well as the correlation between these measurements in relation to gender, were slightly higher in the right side ($p>0.05$). When we correlated the mean height and width of the GC with respect to homologous sides and sexes, they were also higher in males, but this finding was statistically significant ($p<0.05$).

Conclusion: The findings of these measurements of the glenoid cavity represent a contribution not only for anatomy, but especially for orthopedists, when planning shoulder arthroplasty procedures, as well as helping the industry to develop more accurate and functional joint prostheses for the Brazilian population.

Keywords: Glenoid cavity; arthroplasty, replacement, shoulder; shoulder joint; surgery; anatomy; anthropometry.

INTRODUCTION

The scapula is a flat triangular bone located posterolaterally in the rib cage in the projection of the second to seventh rib. Its lateral angle is truncated and is characterized by the presence of the glenoid cavity (GC), which articulates with the head of the humerus, forming the glenohumeral joint. This joint is more prone to dislocation than other joints in the human body, (1) where there is a discrepancy between the morphology of the GC and the humeral head (2).

In a joint, the relationship between the bone surfaces of its components is very important. This is essential for its stability and the understanding of its biomechanical behavior during loading and movement, in terms of the forces transmitted by this joint and its kinematics (2). Considered as the most unstable in the human body, the glenohumeral joint is subject to frequent dislocations, which can result in acute fracture or gradual bone loss, which can lead to recurrent instability, additional injury and pain (3,4).

The scenario of loss of more than 20% of the scapulae GC width is considered a significant bone deficiency (5,6). Thus, bone augmentation procedures are necessary to restore glenohumeral stability, minimizing the risk of recurrence of instability and bone loss by friction (6,7). As far as we know, measurement parameters such as GC height and width are of great importance when planning sizing, positioning, and prosthetic design for total shoulder arthroplasty (8-10). Therefore, our objective was to carry out a study of the maximum height and width measurements of the GC of dry scapulae, correlating them with sex and dimidium.

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Corresponding Author: José Aderval Aragão **E-mail:** adervalufs@gmail.com

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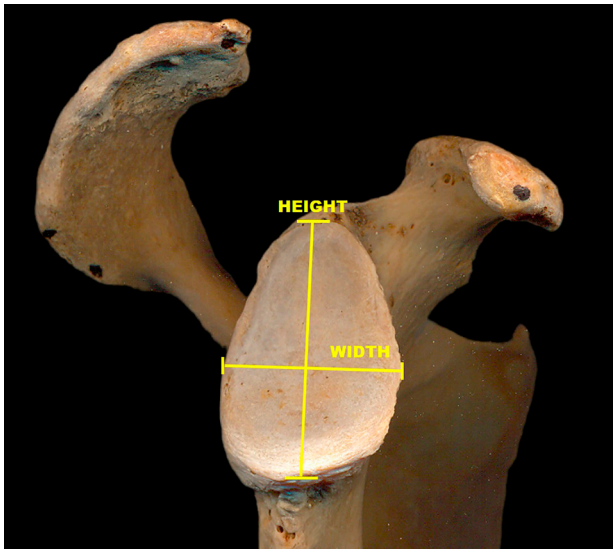
METHODS

Measurements were taken of the maximum heights and widths of 90 GC of scapulae, 54 were male (27 right and 27 left) and 36 female (18 right and 18 left), with a mean age of 51.9 years, all belonging to the Laboratory of Anatomy.

The maximum height of the GC was measured from the supraglenoid tubercle to its lower margin (largest vertical axis), and its maximum width was measured below the notch of the GC, in its longest transverse axis (**Figure** 1). All these measurements were performed using a 0.01 mm precision digital caliper.

Figure 1. Height and width of the glenoid cavity



This research project was approved by the Ethics Committee for Research Involving Human Beings under protocol no. CAAE 0041.0.107.000-08. No free and informed consent statement was applied because this was a study on cadavers. The scapulas were obtained in accordance with Law 8501, of November 30, 1992, which makes provisions regarding the use of unreclaimed cadavers for the purposes of scientific studies or research.

Main Points:

- The glenoid cavity is essential for its stability and the understanding of its biomechanical behavior during loading and movement.
- The glenoid cavity bone augmentation procedures are necessary to restore glenohumeral stability, minimizing the risk of recurrence of instability and bone loss by friction
- The morphometric variations of the scapula glenoid cavity are very important in the assessment of rotator cuff diseases, shoulder dislocation and in determining the appropriate size of the glenoid component in shoulder arthroplasty
- The findings were compared with those performed in different population groups, where these measurements were performed in different ways

Statistical Analysis

Variables were expressed as mean and standard deviation. To analyze the data collected, the t-Student test was used for independent or unpaired samples, to compare the values of the scapula GC measurements in relation to sex and dimidium. Values of $p<0.05$ were considered statistically significant. Data were analyzed using the Bioestat 5.3 Program (Instituto de Desenvolvimento Sustentável Mamirauá, Belém, Pará, Brasil).

RESULT

In general, the height and width measurements of the GC were slightly higher in the right side (Table 1). This finding, however, was not statistically significant ($p> 0.05$).

When correlating the height and width measurements of the GC in the same sex in relation to the right and left sides, it can be observed that these measurements, for the most part, were greater in the right side than in the left side (Table 2). This finding was not statistically significant ($p>0.05$).

Correlating the mean height and width of the GC with the homologous sides and genders, it was found that these measures were greater in males (Table 3). This finding was statistically significant ($p<0.05$).

DISCUSSION

The relationship between the anatomy of the bone elements and the stability of a joint is of great importance in understanding its biomechanical behavior during load and movement, in the forces transmitted through the joint and its kinematics, and this occurs, especially in the shoulder joint, where there is a discrepancy between the shape of the GC of the scapula and the head of the humerus.

The morphometric variations of the scapula GC are very important in the assessment of rotator cuff diseases, shoulder dislocation and in determining the appropriate size of the glenoid component in shoulder arthroplasty (11), as well as in the prognosis in glenohumeral osteoarthritis (12). In the present study, measurements of the height and width of the GC of dry scapulae were carried out in a population sample from the located in the northeast region of Brazil. The findings were compared with those performed in different population groups, where these measurements were performed in different ways: direct from embalmed corpses, direct from dry scapulae, by computed tomography of scapulae removed from corpses or using acrylic resin modems. In our study, performed on dry scapulae, the average height ranged from 25.3 to 40.04 mm in males and from 29.14 to 34.99 mm in females, while the average width in males ranged from 22.7 to 36.09 mm and in females was 19.8 to 26.2 mm, which is also in agreement with the results found by several authors where these measurements were significantly higher in males (Table 4).

After measuring the height and width of the GC in relation to the right and left sides, our results were compared with those of other authors (Table 5).

Table 1. General morphometry of the glenoid cavity according to the dimidium

	n	Dimidium	Minimum	Maximum	Mean	SD	p
Height (mm)	45	Right	31.14	43.83	36.91	3.34	0.399
	45	Left	28.48	42.39	36.73	3.34	
Width (mm)	45	Right	21.81	35.27	26.51	3.41	0.961
	45	Left	21.11	31.89	25.62	3.02	

mm – milimeter
SD – standard deviation
T-Student test; p>0,05; Differences between the averages of height and width in relation to the dimidium.

Table 2. Glenoid cavity morphometry according to sex and dimidium

	Sex	n	Dimidium	Minimum	Maximum	Mean	SD	p
Height (mm)	Male	27	Right	31.16	41.95	37.93	2.83	0.3939
		27	Left	33.52	41.72	38.05	2.69	
	Female	18	Right	31.14	43.83	35.36	2.96	0.2363
		18	Left	24.48	42.39	34.62	3.16	
Width (mm)	Male	27	Right	21.81	35.27	28.4	3.12	0.0906
		27	Left	21.11	31.89	26.95	2.72	
	Female	18	Right	21.91	31.66	24.23	2.47	0.2230
		18	Left	21.41	30.01	23.62	2.28	

SD – standard deviation
mm – milimeter
T-Student test; p>0,05; Differences between the averages of height and width in relation to the dimidium.

Table 3. Correlation of mean height and width of the glenoid cavity between homologous dimidiums and sexes

	Dimidium	Sex	n	Mean	SD	p
Height (mm)	Right	Male	27	37.93	2.83	*0.0028
		Female	18	35.36	2.96	
	Left	Male	27	38.05	2.69	*0.0001
		Female	18	34.62	3.16	
Width (mm)	Right	Male	27	28.4	3.12	*0.0000
		Female	18	24.23	2.47	
	Left	Male	27	26.95	2.72	*0.0001
		Female	18	23.26	2.28	

SD – standard deviation
mm – milimeter
T-Student test; *p< 0,05; Differences between mean height and width in relation to homologous dimidiums and sex.

Observing this table, it can be noted that the values of the present study were similar to those of most studies. In the present study, the mean height of the right GC of the scapula was 36.91±3.34 mm and 36.73±3.34 mm on the left. Meanwhile, the width of the right GC was 26.51±3.41 and the left 25.62±3.02. This shows that the height and width of the right GC is slightly larger than the left one, and that these differences were not statistically significant.

LIMITATIONS

The limitation of this study is the fact that there is a small number of scapulae, as well as an equal number of scapulae in both sexes.

CONCLUSION

Variations in the size of the scapula GC will be of great help for orthopedic surgeons to better understand the pathology of the shoulder and decide the appropriate size of the glenoid component for shoulder arthroplasty in northeastern Brazil. This will help establish relevant anatomical and clinical standards to improve medico-legal identification, make the diagnosis and determine the extent of orthopedic injuries, plan arthroplasty procedures, and develop more accurate and functional joint prostheses.

Table 4. Comparative table of height and width measurements of the scapula GC in relation to sex

Author	Population	Study	Sex	n	Mean height of the GC	Mean width of the GC
Polguj et al. (13)	European	Dry scapulae	Male	33	40.04±2.97	36.09±2.20
			Female	41	29.14±2.14	25.65±1.98
Mathews et al. (14)	European	Embalmed corpses	Male	14	39.5±3.5	30.3±3.3
			Female	22	34.8±2.2	26.2±1.6
Knapik et al. (15)	American	Dry scapulae	Male	813	-	29.6±2.1
			Female	181	-	24.7±1.7
Homem et al. (16)	Brazilian	Scapulae in acrylic resin model	Male	50	36.7±0.03	22.7±0.03
			Female	50	31.0±0.03	19.8±0.03
Chaijaroonkhanarak et al. (17)	Thai	Dry scapulae	Male	166	37.1±2.2	27.6±2.1
			Female	98	33.3±1.9	23.9±1.7
Jia et al. (18)	Chinese	Computed tomography	Male	55	-	29.09±2.27
			Female	29	-	25.52±1.72
Khan et al. (19)	African	Dry scapulae	Male	68	35.3±3.1	24.2±2.7
			Female	96	34.6±2.8	23.7±2.8
Present study	Brazilian	Dry scapulae	Male	54	38.04±2.71	27.49±2.95
			Female	36	34.99±3.04	23.93±2.36

Table 5. Correlation of morphometric measurements of the scapula GC in relation to the dimidium.

