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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.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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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
Fax: +90 342 360 16 17
E-mail: info@eurjther.com

Publisher: AVES
Address: Büyükdere Cad., 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
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Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489–93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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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.

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

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

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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
Fax: +90 342 360 16 17
E-mail: info@eurjther.com

Publisher: AVES
Address: Büyükdere Cad. 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
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Contents

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Original Article

Incidental Maxillary Sinus Pathologies in Asymptomatic Subjects—A CBCT Study

Soundarya Sakthivel, Vidya Ajila, Gogineni Subhas Babu, Renita Lorina Castelino, Shruthi Hegde, Anwesha Biswas

Department of Oral Medicine and Radiology, Nitte (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Mangalore, India

ABSTRACT

Objective: The objective of the present study was to record the prevalence of incidental maxillary sinus pathologies in patients using cone beam computed tomography (CBCT) scans performed for maxillofacial diagnostic purposes.

Methods: This study was carried out retrospectively on CBCT records from January 2017 to July 2019. Pathologic findings were categorized as mucosal thickening, opacification, polypoidal mucosal thickening, others (such as antrolith, septa, or discontinuity of the sinus floor), and no pathologic findings. The incidence of maxillary sinus changes and their correlation with age and gender was analyzed.

Results: A total of 683 scans were identified out of which 252 cases met the inclusion criteria. Pathologies were similar across age groups with a slight male predilection. The incidence of maxillary sinus pathologies overall was 68.2%. Both sinuses showed changes in 39% cases, and 29% cases had unilateral findings while 32% cases had no sinus abnormalities. Mucosal thickening, opacification, and polyps were higher in males on both left and right sides. Females showed an increase in incidence in other findings such as antroliths, septa, and discontinuity of the sinus floor more on the right side. The results were not statistically significant.

Conclusion: Incidental maxillary sinus abnormalities are highly prevalent in asymptomatic dental patients. Oral radiologists should be aware of these incidental findings and comprehensively evaluate the entire captured CBCT volume, which can help in early diagnosis, treatment, and follow-up of the patient.

Keywords: Maxillary sinus, cone beam computed tomography, pathology

INTRODUCTION

Maxillary sinuses are a pair of paranasal sinuses in the head-and-neck region.[1] The evaluation of maxillary sinuses is essential in cases of trauma, sinusitis, and implant placement and can be performed using different imaging methods.[2] Maxillary dental infections can also cause changes in the maxillary sinus. A 1-mm thick sinus membrane is considered normal and is not evident in radiographs, while pathological conditions can cause increased mucosal thickness which is seen as a radiopacity within the sinus.[1] The maxillary sinus can be visualized using conventional radiographs as well as imaging modalities such as computed tomography (CT), cone beam computed tomography (CBCT), and magnetic resonance imaging (MRI).[2]

CBCT is a 3D imaging modality with the advantages of good image quality without distortion and superimposition of surrounding structures. In addition, it is comparatively low cost, and radiation exposure has made it a preferred imaging method.[2] CBCT has a variety of applications in dentistry such as in implantology, endodontics, orthodontics, temporomandibular joint evaluation, and maxillofacial surgery. In many cases, the maxillary sinus falls within the field of view of CBCT, and incidental findings in the sinus can be visualized.[3]

Maxillary sinus can be visualized on the panoramic radiograph, water's view, CT, MRI, and CBCT.[1] CT is considered as the "gold standard" for in depth examination of maxillary sinuses. However, in dentistry, CT machines have limitations that include high cost and high radiation exposure. CBCT addresses these limitations of CT and provides many dental advantages.[3] The area of the maxillary sinus can be within the imaging field when the CBCT is indicated for various reasons such as dental implant site assessment, periapical bony and inflammatory pathologies, endodontic lesions, sinus augmentation, impacted, and supernumerary teeth, and in orthodontic applications. Hence, incidental findings are frequently viewed in the area of the maxillary sinus by maxillofacial radiologists. The extent of incidental findings varies from 10 to 69% in various studies.[4]

The present study was designed to record incidental maxillary sinus pathologies in asymptomatic individuals using CBCT scans performed for maxillofacial diagnostic purposes and to

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ORCID iDs of the authors: S.S.0000-0002-3213-9395; V.A.0000-0002-5744-9322; G.S.B.0000-0001-9383-7886; R.L.C.0000-0002-8696-549X; S.H.0000-0002-0744-5593; A.B.0000-0001-6716-6409.

Corresponding Author: Vidya Ajila **E mail:** ajila_v@yahoo.com

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Figure 1. (A) Coronal CBCT section showing mucosal thickening in the right maxillary sinus. (B) Axial CBCT section showing polypoidal thickening in the left maxillary sinus. (C) Coronal CBCT section showing opacification of bilateral maxillary sinuses.

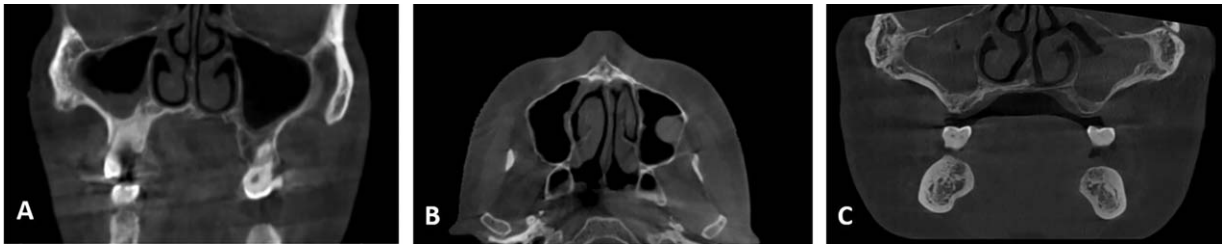
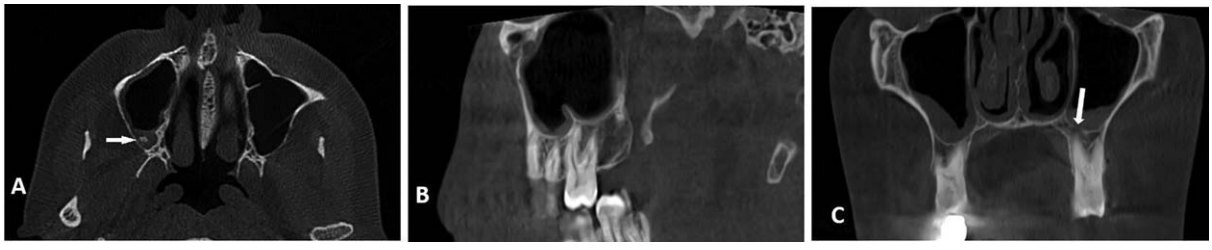


Figure 2. (A) Axial CBCT section showing antrolith (white arrow). (B) Coronal CBCT section with septa in the floor of the left maxillary sinus. (C) Coronal CBCT section showing discontinuity in the floor of the left maxillary sinus (white arrow).



compare and correlate these pathologies with age, gender, and side of the involved maxillary sinus.

METHODS

This retrospective study used CBCT scans of bilateral maxillary sinuses from the archives of the Department of Oral Medicine and Radiology, from 2017 to 2019. Institutional ethical consent was obtained for the study from AB Shetty Memorial Institute of Dental Sciences, Mangalore, India (ABSM/EC/11/2020). Inclusion criteria consisted of asymptomatic subjects with CBCT scans of bilateral maxillary sinuses, where data regarding gender and age were available. CBCT scans of subjects with congenital defects such as cleft palate, known pathologies, or trauma involving the maxillary sinuses as well as subjects below 10 years were excluded.

All CBCT scans had been obtained with the Planmeca Romexis 3D MID model. The exposure factors were field of view ranging from $5 \times 5\,cm^2$ to $17 \times 13.5\,cm^2$, 80-90 kV, 8 mA, and the voxel size of 90-500 μm. All scans were examined by a single trained observer twice with a time interval of two weeks. Axial, coronal, and sagittal planes were evaluated under standardized conditions, and pathologic findings were recorded as present or absent. If present, pathologic findings were categorized as mucosal thickening, polypoidal mucosal thickening, sinus opacification, and others.

Mucosal thickening was measured from the air–mucosal interface to the inner margin of the bone lining the sinus and was noted when the distance was greater than 3 mm. Dome shape opacity within the maxillary sinus was considered as polypoidal mucosal thickening. Polyps and retention cysts are seen as smooth, convex radiopacities and cannot be differentiated. Hence, both were included under polypoidal thickening. Both complete and partial radiopacity of the sinus was included under sinus opacification (Figure 1). Antroliths, septa, and discontinuity of the sinus floor were included under other findings (Figure 2). The data, thus, recorded was tabulated and statistically analyzed.

Main Points

- Maxillary sinus abnormalities are highly prevalent in asymptomatic subjects.
- The most common pathology is mucosal thickening followed by mucosal polyps and sinus opacification.
- Oral and maxillofacial radiologists should record any changes within the maxillary sinus noted during CBCT for dental purposes in order to ensure appropriate diagnosis and management.

Statistical Analysis

The Statistical Package for the Social Sciences version 26.0 (IBM SPSS Corp.; Armonk, NY, USA) was used to analyze the data. Qualitative data were presented by using counts and percentages. The association between categorical variables was tested by using Chi square test. $P < .05$ was considered statistically significant.

RESULTS

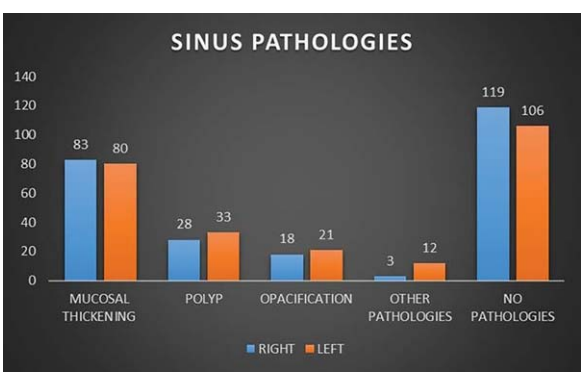
A total of 683 scans were identified out of which 252 met the inclusion criteria and were analyzed. Thus, a total of 504 sinuses

Table 1. Comparison of Pathologies based on Age Groups

			Age					
			≤30		>30			
		N	Count	Column N %	Count	Column N %	Chi square	*P* value
Right mucosal thickening	Absent	169	95	69.90	74	63.80	1.041	.308
	Present	83	41	30.10	42	36.20		
Left mucosal thickening	Absent	172	102	75.00	70	60.30	6.205	**.013**
	Present	80	34	25.00	46	39.70		
Right opacification	Absent	234	124	91.20	110	94.80	1.258	.262
	Present	18	12	8.80	6	5.20		
Left opacification	Absent	231	122	89.70	109	94.00	1.487	.223
	Present	21	14	10.30	7	6.00		
Right polyp	Absent	224	124	91.20	100	86.20	1.565	.211
	Present	28	12	8.80	16	13.80		
Left polyp	Absent	219	118	86.80	101	87.10	0.005	.943
	Present	33	18	13.20	15	12.90		
Right other findings	Absent	244	132	97.10	112	96.60	0.052	.819
	Present	8	4	2.90	4	3.40		
Left other findings	Absent	240	129	94.90	111	95.70	0.097	.756
	Present	12	7	5.10	5	4.30		
Right no findings	Absent	158	84	61.80	74	63.80	0.11	.74
	Present	94	52	38.20	42	36.20		
Left no findings	Absent	159	83	61.00	76	65.50	0.541	.462
	Present	93	53	39.00	40	34.50		

Left mucosal thickening was significantly greater in subjects above 30 years of age

Figure 3.. Sinus pathologies in the study group.



were evaluated for any abnormality. Highest incidence of pathologies was in the 20-29 years age group. On comparison, pathologies were similar across age groups with no statistically significant difference except for left mucosal thickening which was significantly greater in subjects above 30 years of age (Table 1). Male predilection was observed with 61.9% pathologies in males as compared to 38.1% in females, and the difference was statistically significant. The overall incidence of maxillary sinus pathologies was 68.2%. Both sinuses showed changes in 39% cases; 29% cases had unilateral findings, while 32% cases had no sinus abnormalities. Mucosal thickening was noted in the right sinus in 32.9% cases and in the left sinus in 31.7% cases; opacification was noted in the right sinus in 7.1% cases and in the left sinus in 8.3% cases; polyps were noted in the right sinus in 11.1% cases and in the left sinus in 13% cases; and other findings were noted in the right sinus in 3.1% cases and in the left sinus in 4.7% cases (Figure 3). Mucosal

Table 2. Comparison of Pathologies Based on Gender

			Sex					
			Male		Female			
		N	Count	Column N %	Count	Column N %	Chi square	*P* value
Right mucosal thickening	Absent	169	103	66.00	66	68.80	0.2	.655
	Present	83	53	34.00	30	31.20		
Left mucosal thickening	Absent	172	101	64.70	71	74.00	2.329	.127
	Present	80	55	35.30	25	26.00		
Right opacification	Absent	234	141	90.40	93	96.90	3.774	.052
	Present	18	15	9.60	3	3.10		
Left opacification	Absent	231	140	89.70	91	94.80	1.983	.159
	Present	21	16	10.30	5	5.20		
Right polyp	Absent	224	135	86.50	89	92.70	2.291	.13
	Present	28	21	13.50	7	7.30		
Left polyp	Absent	219	133	85.30	86	89.60	0.978	.323
	Present	33	23	14.70	10	10.40		
Right other findings	Absent	244	154	98.70	90	93.80	4.772	**.029**
	Present	8	2	1.30	6	6.20		
Left other findings	Absent	240	148	94.90	92	95.80	0.121	.728
	Present	12	8	5.10	4	4.20		

In the right maxillary sinus, other findings such as antroliths and floor defects were significantly higher in females.

thickening, opacification, and polyps were higher in males on both left and right sides. Females showed an increase in incidence in other findings like antroliths and sinus floor defects such as septa and discontinuity of the sinus floor more on the right side. The difference in occurrence of these findings between males and females was not statistically significant. However, in relation to the other findings in right maxillary sinus which included antroliths and floor defects, the difference was statistically significant with more cases observed in females (Table 2).

DISCUSSION

Preoperative 3D imaging is essential for both diagnosis and formulation of an appropriate treatment plan. Pathologies of the maxillary sinus can be identified in many cases necessitating the modification of dental treatment. CT prior to invasive endoscopic sinus surgery has been associated with decreased risk of complications due to early identification of anatomical variants.[5] Thus, it provides additional information and helps in surgical management.[6] Some cases may need referral to different specialties for treatment.[7] Sinus floor elevation (SFE) is a common procedure in implant placement in the posterior maxillary region with deficient alveolar height. Septa in the sinus floor can lead to accidental perforation of the Schneiderian membrane during SFE. Preoperative CBCT can identify such variations. Alternate surgical approaches such as the use of "W" shaped or two trapdoors instead of the traditional lateral approach can avoid such complications.[8] In the present study, CBCT scans taken for dental purposes which visualized bilateral maxillary sinuses within the field of view were included.

In the present study, the incidence of maxillary sinus pathologies using CBCT was 68.2%, which is in accordance with the study conducted by Rege et al.[9] An incidence up to 73% was noted by Elwakeel et al.[10] Other studies conducted by Cho and Jung,[11] Ritter et al.,[12] and Raghav et al.[4] found the incidence of maxillary sinus pathologies varying from 37 to 59%. This wide difference in incidence can be attributed to the indication for which CBCT was taken, difference in the imaging modality used, and climate and geographical variations.[9,10] Previous studies have used different imaging methods to detect sinus abnormalities. Hansen et al.[13] found incidental maxillary sinus abnormalities in 66% cases using MRI. Vallo et al.[14] found mucosal changes in 19% subjects using panoramic radiography. Malina-Altzinger et al.[15] compared maxillary sinus

abnormalities in panoramic radiographs and CBCT and found increased risk for false diagnosis when using panoramic radiographs alone. Hahnel et al.[16] compared CT and MRI and found that fine bony details were clearer in CT while MRI is preferred for the visualization of sinusitis. Ritter et al.[12] found that CT and CBCT offer similar visualization quality with CBCT having the advantages of high resolution and lower radiation dose.

The prevalence of pathological findings varies from second to sixth decades of life.[10,12] In the present study, subjects showed higher incidence in the third decade, which is in accordance with the study by Raghav et al.[4] Elwakeel et al.[10] found increased incidence in both second and third decades, while Ritter et al.[12] found maximum incidence in subjects above 60 years of age. The present study excluded CBCT scans in children below 10 years of age due to incomplete sinus development and higher incidence of mucosal thickening and opacification.[9] The present study results showed that there is no variation in the incidence of maxillary sinus pathologies with age.

The present study showed increased maxillary sinus pathologies in males as compared to females, although the difference was not statistically significant, which is similar to the results of Ritter et al.,[12] Da Silva et al.,[17] and Rege et al.[9] Vallo et al.[14] mentioned that males have higher incidence of dental pathologies causing increased mucosal thickening in the maxillary sinus. In contrast, the study by Elwakeel et al,[10] had significantly higher incidence of pathologies in females. Periapical infection in the maxillary molars can extend to the sinus causing mucosal thickening with or without cortical perforation of the sinus floor. Thus, CBCT plays a key role in endodontic treatment planning by allowing visualization of the size and location and relation to anatomic structures in a 3D view.[18]

A higher frequency of pathological findings was found on the right side of the sinus as compared with other studies conducted by Ritter et al.[12] and Rege et al.,[9] but the result was not statistically significant.