Author	Population	Dimidium	n	Mean height of the GC	Mean width of the GC
Mamatha et al. (20)	Indian	Right	98	33.67±2.82	23.35±2.04
		Left	104	33.92±2.87	23.05±2.30
Rajput et al. (21)	Indian	Right	43	34.76±3	23.31±3.00
		Left	57	34.43±3.21	22.92±2.80
Gandhi et al. (9)	Indian	Right	64	38.78±4.43	26.47±3.79
		Left	59	40.30±5.12	27.85±3.86
El-din, Ali, (11)	Egyptian	Right	80	38.88±2.63	28.31±2.38
		Left	80	39.01±2.49	27.99 ± 2.55
Akhtar et al. (10)	Indian	Right	126	36.03±3.15	23.67±2.53
		Left	102	35.52±3.12	23.59±2.47
Tiwari et al. (22)	Indian	Right	100	35.94±2.30	16.62±2.82
		Left	106	35.68±2.14	16.14±2.84
Raaj et al. (23)	Indian	Right	50	33.1±4.1	21.4±4.4
		Left	50	31.6±3.4	20.5±2.8
Singh, (1)	Indian	Right	91	33.4±3.0	15.4±2.0
		Left	81	33.9±3.6	15.3±2.2
Present study	Brazilian	Right	45	36.91±3.34	26.51±3.41
		Left	45	36.73±3.34	25,62±3.02

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Original Research

Evaluation of Diagnostic Performance of Bd Max Ebp Assay in Patients with Diarrheal Illness

Ozlem Koyuncu Ozyurt[1], Imran Saglik[2], Betil Ozhak[1], Derya Mutlu[1], Belkıs Levent[3], Levent Donmez[4], Gozde Ongut[1], Dilek Colak[1], Dilara Ogunc[1]

[1] Akdeniz University, Faculty of Medicine, Department of Medical Microbiology, Antalya, Turkey
[2] Uludag University, Faculty of Medicine, Department of Medical Microbiology, Bursa, Turkey
[3] National Enteric Pathogens Reference Laboratory, Ankara, Turkey
[4] Akdeniz University, Faculty of Medicine, Department of Public Health, Antalya, Turkey

ABSTRACT

Objective: Detection of the etiological agents in patients with acute diarrhea is challenging due to a wide variety of pathogens. The aim of this study is to evaluate the diagnostic performance of BD Max Enteric Bacterial Pathogens (EBP) PCR assay in patients with diarrheal illness.

Methods: Between 1 January 2014 and 31 May 2015, stool samples from pediatric or adult patients with diarrhea submitted for routine analysis of bacterial stool pathogens were included in the study. We compared the BD Max EBP PCR assay to culture for the detection of Salmonella spp., Shigella spp., Campylobacter jejuni, and Campylobacter coli and an EIA for Shiga toxins 1 and 2. Discordant results were adjudicated by either antigen detection methods or Film array GI Panel.

Results: When coinfections were excluded, the positive percent agreement values for the BD Max EBP assay (PPA) was 100% and negative percent agreement (NPA) was between 98.0%-99.7%, when compared with culture and EIA. After discrepant analysis, the PPA values for the BD Max EBP assay was 100% and NPA was between 99.5%-100%.

Conclusion: The BD Max EBP assay showed a high correlation rate with conventional and molecular methods for the detection of stool pathogens.

Key words: Osteoarthritis, knee joint, talocrural joint, transverse tarsal joint

INTRODUCTION

Infectious diarrheal diseases cause substantial morbidity and mortality worldwide. A wide variety of pathogens lead to infectious diarrhea, which makes the diagnosis of bacterial pathogens particularly challenging given the large amounts of background normal gastrointestinal flora (1,2).

Viral agents such as the noroviruses are responsible for most of the acute infectious diarrhea, while bacteria are responsible for most cases with more aggresive and inflamatory diarrhea. (3) *Salmonella*, *Campylobacter*, *Shigella*, and Shiga toxin-producing *Escherichia coli* (STEC) are the most common diarrheagenic bacteria and routine stool culture is designed to detect these pathogens in most laboratories (4).

Detection and identification of the pathogens of acute diarrhea are important for both individual patient care and public health investigation. Furthermore, some infectious diarrheal pathogens can lead to long-term complications such as Hemolytic uremic syndrome, Guillain-Barr syndrome (5).

Conventional stool culture is the gold standard for the diagnosis of bacterial gastroenteritis (6). On the other hand stool cultures are either insensitive or labor intensive with long turn around time. For the diagnosis of bacterial diarrhea, a wide variety of culture protocols involving multiple selective media and reagents are available in the microbiological laboratory (1,7). However, the use of antibiotics affects the culture result and frequently causes low yield for identification of enteropathogens (7). Molecular methods can increase sensitivity and specificity compared to stool culture (8).

The aim of this study is to evaluate the diagnostic performance of BD Max Enteric Bacterial Pathogens (EBP) assay in patients with diarrheal illness.

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Corresponding Author: Betil Ozhak **E-mail:** betilozhak@yahoo.com

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METHODS

Between 1 January 2014 and 31 May 2015, stool samples from pediatric or adult patients with diarrhea submitted for routine analysis of bacterial stool pathogens were included in the study. Duplicate specimens from the same patient were not enrolled. Culture and Enzyme Immunoassay (EIA): Fresh stool specimens were inoculated onto Mac Conkey agar, XLD agar for *Salmonella* and *Shigella*, and incubated at 37°C for 24 hours in an aerobic incubator. Lactose, xylose nonfermenting colonies with or without black centers on these media were screened phenotypically on triple sugar iron agar, motility medium, urea agar, Simmon's citrate agar and lysine iron agar. Suspected colonies were tested with Wellcolex™ Color Salmonella Rapid Latex Agglutination Test Kit and Wellcolex™ Color Shigella (ThermoFisher, UK).

E. coli Shiga toxin was detected using by EIA (ProSpecT Shiga Toxin *E. coli* Microplate Assay,Remel,UK), according to the manufacturer's instructions.

Screening for *Campylobacter spp* in stool was performed with *Campylobacter* selective agar and incubated under microaerobic condition at 42°C for 5 days. Suspected colonies were identified by Gram stain examination of the colony along with oxidase test and MALDI-TOF MS.

BD Max EBP automated PCR: The BD Max enteric bacterial panel (EBP) is a multiplex nucleic acid amplification assay which detects DNA from *Campylobacter* spp. (*jejuni* and *coli*), *Salmonella* spp., *Shigella* spp. / Enteroinvasive *E. coli* (EIEC), Shiga toxin1(stx1)/Shiga toxin2(stx2) genes in stool specimens with the BD Max system less than three hours. (BD Diagnostics, Baltimore, MD, USA) (Harrington). The BD MAX™ System is a fully-automated, closed system which allows for simultaneous processing of up to 24 individual tests.

Fresh stool samples were tested daily with the BD Max EBP assay, according to the manufacturer's instructions.

Interpretation

We accepted conventional culture as the reference method for the detection of *Shigella* spp., *Campylobacter spp* and *Salmonella* spp. and EIA as the reference method for the detection of Shiga toxins for the calculation of NPA and PPA of BD Max EBP assay. In addition, BD Max EBP assay positive and conventional method negative results were adjudicated by either antigen detection method (*Campylobacter* EIA) or Film array GI Panel.

Main Points:

- Culture remain the method of choice for diagnosis of bacterial enteritis. On the other hand, nucleic acid amplification tests offer rapid results and markedly improve the detection and identification of stool pathogens.
- In our study, BD Max EBP assay showed excellent performance for the detection of Salmonella spp., Shigella spp., Campylobacter spp and Shiga toxins.
- The BD Max EBP assay showed a high correlation rate with conventional and molecular methods for the detection of stool pathogens.
- The BD Max EBP assay detect DNA and not necessarily viable organisms which may lead to increased appreciation of asymptomatic infections and prolonged shedding.

Stool samples with discordant results between Campylobacter culture and the BD Max EBP assay were tested by using an enzyme immunoassay (RIDASCREEN® Campylobacter,r-biopharm,-Germany) according to the manufacturer's instructions. Samples that gave different results between the BD MAX EBP assay and *Campylobacter* EIA were subject to FilmArray Gastrointestinal (GI) Panel (BioFire -BioMérieux, France).

Samples with discordant results between *Salmonella* and *Shigella* culture or Shiga toxin EIA and the BD Max EBP assay were tested by FilmArray GI Panel following the manufacturer's instructions.

Statistical analysis: Positive percent agreement (PPA) and Negative percent agreement (NPA) and their 95% confidence intervals were calculated, as reported previously (9) .

The method used in the study is a routinely applied method in our hospital. Informed consent was not obtained from the patients because it is not necessary to obtain informed concent for archieve material collected from patient stool. However, data usage permission has been obtained. Ethics committee application was made and ethics committee approval was obtained.

RESULTS

One thousand two hundred twenty four stool samples were included in the study, 46 of which were excluded due to inhibition by BD Max EBP assay.

Culture and Shiga toxin EIA results**:** 14 (1.19%) specimens were positive for *Campylobacter* spp, 22 (1.87%) were positive for *Salmonella* spp and two (0.17%) were positive for Shigella/EIEC by culture. 21 (1.78%) were positive for Shiga toxins by EIA. These were also positive with BD Max EBP assay. Coinfection was not detected by culture. The positivity rate of investigated pathogens was 5.01% (59/1178) by culture and EIA.

Of the 1178 samples, 30 had *Salmonella*, 6 had *Shigella / EIEC*, 37 had *Campylobacter spp*, and 38 had Shiga-like toxin genes (*stx1* and / or *stx2*) by BD Max EBP assay. In addition, BD Max EBP assay identified coinfections in two samples (in one sample *Salmonella* + Shiga-like toxin genes and in another *Campylobacter* + Shiga-like toxin genes).

When coinfections were excluded, the NPA of the BD Max EBP assay was 99.3% for *Salmonella*, 99.7 % for *Shigella* / EIEC, and 98.0% for *Campylobacter* when compared with culture. NPA was 98.5% for Shiga toxins using EIA as a reference method. PPA was 100% for all targets (Table 1).

Results after discrepant analysis: *Campylobacter* spp was isolated from culture in 14 out of 37 samples that were positive by BD Max EBP assay. In 9 out of 23 samples that were found to be incompatible by BD Max EBP assay and culture, the enzyme immunoassay

Table1: Performance of BD Max EBP assay when compared with the reference method (stool culture and Shiga toxin EIA)

	No. of pathogens with BD Max EBP result with reference to culture/EIA				Total number of samples	PPA (95% confidence interval)	NPA (95% confidence interval)
Target type	True Positive	False Negative	False Positive	True Negative			
Salmonella	22	0	8	1146	1176	100	99,31 (98,83-99,78)
Shigella/EIEC	2	0	4	1170	1176	100	99,66 (99,33-99,99)
Shiga toxins	21	0	17	1138	1176	100	98,53 (97,84-99,22)
Campylobacter	14	0	23	1139	1176	100	98,02 (97,22-98,82)

Not:Coinfections were excluded in two samples
EBP: Enteric Bacterial Pathogens, EIA: Enzyme Immun Assay, PPA: Positive Percent Agreement, NPA: Negative Percent Agreement, EIEC: Entero Invasive Eschercia coli

Table 2: Performance of BD Max EBP assay when compared with the reference method (Campylobacter EIA and Film Array GI panel)

	No. of pathogens with BD Max EBP result with reference to EIA and Biofire Film Array GI Panel				Total number of samples	PPA (95% confidence interval)	NPA (95% confidence interval)
Target type	True Positive	False Negative	False Positive	True Negative			
Salmonella	25	0	5	1146	1176	100	99,57 (99,19-99,94)
Shigella/EIEC	6	0	0	1170	1176	100	100
Shiga toxins	32	0	6	1138	1176	100	99,48 (99,06-99,89)
Campylobacter	33	0	4	1139	1176	100	99,65 (99,31-99,99)

Not:Coinfections were excluded in two samples
EBP: Enteric Bacterial Pathogens, EIA: Enzyme Immun Assay, GI: Gastrointestinal, PPA: Positive Percent Agreement, NPA: Negative Percent Agreement, EIEC: Entero Invasive Eschercia coli

(RIDASCREEN® *Campylobacter*, r-biopharm, Germany) was found to be positive. *The remaining 14 discordant samples were studied with* FilmArray GI Panel *and Campylobacter spp was positive in 10 samples. NPA was found as 99.7% for Campylobacter spp.*
Discrepant results between culture or EIA and the BD Max EBP assay for *Salmonella* spp., *Shigella* spp., and Shiga-like toxin genes (*stx1* and/or *stx*2) were tested by using FilmArray GI Panel. The *NPA* of the BD Max EBP assay was 99.6% for *Salmonella*, 100% for *Shigella* / EIEC, and 99.5% for Shiga toxin.