The most prevalent finding was mucosal thickening, which is in accordance with the studies conducted by Elwakeel et al.,[10]Rege et al.,[9] Raghav et al.,[4] DaSilva et al.,[17] and Ritter et al.[12] This increased incidence of mucosal thickening in asymptomatic individuals may be due to odontogenic pathologies, allergens, and microbial infections. These manifest in the maxillary sinus as linear mucosal thickening which may progress to partial and total opacification. Ata-Ali et al.[19] in their systematic review of 23 studies including a total of 11,971subjects found that mucosal thickening, sinusitis, and sinus opacification were the most common sinus pathologies identified in CBCT scans of the maxillofacial region. Amine et al.[8] reported that mucosal thickening as the most frequent finding. Maillet et al.[18] in their study on maxillary sinusitis found an average mucosal thickening of 7.4 mm. Mucosal polyps showed the next higher incidence followed by opacification and other pathologies such as antroliths and floor defects, which is in accordance with the studies conducted by Rege et al.,[9] Rodrigues et al.,[20] Bosio et al.,[21] and Rhodus et al.[22] Some studies have found polypoidal thickenings as the most prevalent finding, while some studies found opacification as the most prevalent finding. This may be due to the system of classification used or the visualization technique used.

There are some limitations in the above study. Since this was a retrospective analysis, data regarding the smoking habits of the subjects were not available which may be a cause for sinus changes in males. Differentiation between polyps and retention cysts could not be done as both are radiographically similar.

CONCLUSION

The present study highlights the high incidence of maxillary sinus changes in asymptomatic individuals and the role of CBCT in the identification of sinus pathologies. This study also emphasizes that oral radiologists should carefully examine and report all changes of the sinus in CBCT images taken for dental purposes so that appropriate management of sinus abnormalities can be done.

Ethics Committee Approval: from AB Shetty Memorial Institute of Dental Sciences, Mangalore, India (ABSM/EC/11/2020).

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Original Article

COVID-19 Seroprevalence among Healthcare Workers in a University Hospital in Southeastern Turkey

Tekin Karslıgil[1], Hüseyin Akdoğan[2]

[1]Department of Medical Microbiology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

[2]Department of Medical Microbiology, Gaziantep University Institute of Health Science, Gaziantep, Turkey

ABSTRACT

Objective: In our study, IgG and IgA antibodies were investigated by using the ELISA method, especially in healthcare workers (HCWs) who were more likely to encounter infection as of June 2020, and it was aimed to determine the level of HCWs being affected by the pandemic.

Methods: A total of 186 volunteer HCWs from different professions working in different departments were included in the study. Serum was obtained by taking 5 mL of blood samples from the volunteers. The presence of IgA and IgG antibodies against the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus was investigated in the sera by using the ELISA method.

Results: The mean age of the participants in the study was 34.22 (±7.85), and 71 (38.2%) were female. One hundred and eighty participants tested for SARS-CoV-2 IgA antibodies, and eight (4.4%) of them were found positive. One hundred and eighty-six participants tested for SARS-CoV-2 IgG antibodies, and five (2.7%) of them were found positive. The highest antibody positivity was detected in the intensive care unit and doctors.

Conclusion: At the end of the study, low seropositivity rates were found. However, the risk of infection in HCWs increased in proportion to the continuation of the pandemic and the increase in cases. HCWs took the necessary precautions to minimize the infection. Investigating the presence of antibodies in HCWs at regular intervals will help to calculate the risk among HCWs.

Keywords: COVID-19, healthcare workers, seroprevalence

INTRODUCTION

COVID-19 is an infectious disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2), which appeared in Wuhan, China, in late 2019 and now causing worldwide outbreak.[1] The World Health Organization (WHO) announced COVID-19 as a pandemic on March 11, 2020.[2] As of October 25, over 43 million cases and over 1 million deaths occurred worldwide.[3] The first case was reported on March 11 in Turkey. Until today (November 1), a total of 377,473/14,125,157 (2.67%) confirmed cases, 10,326 deaths, and 325,486 recoveries were reported in Turkey.[4]

Currently, the detection of SARS-CoV-2 RNAs by using molecular methods is applied as a standard for the diagnosis of COVID-19. In addition, reliable serological methods are needed to identify people who are infected or have had the disease. As a result of the last studies, serological tests based on the detection of antibodies specific for SARS-CoV-2 were identified.[5–8] In a study, receptor binding domain (RBD)-specific IgA, IgM, and IgG kits showed 98.6%, 96.8%, and 96.8% sensitivity and 98.1%, 92.3%, and 99.8% specificity, respectively. After 4-10 days following the onset of symptoms, IgA showed the highest positive diagnostic rate. After 11-41 days after the onset of symptoms, both RBD IgA and IgG had the same positive diagnostic rate as 99.5%, while IgM was low.[9]

The main mode of transmission of COVID-19 occurs from person to person.[10] Healthcare workers (HCWs) are the most important people for clinical monitoring of suspected or confirmed cases. For this reason, they contact with patients more frequently and are more likely to get sick and spread the disease to other HCWs.[11]

Seroprevalence studies on HCWs can provide infection recently or in the past. In addition, determining the frequency of infection among HCWs will help in identifying high-risk departments and important for efficient use of HCWs.[12]

The aim of this study is to investigate the presence of antibodies against SARS-CoV-2 of HCWs working at University Hospital of Gaziantep during the pandemic and the effect of the disease on profession groups and units.

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ORCID iDs of the authors: T.K. 0000-0001-7672-3625; H.A. 0000-0002-0536-4300.

Corresponding Author: Tekin Karslıgil **E-mail**: karsligil@gantep.edu.tr

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METHODS

Study Population

Doctors, nurses, laboratory technicians, radiology technicians, assistant nurses, emergency medical technicians (EMT), and cleaning staff who have been working at the University Hospital of Gaziantep since the beginning of the pandemic were included in this study. These HCWs were randomly and voluntarily selected among the HCWs working in the emergency, infectious diseases, radiology, pulmonary diseases and otolaryngology departments, intensive care unit (ICU), and laboratory.

Serological Analysis

Five milliliters of the blood sample was collected from HCWs who agreed to participate in the study. Sera samples were stored until used. SARS-CoV-2 IgG and SARS-CoV-2 IgA antibodies were detected in sera samples using a semiquantitative anti-SARS-CoV-2 IgA and IgG enzyme-linked immunosorbent assay kits (Euroimmun Medizinische Labordiagnostika, Lübeck, Germany) with an automated device according to the manufacturer's instructions. The results obtained were recorded for statistical analysis.

This study was performed after the ethical approval from Ethics Committee of Gaziantep University and the Republic of Turkey Ministry of Health (06.05.2020, 2020/165).

Participants in the study were informed about the study, and their participation approvals were obtained.

RESULTS

Overall, 186 HCWs including seven different profession groups, who have been working since the beginning of the pandemic, participated in the study, in which 115 (61.8%) were male and 71 (38.2%) female. The mean age of participants was 34.22 (±7.85). In 186 participants, 64 (34.4%) were doctors, 45 (24.2%) nurses, 31 (16.7%) laboratory technicians, 50 (26.9%) working in emergency department, 41 (22%) working in laboratory, and 32 (17.2%) working in ICU. Sixty-four (34.4%) HCWs stated that they smoke. Thirty (16.1%) had chronic diseases (respiratory disease, cardiovascular diseases, diabetes mellitus, gastrointestinal diseases, etc.). Eighty-eight (47.3%) HCWs stated that they experienced mild COVID-19 symptoms such as fatigue and malaise, sore throat, cough, headache, and general pain, during the pandemic period, but only three had a PCR test for severe symptoms (Table 1). They

Table 1. Subject Characteristics

Sex	
Male	115 (61.8%)
Female	71 (38.2%)
Age	34.22 ± 7.85
Profession	
Doctor	64 (34.4%)
Nurse	45 (24.2%)
Laboratory technician	31 (16.7%)
EMT	6 (3.2%)
Radiology technician	7 (3.8%)
Assistant nurse	24 (12.9%)
Cleaning staff	9 (4.8%)
Departments	
Emergency	50 (26.9%)
Infectious diseases	25 (13.4%)
Pulmonary diseases	12 (6.5%)
Otolaryngology	15 (8.1%)
Laboratory	41 (22%)
Radiology	11 (5.9%)
ICU	32 (17.2%)
Smoking	
Yes	64 (34.4%)
No	122 (65.6%)
Chronic diseases	
Yes	30 (16.1%)
No	156 (83.9%)
Symptoms of COVID-19	
Yes	88 (47.3%)
No	98 (52.7%)
Total	186 (100%)

EMT, emergency medicine technician; ICU, intensive care unit.

Main Points

- Seroprevalence studies conducted on HCWs during the pandemic provide information on the extent to which the pandemic affected HCWs.
- In this study, we investigated the presence of SARS-CoV-2 IgA and IgG antibodies in HCWs working in units that can be considered as risky for infection.
- Results of the study showed a low level of seropositivity in HCWs.
- The department with the highest seropositivity was intensive care. The profession with the highest seropositive rate was doctors.
- Regular antibody screening of HCWs is important for both providing information about the status of the epidemic and the effective use of HCWs.

Table 2. Results of IgA and IgG Tests

Result	IgA	IgG
Positive (ratio ≥1.1)	8 (4.4%)	5 (2.7%)
Border (ratio ≥0.8-<1.1)	6 (3.3%)	2 (1.1%)
Negative (ratio <0.8)	166 (92.3%)	179 (96.2%)
Total	180	186

Table 3. Seropositivity by Sex

Sex	IgA*			IgG		
	Positive	Border	Negative	Positive	Border	Negative
Male	4 (3.5%)	4 (3.5%)	105 (93%)	3 (2.6%)	1 (0.9%)	111 (96.5%)
Female	4 (6%)	2 (3%)	61 (91%)	2 (2.8%)	1 (1.4%)	68 (95.8%)

*180 HCWs were evaluated for SARS-CoV-2 IgA.

Table 4. The Presence of Contact, Contact Time, and the Effect of PPE Use on SARS-CoV-2 Antibody Positivity

		IgA*			IgG		
		Positive	Border	Negative	Positive	Border	Negative
Contact							
Yes	161 (86.6%)	7 (4.5%)	6 (3.8%)	143 (91.7%)	4 (2.5%)	2 (1.2%)	155 (96.3%)
No	25 (13.4%)	1 (4.2%)	-	23 (95.8%)	1 (4%)	-	24 (96%)
Contact time							
<2 minutes	10 (6.2%)	-	-	10 (100%)	-	-	10 (100%)
2-5 minutes	44 (27.3%)	2 (4.9%)	2 (4.9%)	37 (90.2%)	1 (2.3%)	1 (2.3%)	42 (95.4%)
>5 minutes	107 (66.5%)	5 (4.8%)	4 (3.8%)	96 (91.4%)	3 (2.8%)	1 (0.9%)	103 (96.3%)
PPE							
Yes	117 (72.7%)	4 (3.5%)	3 (2.6%)	107 (93.9%)	1 (0.9%)	2 (1.7%)	114 (97.4%)
Partially	25 (15.5%)	1 (4.3%)	2 (8.7%)	20 (87%)	1 (4%)	-	24 (96%)
No	19 (11.8%)	2 (10.5%)	1 (5.3%)	16 (84.2%)	2 (10.5)	-	17 (89.5%)

*180 HCWs were evaluated for SARS-CoV-2 IgA.

have previously had the disease and confirmed by PCR. One of them (33.3%) was working in ICU as a nurse, one of them (33.3%) was working in laboratory as a technician, and one of them (33.3%) was working in otolaryngology as a doctor. All of these HCWs were found to be seropositive for both antibodies.

The data obtained at the end of the study were evaluated according to the manufacturer's instructions as negative if ratio <0.8, border if ratio ≥0.8-<1.1, and positive if ratio ≥1.1.

Hundred and eighty participants tested for SARS-CoV-2 IgA antibodies. Of these, eight (4.4%), six (3.3%), and 166 (92.3%)

Table 5. The Range of IgA Results According to Profession Groups and Departments

		Professions						
Departments	**Results**	**Doctor**	**Nurse**	**Laboratory technician**	**EMT**	**Radiology technician**	**Asst. nurse**	**Cleaning staff**
	Positive	-	1	-	-	-	-	-
Emergency	Border	1	1	-	-	-	-	-
	Negative	19	14	-	6	-	5	3
	Positive	1	-	-	-	-	-	-
Infect. dis.	Border	1	-	-	-	-	-	-
	Negative	6	11	-	-	-	6	-
	Positive	1	-	-	-	-	-	-
Pulmonary dis.	Border	-	-	-	-	-	-	-
	Negative	3	2	-	-	-	2	1
	Positive	1	-	-	-	-	-	-
Otolaryngology	Border	1	-	-	-	-	-	1
	Negative	9	2	-	-	-	1	-
	Positive	-	-	1	-	-	-	-
Laboratory	Border	-	-	-	-	-	-	-
	Negative	8	-	28	-	-	-	1
	Positive	-	-	-	-	-	-	-
Radiology	Border	-	-	-	-	-	-	-
	Negative	3	-	-	-	7	-	1
	Positive	1	1	-	-	-	1	-
ICU	Border	-	-	-	-	-	1	-
	Negative	9	12	-	-	-	6	1

EMT: emergency medicine technician; ICU: intensive care unit.

HCWs were detected for SARS-CoV-2 IgA as positive, border, and negative, respectively. Hundred and eight six participants tested for SARS-CoV-2 IgG antibodies. Of these, five (2.7%), two (1.1%), and 179 (96.2%) HCWs were detected for SARS-CoV-2 IgG as positive, border, and negative, respectively (Table 2).

The SARS-CoV-2 IgA was positive in four of 113 (3.5%) male and four of 67 female (6%) HCWs. SARS-CoV-2 IgG was positive in three of 115 (2.6%) male and two of 71 female (2.8%) HCWs (Table 3). No significant difference was found between male and female in terms of SARS-CoV-2 positivity.

One hundred and sixty-one of 186 (86.6%) participants stated that they had contact with a sample or patient (confirmed or suspected), and it was determined that seven (4.5%) were positive for SARS-CoV-2 IgA and four (2.5%) were positive for SARS-CoV-2 IgG. The contact time of the HCWs with the patient or sample (confirmed or suspected) was determined: for <2 minutes, 10 of 161 (6.2%) participants had contact and all of them were negative for both the antibodies; between 2 and 5 minutes, 44 of 161 (27.3%) had contact of which two (4.9%) were positive for SARS-CoV-2 IgA and two (4.9%) were positive for SARS-CoV-2 IgG; and for >5 minutes ,107 of 161 (66.5%) had contact of which five (4.8%) were positive for SARS-CoV-2 IgA and three (2.8%) were positive for SARS-CoV-2 IgG. Of these HCWs, 117 of 161 (72.7%) had full personal protective equipment (PPE), 25 of 161 (15.5%) partially, and 19 of 161 (11.8%) had no PPE, in which 4 (3.5%), 1 (4.3%), and 2 (10.5%) were positive for SARS-CoV-2 IgA and 1 (0.9%), 1 (4%), and 2 (10.5%) were positive for SARS-CoV-2 IgG, respectively. A laboratory

Table 6. The Range of IgG Results According to Profession Groups and Departments

		Professions						
Departments	**Results**	**Doctor**	**Nurse**	**Laboratory technician**	**EMT**	**Radiology technician**	**Asst. nurse**	**Cleaning staff**
	Positive	-	-	-	-	-	-	-
Emergency	Border	-	-	-	-	-	-	-
	Negative	20	16	-	6	-	5	3
	Positive	1	-	-	-	-	-	-
Infect. dis.	Border	1	-	-	-	-	-	-
	Negative	6	11	-	-	-	6	-
	Positive	-	-	-	-	-	-	-
Pulmonary dis.	Border	-	-	-	-	-	-	-
	Negative	4	3	-	-	-	4	1
	Positive	2	-	-	-	-	-	-
Otolaryngology	Border	-	-	-	-	-	-	-
	Negative	9	2	-	-	-	1	1
	Positive	-	-	1	-	-	-	-
Laboratory	Border	-	-	1	-	-	-	-
	Negative	8	-	29	-	-	-	2
	Positive	-	-	-	-	-	-	-
Radiology	Border	-	-	-	-	-	-	-
	Negative	3	-	-	-	7	-	1
	Positive	-	1	-	-	-	-	-
ICU	Border	-	-	-	-	-	-	-
	Negative	10	12	-	-	-	8	1

EMT, emergency medicine technician; ICU, intensive care unit.

technician was positive for both the antibodies despite not making contact (Table 4).