After analysis of discrepant results, use of BD Max EBP assay identified an additional 37 pathogens, thereby increasing the frequency to 8.2% (96/1176), when coinfections were excluded (Table 2).

When the samples with coinfection were examined, there was no growth in culture and EIA tests results were negative. When these samples were studied with FilmArray GI Panel, *Salmonella* and Shiga-like toxin genes were found to be negative in one sample and only *Campylobacter* gene was positive in another sample with *Campylobacter* + Shiga-like toxin genes.

DISCUSSION

Detection of the etiological agents in patients with acute diarrhea is important for appropriate therapy and public health interventions. Culture remain the method of choice for diagnosis of bacterial enteritis. On the other hand, nucleic acid amplification tests offer rapid results and markedly improve the detection and identification of stool pathogens. The use of FDA-approved culture-independent diagnostics in addition to traditional methods is supported by recent research (10).

Harrington et al (9) conducted a multicenter evaluation of the BD Max EBP assay in comparison to culture for the detection of *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, and *Campylobacter coli* and an EIA for Shiga toxins 1 and 2 with stool culture for fresh and preserved stool specimen. Following discrepant analysis, PPA and NPA values were 97.3% and 99.8% for *Salmonella* spp. 99.2% and 100% for *Shigella* spp. 97.5% and 99.0% for *C.jejuni* and *C. coli*, and 100% and 99.7% for Shiga toxins, respectively. They concluded that, the BD Max EBP assay with superior sensitivity compared to conventional methods and excellent specificity, may improve the detection of bacterial stool pathogens and time to reporting of results.

In a prospective study including 971 stool samples, the PPA of the BD MAX EBP assay and stool culture or enzyme immunoassay was **97%** for *Campylobacter* spp. **75%** for *Salmonella* spp., **100%** for *Shigella* spp., and **88%** for Shiga toxins. Furthermore, a NPA of **98%** for *Campylobacter* spp. **99%** for *Salmonella* spp. **99%** for *Shigella* spp. and **99%** for Shiga toxins has been demonstrated.

They found that the use of the BD MAX EBP increased the overall detection rate from 5.26% to 8.06%. Their study highlighted the superior detection rate of molecular assays compared to conventional diagnostic procedures (1)

Biswas et al (11), evaluated the diagnostic accuracy and laboratory turnaround time of three molecular assays. When the prospective samples were evaluated, the sensitivity and specificity of BD MAX EBP assay for Salmonella spp., Shigella spp., and Campylobacter spp. were found to be 99.7-100%.

Anderson et al (2), investigated the performance of the BD MAX EBP in preserved stool specimens that were artificially spiked with pathogen strains at different concentrations. The EBP panel demonstrated superior sensitivity and reliably detected Salmonella, EHEC O157, Shigella, and Campylobacter at concentrations 1 to 2-log10 lower than those needed for culture detection.

Mortensen et al (12), evaluated 86 stool samples with culture and BD Max EBP. Approximately 20% of cultures required additional process steps to exclude potential pathogens. Negative result reporting time with conventional culture was found to be approximately 41-54 hours.

In our study, BD Max EBP assay showed excellent performance for the detection of Salmonella spp., Shigella spp., Campylobacter spp and Shiga toxins. The NPA of BD MAX EBP in our study was similar to previous reports. Since we did not have a BD MAX EBP negative but the reference test positive sample, PPA of BD MAX EBP in our study was slightly higher than previous studies. The reasons for this difference may be due to interlaboratory technical variance, specimen transport and processing practices such as unemployment of enrichment broth.

This is a single center, laboratory-based, prospective study with a high number of samples. The limitation of our study is that the clinical features of patients were not included and also the study was done only in fresh stool samples but not Cary Blair-preserved specimens.

CONCLUSION

We concluded that, the BD Max EBP showed a high correlation rate with conventional and molecular methods for the detection of stool pathogens. In addition, our detection rates increased with BD Max EBP which has high PPV and NPV. On the other hand, BD Max EBP assay detect DNA and not necessarily viable organisms which may lead to increased appreciation of asymptomatic infections and prolonged shedding. For this reason the results should be interpreted with consideration of clinical information.

Ethical Considerations: This study was approved by the Akdeniz University School of Medicine Ethical Committee of Clinical Research (Decision number: 471).

Peer-review: Externally peer-reviewed.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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Original Research

Comparison of Pineal Gland Volume Between Patients with Fibromyalgia and Healthy Controls

Çağlar Karabaş[1], Samet Karahan[2], Havva Talay Çalış[3], Ali Koç[4]

[1]Kastamonu Physical Therapy and Rehabilitation Center, Department of Physical Medicine and Rehabilitation, Kastamonu, Turkey
[2]Kayseri City Hospital, Department of Internal Medicine Division of Rheumatology, Kayseri, Turkey
[3]Kayseri City Hospital, Department of Physical Medicine and Rehabilitation, Kayseri, Turkey
[4]Kayseri City Hospital, Department of Radiology, Kayseri, Turkey

ABSTRACT

Objectives: The pineal gland is an important neuroendocrine organ accounting for the melatonin secretion and chronobiology that regulate circadian rhythm. This study was designed to compare pineal gland volume (PGV) with healthy controls and patients with fibromyalgia syndrome (FM), in which sleep quality and efficiency is reduced.

Patient and Methods: In this cross-sectional study, PGV and functional pineal gland volume (FPGV) of FM patients with age- and sex-matched healthy controls were compared. All MR imaging studies were performed using a 3 Tesla scanner with a multi-channel phased array head coil. The volume of pineal glands and pineal cysts were calculated from 3D MP RAGE images using the formula: volume= AP x transverse x craniocaudal diameter x 0.523.

Results: There was no significant difference in PGV and FPGV between the FM group and healthy controls (p=0.374 and p=0.421, respectively). In the correlation analysis, age was negatively correlated with PGV and FPGV in the FM group (r=-0.496, p=0.010; r=-0.477, p=0.014, respectively). No significant correlation was detected between age, PGV and FPGV in the control group (r= 0.022, p=0.916; r= -0.019, p=0.925, respectively).

Conclusions: Based on the results, there was no significant difference between the FM group and healthy controls regarding PGV and FPGV. However, PGV and FPGV were decreased by advancing age in the FM group in which melatonin therapy is offered as an option.

Keywords: Fibromyalgia syndrome, pineal gland volume, functional pineal gland volume, pineal gland magnetic resonance imaging

INTRODUCTION

The pineal gland is an endocrine organ adjacent to the posterior wall of the third ventricle between the posterior and dorsal habenular commissures, accounting for the melatonin secretion and chronobiology that regulates circadian rhythm (1). Melatonin is a hormone that regulates the sleep/wake cycle. There is a correlation between pineal gland volume (PGV) and melatonin secretion (2). It has been seen that individuals with a smaller PGV secrete less melatonin (3). It was suggested that "functional parenchymal volume" (FPGV), which excludes cysts and calcification from pineal gland volume, is actually responsible for melatonin secretion (1, 4).

In previous studies, PGV was found to be lower in chronic systemic diseases such as obesity, primary insomnia, and schizophrenia, leading to an impaired sleep circadian rhythm when compared to the general population, suggesting that lower PGV may play a role in disease pathogenesis (2, 3, 5).

Fibromyalgia (FM) is a clinical entity involving many symptoms such as chronic generalized pain, fatigue, sleep disorder, cognitive dysfunction, and depressive episodes (6). Wikner et al. compared FM patients with healthy individuals and found lower levels of nocturnal melatonin secretion in FM patients (7). To the best of our knowledge, there is no study showing a relationship between PGV and FM, which influences daily quality of life and leads to depression and sleep disorders.

In this study, we aimed to evaluate the relationship between FM and PGV and FPGV.

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Corresponding Author: Çağlar Karabaş **E-mail:** drckarabas@gmail.com

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MATERIAL AND METHOD

Study Design and Participation

A cross-sectional experimental design was made to compare PGV and FGPV obtained by magnetic resonance imaging (MRI) in FM patients with healthy controls. The study was approved by the lolocal Ethics Committee (Approval No: 2019/564-24.07). The study was conducted in accordance with the Helsinki Declaration and all participants gave written informed consent.

The study included 26 patients (23 women and 3 men), who presented to the Physical Therapy & Rehabilitation and Rheumatology outpatient clinics of the Kayseri City Hospital and were diagnosed as having FM based on the American College of Rheumatology (ACR) 2016 Diagnostic Criteria between September, 2016 and December, 2019 and age-, sex-, and body mass index (BMI)-matched 26 healthy individuals (23 women, 3 men) as controls (8). Patients with chronic systemic diseases, inflammatory rheumatoid disease (rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, etc.), history of malignancy, history of autoimmune disorder, with psychiatric disorders such as depression or schizophrenia, and patients with regular or excessive alcohol consumption were excluded.

All patients with FM were asked to answer the Fibromyalgia Impact Questionnaire (validated in Turkish), which assesses physical function, occupational status, depression, anxiety, sleep, pain, stiffness, fatigue, and well-being (9, 10). The pain was assessed using the Visual Analog Scale (VAS), which is rated by a 0-100 mm ruler (0: no pain, 100, intractable pain). The patients were asked to mark on the ruler according to their pain level. The VAS score was defined as the distance from point 0 (no pain) to the point marked by the patient and recorded in mm (11). All subjects underwent MR imaging in accordance with the protocol. No complication was observed during the imaging studies.

MR Imaging Protocol and Measurement of Pineal Gland Volume

All MRI exams were performed with a 3 T scanner (MAGNETOM®, Skyra; Siemens Healthcare, Erlangen, Germany) using a multi-channel phased array head coil. The protocol was composed of axial T1-weighted three dimensional magnetization-prepared rapid acquisition gradient echo (3D MP RAGE) sequence (Time to echo, 3 ms; Time repetition, 22 ms; Flip angle, 30°; Field of view, 200 mm; matrix, 256 × 256; slice thickness, 0.5 mm), sagittal T1-weighted three dimensional magnetization-prepared rapid acquisition gradient echo (3D MP RAGE) sequence (Time to echo, 2 ms; Time repetition, 20 ms; Flip angle, 30°; Field of view, 180 mm; matrix, 256 × 256; slice thickness, 0.5 mm), and axial T2-weighted fluid attenuated inversion recovery (FLAIR) sequence (Time to echo, 85 ms; Time repetition, 9000 ms; Field of view, 100 mm; matrix, 256 × 256; slice thickness, 3 mm). MRI images were reviewed by a radiologist with 15 years of experience in neuroradiology. Pineal cysts were identified as circular areas isointense to the cerebrospinal fluid. The volume of the pineal glands and pineal cysts were calculated from the 3D MP RAGE images using the formula:

Volume= X x Y x Z (AP x transverse x craniocaudal diameters) x 0.523.

For patients with pineal cysts, cyst volume was subtracted from the gland volume in order to find the functional gland volume.

Main Points:

- This is the first study assessing PGV in patients with FM and healthy controls using MR imaging.
- There is no significant difference in PGV and FPGV between FM and control groups.
- Age was negatively correlated with PGV and FPGV in the FM group while no correlation was detected in the control group.