The department with the highest SARS-CoV-2 IgA positivity was in the ICU (three of 32, 9.4%), and the positive HCWs had different professions. In the radiology department, no HCW with SARS-CoV-2 IgA positive was found. The profession with the highest SARS-CoV-2 IgA positivity was doctors (four of eight, 50%) and they do not work in the same department (Table 5). The department with the highest SARS-CoV-2 IgG positivity was in the otolaryngology department (two of five, 40%) and both were working as doctors. In the radiology, emergency, and pulmonary diseases departments, no HCW with SARS-CoV-2 IgG positive was found. The profession with the highest SARS-CoV-2 IgG positivity was doctors (three of five, 60%) (Table 6).

DISCUSSION

In our study, we found that the seropositivity prevalence of both the antibodies was low in HCWs. The SARS-CoV-2 IgA seropositivity rate was higher than that of SARS-CoV-2 IgG. Likewise, in the border value range, SARS-CoV-2 IgA was higher than that of SARS-CoV-2 IgG. As a result of some studies, the seropositivity rates of SARS-CoV-2 antibodies on HCW were found to be at low levels. For example, Korth et al.[13] found a lower level of SARS-CoV-2 IgG positivity in their study on 316 HCW (5/316, 1.6%). In another study conducted in China, 18 of 105 (17.14%) HCWs who contacted four confirmed patients were found to be seropositive.[14] As a result of the study conducted in a large study group in Sweden, 410 (19.1%) HCWs were found to be positive for SARS-CoV-IgG.[15] In the study conducted by Garcia-Basterio et al.,[16] 54 of 578 HCWs were found to be seropositive, and a higher level of

SARS-CoV-2 IgA (8.1%) and SARS-CoV-2 IgG (7.6%) was found. In addition, they detected 6.2% positivity of SARS-CoV-2 IgM. When the effects of SARS-CoV-2 IgA and IgG positivity on sex were examined, no significant difference was observed ($P > .05$). Xu et al.,[17] in their seroprevalence study on a study group including HCWs, stated that there was no significant difference in seropositivity rates between sexes. Rudberg et al.[15] found that there was no difference between seropositive and seronegative individuals in terms of sex as a result of their studies. These results showed that both sexes were equally likely to be infected.

No significant difference was observed for both SARS-CoV-2 IgA and IgG between departments and seropositivity ($P > .05$). While the department with the most SARS-CoV-2 IgA positive HCW was ICU, it was otolaryngology for SARS-CoV-2 IgG. This may be because the virus affects the respiratory tract, and this department directly contacts the nose and throat of the patients. In the radiology department, all HCWs were negative for both the antibodies. Iversen et al.,[18] as a result of their studies, showed that HCWs working in a dedicated COVID-19 department had a higher seroprevalence compared to other departments.

There was no significant difference in seropositivity for both SARS-CoV-2 IgA and IgG among professional groups ($P > .05$). However, the doctors reported most positivity among the HCWs who were positive for both the antibodies, while the EMT and radiology technicians were all negative for both the antibodies. However, this situation showed that the risk level of the professions with close and continuous contact, as in departments, is higher than the others. As a result of the research conducted by Chen et al.,[14] seven of 17 doctors who contacted four patients were found to be positive. In other studies, it was found that those working in the frontline or high and intermediate-risk groups in the hospital had higher seroprevalence than others.[13,18]

There was also no significant difference between the use of PPE and contact with the patient or sample (confirmed or suspected) and seropositivity ($P > .05$). However, the full use of PPE and the reduction in contact time significantly reduced the rate of seropositivity as it reduced the possibility of infection. In addition, these measures reduced the transmission of infection from the HCW to HCW.

CONCLUSION

In our study, we investigated the rate at which HCWs encountered COVID-19 at the beginning of the pandemic. Although the rates were found to be low at the end of our study, as the number of sick individuals in the community increases, the risk of infection in HCW will increase. Therefore, we think that HCWs should be more careful in their fight against the virus. In addition, we think that antibody tests will be effective in detecting seroprevalence in healthcare professionals and the community.

Ethics Committee Approval: This study was performed after the ethical approval from Ethics Committee of Gaziantep University and the Republic of Turkey Ministry of Health (06.05.2020, 2020/165).

Informed Consent: Participants in the study were informed about the study, and their participation approvals were obtained.

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Original Article

Comparison of the Tricuspid Valve Function with or without Tricuspid Valve Detachment in Closure of Ventricular Septal Defect VSD Closure with Tricuspid Valve Detachment

Mehmet Asam[1], Erkan Kaya[2]

[1]Paediatric Cardiovascular Surgery, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

[2]Cardiovascular Surgery, SANKO University Faculty of Medicine, Gaziantep, Turkey

ABSTRACT

Objective: Ventricular septal defect (VSD) is defined as a defect in the interventricular septum. It is the second most prevalent congenital heart disease following bicuspid aortic valve and makes up 5% of congenital heart diseases. Although most VSDs tend to close on their own in the first year of life, larger defects should be percutaneously or surgically closed to prevent right ventricular strain and right ventricular failure. Considering the frequency of the procedure, a safe and effective closure without tricuspid valve and atrioventricular node injury is vital.

Methods: We retrospectively included 165 patients with a diagnosis of VSD who underwent surgical closure. Depending on the excised leaflet of the tricuspid valve, the patients were divided into two groups: 86 patients (Group 1) had their anterior leaflet excised, while 79 patients (Group 2) had their posterior leaflet excised. The diagnosis was based on the results of preoperative catheter angiography and echocardiography. Echocardiography was repeated on the 1st week, 1st month, and 6-12th month to evaluate postoperative residual VSD and postoperative tricuspid regurgitation.

Results: The aortic cross-clamp time, cardiopulmonary bypass time, duration of intubation, length of stay in intensive care unit and hospital, postoperative residual VSD, postoperative tricuspid regurgitation, and postoperative morbidity and mortality were evaluated in patients in Groups 1 and 2. Tricuspid regurgitation or dysfunction was not detected in any group. Furthermore, no other parameters differed between two groups.

Conclusion: Our study has shown that elaborate tricuspid leaflet incision for adequate visualization allows a safe and effective closure of VSD.

Keywords: Ventricular septal defect, tricuspid valve, tricuspid regurgitation

INTRODUCTION

Ventricular septal defect (VSD) is the most common congenital heart defect after bicuspid aortic valve, and it is seen at 2-6 per 1,000 live births.[1] VSDs are likely to close spontaneously in the first year of life. However, since larger defects will cause excessive volume burden to the lungs and right heart failure, treatment of these defects is often surgical.[2]

The main point in VSD surgery is the safe and complete closure of the defect without damaging the tricuspid valve and atrioventricular node. Therefore, sufficient surgical field of vision is required to ensure a successful closure.[3] Retraction of the tricuspid valve may be sufficient, but the retraction procedure alone is insufficient for a clear assessment of the boundaries and relationships of some defects, resulting in a lower chance of surgical success.[4] Excessive retractions of the tricuspid valve for maintaining surgical vision can cause injury to the leaflets and/or cordal structures.[5] In order to avoid injury, reveal the true limits of the defect, and ensure successful closure, incision of the tricuspid valve leaflets in the radial and circumferential style is preferred.[6–9]

In this study, we aimed to compare the results of tricuspid valve functions at the post-operative period in patients with the diagnosis of isolated VSD, who have or have not undergone tricuspid valve leaflet detachment procedure by radial and circumferential incision in our clinic between 2007 and 2015.

METHODS

In the Department of Cardiovascular Surgery of Gaziantep University Faculty of Medicine Şahinbey Practice and Research Hospital, 165 patients diagnosed with VSD and surgically closed VSD were retrospectively examined between the years of 2007 and 2015. In patients operated in Group 1, VSD was

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ORCID iDs of the authors: M.A. https://orcid.org/0000-0003-2261-8680; K. E. https://orcid.org/0000-0002-5369-4689.

Corresponding Author: Mehmet Asam **E-mail:** drmehmetasam@gmail.com

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Table 1. Preoperative Catheter, Preoperative Echocardiographic Values and Anatomical Location of VSD

	Group 1	Group 2	*P*
PAB (mmHg) (ÇN: terimlerin İngilizce versiyonları gelecek PAP gibi)	30 ± 2.08	29 ± 2.18	>.05
QP/QS	3.7 ± 1.98	3.9 ± 1.68	>.05
EF (%)	65.7 ± 2.08	63.1 ± 1.5	>.05
FK (%)	45.2 ± 1.65	43.0 ± 1.36	>.05
LVH (mL)	22.6 ± 4	21.6 ± 1.77	>.05
LVEDD (mm)	31.3 ± 1.2	30.2 ± 1.3	>.05
LVESD (mm)	12.9 ± 1.18	15.3 ± 1.08	>.05
Perimembranous outlet VSD	76	65	>.05
Perimembranous inlet VSD	4	5	>.05
Muscular outlet VSD	3	2	>.05
Muscular trabecular VSD	2	1	>.05
Subarterial VSD	1	6	>.05

operated by separating tricuspid valve leaflets, and in Group 2, VSD was directly closed. All patients in Groups 1 and 2 were operated by the same surgical team. Intensive care and inpatient follow-up of the patients were also performed by the same surgical team. Ethics committee approval was received for this study from the ethics committee of Gaziantep University Faculty of Medicine Sahinbey Practice and Research Hospital (18.05.2015-2015/167).

Surgical Technique

All patients were operated under general anesthesia. The patient's skin was encased by sterile covering after surgical cleaning and prepared for the operation. Median sternotomy was performed. Following bicaval and aortic cannulation, a cross-clamp was placed and cardiac arrest was achieved using crystalloid cardioplegia. Moderate hypothermia (32-34°C) was achieved in all patients. VSD was closed by right atriotomy in 158 patients by pulmonary artery way in three patients and by transaortic way in four patients. VSDs were closed using Dacron patch material or autologous pericardial patch material fixed with gluteraldehyde. When closing VSDs, 5/0 or 6/0 prolene suturing and continuous suture technique were used in all patients. In patients where the tricuspid anterior-septal leaflet was cut prior to VSD closure, the circumferential incision was repaired using the continuous suturing method, and the radial septal incision was repaired using the interrupted suture technique with 6/0 prolene sutures. In order to understand the tricuspid leak after the procedure, pulmonary artery occlusion right ventricle was filled quickly with SF by using a 50 cc syringe, and it was evaluated whether there is any leak or not. The right atrium was closed with two rows of prolene sutures using a continuous suture technique, and the aortic cross clamp was removed. After warming up and air removal procedures, the patient was separated from cardiopulmonary-bypass. After controlling bleeding, pacemaker wires and chest drainage tubes were placed, and the operation was terminated by closing the layers according to the anatomy.

Main Points

- Ventricular septal defect is the most common congenital heart defect after bicuspid aortic valve, and it is seen at 2-6 per 1000 live births.
- In our study, no statistically significant difference was found between the two groups in terms of tricuspid regurgitation.
- The results of the surgical applications performed by separating the anterior and the septal leaflet either circumferentially or radially are close to perfection.

The Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) program was used for statistical calculations. For parametric variables, two independent group comparisons, student t test, and nonparametric variables Mann–Whitney U test were performed. Results with $P < .05$ were interpreted as statistically significant. In cases where both variables are categorical, Chi-square, Fisher's exact, or Mantel–Hansel chi-square tests were performed, and again $P < .05$ was interpreted as statistically significant.

RESULTS

Preoperative echocardiographic examination revealed no significant aortic insufficiency in both the groups. Preoperative echocardiography revealed no tricuspid insufficiency in Group 1, and five patients with moderate tricuspid insufficiency in Group 2. There was no statistical difference when both the groups were evaluated in terms of preoperative echocardiographic features. Also, when preoperative catheter findings of both the groups were compared, no statistically significant difference was found (Table 1). When we look at the location of

Table 2. Cardio-Pulmonary By-pass Time, Aortic Cross Clamp Time, Extubation Time of the Patients According to the Groups, Intensive Care Time, Chest Tube Length of Stay, Post-surgery Hospitalization Time, and Post-Operative Echocardiography Values of the Patients

	Group 1 (mean ± SD)	Group 2 (mean ± SD)	*P*
CPB (minute)	74.7 ± 11.2	72.3 ± 12.3	>.05
ACC (minute)	52.3 ± 9.7	44.5 ± 12.1	>.05
Intubation (hour)	7.9 ± 3.6	8.2 ± 2.94	<.05
Intensive care times (day)	1.8 ± 0.23	1.7 ± 0.48	>.05
Chest tubes length of stay (day)	1.4 ± 0.27	1.9 ± 0.14	>.05
Discharge time (day)	5.75 ± 1.87	6.58 ± 0.77	>.05
Postoperative ECO EF (%)	60.8 ± 8.71	64.76 ± 8.23	>.05
FK (%)	29.7 ± 6.3	30.1 ± 5.8	>.05
LVH (mL)	18.2 ± 10.7	17.5 ± 8.59	>.05
LVEDD (mm)	24.3 ± 6.13	22.8 ± 5.7	>.05
LVESD (mm)	16.7 ± 3.3	14.6 ± 4.81	>.05

VSD, there was no statistically significant difference between the two groups (Table 1).

No statistically significant difference was found between the patients in both the groups in terms of cardiopulmonary bypass and aortic cross-clamp times (Table 2).

When the average extubation time, length of stay in the ICU, duration of the chest drainage tubes, and length of hospital stay were compared, there was no statistically significant difference between the two groups (Table 2).

When both the groups were compared in terms of A-V full block, there was no statistically significant difference between the two groups ($P > .05$). In both the groups, deep wound infection and mediastinitis were not observed in the post-operative period. There was no early mortality in either group.

There was no statistically significant difference in the echocardiographic examinations performed in the first post-operative period, when both th were compared (Table 2). In both the groups, no aneurysm formation on the patch placed on VSD that is narrowing the ventricular outflow tract was observed.

The mean follow-up time of the patients was 23 ± 7.19 months in Group 1 and 21.3 ± 5.64 months in Group 2. When the groups were compared in terms of residual ventricular septal defect, there was no statistically significant difference between the two groups, and residual ventricular septal defects were hemodynamically insignificant.

In final post-operative echocardiographic examinations, mild tricuspid regurgitation was detected in three patients in Group 1 and five patients in Group 2. There were no patients with severe insufficiency. No statistically significant difference was found between the two groups in terms of tricuspid regurgitation. At the follow-up period, infective endocarditis was not detected clinically and echocardiographically in both the groups.

DISCUSSION

Considering that VSD is the second most common disease among congenital heart defects is seen with other congenital heart defects, or is a component of some congenital malformations, it can be said that its incidence is gradually increasing. While the incidence is so high, it is important to close the defect safely and completely without any damage to the tricuspid valve or A-V node.

Most VSDs tend to close spontaneously in the first year of life, and this rate draws a curve that decreases with age from the first months. Other than spontaneous closure, invasive closure is preferred in VSD treatment. Although some of the VSDs have been successfully closed with transcatheter or hybrid approaches thanks to the advances in invasive cardiology in recent years, many centers still prefer transcatheter methods in muscular defects or in closing post-operative residual defects, especially due to the high A-V full block and aortic valve injury rates that appear after VSD closure, and surgical treatment is still the most commonly used method of VSD closure.

Patch material is often used when closing VSD, and very small defects can also be closed primary. The most commonly used patch materials during VSD closure are synthetic materials. Dacron material is used most frequently among synthetic materials, and polytetrafluoroethylene (PTFE) used secondly.

As the closure of residual VSDs is delayed in the post-operative period, patients are at risk for infective endocarditis and

continued pulmonary overload. At the same time, due to excessive pulling motion to increase the surgical field of vision during the closure of VSD, tricuspid insufficiency, right atrial dilatation, atrial arrhythmias, and right heart failure may occur due to tricuspid valve and chorda damage. It is very important to observe the exact defect boundaries and neighborhoods during the VSD closure to avoid the occurrence of residual defect, tricuspid valve, and A-V node damage.

In 1962, Hudspeth et al.[7] described the incision or partial detachment of the tricuspid valve to provide sufficient visibility during VSD closure. In this publication of the authors consisting of eight patients, the tricuspid valve was removed from the annulus with a circumferential incision, thereby demonstrating an increased visibility.

Gaynor et al.[6] separated the tricuspid valve from the annulus by making a circumferential incision along the tricuspid anterior and septal leaflet and reported that after this procedure, tricuspid insufficiency, rhythm problems, and residual defect have not been occurred. They also stated that there was no statistically significant difference in terms of cardiopulmonary bypass time, aortic cross-clamp time, and post-operative hospital stay. They also emphasized that in the group in which the tricuspid valve was removed, the rate of reoperation was lower than in the other group, and the tricuspid regurgitation (TR) rate was minimal in the group where the tricuspid valve was removed, but in the other group, six patients had moderate tricuspid regurgitation. In the literature, there are similar results from different centers.[10–13] In their study, Kapoor et al. described a technique in which they performed a chordal detachment from the septum and performed a cordal reimplantation after closing the defect; as a result, they detected residual VSD in only one patient and did not provide any information about TR. However, there are no long-term results regarding this technique, and we think that severe tricuspid valve deficiency will occur in the case of detachment after cordal reimplantation of the septum.[11]

Bol-Rapp et al. applied tricuspid detachment to 39 of 149 patients and showed that the cross-clamp time was slightly longer than the other group. In addition, reoperation was performed due to residual VSD in a patient who did not underwent detachment procedure.[10]

In the 215 case series with isolated VSD, Scully et al.[12] reported that they performed retraction to see the defect limits clearly in the majority of patients, but if they could not see this limit clearly, separating the septal and/or anterior leaflet from the annulus is a very useful technique to see the defect limits.

Russell et al. claim that VSD closure with detachment has very successful results, with very low residual VSD, heart block, or tricuspid regurgitation.[13]

In the 600-patient case series published by Zhao et al., they applied tricuspid valve detachment to 122 patients.[14] They stated that there was no difference between the two groups in terms of total bypass time and aortic cross-clamp time, and in their study, it has been observed that while there were three patients with heart block, 10 patients with residual VSD, and 12 patients with first degree TR in group without detachment procedure, there was only insignificant TR in nine patients in the detachment group.

In the current publications, approximately 25-50% of surgeons make a tricuspid valve incision.[14] In our opinion, the most important concern in this regard is the tricuspid regurgitation that may occur in the post-operative period. Therefore, which patient should have detachment procedure depends on the location of the defect, the valve and chorda structure, and other intracardiac anatomical variations, and most importantly the surgeon's experience.

In our series, there is no statistically significant difference between the group in which the detachment process is applied and the other group in terms of results, and these results are parallel with the literature.

There are numbers of publications related to septal leaflet detachment; in the series of 33 patients who underwent the anterior leaflet separation procedure published by Maile et al., only one patient who they continued to follow had a residual defect, one patient had complete block, and two patients had right bundle branch block.[15]

All these studies are nonrandomized and retrospective. Therefore, patients do not have long-term follow-up echoes.

CONCLUSION

In VSD closure operation, which is the most common pediatric cardiac surgery operation, the important thing is to make the correct surgical timing. Consensus should be provided between the pediatric cardiologist and surgeons for both catheter closure and timing of surgical closure. The results of the surgical applications performed by separating the anterior and the septal leaflet either circumferentially or radially are close to perfection, and as stated earlier, the determining factors in success of these procedures are the well-known intracardiac anatomy and the correct evaluation of the echo and catheter findings, and successful results will continue in line with the increased surgical experience. In our retrospective and nonrandomized study, the data that we obtained could contribute to the circumferential and radial tricuspid valve detachment surgery method. We believe that it can be more meaningful and useful after results of further prospective and randomized studies.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University Faculty of Medicine Şahinbey Practice and Research Hospital (18.05.2015-2015/167).

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Analysis and/or Interpretation - M.A.; Literature Search - M.A.; Writing Manuscript - M.A.; Critical Review - E.K.

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Original Article

Thyroglossal Duct Cysts: A Clinico-Surgical Experience of 100 Cases

İsmail Aytaç, Orhan Tunç

Department of Otorhinolaryngology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

ABSTRACT

Objective: Thyroglossal duct cysts occur if a thyroglossal duct does not disappear after the complete embryonic development of the thyroid gland and becomes cystic. This study aimed to examine the clinical features, physical examination findings, and treatments of 100 patients who underwent surgery with the diagnosis of thyroglossal duct cyst in the midline of the neck.

Methods: This was a retrospective study based on anamnesis forms, clinical examinations, and radiographic imaging of 100 cases diagnosed with and operated on for thyroglossal duct cyst in our clinic.

Results: Of the 100 patients, 53 were males and 47 were females, with their ages ranging from 1 to 62 (mean 18.15 ± 15.8) years. The most common complaints were neck swelling (88%) and intermittent discharge (11%). Concomitant infection and fistula were present in 52 and 30% patients, respectively. The most common localization observed in 67 (67%) patients was in the infrahyoid area. Infection and abscess were observed in six cases (6%) during the early post-operative period. Papillary thyroid carcinoma, in addition to the cyst, was found in four cases. Recurrence was observed in three (3.7%) of the 81 primary cases. Sistrunk procedure was employed in all study patients.

Conclusion: Thyroglossal duct cyst is the most common observed congenital mass in the neck. It must be considered in the differential diagnosis of patients admitted due to discharge and swelling in the midline of the neck. The generally accepted treatment of thyroglossal duct cysts is surgery, with Sistrunk surgery being the most appropriate surgical technique owing to its low complication and recurrence rate. It should also be known that these cysts have a risk of malignant transformation. The most common post-operative complications observed in our study were infection and abscess.

Keywords: Thyroglossal, cysts, fistula, midline neck swelling, sistrunk

INTRODUCTION

The thyroglossal duct forms during the embryonic development of the thyroid gland. While the thyroid rudimentary base descends caudally in the neck, it is believed that the tongue forms a channel attached to the origin point at the foramen cecum level.[1,2] Thyroglossal duct typically undergoes atrophy during the 7-10th gestational week, following the primitive thyroid's transition to the last pretracheal position in the neck's lower half. Thyroglossal duct cysts occur if the thyroglossal duct does not disappear after the thyroid gland completes its embryonic development and becomes cystic.[3,4] This developmental abnormality is observed in approximately 7% of the population.[1,3,5] Thyroglossal duct cysts are typically present in the neck's midline and are painless swellings that move with tongue movement; they can also be fistulized to the skin, in which case, they are called thyroglossal fistula.[3]

Thyroglossal duct cysts, although not infrequent in adults,[6] are the most common cervical lesions encountered in infancy and childhood.[5] They constitute >75% of the midline neck masses in childhood.[7] Cysts can occur anywhere along the migration path of the thyroglossal duct of a developing thyroid gland. Histologically, it is covered by the respiratory epithelium, squamous epithelium, or a combination of both. Microscopic foci of ectopic thyroid tissue are variable.[8] Thyroglossal duct cyst is commonly observed between the hyoid and thyroid gland, but differences are present in the admission levels for the same. Approximately 20–25% are present at the suprahyoid level, 15–20% at the hyoid level, and 25–65% at the infrahyoid level.[3]

Cyst infection and accompanying fistula are common, especially during the first decade of life. If a fistula is present, the fistulized skin must be removed along with the cystic structure and hyoid bone corpus.[1]

Approximately 1% patients with thyroglossal duct cysts may have an associated malignancy, which may of different types (papillary, follicular, anaplastic thyroid cancer, Hurtle cell carcinomas, etc.).[9] However, the most common type of malignancy is papillary thyroid carcinoma.[4,10] Due to the risk of malignancy, the treatment of this condition is surgical.[11] Sistrunk operation is the gold standard in surgery for this condition, involving the removal of hyoid bone corpus together with the entire cyst tract.[12–14]

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ORCID iDs of the authors: İ.A. 0000-0002-0947-366X; T.O. 0000-0001-7764-1138.

Corresponding Author: İsmail Aytaç E-mail: dr.iaytac@gmail.com

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In this study, we examined the symptoms, findings, revision rates, post-operative complications, and follow-up of patients who underwent Sistrunk surgery with the diagnosis of thyroglossal cyst or fistula in our clinic.

MATERIAL AND METHODS

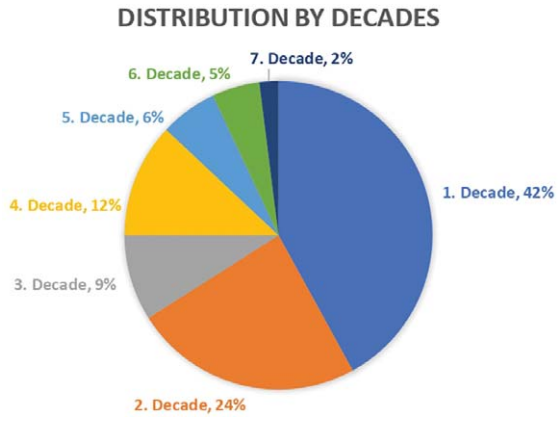
We included 100 patients who underwent surgery with the diagnosis of thyroglossal duct cyst in our hospital's clinic between 2003 and 2020. Patient profiles were examined for sex, age, admission complaints and findings, cyst location with respect to the hyoid bone and neck midline, concomitant infection, thyroglossal duct cyst fistulization, imaging findings, operation performed, post-operative recurrence and complications, post-operative pathology results, and patient follow-ups. Additionally, the obtained findings were evaluated with the existing literature. Descriptive statistical analysis, χ^2 and Crosstabs, was performed using Statistical Package for the Social Sciences (SPSS) version 22 (IBM SPSS Corp., Armonk, NY, USA). The statistical significance was set at $P < .05$. This study was conducted in accordance with the principles of the Declaration of Helsinki. Additionally, approval for this study was obtained from the Ethical Committee of the Faculty of Medicine of Gaziantep University (2020/329).

RESULTS

Of the 100 patients who underwent surgery, 53 (53.3%) were males and 47 (47%) were females. Their ages were 1-62 (mean 18.15 ± 15.837) years. Admission was most frequently observed in the first decade of life (46%) (Figure 1). The most common complaints at the time of first admission were swelling in the neck (88%) and discharge in the neck (11%), and more rarely, difficulty in swallowing (1%). Additionally, 30% patients had a fistula mouth in the neck or under the chin. Examination findings revealed that 30 patients had a fistula and 70 had a cyst. Additionally, 52% patients had an infection. Patients with cyst formation had a well-circumscribed, mobile, and painless swelling, while those with fistulization had a fistula mouth on the skin. Neck ultrasonography (USG) imaging records were available for all patients. USG findings revealed a cystic or fistulized cystic lesion with debris and dense contents. All patients underwent Sistrunk surgery under general anesthesia.

In the follow-up of 81 patients whose primary surgery was performed by us, recurrence was observed in only three patients (3.7%), and these patients were reoperated at the same incision site. Additionally, 19 patients previously had been operated in another center. The excision of the hyoid bone corpus was either not performed or was performed insufficiently in these patients. Hence, hyoid bone corpus excision and Sistrunk operation were performed in all these patients. As recurrence was observed in two patients operated in an external center and reoperated by us, these patients had to undergo surgery for a third time as well. No complications were observed later during their follow-ups.

Figure 1. Distribution of thyroglossal duct cysts in patients according to decades of life (100 patients).



Infection was observed in six of the 100 patients during the post-operative follow-up period (6%). Three of these patients required surgical drainage, while the other three were treated using antibiotherapy without drainage. Histopathological examination results were in accordance with thyroglossal duct cysts for all patients. The results of four patients revealed signs of malignancy (papillary thyroid carcinoma on the thyroglossal background) in addition to the cyst. Three of these patients underwent thyroidectomy in the same session, while one underwent thyroidectomy later. When the USG results were evaluated, the location of the cyst was at the infrahyoid level in 67 patients (67%), the suprahyoid level in 20 patients (20%), and the hyoid level in 13 patients (13%).

The most common complaints at admission were painless swelling in the neck midline (88%) and intermittent discharge (11%). Additionally, only one patient with a suprahyoid cyst had difficulty in swallowing. When the relationship between the localization of the cyst and the complaints at admission was evaluated, no significant relationship was found (Table 1).

The USG dimensions of thyroglossal duct cysts were 2-47 mm, the mean size being 19.38 ± 10.180 mm. When the relationship between the cyst's size and the recurrence rates was evaluated, no statistically significant relationship was found ($P > .05$).

Main Points

- Thyroglossal duct cyst is the most common congenital neck mass in the midline of the neck.
- Thyroglossal duct cysts are typically present in the neck's midline and are painless swellings that move with tongue movement.
- Cyst infection is a common condition often accompanied by the presence of a fistula.
- Malignant degeneration can occur; hence, it must be treated surgically.
- Sistrunk operation is the gold standard in surgery for this condition, involving the removal of hyoid bone corpus together with the entire cyst tract.

DISCUSSION

Thyroglossal duct cyst is the most common congenital neck mass in the midline of the neck, which is caused by dilatation

Table 1. Comparison of Cyst Localization and Admission Complaints

Admission complaint	Hyoid localization	Infrahyoid localization	Suprahyoid localization	Total
Discharge	2	6	4	12
Neck swelling	11	61	15	87
Difculty in swallowing	0	0	1	1
Total	13	67	20	100

and persistence of epithelial tract remnants in thyroid migration during embryogenesis.[4,14] These anomalies, generally observed as cysts, are called thyroglossal fistulas if they open to the neck epithelium.[7]

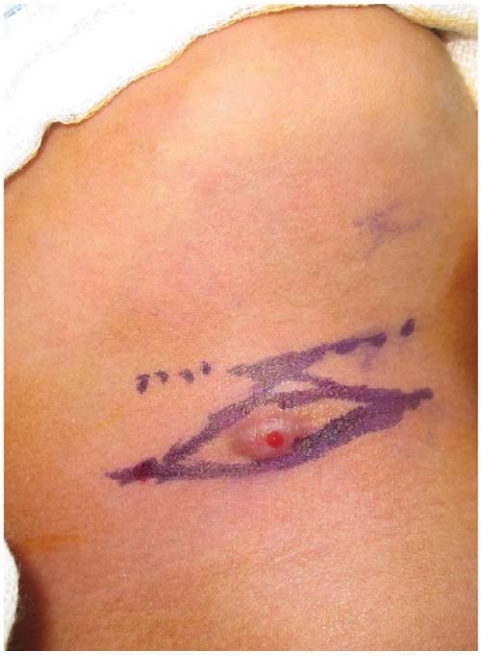
The exact incidence of thyroglossal canal remnants is unknown. However, it has been reported that a thyroglossal duct cyst or remnant is observed in about 7% of the population.[1,4,5]

Although these anomalies, observed equally in both sexes, can occur in any decade of life, they are more noticeable in the childhood, especially in the first 5 years of life.[6,15] In the present study, the age range was 1-62 years, and in correlation with the literature, 46% of the patients were in their first decade of life. Additionally, 53 (53%) patients were males and 47 (47%) were females.

According to research, approximately 50% of thyroglossal duct cysts become infected, which is the main reason for patients consulting a physician.[3,7,16] Consistent with the literature, infected cysts were observed in 52 patients (52%) in the present study. Cyst infection is a common clinical picture often accompanied by a fistula.[17] In a study conducted by Abuabara et al.,[18] it was reported that one-fourth of the adult patients presented with a drainage sinus caused by spontaneous drainage or surgical drainage of the abscess. Additionally, Ren et al.[15] reported cutaneous fistula in 10% patients. In this study, thyroglossal fistula was observed in 30 (30%) patients, with 28 of these having an active infection. In patients having a fistula, the fistulized skin was excised along with the cystic structure and the hyoid bone corpus (Figure 2). Consistent with the literature,[1,7,14] the fistula appeared during the first decade of life in half of the patients.

When the cyst is infected, pain, difficulty in swallowing, and skin hyperemia may be observed.[19] Thyroglossal cysts are rarely associated with dysphagia or airway obstruction.[20] Although the order of priority varies between studies, the most common complaints were painless swelling and intermittent discharge.[7] A comprehensive meta-analysis study performed on 1,015 cystic cervical masses reported infection, abscess, fistula, dysphagia, and airway obstruction to be the most common clinical presentations and symptoms.[11] The most common complaint in our patients was painless swelling in the neck midline (88%) and intermittent discharge (11%), with difficulty in swallowing being reported in only one case with a suprahyoid cyst. Due to its embryological origin, the thyroglossal duct cyst is usually present in the midline of the neck, in the form of a somewhat hard mass that moves as the tongue is pulled out.[4,11,21] These cysts can be found anywhere along the thyroglossal duct, from the base of the tongue to the suprasternal notch.[18] However, in approximately three-fourth of the cases, they are observed under the hyoid bone in the neck.[6,20,22] Based on the rates determined in previous studies, the most common locations of cysts are the infrahyoid region (61%), the suprahyoid region (24%), the suprasternal region (13%), and the intralingual region (2%).[5,9] In accordance with the literature, 67 patients (67%) in our study had infrahyoid cysts, 20 (20%) had suprahyoid cysts, and 13 (13%) had hyoid cysts. An intralingual cyst was not observed in any of the patients. The differential diagnosis should consider dermoid cyst, cystic hygroma, branchial cyst, thyroid pyramidal lobe hyperplasia and cysts, lipoma, ectopic thyroid tissue, lymphadenopathy, sebaceous cysts, hamartoma, hemangioma, teratoma, and primary or metastatic neoplasms.[5,7,11] USG, computed tomography (CT), and magnetic resonance imaging (MRI) are important auxiliary diagnostic methods for determining the cyst's size and its surrounding tissues. On USG, it is observed as homogeneous hypoechoic or heterogeneous lesions. On CT, it

Figure 2. Thyroglossal duct cyst incision line and inclusion of fistulized skin.



is observed as a hypodense mass and thickening of the wall. On MRI, it is more homogeneous in T1 than normal cysts and fluids.[23] Neck USG is a noninvasive and appropriate imaging method for diagnosing thyroglossal cysts.[11,18] However, USG may not reveal the depth of the hyoid and infrahyoid thyroglossal duct cysts and cannot reliably evaluate the base of the tongue in suprahyoid thyroglossal duct cysts. CT scan is better for understanding the relationship between the cyst and the hyoid. MRI is preferred for lesions close to the base of the tongue.[21] Thyroid scintigraphy can be performed to differentiate the presence of ectopic thyroid tissue.[24] The accepted treatment for thyroglossal duct cysts is surgery. Recurrent infections, unwanted cosmetic appearance, malignant degeneration, and fistula formation are surgical indications for thyroglossal duct cysts and fistulas.[11,25] Sistrunk surgery is frequently performed these days, which involves making a horizontal incision on the lower edge of the mass and carefully dissecting the cyst from the skin. After the cyst is freed, the cyst tract, along with the hyoid bone corpus, is dissected toward the tongue base. To prevent recurrence, dissection is continued up to the foremen cecum and the cyst is completely removed along with the hyoid corpus and the tract by placing a suture.[4,12–14] Post-operative recurrence rate after this procedure is 1.5–4%.[23] However, the recurrence rate has been reported to be 38% in cases where the hyoid bone is not resected.[19] If the hyoid corpus is not removed, this rate may increase up to 85%.[19,23,26] During surgery, care should be taken not to damage the vagus, hypoglossus, spinal accessory nerve, and other important structures adjacent to the tract.[19] Sistrunk surgery was performed in all of the study patients in our clinic. It has been reported that approximately 1% of patients with thyroglossal duct cysts present with malignancy, the most common being papillary thyroid carcinoma.[3,4,11,25] In this study, papillary thyroid carcinoma occurred four patients with thyroglossal duct cysts, and Sistrunk + thyroidectomy surgery was performed on these patients.