Statistical Analysis

To evaluate the effect size, Cohen's d coeficient was calculated for between- group variables that showed a significant change. An effect size of 0.20 to <0.50 was regarded as small, 0.50 to <0.80 as medium, and >0.80 as large (12). To detect a clinically important difference of 30 mm3 on the PGV between groups, with an estimated SD of 20 mm3, 80% power, and 5% significance level using the Mann- Whitney U test, 24 participants were needed in each group. To account for dropout, 2 participants were recruited. Statistical analyses were performed using SPSS version 23.0 (IBM, Armonk, NY, USA). Categorical variables were presented using descriptive statistics (frequency counts and percentages), while numeric items were summarized using mean ± standard deviation or median (IQR25-75). The normal distribution of data was assessed using the Shapiro-Wilk test and histogram. The chi-square test was used in comparisons between groups. A Student's t test was used to compare variables with normal distribution while the Mann Whitney U test was used to compare variables with skewed distribution between groups. Spearman's correlation analysis was performed for data with skewed distribution and quantitative data. The variables used in Spearman correlation analysis were age, gender, BMI, symptom duration, VAS, Fibromyalgia Impact Questionnaire (FIQ), FGV, and FGPV. Statistical significance of correlation coefficients estimated was assessed by a determination coefficient of 0.01 and 0.05. A p value < 0.05 was considered as statistically significant.

RESULT

In both the FM and control groups, there were 23 women (88.5%) and 3 men (11.5%). The mean age was 40.62 ± 9.02 years in the FM group and 37.42 ± 6.06 years in the control group (p=0.140). There were no significant differences in age, gender, and body mass index between the groups. Table 1 presents the demographic and clinical characteristics of the participants.

No significant difference was detected in PGV and FPGV between the FM and control groups (p=0.374 and p=0.421, respectively; Table 2). In correlation analysis, age was negatively correlated with PGV and FPGV in the FM group (r=-0.496, p=0.010; r=-0.477, p=0.014, respectively). No significant correlation was detected between age, PGV and FPGV in the control group (r= 0.022, p=0.916; r= -0.019, p=0.925, respectively). In the FM group, no significant correlation was detected between BMI, PGV and FPGV (r= -0.253, p=0.213; r= -0.236, p=0.245, respectively). In the FM group, no significant correlation was detected between duration of symptoms, PGV and FPGV (r= -0.112, p=0.587; r= -0.106,

p=0.606, respectively). In addition, no significant correlation was detected between BMI, PGV and FPGV in the control group (r= 0.154, p=0.453; r= 0.127, p=0.536, respectively). In the correlation analysis among VAS, PGV and FPGV, it was found that PGV and FPGV were reduced by increasing FIQ in the FM group but the difference did not reach statistical significance (r= -0.352, p=0.078; r= -0.344, p=0.085, respectively). Again, no significant difference was detected between FIQ and PGV and FPGV in the FM group (r= -0.261, p=0.199; r= -0.239, p=0.240; Table 3). Figure 1a and 1b present correlation between age and PGV and FPGV in the FM group. Figure 1c and 1d present correlation between age and PGV and FPGV in the control group. Figure 2a and 2b present correlation between VAS and PGV and FPGV while Figure 2c and 2d present correlation between FIQ and PGV and FPGV in the FM group.

Figure 1. a and b, correlation between VAS, PGV and FPGV in FM patients; c and d, correlation between FIQ, PGV and FPGV in FM patients

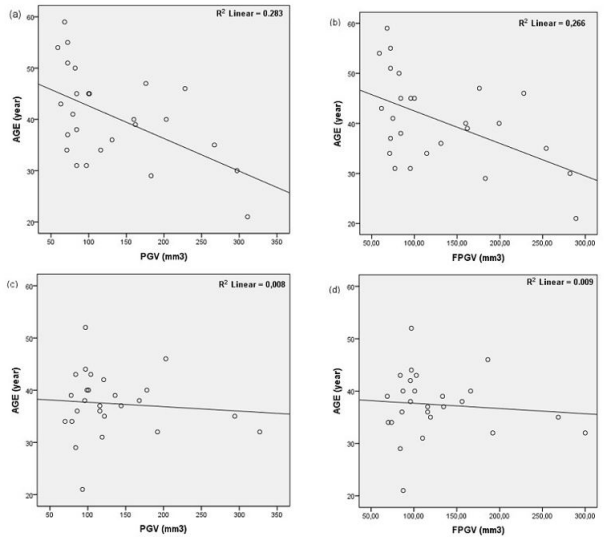
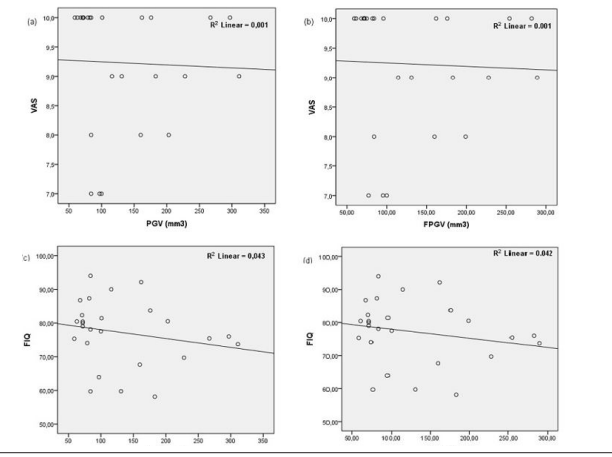


Figure 2. a and b, correlation between age, PGV and FPGV in FM patients; c and d, correlation between age, PGV and FPGV in healthy controls



DISCUSSION

To the best of our knowledge, this is the first study assessing PGV in patients with FM and healthy controls using MR imaging. In the study, the major finding is that there were no significant differences in PGV and FPGV between FM patients and healthy controls. However, age was negatively correlated with PGV and FPGV in the FM group while no correlation was detected in the control group.

Melatonin is a hormone secreted in lower concentrations during daytime and higher concentrations during nighttime by the suprachiasmatic nucleus (13). PGV is positively correlated with the number of pinealocytes responsible for melatonin secretion and is highly variable among healthy individuals. The reduction in PGV affects pinealocyte count, resulting in low melatonin level. In previous studies, a correlation was detected between PGV and melatonin level (14). Thus, it is thought that PGV may affect etiopathogenesis in chronic diseases that cause sleep/wake cycle and lead affective disorders (2, 14, 15). In a study by Bumb et al. (3), PGV was assessed in patients with primary insomnia and healthy volunteers using MR imaging. It was found that PGV was significantly lower in patients with primary insomnia compared to healthy volunteers. In a study by Fındıklı et al. (16), PGV was found to be lower in patients with schizophrenia when compared to healthy volunteers. However, in the same study, no significant difference was detected in PGV when patients with unipolar depression and bipolar disorder were compared with healthy controls. In a study by Takahashi et al. (17), no significant differences were detected in both total pineal volume and pineal parenchymal volume (excluding pineal cyst volume) between patients with major depression and bipolar disorders and healthy controls. In our study, no significant difference was detected between the FM group and health controls regarding PGV and FPGV.

In a study performing Micro-structural analysis of the pineal gland using the trueFISP imaging technique, both parenchymal volume and volume of cystic structure in the pineal gland were analyzed. In the study, pineal parenchymal volume was obtained by subtracting cystic volume from total PGV. In conclusion, it was found that both PGV and pineal parenchymal volume were reduced by advancing age. However, another striking finding was that total cystic volume or number of cysts had no correlation with age in the study (18). In our study, we found that there was no correlation between age and pineal cystic volume or number of pineal cysts in both FM patients and controls in agreement with the literature.

In the above-mentioned study by Bumb et al. (3), it was also found that there was a negative correlation between PGV and age in patients with primary insomnia but not in healthy controls. In our study, a negative correlation was found between age and PGV in FM patients but no such relationship was detected in healthy controls. It is known that sleep quality is an important age-related change. Older individuals have shorter and more inefficient sleep (19). In a postmortem study on 80 cadavers, effects of age, height, and weight were investigated on PGV and suggested that effects of age on PGV can be negligible (20).

Table 1: Demographic and clinical characteristics of the participants

	Fibromyalgia (n=26)	Healthy Control (n=26)	p
Age (years), mean ± SD	40.62 ± 9.02	37.42 ± 6.06	0.140
Gender (F/M), n (%)	23/3 (88.5/11.5)	23/3 (88.5/11.5)	1.00
BMI (kg/m2), mean ± SD	28.54 ± 4.06	26.78 ± 5.00	0.169
Duration of symptoms (month), median (IQR 25-75)	36 (12 -60)		
VAS pain score, mean ± SD	9.23 ± 1.07		
FIQ score, mean ± SD	77.20 ± 9.63		

FIQ: Fibromyalgia Impact Questionnaire; VAS: Visual Analog Scale; BMI: Body Mass Index; F: female; M: male; SD: standard deviation; IQR: interquartile range; (P < 0.05 considered statistically significant)

Table 2: Pineal gland volume and functional pineal gland volume in the control group and fibromyalgia group

	Fibromyalgia (n=26)	Healthy Control (n=26)	p
PGV, median (IQR 25-75	98.5 (72 - 177.75)	110 (91.25 - 150)	0.374
FGPV, median (IQR 25-75)	95.5 (72 - 177.75)	101.91 (86.93 - 139.99)	0.421
Cyst volume, median (IQR 25-75)	0.0 (0.0 - 4.44)	1.89 (0.0 - 9.99)	0.291
Number of cysts (yes/no), n (%)	11/15 (42.3/57.7)	14/12 (53.8/46.2)	0.579

PGV: Pineal Gland Volume; FGPV: Functional Pineal Gland Volume; SD: standard deviation; IQR: interquartile range; (p<0.05 considered statistically significant).

Table 3: Spearman correlation analysis among age, body mass index, PGV, and FGPV in fibromyalgia and healthy control groups and correlation analysis among Duration of symptoms, VAS, FIQ, PGV, and FPGV in the fibromyalgia group

	Fibromyalgia Group				Healthy Group			
	PGV		FPGV		PGV		FPGV	
	r_s	p-value	r_s	p-value	r_s	p-value	r_s	p-value
Age	-0.496**	0.010	-0.477**	0.014	0.022	0.916	-0.019	0.925
BMI	-0.253	0.213	-0.236	0.245	0.154	0.453	0.127	0.536
Duration of symptoms	-0.112	0.587	-0.106	0.606				
VAS	-0.352	0.078	-0.344	0.085				
FIQ	-0.261	0.199	-0.239	0.240				

Pineal Gland Volume; FGPV: Functional Pineal Gland Volume; BMI: Body Mass Index; VAS: Visual Analog Scale; FIQ: Fibromyalgia Impact Questionnaire; r_s: Spearman's correlation coefficients. (p < 0.05 considered statistically significant).

In our study, we failed to find a correlation between age and PGV in healthy controls; thus, we think that such a relationship can be neglected in healthy controls. However, a negative correlation was detected between age and PGV in the FM group in our study. We think the correlation between age and PGV in the FM group is relevant in fibromyalgia syndrome in which sleep quality and efficient are decreased.

This study has some limitations. Firstly, we did not assessed melatonin levels when assessing PGV. However, in previous studies, nocturnal melatonin level was assessed in FM and found to be normal or low (7, 21).We designed this study without melatonin assessment due to concerns regarding cost as there is no previous study investigating correlation between FM and PGV. In PGV, it is known that pineal calcifications and pineal cysts are hormonally inactive part (17). In our study, volume caused by pineal calcifications was neglected although pineal cyst volume was taken into account. Computed tomography (CT) is required for precise assessment of pineal calcifications (22). We did not obtained CT scans due to additional cost and complications caused by ionizing radiation.

CONCLUSION

This is the first study assessing PGV in FM patients and healthy controls using MR imaging. Based on results, there is no significant difference in PGV and FPGV between FM and control groups.

However, age has an impact on PGV and FPGV in the FM group. However, we concluded that age has negligible effect on PGV and FPGV in healthy controls. There is a need for further studies with larger sample size, which simultaneously evaluate melatonin level, in order to assess PGV in patients with FM.

Compliance with Ethical Standards: The study was approved by the local University Ethics Committee (Approval No: 2019/564-24.07).