There are many reasons for unsuccessful surgical treatment of thyroglossal duct cyst. It has been demonstrated that an incorrect surgical procedure, such as a cystectomy alone, is the primary reason behind the high recurrence rate.[28] Sistrunk[12] suggested that various factors, such as the patient's young age, infection, and cyst's puncture during surgery, also caused recurrence. Ein et al.[29] examined 270 patients who underwent surgery for thyroglossal duct cysts and reported that infection and drainage of the cyst were the most important factors leading to recurrence. Dedivitis et al.[30] reported that the diagnosis of thyroglossal duct cyst could be made clinically, and that Sistrunk operation showed good results with low complication (11.62%) and recurrence (2.32%) rates; they also stated that antibiotic treatment could be avoided, and that hospital stay was short. Considering this information, thyroglossal duct cysts should also be considered in the differential diagnosis of patients presenting with neck swelling or mass. Additionally, it should not be forgotten that Sistrunk surgery is the gold standard in the treatment of this condition. Sistrunk surgery was performed for all cases in our clinic. The recurrence rate was 3.7% in patients whose primary surgery was performed by us, wherein these operations were performed by various surgeons as the clinic where the study was conducted is also an educational hospital. It is believed that the recurrence rate may be even lower if the surgeries are performed by the same surgeon.

CONCLUSION

Thyroglossal duct cyst is the most common congenital neck mass in the midline of the neck. Anamnesis and physical examination as well as radiological examinations are typically used for diagnosis. Cyst infection is a common condition often accompanied by the presence of a fistula. Although rare, malignant degeneration can occur; hence, it must be treated surgically. The most appropriate surgical technique is the Sistrunk surgery, which has fewer cases of recurrences.

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Original Article

Examination of the Level of Conus Medullaris Termination Using Magnetic Resonance Imaging

Merve Kalindemirtaş[1], Mustafa Orhan[1], Ayşe Bahşi[2], İlhan Bahşi[1]

[1]Department of Anatomy, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

[2]Clinic of Physical Medicine and Rehabilitation, Dr. Ersin Arslan Education and Research Hospital, Gaziantep, Turkey

ABSTRACT

Introduction: Recognition of the level of the conus medullaris termination (CMt) is of clinical importance for avoiding iatrogenic injuries during spinal anesthesia and lumbar puncture. Although CMt levels have been examined in a variety of studies, they vary in classical textbooks and literature studies. The aim of this study was to investigate the level of CMt and its correlation to gender, age, and body mass index (BMI) using magnetic resonance imaging (MRI) in healthy individuals and those with lumbar disc herniation.

Methods: The lumbar MRIs of 341 subjects, including healthy individuals (F: 123, M: 68) and those with lumbar disc herniation (F: 105, M: 45), were retrospectively examined, and the CMt levels were determined.

Results: It was found that CMt levels were most commonly located at upper 1/3 of the L1 vertebral body in both healthy individuals and those with disc herniation groups. No statistically significant difference was observed between the two groups evaluated. In addition, no significant mean level of CMt, weight, height, and BMI difference existed between the two groups.

Conclusion: In the literature, the highest level of CMt is seen as being at the intervertebral disc between T11 and T12 vertebrae, while the lowest level of the CMt is seen as being at lower 1/3 of the L3 vertebral body. Consequently, we are of the opinion that the L3-4 or L4-5 intervertebral spaces should be preferred to lower the complication rate in procedures such as spinal anesthesia and lumbar puncture.

Keywords: Conus medullaris, level of conus medullaris termination, magnetic resonance imaging

INTRODUCTION

The spinal cord extends from the foramen magnum to the sacrum in the fetus at the beginning of the second trimester and, subsequently, ascends.[1–3] At the time of birth, the level of the conus medullaris termination (CMt) is at the L2 vertebral body or above.[3] In its final position, it is generally located around the L1 vertebral body during the adult life.[4] This change is explained by the fact that the CMt does not truly "*ascend*" within the vertebral canal and that there is a differentially increased growth of the vertebral column (more rapid) relative to the spinal cord (slower).[1]

Recognition of the CMt level is of clinical importance for avoiding iatrogenic injuries during spinal anesthesia, lumbar puncture, and tethered cord syndrome.[5] Diagnostic lumbar puncture is one of the invasive tests frequently used in medicine.[6] Although serious complications are rarely encountered during this procedure, many temporary or permanent damages may occur.[7–9] More dramatically, complications with fatal outcomes have also been reported in the literature.[10]

One of the most important concerns during the needle placement for the spinal anesthesia is the damage of the conus medullaris (CM).[11] Damage to the spinal cord can be caused by incorrect identification of the lumbar vertebrae.[7,8] On the other hand, even if the lumbar space is correctly identified, patients with lower CMt levels can be expected to be at higher risk of damage to the CM.[11] Manzone et al.[3] stated that the normal rate of medullary ascent during the fetal period until reaching its final level has not been fully clarified.

Because of its clinical importance, the level of CMt has been examined in many previous studies. In these previous studies, it has been examined using ultrasounds,[12–16] magnetic resonance imaging (MRI),[3,5,11,17–42] cadavers,[43–47] both cadavers and MRI,[48–51] intraoperative neurophysiological testing (mapping and monitoring),[52] fetus MRIs,[1,53] and a combination of cadaver dissection, ultrasonography, and MRI.[54] By the same token, it is believed that the actual position of CMt is probably better assessed using MRI than cadavers.[11,34] Furthermore, CMt levels have been studied in various pathologies, such as adolescent

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ORCID iDs of the authors: M.K. 0000-0003-2266-3967; M.O. 0000-0003-4403-5718; A.B. 0000-0002-2852-9788; İ.B. 0000-0001-8078-7074.

Corresponding Author: İlhan BAHŞİ **E-mail:** dr.ilhanbahsi@gmail.com

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idiopathic scoliosis as a peripheral neuropathy,[20] lumbar spinal stenosis,[18] ankylosing spondylitis,[31] skeletal dysplasia,[35] severe idiopathic scoliosis,[38] and Chiari I malformation.[40]

According to a study by Nasr,[50] although numerous MRI and cadaver studies about CMt levels have been carried out, a few studies have made comparisons in terms of age and gender. On the other hand, in the literature, the relationship between the level of CMt and the body mass index (BMI) has been evaluated in very few studies.[19,33] Moreover, Lin et al.[11] stated that despite the fact that disc herniation is common in the general population, the effect on the level of CMt of the disc herniation has not been well investigated.

The aim of this study is, therefore, to investigate CMt levels and their correlation to gender, age, and BMI using MRI in both healthy individuals and those with lumbar disc herniation.

METHODS

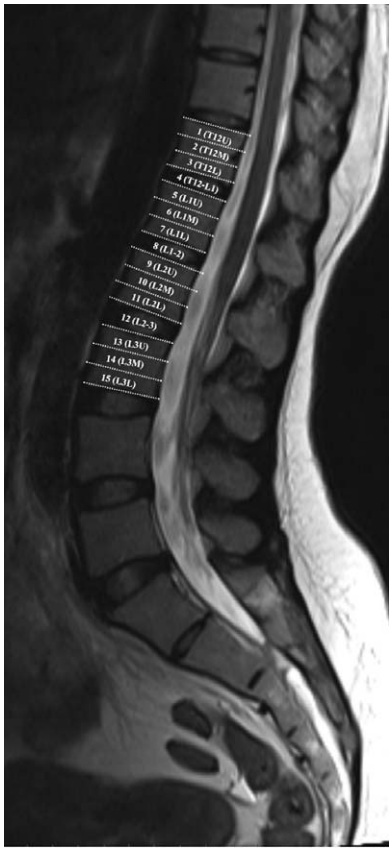
The images of patients admitted to the Physical Therapy and Rehabilitation Outpatient Clinic of Dr. Ersin Arslan Education and Research Hospital for the diagnosis and/or treatment of lower back pain, hip pain, and lumbar radiculopathy pain between 2017 and 2019 were retrospectively examined. Data regarding the diagnosis, gender, age, weight, and height of these patients were obtained from the patient registries and MRI reports. BMIs were calculated based on the height and weight information available. The approval of Gaziantep University Clinical Trials Ethics Committee was obtained before the study commenced (Decision date and number: 2019/14).

In this study, MRIs of 750 patients were evaluated. A total of 409 patients were excluded from the study due to MRIs featuring artifacts that would prevent the detection or measurement of the reference points, it not being possible to clearly observe the CMt in the MRI, the patient having a tumor, infection, ischemia, congenital spine abnormality, or deformity, there being missing or inconsistent demographic information, or the patient having previously undergone surgery in the lumbosacral area. Individuals with no pathologies who presented to the clinic without any complaint (they are considered as healthy individuals) and patients with protruding disc herniation were included in the study. One hundred ninety-one individuals (F: 123 and M: 68) were examined in the normal group, and 150 individuals were examined in the group diagnosed with protruding disc herniation (F: 105 and M: 45).

Main Points

- Recognition of the CMt level is clinical importance for avoiding iatrogenic injuries during spinal anesthesia, lumbar puncture and tethered cord syndrome.
- Although the levels of CMt have been examined in a variety of studies, the levels given in classical textbooks and the literature are very different.
- In the literature, the highest level of CMt is seen as being at the intervertebral disc between T11 and T12 vertebrae, while lowest level of it is at the L3L.
- We believe that the L3-4 or L4-5 intervertebral spaces should be preferred to lower the complication rate in procedures such as spinal anesthesia and lumbar puncture.

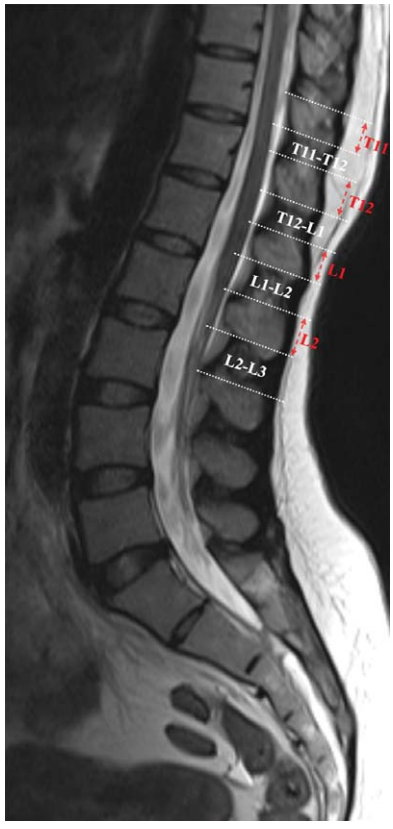
Figure 1. Demonstration of the method for determining the level of CMt with vertebral bodies and intervertebral discs.



MRIs were taken with a GE Signa (GE Medical Systems, Milwaukee, WI, USA) 1.5 Tesla instrument. The slice thickness was 5 mm in sagittal and 4 mm in transverse images. The T1 and T2 sequences of the images were examined using the RadiAnt DICOM Viewer 5.5.1 program. The levels of CMt were determined by being correlated with the transverse section and examined on the midsagittal section. All scans were obtained in the supine position. In each MRI used, the CMt level was clearly visualized on the midsagittal section.

The level of CMt was examined according to its relationship with adjacent vertebrae or intervertebral discs, as in the previous studies.[5,11,17,18,21] The level of CMt was defined at the level of the surrounding vertebrae or intervertebral discs. Each vertebral body at the level of CMt was divided into three equal parts (*U:* upper 1/3, *M:* middle 1/3, and *L:* lower 1/3). When the CMt level coincided with the intervertebral disc, it was considered a separate part. In other words, CMt level was determined at four different levels as three different parts of a vertebral body and an intervertebral disc (Figure 1). For statistical analysis, numbers

Figure 2. Demonstration of the method for determining the level of CMt with spinous processes and interspinous spaces.



1-15 were given from the upper 1/3 of the T12 vertebral body (T12U) to the lower 1/3 of the L3 vertebral body (L3L), similar to the study by Liu et al.[24] Additionally, using a transverse line from the level of CMt to the spinous process of the vertebrae, the level of CMt was also determined according to its relationship with the spinous process (Figure 2).

Statistical Analysis

Conformity to normal distribution of numerical data was tested using the Shapiro–Wilk test. The Student-t test was used to compare the normally distributed variables in two groups. Correlations between categoric variables were tested with the Chi-square test and correlations between numerical variables were tested with Pearson's correlation coefficient. SPSS 22.0 software package was used for the analyses (IBM SPSS Corp.; Armonk, NY, USA). $P < .05$ was considered significant.

RESULTS

The CMt levels of 228 females (mean age: 39.52 ± 11.10) and 113 males (mean age: 37.67 ± 12.23) between the ages of 18 and 66 were examined in both healthy individuals and those with disc herniation. No statistically significant difference existed between age and genders ($P = .164$). The mean level of CMt and mean values of the age, weight, height, and BMI of the individuals examined are given in Table 1. There was a statistical difference between the healthy individuals and those with disc herniation in terms of age ($P = .001$). No statistically significant difference at the level of CMt, weight, height, and BMI existed between the two groups ($P = .468$, .235, .094, and .060, respectively).

The mean value of the levels of CMt was 5.81 ± 2.26 in the healthy individuals and 5.63 ± 2.27 in patients diagnosed with lumbar disc herniation. It was found that CMt levels most commonly located at L1U with a standard deviation shorter than the height of the vertebral body. There was no statistically significant difference between the genders in terms of CMt levels (Table 2). Results of the CMt levels in both genders and in both groups are given in Table 3 and Figure 3.

In the healthy individuals, it was detected that there were no correlations between the level of CMt and weight and BMI, that there was a very weak positive correlation between the CMt level and age ($P = .017$, $r = 0.173$), and that there was a very weak negative correlation between the CMt level and height ($P = .017$, $r = -0.179$). This result demonstrates that the mean level of CMt tends to lower as age increases, and it tends to rise as height increases.

In the group of patients diagnosed with lumbar disc herniation, no correlations were detected between the CMt level and weight, height, and BMI values ($P = .309$, .747, and .201, respectively), while a weak positive correlation with age was observed ($P = .022$, $r = 0.188$). This result demonstrates that as the age increases, the level of CMt falls down.

Finally, using a transverse line from the level of CMt to the spinous process of the vertebrae, the level of CMt was also determined according to its relation with the spinous process. The highest level of CMt is seen as being at the level of the spinous process of T11 vertebrae, while the lowest level of the CMt is seen as being at the between the spinous processes of L2 and L3 vertebrae. The mean levels of CMt in both groups were at the level of spinous process of the L1 vertebrae. No correlations were detected between the level of CMt according to its relation with the spinous process and weight, height, and BMI values ($P = .450$, .353, and .186, respectively), while a weak positive correlation with age was observed ($P = .039$, $r = 0.169$), almost the same way with its relationship of the vertebral bodies and intervertebral discs. Additionally, a positive, very strong correlation was detected between the two methods ($P = 0.001$, $r = 0.853$).