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

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Original Research

Attenuation of Senescence-Induced Oxidative Exacerbations in Aged Rat Testis by Ferula Elaeochytris Root Extract

Atila Yoldaş[1], Nadire Eser[2], Aslı Yaylalı[3], Umut Kökbaş[4], Ahmet Sarper Bozkurt[5]

[1]Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Anatomy, Kahramanmaras, Turkey

[2]Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Pharmacology, Kahramanmaras, Turkey

[3]Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Histology and Embryology, Kahramanmaras, Turkey

[4]Cukurova University, Department of Medical Biochemistry, Faculty of Medicine, Adana, Turkey

[5]Gaziantep University, Faculty of Medicine, Department of Physiology, Gaziantep, Turkey

ABSTRACT

Objective: Age brings about changes to the oxidant and antioxidant balance of male testis that give rise to loss of fertility. The *Ferula elaeochytris* root extract (FE), contents antioxidant and anti-inflammatory component, have been used to treat infertility by local people for centuries. The main objectives of this study were to determine whether FE was effective on sperm quality, spermatogenesis, apoptosis and oxidative stress in aged rat.

Methods: Four groups were formed with 40 rats; young Control (YC), Aged Control (AC), Ferula elaeochytris administrated aged rat (A+FE) and vitamin E administrated aged rat (A+VE). Vitamin E and FE was administered orally for 8 weeks.

Results: The administration of FE significantly increased serum TAS, testetorone levels and decreased testicular *malondialdehyde (*MDA) activity, that these changes were accompanied by the reduced serum TNF-α, and TOS levels. Also, the apoptosis germ cell, the tubular diameter, the germinal epithelium height and Johnson's score a have been regulated after administration of FE ($p < 0.05$). Meanwhile, in the present context, in aged group the sperm count, motility, testicular weight declined significantly. FE showed showed significantly increased effect on the motility and sperm count.

Conclusive: These findings support that aging induces stress oxidative and inflammation, and FE could protect the testis against these damaging effects via its anti-oxidative, anti-inflammatory action and modulate spermatogenesis.

Keywords: Ferula elaeochytris, Aging, Antioxidant system, Spermatogenesis.

INTRODUCTION

In parallel with the increasing world population, the world's elderly population has promoted considerably in the last 20 years and it is obvious that it will gradually increase in the coming years. It is well known that aging is a long process that causes changes in most organ structures in the body. Although environmental and genetic factors are many factors that accelerate the aging process, it is widely accepted that aging is the most important cause of oxidative damage, which is caused by reactive oxygen species (ROS)[1]. The expression of enzymatic and non-enzymatic antioxidants (tocopherol, glutathione, etc.) systems that protect cells from ROS decreases with aging, and therefore the mechanism of protection from oxidative stress slows down [1,2].

Despite the structural changes in all tissue structures due to chronic oxidative stress with aging process, the testicles are more sensitive because they produce steroids and have a weak antioxidant system[3]. Therefore, in addition to chronic diseases associated with aging, prevention of reproductive aging has become important recently. In order to reduce chronic oxidative stress in the aging process, more importance has been given to natural herbal resources that will reduce the accumulation of oxidative stress, as well

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Corresponding Author: Ahmet Sarper Bozkurt **E-mail:** ahmetsarperbozkurt@hotmail.com

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as suggestions such as exercise, sports and diet that change life habits. Ferula species, grows naturally from the Mediterranean region to central Asia, is well known to contain substances that beneficial effect such as antimicrobial, antitumor, anticoagulant, antihyperlipidemic, antioxidant, anti-inflammatory, aphrodisiac, cytotoxic, antidiabetic, antispasmodic, anti-ulcerative and hepatoprotective effects [1].

Ferula elaeochytris, a species of Ferula genus, has been consumed for centuries by the local community as an aphrodisiac as well as for stimulating the mating of goats and sheep. Previous studies have found strong evidence that FE has some therapeutically beneficial components effected anti-inflammatory, phytoestrogen, antiproliferative, antioxidant[2], antidiabetic activities and positive effect on diabetes mellitus-induced erectile dysfunction and age-related erectile dysfunction[3] and age-related erectile dysfunction[4]. Ferula species have been reported to find large amounts of flavonoid compounds, as well as tocopherol, a type of vitamin E, and C[5]. Therefore, the study aimed to investigate whether FE can prevent some testicular damage due to aging, by comparing it with vitamin E, which has a protective effect on the testicle.

METHODS

Preparation of Plant Extract

Extract was extracted using the Soxhlet method and stored in the refrigerator after extracting.

Experimental Animals

Twenty young male Sprague-Dawley rats (4 months; body weight 360 to 375 g), aged rat (24 months: body weight (390-420) were used in the study.

Experimental Procedure

A total of 40 rats were used. There were 10 rats in each group and the study was divided into 4 groups. The dose of *F. elaeochytris* and vitamin E were administered (40 mg/kg, 50 IU/kg/day p.o. for 8 weeks 50Ukg, respectively)as described in previous studies[6].

Young Control : Adult (4 months-aged) rats, Control.
Aged Control: old (24 months- aged) rats, Control.
Aged+Ferulo Elaeochytris : old (24 months-aged) rats administered F. *elaeochytris*
Aged+Vitamin E: old (24 months-aged) rats administered vitamin E

Main Points:

- Ferula extract boosted the spermatogenesis via modulation of oxidative stress, decreased the apoptosis and proinflammatory cytokines such as TNF-α.
- Ferula extract is a versatile compound and can be used in advanced stages in the treatment of many diseases associated with hormonal disorders and oxidative stress.
- Examination of the effects of Ferula extract, which is used in the treatment of many diseases today.

Serum and Tissue Preparation

When the experimental part of the study was completed, no animal deaths were observed. At the end of the experiment, blood samples were centrifuged and their serums were collected. Some of the testicles were fixed in Bouin's solution for use in histopathological studies.

Sperm Quality

The evaluation of spermtazoa was performed as in previous studies [7]. Sperm analysis was used with the microscope to evaluate spermatozoa parameters. Motility parameters from at least 10 areas were analyzed and 1000 sperms per sample were evaluated. Meanwhile, spermatozoa was treated with 1% Eosin to evaluate for sperm Vitality. While living spermatozoa that absorb the dye appear red, inanimate ones are colorless.

Histological Study

Twenty seminiferous tubules were taken into account for each rat under the light microscope, and the evaluations were evaluated after 5 Research Spermatogenesis Johnson scores. The Johnson score applies a score of 1 to 10 for each seminiferous tubule in each seminiferous tubule cross-section[8]. In priefly, the scoring was as follows: score 10: Germinal epithelium is multirow, there are many spermatozoa, score 9: Germinal epithelium is disorganized and agglomerated towards the lumen, there is spermatozoa, 8: Germinal epithelium is multi-rowed, but there are less than 10 spermatozoa in the lumen, 7: No spermatozoa, There are many spermatids, 6: No spermatids, less than 10 spermatids, 5: No spermatozoa, no spermatids, there are spermatocytes, 4: No spermatozoa, no spermatids, less than 5 spermatocytes, 3: Only spermatogonia as germ cells, 2: No germ cells, only Sertoli cells, 1: No cells in the seminiferous tubulus.

DNA fragmentation-associated apoptosis was determined by the TUNEL assay. Apoptotic germ cell ApopTag Plus Peroxidase was designed using the InSitu Apoptosis Detection Kit (Chemicon, catno: S7101, USA) in accordance with the kit's procedures, and cells showing brown nuclear staining were considered positive in the evaluation of TUNEL staining. All prepared preparations were examined, photographed, and examined under a research microscope (Olympus, BX51, Japan). Staining index was determined by counting nuclear staining in at least 500 cells in 10 randomly selected microscopic fields.

Biochemical Analysis

Serum *catalase* (CAT), glutathione (GSH), and *malondialdehyde (*MDA) levels were determined by the methods described by Uchiyama and Mihara [9], respectively. Also, the activity of Total antioxidant status (TAS) and Total oxidant status (TOS) (Product Code: RL0017 and Product Code: RL0024, Rel Assay Diagnostics® Mega Tıp Ltd., Gaziantep, Turkey), as well as the levels of Testosterone (DRG testosterone ELISA, cat num. EIA-1559) and Tumor necrosis factor- alfa (TNF-α) (cat. ab46070, UK) in serum were determined according to the ELİSA kit providers instructions.

Statistical Analysis

Data were analyzed using the SPSS program version 24.0 (SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± SD.

One-way ANOVA analysis of variance was performed, followed by post hoc Tukey tests to find changes between individual groups. Significant value was determined as $P < 0.05$.

RESULT

Effect of FE on Body and Testis Weights

While In YC and AC groups rat the body weight of rat increased by the end of the 8-weeks period, the testis mean weight decreased (Table 1). Moreover, there was described to statistical difference in body weight between the AC and A+FE ($p<0.05$). The testis weight increased in the A+FE group compared to AC, which was statistically significant ($p<0.05$) (Table 1). Result indicated the AG+FE exhibited significant decreased in food intake the ($p<0.05$), as compared to the AG. However, the water intake was increased in A+FE compared the AG (Table 1).

Effect of FE on the Germinal Epithelium, the Tubule Diameter and Spermatozoa Values

In testis samples, the height of the germinal epithelium and the tubule diameter were evaluated under the microscope shown in (Fig 1/A). The height of the germinal epithelium was not significantly reduced in the AC compared to that in the YC ($p>0.05$). However, in AC group, the tubule diameter was showed a significant decrease compared to the YC ($p<0.05$). Meanwhile, the tubule diameter and the germinal epithelium height were observed to increase A+FE and A+VE as compared to the AC (Table 2)(Fig.1/A/B). The motility and number of sperm were observed to reduce in aged rat ($p>0.05$). However, There was no difference between sperm viability of young and old rats ($p>0.05$). The FE and Vitamin E significant improved sperm motility and count in aged rat compared the AC (Table 2).

Table 1. The effect of FE on food, Water intake and body, testicular weight

Groups	Initial body weight (gr)	Final body weight (gr)	Absolute testis weight (mg)	Relative testis weight (as%) body weight	Food intake (g/day)	Water intake (ml/day)
YC	289.1±10.6	318.3±10.3	1.62±0.04	0.49± 0.002	19.78± 6.65	28.5± 4.54
AC	372.6±11.5[a]	394.3±11.1[a]	1.48±0.031[a]	0.37± 0.007[a]	28.58± 7.05 [a]	29.8± 3.83
A+FE	373.4±10.9	348.9±9.3[b]	1.55±0.07[b]	0.44± 0.006[b]	25.18± 4.34 [b]	41.8± 6.36[b]
A+VE	371.5±12.1[b]	373.2±12.1[b]	1.61±0.03[b]	0.43± 0.006[b]	33.78± 4.78	42.4± 5.72[b]

Mean ± SE (8 values).
[a]Significant difference compared with the young controls.
[b]Significant difference compared with the aged rat

Table 2. Data of investigated tubular diameter, germinal epithelium height, sperm number, sperm motility, sperm viability in rat groups

Groups	Tubular diameter (µm)	Germinal epithelium height(µm)	Sperm Count(10^6)	Sperm Motility(%)	Sperm viability(%)
YC	274.2 ± 48.1	84.5 ± 12.16	123 ± 22.4	73.2± 4.2	68.6± 3.3
AC	248.5 ± 35.3[a]	77.4± 8.25	79 ± 19.3[a]	51.3± 6.8[a]	65.9± 4.4
A+FE	263.8 ± 19.6[b]	82.1± 9.46	96 ± 13.8[b]	62.5± 5.2[b]	67.5± 7.2
A+VE	265.4 ± 22.9[b]	81.9± 10.96	100 ± 15.2[b]	65,7± 5.5[b]	66.3± 5.8

Mean ± SE (7 values).
[a]Significant difference compared with the young controls.
[b]Significant difference compared with the aged rat

Effect of FE on the Spermatogenesis

Histopathological evaluation at week 8 revealed that spermatogenic activity in the AC group was dramatically decreased compared to YC. On the other hand, administration of FE to aged rats restored spermatogenic activity ($P > 0.05$; Figure 1/C/D). To confirm the role of FE and also to compare it with vitamin E in germ cell apoptosis in aged rats, apoptosis of spermatogenic cells was also evaluated with TUNEL ($P > 0.05$; Figure 1/E). TUNEL results clearly showed increased germ cell apoptosis in AC testis (Fig. 2/A). Compared to the AC group in the A+FE group (Figure 2/B), the number of apoptotic cells decreased and apoptosis was most common in spermatogonia (Figure 2/C) and spermatocytes (Figure 2/D). Statistically significant difference was found between AC and A + FE group ($p <0.05$) and also between AC and A+VE group ($p <0.05$; Fig. 2/E).

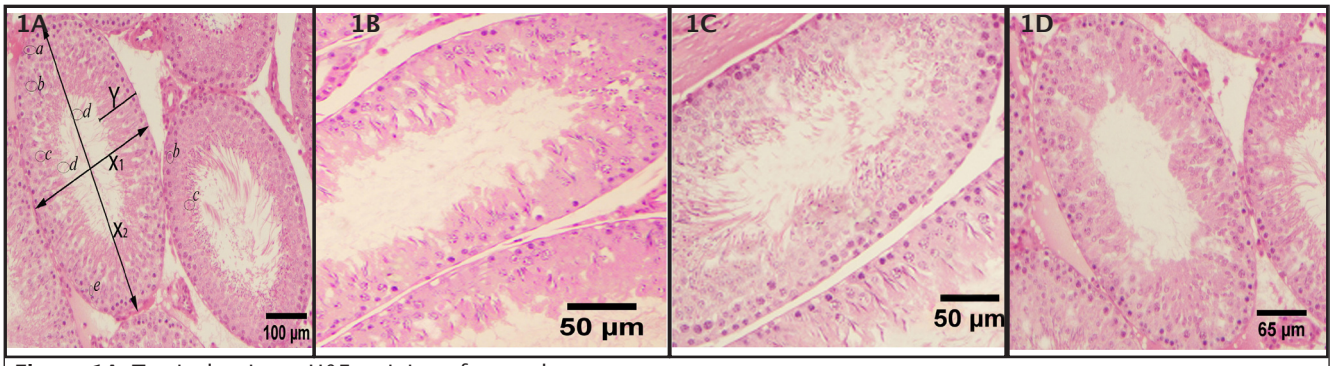

Figure 1A. Testicular tissue H&E staining of control young rats
Normal spermatogenetic activity and many spermatozoa were present in the lumen of most seminiferous tubules, as well uniform seminiferous tubules and regular germ cells;**a:** spermatogonia, **b:** spermatocyte, **c:** spermatid, **d:** spermatid, **e:** sertoli cell, **X1,2:** tubular diameter, **Y:** germinal epithelium heigh;
Figure 1B. Testicular tissue H&E staining of aged rats
Few Spermatogenetic activity with few spermatozoa and degenerative changes in the seminiferous tubules
Figure 1C. Testicular tissue H&E staining of aged of FE-treated aged group
Normal spermatogenetic activity with many spermatozoa in the lumen of most seminiferous tubules. Spermatogenetic activity in FE-treated aged rats was increased compared to aged rats
Figure 1D. Testicular tissue tunnel staining of VE-treated aged group
Normal spermatogenetic activity with many spermatozoa in the lumen of most seminiferous tubules. Spermatogenetic activity in VE-treated aged rats was increased compared to aged rats

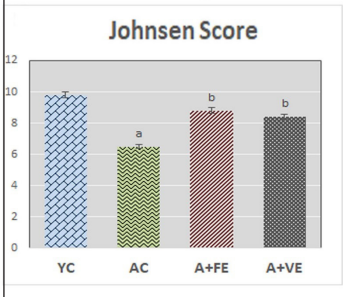

Figure 1E. Comparison of johnson score in all groups
[a] Significant difference compared with the young controls.
[b] Significant difference compared with the aged rat was seen

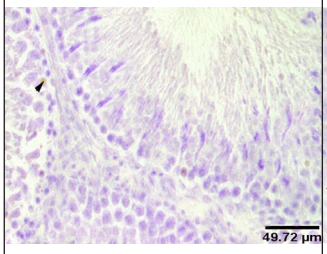

Figure 2A. Testicular tissue tunnel staining (arrow head) of control young rats

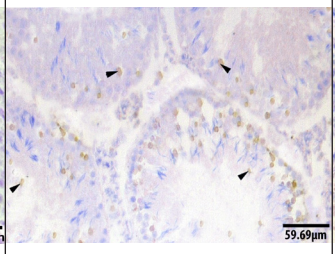

Figure 2B. Testicular tissue tunnel staining (arrow head) of aged rats

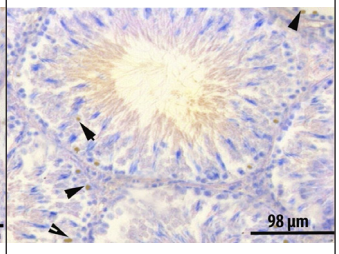

Figure 2C. Testicular tissue tunnel staining (arrow head) of FE-treated aged group

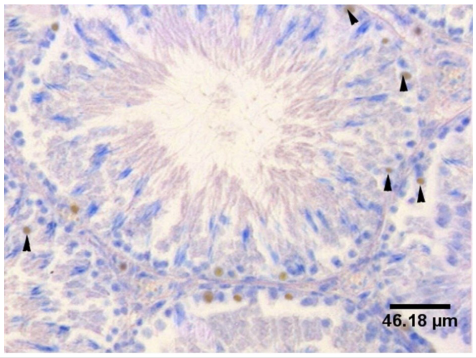

Figure 2D. Testicular tissue tunnel staining (arrow head) of VE-treated aged group

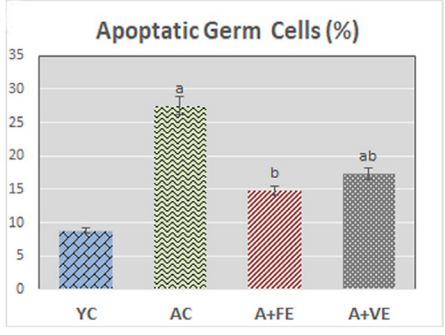

Figure 2E. Effect of FE and VE on apoptotic index of spermatogenetic cells in aged
[a] Significant difference compared with the young controls.
[b] Significant difference compared with the aged rat.

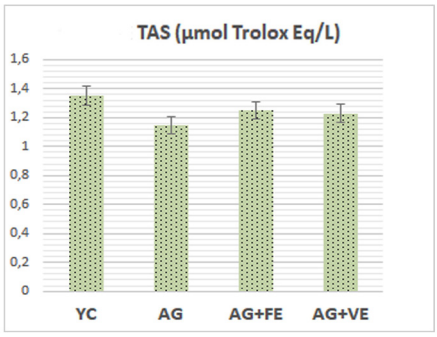

Figure 3A. Serum total oxidantstatus (TAS) in groups

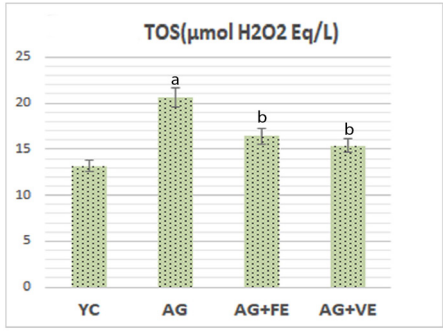

Figure 3B. Serum totola antioxdan capacity (TOS)in groups
[a] Significant difference compared with the young controls,
[b] Significant difference compared with the aged rat

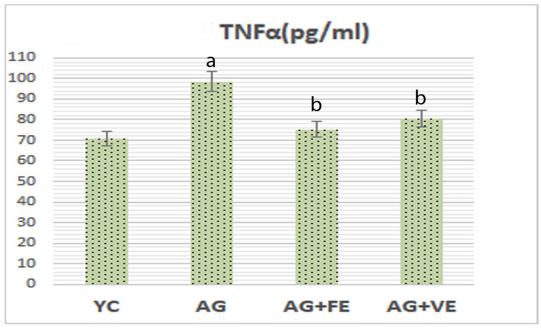

Figure 3C. Serum tumor necrosis factor alpha (TNF-α)
[a] Significant difference compared with the young controls,
[b] Significant difference compared with the aged rat

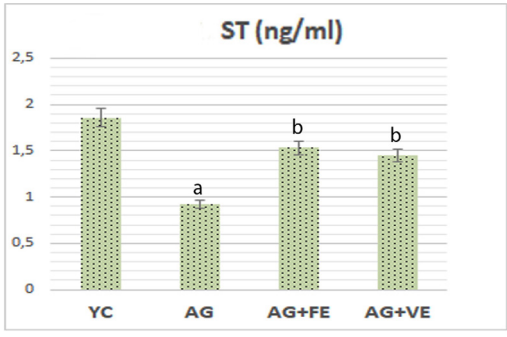

Figure 3D. Serum testosterone (ST) (d) levels in the control
[a] Significant difference compared with the young controls,
[b] Significant difference compared with the aged rat

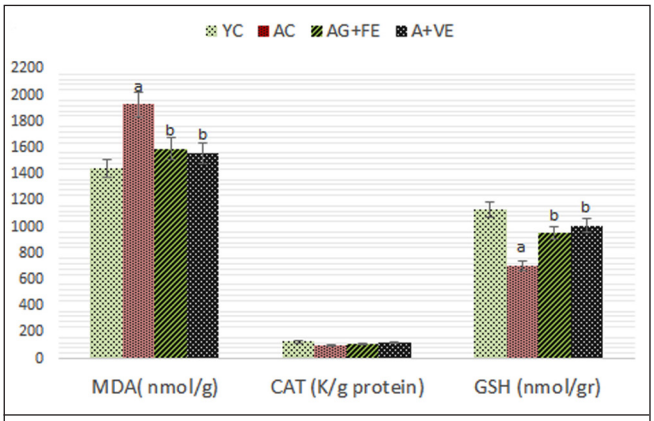

Figure 4. The malondialdehyde (MDA), the catalase (CAT) and the glutathione (GSH) levels in the testicular tissue of the control rats, VE and FE administrated group rat, [a]p < 0.05 compared with Young Control; [b]p < 0.05 compared with control group)

Effect of FE on Serum Testosterone and Tnf –A and Oxidant/ Antioxidant Markers

Although the serum TAS level decreased in AC compared to the YC, there was no statistically difference between two groups (P>0.05;Fig. 3/A). Meanwhile, as a sign of more oxidative damage to tissue, serum TOS levels was showed significantly higher in AC, compared to YC rats. However, as a sign of restoration of tissue damage due to oxidative stress, both A+FE and A+VE rats showed significantly higher serum TAS concentrations compared to AC rats (P<0.001;Fig.3/B).

The results illustrated that serum level of testosterone was importantly decreased in the AC rats compared with the YC rats ((P<0.001). Meanwhile when compared in YC, TNF- α levels was seen considerably to increase (P< 0.05) in AC (Fig.3/C). At the end of 8 weeks, it was seen that administration of FE and VE significantly increased the testosterone serum level and also the serum TNF -α level was significantly regulated (P< 0.05) (Fig. 3/D).
When the evaluated in testis tissue, although the CAT level of the AC was seen to be lower than YC, there was no statistical difference between the two groups (P>0.05). However, testicular GSH level, which had decreased in AC the testicular, it was observed to increase a significant degree in the A+FE and A+VE (P<0.001), compared to AC (Fig. 4). The AC group showed significantly higher tissue MDA levels compared to the YC group, representing greater oxidative damage to lipids (P<0.05).

DISCUSSION

The aging process and protection or elimination of problems caused by this process have become an important field of research. It have been a well-known fact that natural antioxidant production of an organism decreases with aging. Therefore, this study was designed to investigate whether FE has a protective effect on testicles during aging.