DISCUSSION

Ko[55] stated that although the CM is located between the level of the T12-L1 intervertebral disc and L1-2 intervertebral disc, it contains approximately 10 spinal nerve pairs (L1-S5). Kingwell et al.[56] reported that the neurological structures affected by CM lesions are significantly different from those affected by cervical or thoracic spinal cord lesions. On the other hand, it is stated that although the literature on thoracic and lumbar spinal cord traumas are extensive, the number of studies investigating CM lesions is limited. Additionally, Kingwell et al.[56] stated that, in cases where CM lesions have been investigated, it is assumed

Table 1. The Level of CMt, Age, weight, Height, and BMI Values of the Healthy Individuals and Patients with Disc Herniation

	Healthy individuals				Patients with disc herniation			
Parameter	**M**	**F**	**T**	*P*	**M**	**F**	**T**	*P*
Level of CMt	5.56 ± 2.36	5.94 ± 2.20	5.81 ± 2.26	.291	5.31 ± 2.53	5.76 ± 2.15	5.63 ± 2.27	.266
Age	34.68 ± 11.20	37.07 ± 11.02	36.22 ± 11.11	.154	42.20 ± 12.43	42.38 ± 10.55	42.33 ± 11.10	.927
Weight (kg)	80.02 ± 12.46	70.08 ± 14.41	73.66 ± 14.52	.001*	76.82 ± 12.83	74.85 ± 12.18	75.44 ± 12.37	.372
Height (mm)	172.98 ± 5.97	160.40 ± 6.83	164.82 ± 8.88	.001*	171.18 ± 7.05	159.72 ± 6.01	163.22 ± 8.25	.001*
BMI (kg/m^2)	26.80 ± 4.28	27.26 ± 5.39	27.10 ± 5.02	.557	26.17 ± 3.83	29.43 ± 5.10	28.44 ± 4.97	.001*

*Significant difference.
BMI, body mass index; M, male; F, female; T, total; kg, kilogram; mm, millimeter; m: meter.

Table 2. A Comparison of the Mean Level of CMt Values Between the Group the Healthy Individuals and Patients with Disc Herniation

	F	M	*P*
Healthy individuals	5.94 ± 2.20	5.56 ± 2.36	.261
Patients with disc herniation	5.76 ± 2.15	5.31 ± 2.53	.266
Total	5.86 ± 2.17	5.46 ± 2.42	.125

M, male; F, female.

that the CMt at the level of L1 or L2 vertebrae despite its level being known to vary.

Procedures such as lumbar puncture, spinal, or epidural anesthesia should be performed taking the level of CMt into consideration.[5,24] It is essential to be familiar with the morphology and variations of CM to avoid puncturing the CM with the needle.[57] CM damage may result in complications including as pain,[8,58,59] loss of reflexes,[8] motor loss in the lower extremities,[8,60] urinary dysfunction,[8,58] sexual dysfunction,[56,58] loss of sensation,[8,60] dysesthesia,[8] paresthesia,[58] paraplegia,[60] drop foot,[8,9] lower motor neuron syndrome,[56] flaccid muscle tone,[56] atrophic changes,[56] and spinal cord ischemia.[61] Although these complications are generally temporary, it has been reported that permanent complications can occur.[7] Loss of sensation, loss of motor function, hypoesthesia, urinary dysfunction, loss of balance while walking usually heal within a few weeks[62] and drop foot, and hypoesthesia usually heal within a few months.[7] Additionally, pain, hypersensitivity, and paresthesia[63] are complications that are usually permanent. Moreover, Greaves[10] stated that fatal complications may also occur. Reynolds[8] reported seven different patients who were found to have suffered neurological damage following spinal and combined spinal epidural anesthesia. Reynolds[8] remarked that the needle administered to the spinal region should not be administered above the level of L3 vertebra. Despite the fact that most CM-related complications result from the procedure of the procedure at higher levels, it is also known that the level of CMt may be lower than expected. Actually, the lowermost levels of CMt were reported at L3U,[5,24,34] L3M,[64,65] and L3L[48] in healthy individuals. In the present study, the lowermost levels were detected at L3M in the healthy individuals and at L3L in the patients with disc herniation.

The information on the level of CMt differs between anatomy and neurology textbooks and the literature. In the textbooks, the mean levels of CMt are stated to be at L1M,[66] L1 or L2 vertebrae,[67–69] L1L,[70] L1 vertebrae,[71–73] L2 vertebrae,[74] and intervertebral disc between the L1 and L2 vertebrae.[75] However, mean levels of CMt were stated to be at the level of L1 vertebra in the 39th edition of *Gray's Anatomy*[76] and *Gray's Clinical Neuroanatomy*,[71] between the L1 and L2 vertebrae in the 40th edition of *Gray's Anatomy*,[77] and at the level of L1M in the 41st edition of *Gray's Anatomy*,[4] showing variations. In the literature, the level of CMt has been examined in many studies[5,11,17–19,21,24–31,33,34,36–38,45,48–51,78] (Table 4). While some of these studies[5,17,19,21,24,25,28,37,48–51] examined healthy individuals, some also included patients with pathologies such as lumbar spinal stenosis,[18] adolescent idiopathic scoliosis,[20] ankylosing spondylitis,[31] and Chiari I malformation.[40] Others compared healthy individuals with a variety of groups.[11,27] In these studies, a very wide range of levels of CMt were reported. The highest possible point of the level of CMt in the healthy individuals was reported by Sevinç et al.[36] and Demiryürek et al.[5] to be T11-12 intervertebral disc, and the lowest level of CMt was reported in the present study and by Kwon et al.[48] to be the L3L. In the literature, the level of CMt has not been detected to be lower than the L3 vertebra. We also think that, similarly to Kwon et al.[48] and Yedavalli et al.,[57] the L3-L4 or L4-L5 intervertebral spaces are more suitable for the lumbar puncture. There are mobile nerve roots in this region located in the cerebrospinal fluid. Therefore, the risk of injury from needle sting is low.[57]

Table 3. The Levels of CMt Values in the Healthy Individuals and Patients with Disc Herniation

		Healthy individuals			Patients with disc herniation		
Level no.	Level	M	F	T	M	F	T
1	T12U	3 (4.4%)	3 (2.4%)	6 (3.1%)	2 (4.4%)	3 (2.9%)	5 (3.3%)
2	T12M	3 (4.4%)	4 (3.3%)	7 (3.7%)	2 (4.4%)	5 (4.8%)	7 (4.7%)
3	T12L	7 (10.3%)	7 (5.7%)	14 (7.3%)	6 (13.3%)	6 (5.7%)	12 (8.0%)
4	T12-L1	9 (13.2%)	18 (14.6%)	27 (14.1%)	7 (15.6%)	17 (16.2%)	24 (16.0%)
5	L1U	11 (16.2%)	26 (21.1%)	37 (19.4%)	9 (20.0%)	17 (16.2%)	26 (17.3%)
6	L1M	13 (19.1%)	18 (14.6%)	31 (16.2%)	9 (20.0%)	16 (15.2%)	25 (16.7%)
7	L1L	9 (13.2%)	13 (10.6%)	22 (11.5%)	3 (6.7%)	16 (15.2%)	19 (12.7%)
8	L1-L2	7 (10.3%)	13 (10.6%)	20 (10.5%)	3 (6.7%)	16 (5.7%)	19 (12.7%)
9	L2U	4 (5.9%)	17 (13.8%)	21 (11.0%)	2 (4.4%)	6 (%)	8 (5.3%)
10	L2M	1 (1.5%)	3 (2.4%)	4 (2.1%)	1 (2.2%)	2 (1.9%)	3 (2.0%)
11	L2L	-	1 (0.8%)	1 (0.5%)	-	1 (1.0%)	1 (0.7%)
12	L2-L3	-	-	-	-	-	-
13	L3U	-	-	-	-	-	-
14	L3M	1 (1.5%)	-	1 (0.5%)	-	-	-
15	L3L	-	-	-	1 (2.2%)	-	1 (0.7%)
Total		68 (100%)	123 (100%)	191 (100%)	45 (100%)	105 (100%)	150 (100%)

M, male; F, female; T, total.

These intervertebral spaces can be found through palpation, following the line starting from the top of the iliac crest and intersecting with the spine at a straight angle.[78]

Relationship between the CMt Level and Age

Whereas many studies[5,17,22,24,25,27,29,33,34,48-50] report not having found any correlation between the level of CMt and the age, Ugale et al.[41] reported that the level of CMt tends to rise with age. Karabulut et al.[21] reported that the CMt level tends to rise with age in the females, while no correlation with the age was found in the males. Conversely, it was found in some studies[28,37,78] that the level of CMt falls down as age advances, similarly to the present study. This probably results from the fact that the nucleus pulposus undergoes dehydration and degeneration, or the height of the vertebral body decreases with age. Nevertheless, the level of CMt displayed a significantly positive but very weak correlation with the age in the present study, similar to the previous studies.[37,78] Therefore, we believe that larger populations should be studied to better understand the correlation between age and CMt level.

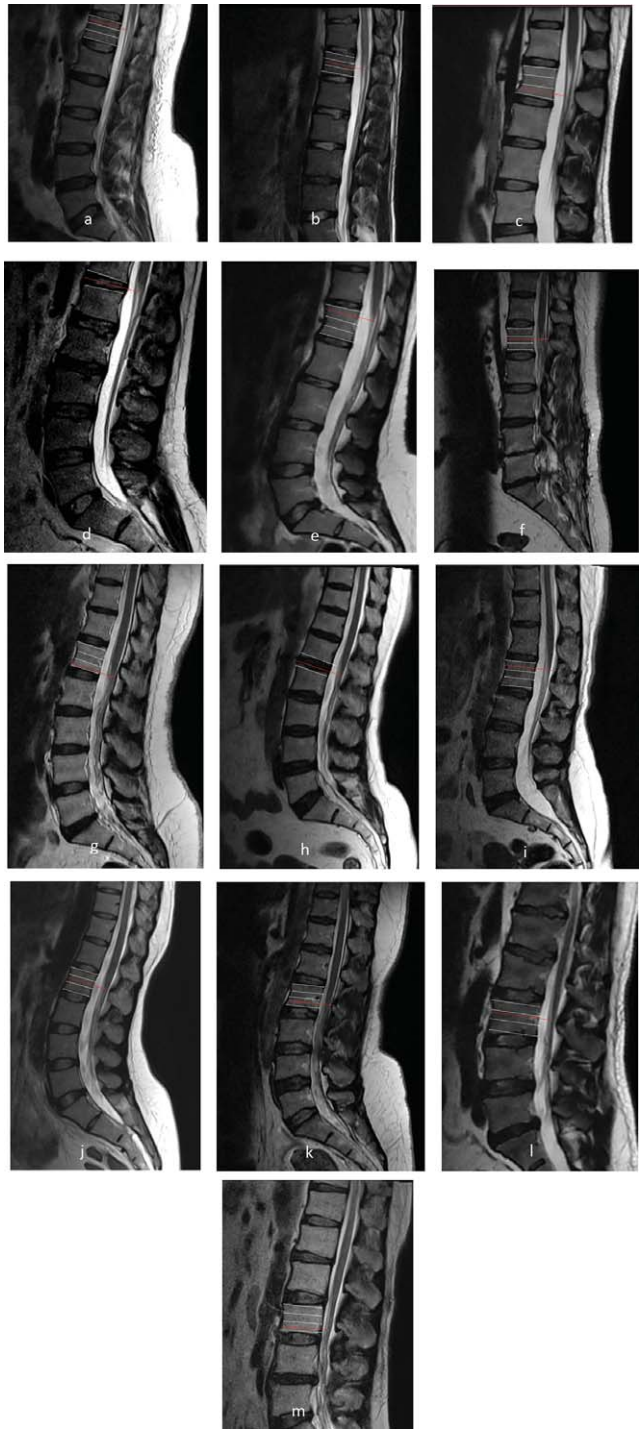
Relationship between the CMt Level and Gender

Lin et al.[11] stated that, interestingly, all reports on spinal anesthesia-related spinal cord damage published to date have included female patients, and that this is probably due to the fact that females are affected more by changes in bone density as a result of vertebral body pathologies. Similar to this opinion, all of the seven complications reported by Reynolds[8] were detected in females. Lin et al.[11] detected no statistically significant correlation between gender and level of CMt in healthy individuals and patients with lumbar vertebral compression fracture. However, they detected a statistically significant difference in patients with disc herniation, disc bulging, and thoracic vertebral compression fracture and found that the average level of CMt was lower in females. Additionally, some other studies[5,24,32,37,46,64,65,78] determined that the average level of CMt was significantly lower in females statistically. On the other hand, many studies[17,19,22,25,27-29,33,34,36,44,45,48-50] did not reveal any statistically significant difference between the genders, as is the case with the present study examining both healthy individuals and patients with disc herniation. Mbaba et al.[25] reported that the probable reason for these variations are to be as the different sample sizes studied and geographical variations in the literature.

Relationship between CMt Level and BMI

To the best of our knowledge, there are very few studies[19,33] in the literature that examine the relationship between the level of CMt and BMI. In the present study, there was no significant relation between BMI and the level of CMt, similar to

Figure 3. Demonstration of the levels of CMt (a: T12U, b: T12M, c: T12L, d: T12-L1, e: L1U, f: L1M, g: L1L, h: L1-L2, i: L2U, j: L2M, k: L2L, l: L3M, m: L3L).



the results obtained by Binokay et al.[19] and Rostamzadeh et al.[33] Furthermore, Schlotterbeck et al.[79] reported that an increase in BMI did not affect the success of lumbar puncture. On the other hand, care should be taken while performing lumbar puncture in obese individuals, as the landmarks used to determine the anatomy of the lumbar region, such as the Tuffier's line and the spinous process, may not be clearly defined.[78,80]

Relationship between CMt Level and Disc Herniation

The level of CMt has been examined in many pathological cases in the literature. The level of CMt may extend to L4

Table 4. A Comparison of the Level of CMt Values with Those in the Literature

Study	Specimen	Gender	n	Age	Level no	Mostly	T11-12	T12U	T12M	T12L	T12-L1	L1U	L1M	L1L	L1-2	L2U	L2M	L2L	L2-3	L3U	L3M	L3L
Demiryürek et al.[5]	MRI	M	296	20-69		T12-L1	-	2 (0.68%)	7 (2.36%)	14 (4.73%)	83 (28.04%)	40 (13.51%)	49 (16.55%)	37 (12.5%)	50 (16.89%)	6 (2.03%)	2 (0.68%)	3 (1.01%)	1 (0.34%)	2 (0.68%)		
		F	343			L1-L2	1 (0.29%)	-	4 (1.17%)	10 (2.92%)	60 (17.49%)	41 (11.95%)	56 (16.33%)	61 (17.78%)	70 (20.41%)	23 (6.71%)	10 (2.92%)	6 (1.75%)	1 (0.29%)	-		
Lin et al.[11]	MRI*	M + F	65	38 ± 9.7	6.5 ± 1.85			-														
	MRI†	M + F	130	54 ± 13.9	6.6 ± 1.96																	
Liu et al.[24]	MRI†	M + F	585	20-74	6.74 ± 2.08****			1 (0.17%)	3 (0.51%)	21 (3.59%)	64 (10.94%)	90 (15.38%)	94 (16.07%)	103 (17.61%)	82 (14.02%)	74 (12.65%)	31 (5.30%)	13 (2.22%)	8 (1.37%)	1 (0.17%)		
	MRI□				6.55 ± 2.09****			1 (0.17%)	9 (1.54%)	26 (4.44%)	63 (10.77%)	102 (17.44%)	87 (14.87%)	99 (16.92%)	92 (15.73%)	56 (9.57%)	35 (5.98%)	11 (1.88%)	3 (0.51%)	1 (0.17%)		
	MRI**				6.81 ± 2.11****			1 (0.17%)	6 (1.03%)	21 (3.59%)	51 (8.72%)	95 (16.24%)	99 (16.92%)	93 (15.90%)	79 (13.50%)	81 (13.85%)	37 (6.32%)	13 (2.22%)	9 (1.54%)	-		
Saifuddin et al.[34]	MRI	M	231	46 (16-85)	7.1****				9 (1.70%)	20 (3.97%)	33 (6.55%)	57 (11.31%)	69 (13.69%)	127 (25.2%)	82 (16.27%)	60 (11.9%)	27 (5.36%)	14 (2.78%)	5 (0.99%)	1 (0.2%)		
		F	273		6.6****																	
Karabulut et al.[21]	MRI	M	607	40.57 ± 15.38				1 (0.1%)	8 (1.0%)	6 (0.7%)	81 (7.8%)	132 (14.3%)	165 (17.9%)	142 (15.4%)	272 (29.5%)	54 (5.9%)	47 (5.1%)	13 (1.4%)	1 (0.1%)			
		F	314	43.72 ± 15.01																		
Morimoto et al.[27]	MRI*	M + F	310	15-44		L1M		2 (0.7%)	2 (0.7%)	12 (3.9%)	55 (17.7%)	63 (20.3%)	65 (21%)	54 (17.4%)	41 (13.2%)	11 (3.6%)	5 (1.6%)	-				
	MRI††		28			L1U		-	1 (3.6 %)	4 (14.3 %)	8 (28.6%)	10 (35.7%)	3 (10.7%)	1 (3.6%)	-	1 (3.6%)	-	-				
	MRI†‡		41			L1L		-	-	1 (2.44 %)	2 (4.88%)	4 (9.76%)	3 (7.32%)	12 (29.27%)	6 (14.63%)	6 (14.63%)	6 (14.63%)	1 (2.44%)				
Kwon et al.[48]	MRI*	M	140	42.3 ± 16.0		L1M				1 (0.5%)	28 (13.3%)	32 (15.2%)	52 (24.8%)	45 (21.4%)	34 (16.2%)	6 (2.9%)	9 (4.3%)	3 (1.4%)	-	-	-	-
		F	108																			
	Cadaver*	M	49	56.0 ± 14.9		L2U				2 (1.7%)	8 (6.8%)	6 (5.1%)	11 (9.3%)	11 (9.3%)	16 (14.0%)	23 (19.0%)	14 (12.0%)	10 (8.5%)	11 (9.3%)	1 (0.8%)	4 (3.4%)	1 (0.8%)
		F	18																			
Arai et al.[17]	MRI*	M + F	602			L1M																
Moussallem et al.[29]	MRI*	M	70	≥18		L1U		1	5	7	7	24	5	6	12	3						
		F	71					2	1	5	12	22	15	5	6	3						
Rostamzadeh et al.[33]	MRI*	M	199			L1L																
		F	151																			
Mbaba et al.[25]	MRI*	M	92	39.56		L1M			4 (4.17%)	-	1 (1.04%)	11 (11.46%)	35 (36.47%)	14 (14.59%)	3 (5.13%)	13 (13.54%)	11 (11.46%)	3 (3.13%)	-	1 (1.04%)		
		F	85						3 (3.13%)	3 (3.13%)	2 (2.5%)	7 (8.75%)	39 (48.75%)	4 (5.0%)	6 (7.5%)	8 (10%)	5 (6.25%)	3 (3.13%)	-			