In many studies, it has been reported that we, as well as others [10], have reported body weight increases during aging [11,12] . Recently, many studies have been conducted to prevent the body weight gain such as genetic, metabolic, hormonal, behavioural, social, and cultural aspects[12]. It is well known that there are many studies reporting the anti-obesity effects of the plant extract such as green tea, Vitaceae, Melanthiaceae and also Ferula species [13] on humans and animals [14]. The study was indicated that FE consumption regulated to the gain body weight. In addition, FE was found to decrease food intake while increasing the water intake in aged rat.

In this study was seen that spermatogenesis activity increased in FE administered group rat compared to the control group. Although the mechanism of action of Ferula and its compounds on the testis needs to be explained, FE is known to have rich antioxidative and anti-inflammatory compounds such as Khusino, alf pinen, beto ionone [15]. Meanwhile, numerous studies are stated that sperm cells are particularly susceptible to reactive oxygen species during spermatogenesis. Aging with accumulating radical oxygen species causes apoptosis in testis as well whole body. For the reasons, the apoptosis marker increased with aging causes to change in the testis germ cell that can lead to decrease spermatogenesis, and also sperms quality [16]. The data of the current study showed that the FE improved the number, motility, of sperms and also may played a protective role against aged-induced apoptosis in germ cell in aged rat. The results obtained from this study were consistent with the results of researchers about ferula species previous reports [17].

Furthermore, it was well also known that the tubule diameter and the thickness of the germinal epithelium layer is an indicator for the status of spermatogenesis and also in aging and some chronic diseases, tubule diameter is reduced and the thickness of the germinal epithelium layer decreases[18]. Our results also indicate that, unlike spermatogenesis activity and the tubule diameter, when the height of the germinal epithelium was analysed, there was only a slight difference between the aged rats and the young control rat as described by researches [19]. However, FE treatment led to recovered in the tubule diameters and germinal layers in aged rat.

One of the most important findings of the study was that serum TNF and T levels were regulated after FE administration. As known, aging is associated with increased TNF activity and low testosterone level in the blood. The deficiency of testosterone or TNF elevation is also known to increase in age-related diseases for example hipogonadizm, metabolic syndrome, diabetes, Alzheimer disease, cardiovascular disease and also erectile dysfunction (ED) [20]. Meanwhile, there is strong evidence that testosterone modulates TNF alpha, an important cytokine responsible for the immune system. We observed that age-related decreased the serum TNF and testosterone level as reported by the researchers[21]. Additionally, serum testosterone level was seen to boost in the FE administered group as compared to the aged rat and the vitamin E administered aged rat. Researchers reported that after extract of some Ferula genus was administered, boosted the serum testosterone level and improved sexual functioning in young rat and mice [22], and also reduced TNF and IL 6 . Thus, FE may be an alternative option for use in the testosterone replacement therapy in the future.

Lipid peroxidation is an oxidative stress indicator resulting from MDA. Some researchers stated that testis MDA level changed with age [16], while some claimed in contrast [6]. In our study, MDA levels of aged rat testis tissue were found to be significantly higher than the aged control group ($p<0.001$). Meanwhile, FE-supplemented aged rats showed significantly lower testicular MDA level, as reported for ferula in rat testicular damage [23]. Glutathione-S-transferase (GST) is an antioxidant enzyme that provides to conjugation the electrophilic and hydrophobic compounds with glutathione, which is generally easier to remove and convert to less toxic metabolites. Studies also show that GST is the enzyme most affected by the aging-related antioxidant changes occurring in the testis and that it decreases greatly in the testicular tissue during aging [16]. It is known that most of the flavonoids have the ability to activate glutathione-S-transferase (GST). This mechanism is accomplished by Glutathione reductase (GR) which is a flavoprotein. GR uses β-nicotinamide dinucleotide phosphate (NADPH) as a hydrogen donor and catalyzes oxidized glutathione (GSSG) to reduced glutathione (GSH). In this study, GSH was observed to decrease with age in the testicular tissue[24]. However, it has been seen that FE restored the decrease in GSH level in the aged rat as reported by authors. This compounds may have been increased the GSH levels in the FE administrated group. Moreover, as in previous studies [6,25], it was found in our study that vitamin E increased the GSH levels statistically in aged group. However, when the rat fed with FE and the rat given VE were compared statistically, it was determined that there was no difference between the two groups.

The current study had several limitations. The results obtained are the study conducted on only one species of the experimental animal. Reliability coefficients can be further increased by increasing different animal species and numbers in this study.

CONCLUSION

The present results indicated that FE clearly inhibited histopathological damage in testicles caused by senescence and preserved spermetegenosis and improved serum testosterone levels. Meanwhile FE reduces inflammation and oxidative stress in the aged rat, it slows down apoptosis in tesitis. The positive findings that FE can reduce testicular dysfunctions due to aging are promising for future studies.

Peer-review: Externally peer-reviewed.

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Letter to the Editor

Food Insulin Index: Implications for Type 2 Diabetes Mellitus

Sergei V. Jargin[1]

[1] Department of Pharmacology, Peoples' Friendship University of Russia, 117198 Moscow, Russia

The education of diabetic patients about their disease is an integral part of prevention, treatment and care (1). Realistic perceptions about nutrition are important for the control and prevention of complications (2). This letter questions the usefulness of the food insulin index (FII) concept for the management of type 2 diabetes mellitus (T2DM) with overweight. FII has been proposed to quantify postprandial insulin response to a food in comparison with an isoenergetic portion of a reference food such as white bread or glucose (3, 4).

Long-term hyperinsulinaemia is known to contribute to insulin resistance and weight gain. Although carbohydrates are a major stimulus for insulin secretion, it is not the only one. Protein-rich foods also elicit insulin response especially when combined with carbohydrates. Other nutritional and endocrine factors can stimulate insulin secretion: certain amino and fatty acids, glucagon and cholecystokinin, incretins incl. glucagon-like peptide 1 (GLP-1) (3, 5). The high FII and insulin load were associated with the overweight in young people estimated by skin fold thickness measurements (6). In a cross-sectional study of healthy subjects, FII was associated with higher triglycerides and inversely related to high-density lipoprotein cholesterol (HDL-C) levels in obese individuals (4). Several recent studies on this topic have been carried out in Iran, where the diet is comparatively rich in carbohydrates (3, 7-10). In particular, a diet with high FII was related to obesity in women and had a borderline association with insulin resistance (7, 8). Elderly men with high insulin load (estimated by multiplying FII by the energy content of corresponding foods) had elevated fasting blood sugar. There was no association between insulin load, HDL-C and body mass index (10).

In type 1 diabetes, FII-based algorithm improved postprandial hyperglycaemia in comparison with the traditional carbohydrate counting (11). The ranking of foods according to FII in lean, young healthy subjects may not be directly applicable to older patients with T2DM (3, 5). Studies have found no significant association of FII with low density lipoproteins, glycated haemoglobin, C-peptide, cardiovascular risks, obesity and metabolic syndrome (3, 4, 9). Apparently, FII values of various products do not provide clear guidance for dietary recommendations for patients with T2DM and overweight. For example, FII of boiled potatoes is relatively high (121 compared to 100 of white bread) (5) but the necessity of its restriction in T2DM is known. Some papers reported a relatively high FII of milk (90-98) (12), other researchers indicated lower values: ~60 (13) or 33 (5). According to a review, dairy products are favourable or at least neutral in T2DM and metabolic syndrome (14). According to different sources, the level of fasting glucose decreased or remained stable after consumption of dairy products (15). Finally it should be noted that the impact of diets with relatively low glycaemic index (GI) but high FII are partly analogous to that of incretin mimetic drugs such as GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. The latter inhibit degradation of GLP-1, which increases insulin secretion contributing to the glycaemic control (16). In this regard, a diet with relatively low GI and high FII is not a priori unfavourable for T2DM patients. In the author's opinion, the FII concept does not significantly affect dietary recommendations for T2DM patients with overweight.

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Corresponding Author: Sergei V. Jargin **E–mail:** sjargin@mail.ru
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Short Communication

A Case of 'Multi-Inflammatory Syndrome in Children' Complicated with Cardiogenic Shock

Nazmiye Serap Biçer[1], Zeynep Türe[2], Birkan Ülger[3], Aliye Esmaoğlu[3]

[1] Erciyes University, Faculty of Medicine, Department of Internal Medicine, Kayseri, Turkey

[2] Erciyes University, Faculty of Medicine, Department of Infectious Diseases, Kayseri, Turkey

[3] Erciyes University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kayseri, Turkey

ABSTRACT

SARS Cov-2 infection causes Multi-Inflammatory Syndrome in Children (MIS-C), a serious condition that affects children. We report an 18-year-old Turkish male who was diagnosed with MIS-C and successfully treated. He was diagnosed with MIS-C and required invasive mechanical ventilation due to cardiogenic shock, after what he recovered. With a high temperature, rash, and conjunctival hyperemia, the patient was taken to the emergency department. He had no symptomatic COVID-19 in his medical history, although he had had contact with a COVID-19 positive patient in the near past. Physical examination revealed an erythematous maculopapular rash on the back and neck, as well as hepatosplenomegaly. SARS-CoV-2 IgM and IgG positivity were detected in the rapid antibody test. Following the procedure, rectal bleeding and tachypnea developed. Inflammation indicators and pro-BNP levels both increased. With echocardiogenic examination, the ejection fraction decreased from 50-55 percent to 35%. He needed invasive mechanical ventilation. As a result, the case was classified as MIS-C with predominant cardiac and gastrointestinal involvement. The patient was discharged after a successful multidisciplinary approach. Although COVID-19 infection in children and adolescents is asymptomatic or minimally symptomatic, clinicians should be aware of post-infection autoimmune complications.

Keywords: COVID 19, MIS-C, SARS-CoV 2, Cardiogenic Shock

INTRODUCTION

The World Health Organization (WHO) confirmed a new pandemic caused by SARS-CoV-2 on March 11, 2020 (1). According to WHO statistics, COVID-19 has affected approximately 250 million people in 222 countries, resulting in the deaths of almost five million people (2). Despite the fact that the disease is characterized by respiratory tract infections, the virus has evolved to damage systems other than the lungs (3). The incidence of COVID-19 in children is relatively lower than in adults, and it is assumed that children overcome this disease mildly (4).

SARS-CoV-2 has the potential to cause Multi-Inflammatory Syndrome in Children (MIS-C), which is a rare but serious illness in children (5). The abnormal immunological response against the virus is assumed to be the origin of MIS-C, which has clinical similarities to Kawasaki Disease (KD), Macrophage Activation Syndrome (MAS), and Cytokine Release Syndrome. MIS-C is responsible for 1% of hospital admissions in the pediatric population during the pandemic period. Most of the patients are required intensive care support and the mortality rate is around 1.5-2% (6). The majority of the cases had a negative PCR test but a positive serology. This condition offers to the theory that it is linked to immunological dysfunction after the acute infection has cleared. Myocardial injury mechanisms have yet to be identified. Systemic inflammation-related injury, acute viral myocarditis, hypoxia, stress cardiomyopathy, and ischemia caused by coronary artery involvement are all potential causes of myocardial damage. Fever, hypotension, rash, myocarditis, and gastrointestinal problems are some of the clinical symptoms. Inflammation is on the increase, according to laboratory results. Symptoms of respiratory disorders may not be observed in this disease (7).

In our report, we present a MIS-C case that required invasive mechanical ventilation due to cardiogenic shock.