Table 4. (Continued)

Study	Specimen	Gender	n	Age	Level no	Mostly	T11-12	T12U	T12M	T12L	T12-L1	L1U	L1M	L1L	L1-2	L2U	L2M	L2L	L2-3	L3U	L3M	L3L
Nasr[49]	MRI*	M	100	43.2 ± 11.9		L1L																
		F	100	39.8 ± 12.1		L1-L2																
	Cadaver*	M	40		L1L ± 1.91	L1L																
		F	20		L1L ± 1.87	L1L																
Nasr[50]	MRI*	M	100	43.2 ± 11.9		L1L																
		F	100	39.8 ± 12.1		L1-L2																
	Cadaver*	M	40		L1L ± 1.91	L1L																
		F	20		L1L ± 1.87	L1L																
Mourlion et al.[28]	MRI*	M	39	48.97 ± 14.66		L1L																
		F	38	45.57 ± 13.33		L1L																
Kim et al.[78]	MRI*	M	347	≥20		L1L																
		F	343			L1-L2																
Soleiman et al.[37]	MRI*	M	338	49.43 (7-85)	5.94	L1U																
		F	297		6.58	L1U																
Qu et al.[31]	MRI*	M	80	36.6 (17-57)		L1M		-	-	3 (3%)	6 (6%)	18 (18%)	22 (22%)	21 (21%)	19 (19%)	8 (8%)	2 (2%)	1 (1%)	-			
		F	20																			
	MRI††	M	86	34.6 (17-65)		L1M		1 (1%)	1 (%1)	6 (6.3%)	13 (13.5%)	8 (8.3%)	22 (22.9%)	16 (16.7%)	16 (16.7%)	8 (8.3%)	3 (3.1%)	1 (1%)	1 (1%)			
		F	10																			
Sun et al.[38]	MRI*	M	58	14.9		L1L				2 (1.7%)	5 (4.2%)	18 (15%)	20 (16.7%)	36 (30%)	16 (13.3%)	13 (10.8%)	6 (5%)	3 (2.5%)	1 (0.8%)			
		F	62																			
	MRI□□□	M	32	14.7 (12-18)		L1L			1 (0.4%)	4 (1.7%)	7 (2.9%)	28 (11.7%)	41 (17.1%)	70 (29.2%)	43 (17.9%)	20 (8.3%)	18 (7.5%)	4 (1.7%)	4 (1.7%)			
		F	208																			
Sevinç et al.[36]	MRI*	M	157	45 ([18_80])		T12-L1	-	-	2 (1.3%)	4 (2.5%)	33 (21%)	16 (10.2%)	29 (18.5%)	19 (12.1%)	29 (18.5%)	12 (7.9%)	13 (8.3%)	-	-			
		F	207			L1-L2	1 (0.5%)	2 (1%)	1 (0.5%)	8 (3.9%)	18 (8.7%)	22 (10.6%)	38 (18.4%)	40 (19.3%)	49 (23.7%)	11 (5.3%)	15 (7.2%)		2 (1%)			

Table 4. (Continued)

Study	Specimen	Gender	n	Age	Level no	Mostly	T11-12	T12U	T12M	T12L	T12-L1	L1U	L1M	L1L	L1-2	L2U	L2M	L2L	L2-3	L3U	L3M	L3L
Binokay et al.[19]	MRI*	M	443	47.5 ± 16.5		L1M		8 (1.8%)	16 (3.6%)	42 (9.5%)	36 (8.1%)	76 (17.2%)	97 (21.9%)	76 (17.2%)	27 (6.1%)	33 (7.4%)	15 (3.4%)	11 (2.5%)	1 (0.2%)	5 (1.1%)		
		F	756	48.5 ± 14.5		L1M		5 (0.7%)	23 (3%)	61 (8.1%)	64 (8.5%)	125 (16.5%)	203 (26.9%)	132 (17.5%)	40 (5.3%)	42 (5.6%)	30 (4.0%)	22 (2.9%)	1 (0.1%)	8 (1.1%)		
Ba et al.[18]	MRI‡‡‡	M	124	48.8	6.84 ± 2.17****	L1L			7 (3%)	15 (6.4%)	31 (13.2%)	30 (12.9%)	16 (6.8%)	53 (22.6%)	37 (15.8%)	9 (3.8%)	8 (3.4%)	5 (2.1%)	16 (6.8%)	7 (3%)		
		F	110																			
Naqshi et al.[30]	MRI*	M + F	100	18-65		L1L					4 (4%)	10 (10%)	15 (15%)	38 (38%)	5 (5%)	14 (14%)	7 (7%)	6 (6%)	1 (1%)			
Moon et al.[26]	MRI	M + F	187	2-94		L1L		1	0	4	12	25	33	40	28	23	11	8	2			
Gatonga et al.[45]	Cadaver	M + F	112	20-80		L2U				(5.5%)	-	(12.7%)	(3.6%)	(18.2%)	-	(9.1%)	(20.0%)	(25.5%)		(3.6%)	(1.8%)	
Van Schoor et al.[51]	MRI□□		26	13-20		L1L																
	MRI***		55	21-29		L1L																
Present study	MRI*	M	68	34.68 ± 11.20	5.56 ± 2.36			3 (4.4%)	3 (4.4%)	7 (10.3%)	9 (13.2%)	11 (16.2%)	13 (19.1%)	9 (13.2%)	7 (10.3%)	4 (5.9%)	1 (1.5%)				1 (1.5%)	
		F	123	37.07 ± 11.02	5.94 ± 2.20			3 (2.4%)	4 (3.3%)	7 (5.7%)	18 (14.6%)	26 (21.1%)	18 (14.6%)	13 (10.6%)	13 (10.6%)	17 (13.8%)	3 (2.4%)	1 (0.8%)				
	MRI†	M	45	42.20 ± 12.43	5.31 ± 2.53			2 (4.4%)	2 (%4.4)	6 (13.3%)	7 (15.6%)	9 (20%)	9 (20.0%)	3 (6.7%)	3 (6.7%)	2 (4.4%)	1 (2.2%)	-	-	-	-	1 (2.2%)
		F	105	42.38 ± 10.55	5.76 ± 2.15			3 (2.9%)	5 (4.8%)	6 (5.7%)	17 (16.2%)	17 (16.2%)	16 (15.2%)	16 (15.2%)	16 (15.2%)	6 (5.7%)	2 (1.9%)	1 (1.0%)	-			

*Normal.
†Disc herniation.
‡Neural position.
□Flexion.
**Extension.
††Patients with sacralization of the fifth lumbar vertebrae.
‡‡Patients with lumbarization of the sacrum.
□□Adolescence.
***Early adulthood.
†††Thoracolumbar kyphosis.
‡‡‡Lumbar spinal stenosis.
□□□Adolescent idiopathic scoliosis.
****Standardization was ensured using numbers specified in our study instead of numbers in these studies.

vertebrae in adults with asymptomatic and undetected diastematomyelia.[9] Besides that, tight filum terminale syndrome is associated with an abnormally positioned CM below the intervertebral disc between the L2 and L3 vertebrae.[81] On the other hand, although lumbar disc herniation is the most common type of disc herniations,[82] a detailed review of the literature showed that there are few studies that investigate the relationship between the level of CMt and disc herniation.[11] Similarly, to Lin et al.,[11] the present study also detected that there was no statistically significant difference on the CMt level between healthy individuals and patient with disc herniation.

Relationship between Level of CMt Level and the Spinous Process

In lumbar applications, it is required to advance forward between the spinous processes to access the lumbar cistern. Doherty and Forbes[6] stated that one of the most important bony landmarks during this procedure is the spinous process of the L4 vertebra. It is observed in the literature that the CMt level is usually evaluated based on the relationship between the vertebral bodies and the intervertebral discs. However, no study investigating the relationship between the spinous process and CMt level was found, despite the fact that the spinous process is one of the most important bony landmarks. In the present study, both vertebral bodies and intervertebral discs and spinous processes were used to determine the level of CMt. A positive, very strong correlation was detected between the two methods ($P = .001$ and $r = 0.853$). This correlation demonstrates that the two methods support each other.

Limitations

As this is a retrospective study, the ethnic features of the individuals were not evaluated. The evaluations were performed using a standard section thickness of 5 mm for the sagittal sections and 4 mm for the transverse sections. The CM may seem to be terminating on a slightly higher level if it is located between these image sections. To prevent this, the sagittal and transverse sections were examined in correlation with each other.

CONCLUSION

Although the levels of CMt have been examined in a variety of studies, the levels given in classical textbooks and the literature are very different. In the literature, the highest level of CMt is seen as being at the intervertebral disc between T11 and T12 vertebrae, while the lowest level of it is at the L3L. We are of the opinion that the L3-4 or L4-5 intervertebral spaces should be preferred to lower the complication rate in procedures such as spinal anesthesia and lumbar puncture.

Ethics Committee Approval: The approval of Gaziantep University Clinical Trials Ethics Committee was obtained before the study commenced (decision date and number: 2019/14).

Informed Consent: N/A

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Original Article

Publication Status of Mouse Embryonic Fibroblast Cells in Scientific Journals

Ahmet Sarper Bozkurt

Department of Physiology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

ABSTRACT

Objective: A bibliometric analysis was performed to investigate trends in mouse embryonic fibroblast (MEF) cells as a topic and also to compare the contributions of countries, journals, authors, keywords and citations. The elaboration of a strategic plan for the development of scientific research in stem cells and especially MEF as a research topic was analyzed.

Methods: A bibliometric study was carried out on Web of Science (WOS) and Scopus databases comprising the period between 1991 and 2020. These papers were analyzed and evaluated in terms of all years, field parameters (authors, keywords, affiliations, funding agencies, authors' nationalities, article type, networks, h-index of journals and authors) and citations.

Results: As a result of the conducted research, it was found that the WOS and Scopus had published 1,255 and 6,562 papers, respectively, on 'Mouse Embryonic Fibroblast' as topic and article title, abstract and keywords. The articles are analyzed by bibliometric techniques in this study.

Conclusion: On analysis of papers related to the main parameters of MEF, it has been ascertained that the MEF-related articles published in the journals within the scope of Science Citation Index (SCI)/Science Citation Index Expanded (SCI-E) was quite high and is growing rapidly. The medical publication feature has been characterized by international visibility and extensive networking with many foreign research structures. The number of citations was increasing significantly. The publishing patterns were different depending on the area of interest. These data are useful in studying the dynamical nature of science and can help in planning newer studies for researchers.

Keywords: Mouse, embryonic, fibroblast, bibliometric

INTRODUCTION

With increased life expectancy, the researchers are exploring new methods for diagnosed people. Stem cell technology has a great potential for the treatment of infarction tissues. Stem cell technologies and gene editing techniques are two of the most promising developments in a variety of fields. Primitively, the research based on fibroblast cells was described at the end of the 19th century. These cells are the most common type of cell from the connective tissue in animals, presenting an elongated morphology and extended cellular processes, with a fusiform shape.[1,2] Fibroblasts are mesenchymal-derived cell types, important and major cell types in extracellular matrix, epithelial differentiation, and wound healing. A fibroblast is the most common cells of connective tissue and synthesizes the extracellular matrix and collagen.[3,4] Practically, fibroblasts were more effective in cell therapy than mesenchymal stem cells, as they may not require isolation of specific subtypes of cells.[5] The first mouse embryonic fibroblast (MEF) publications are found in 1986[6] and 1956[7] in Web of Science and Scopus, respectively. MEF cells have a structure similar to stem cells, which are frequently used as feeder cells because they harbor various growth factors to promote the self-renewal of embryonic stem cells as well as their undifferentiated growth.[8] The MEF encompasses significant opportunities for both basic research and clinical applications in the areas of regenerative medicine and tissue engineering. Fibroblast growth factors (FGF) have been identified in mice. It is named FGF1-FGF23. FGFs are mostly found in developing and adult tissues. FGFs have various biological activities both in vivo and in vitro conditions such as angiogenesis, cell migration and tissue wound healing. Another effect of the FGF family has been stated to be important for neuronal signal transmission in the central and peripheral nervous systems.[9] The establishment of MEF from large animals that model human diseases is significant.[10] Many cell types remodel the extracellular matrix:; osteoblasts,[11] astrocytes,[12] vascular endothelium,[13] macrophages,[14] and pericytes.[15] Today, studies related to tumor and the development of some diseases and cell programming research in humans are strongly influenced by mouse models. The laboratory mice with their ease of genetic manipulation have been used in biomedical research for understanding the complexity of different human pathogenesis. But there are both similarities and discrepancies between mouse and human studies.[16] In this study, the MEF topic is investigated to understand and plan a new study. The originality, creativity, curiosity, novelty, potentiality, and capacity of wondering and questioning with respect to unusual observations, capacity to interact, and to create favorable scientific environment are steps of research plan. The bibliometric analysis of MEF was considered cautiously at this stage. The qualitative bibliometric analyses of the topic decreased the

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ORCID iD of the author: A.S.B. 0000–0002–7293–0974.

Corresponding Author: Ahmet Sarper Bozkurt E–mail: asbozkurt@gantep.edu.tr

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Table 1. Main Statistical Information of MEF Articles in WOS and Scopus

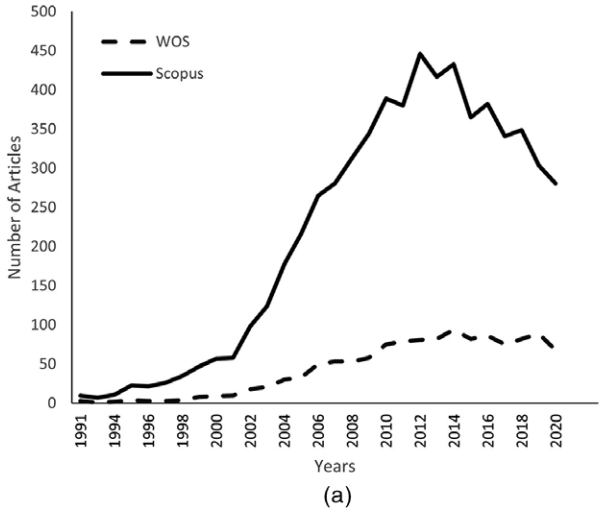
Description	WOS	Scopus
Timespan	1991:2020	1997:2020
Sources (journals, books, etc.)	567	1,179
Documents	1,255	6,000
Average years from publication	7.89	8.26
Average citations per documents	32.14	44.29
Average citations per year per doc	3.032	4.035
Document types		
Article	1,171	5,766
Article: book chapter	15	57
Article: proceedings paper	15	67
Correction	1	7
Review	5	71
Document contents		
Keywords plus (ID)	4,618	28,688
Author's keywords (DE)	3,050	9,004
Authors		
Authors	5,879	26,898
Author appearances	7,733	42,297
Authors of single-authored documents	9	57
Authors of multiauthored documents	5,870	26,841
Authors collaboration		
Documents per author	0.213	0.223
Authors per document	4.68	4.48
Coauthors per documents	6.16	7.05
Collaboration index	4.71	4.52

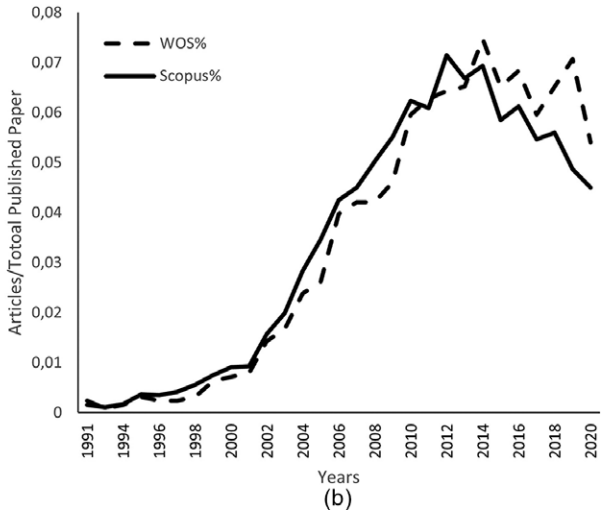
Main Point

- Such studies open a variety of new areas for scientists and manifest health-informatics importance.
- The publications on MEF as topic has shown an increasing trend.
- This study is expected to contribute to the preparation of more original studies on the related subjects, besides preventing repetitive studies relating MEF cells.

risks in research. The paucity or absence of significant publications, global productivity of a researcher, repetition of articles, impact factor, h-index, citation index of papers, the best journals of the discipline were analyzed carefully by this method. The impact factors of papers published in international journals indexed in WOS and Scopus, rank of scientists, associated coauthors were analyzed. The use of artificial intelligence and big data in medicine is growing rapidly in recent years. The bibliometric and phrase frequency analysis that covered the popularity of authors, journals and organizations, publication properties, and the frequency of words and phrases in title keywords and abstracts appeared in the literature. To the best of

Figure 1. The increase in (a) the number and (b) ratio of articles in WOS (dashed line) and Scopus (straight line) databases.





our knowledge, this is the first study aimed at investigating the research on MEF originating on a global basis. The aim of this study was to provide a primer point of scientific results for high-quality research and highlight possible potentials in this field, thereby promising future collaborations for catalyzing the scientific innovation.