CASE REPORT

An 18-year-old Turkish male patient was taken to the emergency department with a four-day high fever and a red, extensive rash that began the day after the fever. Before his symptoms began, he was a student with no chronic diseases, drug or substance addiction history. His vital signs were high fever: 39.1 ^{0}C, pulse: 131 / min, respiratory rate: 20 / min, and blood pressure: 108/60 mm Hg. On physical examination, the patient appeared to be in good health, aware, and oriented, with bilateral conjunctival hyperemia and an extensive maculopapular rash on the erythematous floor of the back, trunk, and arms (Figure 1). The liver edge was palpable under the right costal margin on abdominal examina-

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Corresponding Author: Zeynep Türe **E-mail:** dr.zeynepture@gmail.com

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tion. The results of the whole physical examination were normal. The examinations performed at the time of admission revealed lymphopenia and an increase in acute phase reactants. Liver and renal function tests showed no abnormalities (Table 1).

The spleen was 15.5 cm in diameter and had a homogenous density when abdominal imaging was done to determine the origin of the fever. The liver measured 19 cm in diameter and had a homogeneous density. The thoracic tomography revealed no infiltration. In the nasopharyngeal smear, a real-time reverse transcription-polymerase chain reaction (RT-PCR) results came back negative. He was transferred to the Clinic of Infectious Diseases. The rapid antibody test performed in the clinic provided a positive result.

Treatment with Favipravir and low molecular weight heparin were started. Ejection fraction (EF) was 50-55 percent on transthoracic echocardiography performed at the bedside, with mild global hypokinesia, 1st-degree mitral insufficiency, 1st-degree tricuspid insufficiency, and enlargement of the right spaces. Cardiac troponin was 0.283 ng/mL, and pro-BNP was higher than 35000 pg/mL. Two days later, the patient complained of diarrhea and rectal bleeding. The patient's fever persisted, so empiric piperacillin-tazobactam treatment was initiated after blood, stool, and urine cultures were taken. Two g / kg intravenous immunoglobulin (IVIG) was started with the pre-diagnosis of MIS-C, with cardiac and gastrointestinal involvement predominant. Pulse-methyl-prednisolone was also started, at a dose of one gram per day for three days. The patient was transferred to the intensive care unit due to hypotension and tachycardia. On echocardiography done in the intensive care unit, the ejection fraction was 35 percent, and diffuse global hypokinesia and perimyocarditis were detected. With the pre-diagnosis of shock, noradrenaline and dobutamine treatment proceeded. The patient's antibiotherapy was changed to meropenem plus vancomycin after a rise in acute phase reactants (CRP: 222 mg/l, procalcitonin: 13 ng/ml). The patient needed invasive mechanical ventilation on the same day. Because of his anuric course and chronic acidosis, he was administered with continuous renal replacement. Cardiopulmonary resuscitation was performed for 17 minutes after cardiac arrest occurred. The patient was monitored in Synchronized Intermittent Mandatory Ventilation (SIMV) mode with high PEEP and oxygen support. The patient was extubated with reduced support after four days of intubation. After the negative culture results, the meropenem and vancomycin therapies were terminated. Control transthoracic echocardiography, EF was 45% with mild global hypokinesia, 1st-degree mitral insufficiency, 1st-degree tricuspid failure. He was discharged with a beta-blocker and furosemide medications on the 16th day of his hospitalization.

Written consent was obtained from the patient to be presented in the case presentation.

Figure 1. Maculopapular eruptions on erythematous on the arm (A), back (B) and trunk (C) at the time of admission and intensive care follow-up

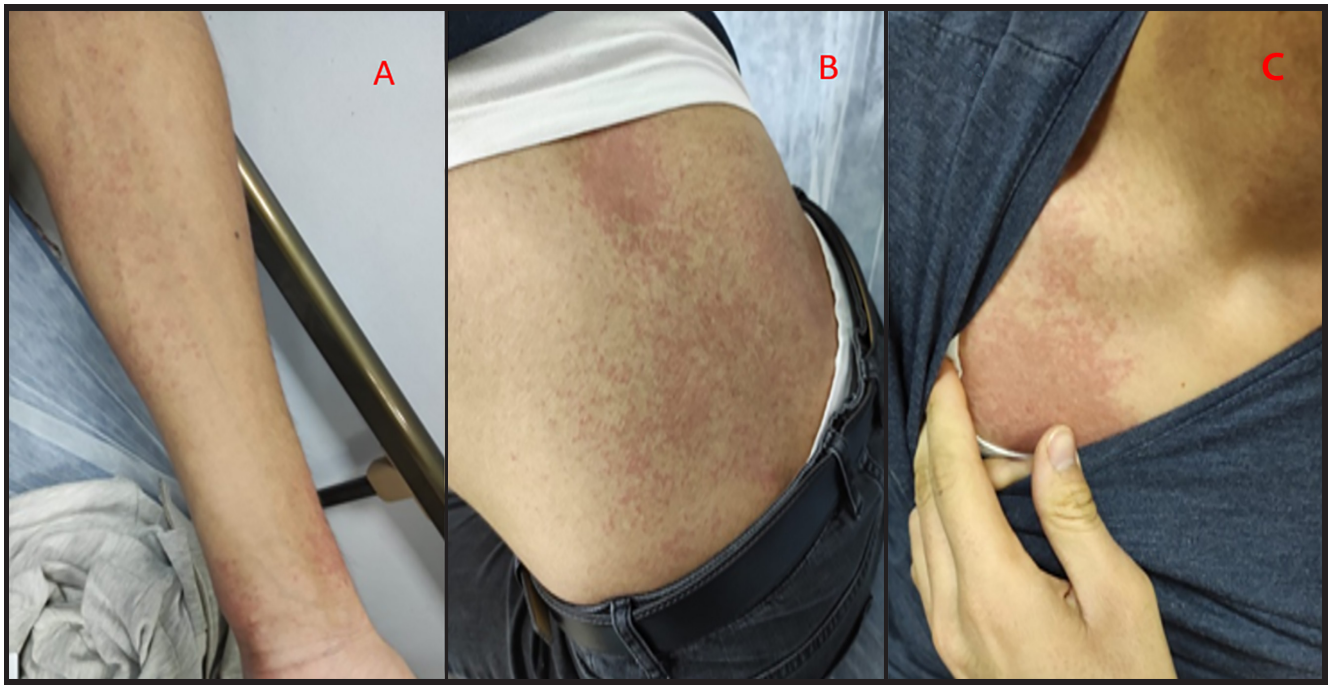

Table 1. Laboratory parameters of the patient on the admission, follow-up and discharged period

	Day 1	Day 2	Day 7	Day 16 (discharged)
White Blood Cell Count (4,8-10,7x10³/µL)	4.72	8.03	12,02	8,54
Lymphocyte (1,3-2,9 x10³/µL)	0.38	0.29	0,74	1,55
Hemoglobin (g/dL)	14,2	13,6	12,4	11,8
Hematocrit	42,1	40,8	38,2	36,3
Platelet (130-400x10³/µL)	155	127	133	207
Ferritin (20-500ng/ml)	341	443	546	469
Pro-BNP (0-125 pg/ml)	> 35.000	> 35.000	-	225
Troponin T (0-0,014 ng/ml)	0,159	0,283	0,168	0,185
Fibrinogen (180-350 mg/dl)	701	-	358	230
D-dimer (0-550µg/l)	1170	1770	2970	2700
INR	1,53	1,23	1,16	1,13
CRP (0-5 mg/dL)	115	223	60	6.6
Procalcitonin (0,5-2ng/ml)	0,68	5.57	4.44	0.11
ALT (0-40u/L)	15.8	18	1337	99
AST (0-40u/L)	35.2	22	687	34
Bilirubin (mg/dL)	0,72/0,41	0,98/0,56		0,55/0,18
Albumin (g/dL)	2,84	2,56	3,15	3,45
GGT (u/L)	23	17	18	25
Creatinine (0.5-1.2 mg/dl)	0.94	1.2	0.89	0.7

BNP, brain natriuretic peptide; INR, international normalized ratio; CRP, C-Reactive protein; ALT, alanine aminotransferase; AST: aspartate aminotransferase; GGT; gamma glutamine transferase

Table 2. Some of MIS-C cases reported in the literature, clinical symptoms, treatment and prognosis

Number of the case	Age/ Gender	Clinical Symptoms	ICU need	Cardiac Involvement	Treatment	Outcome
1 [11]	36/F	Fever, abdominal pain, vomiting, diarrhea diffuse rash and arthralgia	No	Yes	IVIG, methylprednisolone	Alieved
2[12]	5month old/F	Fever and intermittent tachycardia	Yes	Yes	Methylprednisone	Alieved
3 [13]	12/F	Fever, breathlessness, skin rashes, mucosal excoriations, conjunctivitis and diarrhea	Yes	No	Methylprednisone	Alieved
4 [14]	8/F	Fever, rash, respiratory distress, hemodynamic instability, hyperglycemia, ketosis and metabolic acidosis	Yes	Yes	IVIG, infliximab, methylprednisolone	Alieved
5 [15]	16/F	Abdominal pain, vomiting, fever, headache, myalgia and cough	Yes	Yes	IVIG, aspirin, methylprednisolone and norepinephrine	Alieved
6 [16]	22/M	Asthenia, chills, diffuse myalgia, abdominal pain and diarrhea	Yes	Yes	IVIG and tocilisumab	Alieved
7 [17]	15/M	headaches, sore throat, and fever as well as one day of neck pain and stiffness	Yes	Yes	IVIG, dexamethasone and aspirin	Alieved

ICU, intensive care unit F, female; M, male; IVIG, intravenous immunoglobulin

DISCUSSION

Among the problems seen during and after SARS-CoV-2 infection are the amount of viral replication and an uncontrolled autoimmune response to viral replication (8). In addition to the viral cytopathic effect, Type 2 and Type 4 hypersensitivity responses play a role in the formation of the autoimmune response (8). Fever, rash, conjunctivitis, mucocutaneous ingestion, hypotension, and cardiac failure were the most commonly reported symptoms in a study of 9 MIS-C patients. Troponin and pro-BNP levels were shown to be higher in the same study (9). The high temperature, rash, hypotension, conjunctivitis, and cardiac failure seen in this case are similar to those seen in other cases in the literature.

The mortality rate was 2% in a review of 8 MIS-C trials with 440 individuals. The median age of the patients in this study ranged from 7.3 to 10. At the same time, most of the symptoms were gastrointestinal symptoms (87%), other symptoms reported as dermatological/mucocutaneous (73%), cardiovascular symptoms (71%), respiratory (47%), neurological (22%), and musculoskeletal (21%). In 26% of the patients, mechanical ventilation was required, and 6% of the cases required extracorporeal membrane oxygenation (10). According to the literature, the frequency of MIS-C cases requiring invasive mechanical ventilation and being discharged in the follow-up is extremely rare. Table 2 lists some of the MIS-C cases that have been reported (11-17). In this case report, a multidisciplinary approach was used to successfully discharge a patient with cardiological, gastrointestinal, dermatological/mucocutaneous symptoms, and invasive mechanical ventilation.

The diagnosis of MIS-C can be made using a variety of diagnostic criteria. According to WHO criteria, the presence of inflammatory markers between 0-19 years of age for more than three days, the presence of 2 clinical findings, and COVID-19 serological or PCR test are considered cases. According to the Centers for Disease Control and Prevention, patients under the age of 21 are actually covered (18). Both clinical and laboratory criteria are met in this case.

The cause for admittance to the hospital in this case was a high fever and rash. In an Italian research, 88 individuals diagnosed with Covid-19 were studied, excluding drug-related rashes, and skin findings were reported in 20.4 percent of the cases (18 patients). 14 patients had an erythematous rash, three had extensive urticaria, and one had varicella-like vesicles (19). Rashes on the skin are one of the most commonly reported symptoms in the literature, and these rashes are thought to be autoimmune in origin (11,13,14). Maculopapular rashes that appear during or after the disease process should be considered a rare COVID-19 clinical finding.

LESSONS LEARNED

Although COVID-19 infection in children and adolescents is asymptomatic or minimally symptomatic, clinicians should be aware of the possibility of autoimmune consequences. Patients admitted to the emergency department during the pandemic should be monitored for SARS CoV-2-related symptoms and complications, even if they do not have typical respiratory symptoms.

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