METHODS

In order to understand the work done with MEF cells from the repetitive publications, also to determine the international limits of the work done with these cells and to guide future studies, the bibliometric method is used. The bibliometric analysis provides more objective and reliable statistical results,[17] by having the analyses of documentation related to the field of interest.[18] Through the evolution of t digital age, the scientific community has been investigating and representing a huge number of papers in all fields. Many online scientific databases such as Web of Science, Science Direct, Scopus, Researchgate, and Google Scholar were the sources of article information. Precisely, theWeb of Science and the Scopus are the two most extensive databases chosen for this analysis. A new research paper is published every 30 seconds, and there are 10,000 updates to PubMed every day. WOS is maintained by Clarivate Analytics to explore the systematic literature reviews of different scientific domains. It includes different citation databases with journal conference proceedings and books. Scopus, an another alternative database for retrieving bibliometric datasets, considers wider timespan than WOS.. The well-known and widely used databases of WOS and Scopus are advantaged for searching the literature on different scientific fields.[19] WOS had been the only citation database which covered all domains of science for many years. Other widespread used database of Scopus database in Elsevier Science started at 2004. As previously explained, the disadvantages of these databases are higher rate of duplicated citation, self-citation records, and certain lack of standardization procedures. WOS is considered the source of bibliometric data with the highest quality of information. The WOS database was used to search for documents containing the 'Mouse Embryonic Fibroblast' as a topic with quotes. To collect the MEF-related articles, we queried the WOS indexing database on September 20, 2020. A bibliometric analysis was conducted to look into the article's publication date, journal's name, authors' institutions, organizations, article and author's citations and the related countries. The number of publications is currently increasing, and it is becoming increasingly infeasible to remain updated with everything that is being published. We searched for all the documents in WOS including MEF as topic, obtaining 1,255 entries. We performed bibliometric analysis by using main information about publication and its citation performance parameters for productivity and research performance. The inappropriate data of some journals and irrelevant articles were eliminated and the dataset was rearranged for bibliometric analyses. This set covers documents published until September 2020, since the WOS Core Collection indexes only those MEF publications which appeared after 1991. This is a well-known limit because the keywords of the documents published before the nineties are generally not indexed.[20] The number of articles related to MEF was 1,255, including 1,171 articles, 15 proceedings papers, and five reviews in this reference interval. The limitation of this study is mainly related to the used tools and methods of bibliometric analysis. We included articles from WOS and Scopus, but the most common medical database PubMed is not included. The analyses and results in this study provide significant insights into the evolving trends over the last decades.

Statistical Analysis

Data were collected and exported into bibliometric R-package for generating descriptive analyses, statistical graphs, and science maps[21]

RESULTS AND DISCUSSION

Towards the end of 1990s, the volume of documents increased annually, with an average production equal to 7.92 documents

Table 2. The Impact of Authors in MEF Topic in WOS

Author	h-index	TC	NP	PY
Zhang Y	11	472	23	2006
Li Y	11	453	16	2008
Wang Y	9	217	15	2011
Li X	9	358	14	2008
Wang L	9	446	13	2004
Zhang J	8	281	17	2005
Liu Y	8	289	12	2006
Batiha Ges	7	106	11	2019
Beshbishy Am	7	106	11	2019
Wang Z	6	122	13	2008
Özdemir A	5	175	17	2015
Kaplancikli Za	5	133	13	2015
Zhang	5	142	11	2009
Atli O	5	146	11	2014
Sever B	4	48	12	2017

TC, total citations; NP, number of publication; PY, publication year.

Table 3. Country Scientic Production in Scopus

Country	Frequency	Country	Frequency
USA	7,200	Australia	344
China	2,251	India	268
Japan	2,224	Netherlands	253
South Korea	1,047	Sweden	227
Germany	1,021	Iran	208
Canada	793	Switzerland	206
UK	742	Turkey	205
France	572	Singapore	199
Spain	488	Belgium	152
Italy	461	Austria	144

per year. The main information and statistics of analyzed articles are reported in Table 1. A number of publications increase and reach at least 50 documents per year in WOS. The total number of papers in Scopus is higher than WOS. But the increase in the ratio of articles to the total published documents is similar to WOS as shown in Figure 1. The main factors for the growth in the number of articles are the increasing number of researchers and the development of laboratory opportunities. When searching for MEF, we found that there was only one document before 1991 in WOS. The number of articles on WOS and Scopus database is compared in years between 1991 and 2020 in Figure 1. The search results revealed 1,255 articles in WOS and 6,562 articles in Scopus. The articles in WOS showed an average citation of 32.14 per article in the period between 1991 and 2020 and were written by 4.68 authors with a mean of 0.212 articles per author as shown in Figure 1. The articles in Scopus showed an average citation of 44.29 per article in the period between 2007 and 2020 (latest 6,000 papers were selected) and were written by 4.48 authors with a mean of 0.223 articles per author. In Table 2, the impact of authors with h-index and publications data are shown. There were five Turkish authors in the author's impact list of WOS in MEF topic. The authors' Hirsch index (h-index) is an author-level publication metric that attempts to measure both the productivity and citation impact of a scientist or scholar.[22,23] The h-index of the following scientists with citations is given in Table 2. The most productive countries were analyzed using Scopus database and are tabulated in Table 3. The generated citations were used to find the frequency of the most cited references or the most cited first authors or the countries. The most cited countries sorted by total citations in WOS are shown in Table 4. The total citations should increase with the increase in the number of publications, in general; however, a high number of publications do not represent high citation numbers in this study. The cited references have problems in the format due to nonstandardized format in databases. In Figure 2, the

Table 4. Most Cited Countries in WOS

Country	Total citations	Average article citations
USA	19,705	57.28
Japan	3,821	31.07
China	2,762	14.93
Korea	1,976	16.2
United Kingdom	1,767	49.08
India	963	16.05
Israel	962	160.33
Singapore	918	76.5
Canada	866	26.24
Spain	786	34.17
Sweden	713	64.82
Germany	659	21.97
Australia	594	37.12
France	513	34.2

Figure 2. Most relevant journals of MEF.

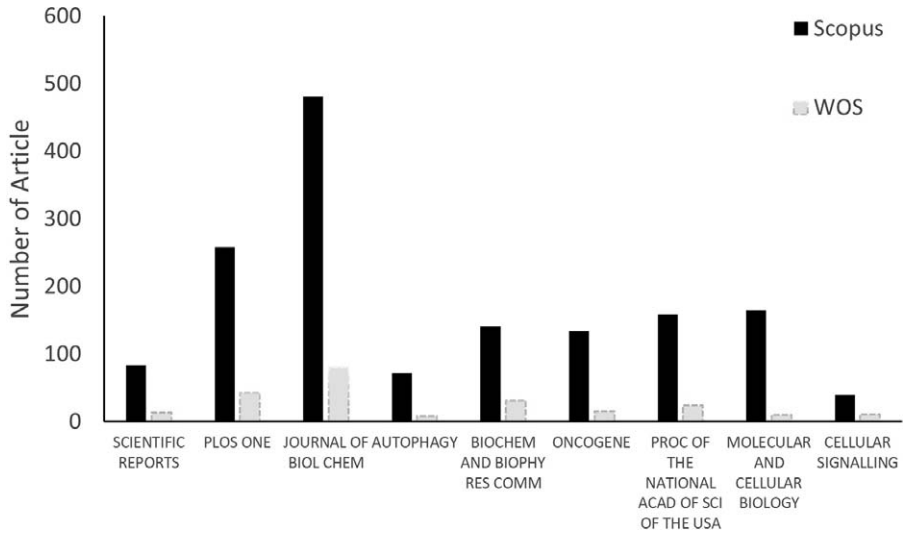


Table 5. Most Global Cited Documents in Scopus

Author, paper	Total citations	TC/year
Stambolic V, 1998, Cell	1,945	84.56
Zinszner H, 1998, Genes Dev	1,558	67.73
Okita K, 2008, Science	1,494	114.92
Xu C, 2001, Nat Biotechnol	1,492	74.60
De Brito Om, 2008, Nature	1,341	103.15
Burma S, 2001, J Biol Chem	1,268	63.40
Jacobs Jl, 1999, Nature	1,266	57.545
Scorrano L, 2003, Science	1,147	63.72
Wang Jl, 2000, Proc Natl Acad Sci USA	1,059	50.42
Zhao T, 2011, Nature	992	99.20

most productive source was the "Journal of Biological Chemistry" in WOS and Scopus. The number of articles on MEF in journals is comparatively given in Figure 2. WOS and Scopus databases are permanently improving. The significant advantage of choosing one of these two sources depends on the subject's area. Among the most cited articles, countries are given in Table 5. The first ranked article is "Stambolic V, 1998, Cell" with 1,945, followed by "Zinszner H, 1998, Genes Dev" with 1,558 global citations. The various most frequent keywords and their importance within the collection of articles are given in Table 6. As expected, the most frequent phrases (common keywords) in the WOS and Scopus database results were cytotoxicity, MEF, apoptosis, and autophagy, as that MEF was also the main search term. The keywords in articles could reflect the change of hot spots and concerns in databases with their own priorities. The thematic evolution due to authors' keyword is given in Figure 3. From the analysis of publications and the publishing profile, the thematic evolution due to authors' keyword is presented. And Interestingly, the keywords and the trend were given in current research. After analyzing each subperiod, it is possible to trace the temporal evolution of MEF research keywords. The diagram is used to show profiles, connections, and developments of the themes in this time interval.

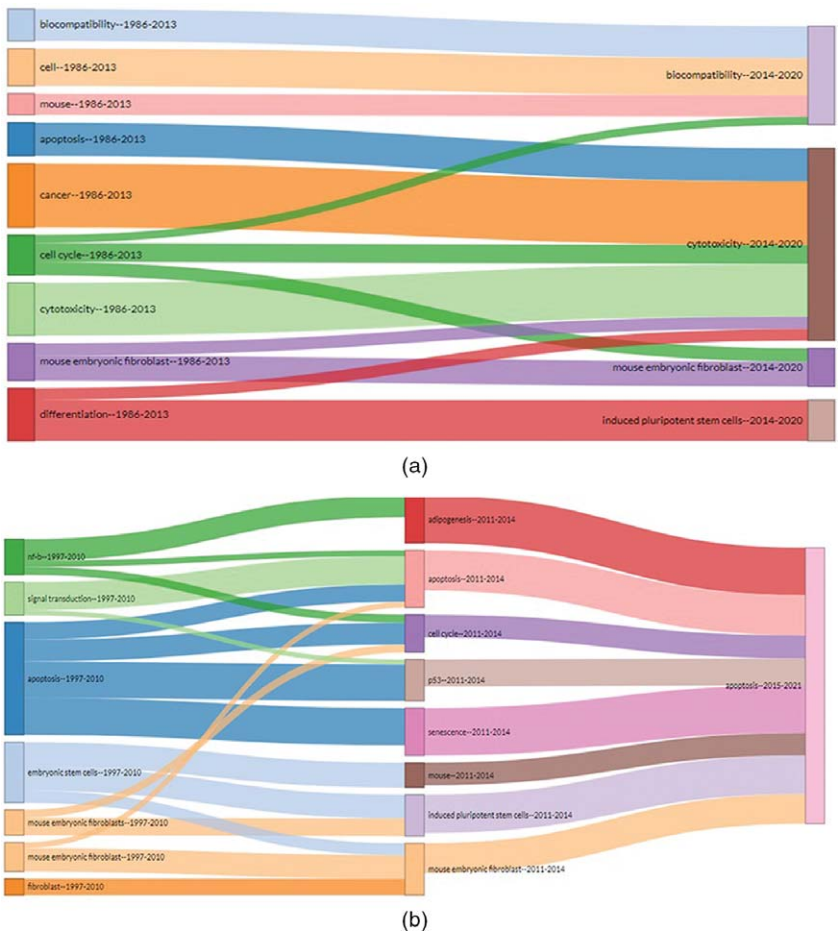
CONCLUSION

This paper focused at presenting a systematic analysis of MEF topic-related articles published on WOS and Scopus databases through a bibliometric approach. In this study, the bibliometric profile of MEF in scientific publications was documented in two

Table 6. Most Frequent and Most Important Phrases (Most Frequent Keywords)

WOS			Scopus		
Item	**Freq**	**Year**	**Item**	**Freq**	**Year**
Cytotoxicity	58	2016	Apoptosis	88	2018
Mouse embryonic broblast	37	2012	Autophagy	87	2018
Autophagy	35	2015	Mouse embryonic broblasts	67	2018
P53	27	2012	Cytotoxicity	59	2017
Differentiation	23	2009	Mitochondria	49	2018
Embryonic stem cells	23	2009	Cancer	34	2017
Mouse	21	2013	Induced pluripotent stem cells	33	2018
Biocompatibility	19	2016	Reprogramming	33	2018
Embryonic stem cell	19	2009	Senescence	25	2019
Fibroblast	16	2016	Mtor	23	2017
DNA damage	16	2010	P53	22	2017
Cell	16	2013	Cell proliferation	20	2016

Figure 3. The thematic evolution due to authors' keyword (a) in WOS and (b) in Scopus.



databases. The indicators were used to describe the comprehensive picture of the documents, the scientific activity, capacity, and orientation. The results drawn are salient as they provide requisite suggestions for scholars and publishers. The number of articles on MEF as a topic has shown an increasing trend in this period. The most frequently used keywords are apoptosis, mouse embryonic fibroblast, autophagy, P53, differentiation, and embryonic stem cells. In summary, both the articles and reviews in this research are an excellent source of information on the current knowledge in the fibroblast growth factor signaling pathway in metabolic regulation, development, disease, and repair after injury field. An analysis on the other textual information could be considered to enrich our findings on MEF. A large amount of research indicate several features of MEF, and furthermore, in order to understand the fibroblast and especially MEF, several additional in vitro and in vivo experiments are needed. This study provided significant insights into the evolution of the topics discussed throughout the years. These types of methodological practices will greatly contribute to the research fields.

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Original Article

Endoscopic Retrograde Cholangiopancreatography in Patients with Post-Operative Bile Duct Injuries: Experience of a Tertiary Center in Turkey

Tolga Düzenli[1], Hüseyin Köseoğlu[1], Elif Sümeyye Aktı[1], Barış Yılmaz[2]

[1]Department of Gastroenterology, Hitit University Erol Olçok Training and Research Hospital, Çorum, Turkey

[2]Department of Gastroenterology, Biruni University Hospital, İstanbul, Turkey

ABSTRACT

Objective: The aim of this study was to determine the effectiveness and safety of endoscopic retrograde cholangiopancreatography (ERCP) in the diagnosis and treatment of post-operative bile duct injuries and to share our experience of a tertiary referral center.

Methods: Patients who underwent ERCP in our hospital due to biliary injuries after biliary surgery between January 2017 and March 2020 were included in this study. Demographic data, etiologies, clinical conditions, endoscopic treatment methods, and results of the patients were analyzed.

Results: A total of 30 patients (16 females and 14 males) were included in this study. Twenty-six patients experienced bile leakage or stenosis after cholecystectomy, and four patients had hepatic hydatid cyst surgery. ERCP was successful in 25 patients (83.3%), but four (13.3%) patients underwent surgery and one patient (3.3%) underwent percutaneous transhepatic cholangiography after failed ERCP. Among the patients who had biliary stenting, biliary leakage was recovered in all of the patients, and repeat ERCP revealed that 18.2% of the patients had stones or mud in the common bile duct. The median time to ERCP was 6.5 days, and there was no difference between early (first 10 days) or late (10-30 days) ERCPs in terms of effectiveness and safety.

Conclusion: ERCP is a safe and effective method that should be considered before percutaneous procedures and surgery, whether surgery to ERCP time is early or late. Biliary stenting effectively recovers biliary leakage, and stent removal by repeat ERCP should be performed to check the common bile duct for stones or mud, instead of solely stent removal.

Keywords: Post-operative bile leakage, ERCP, cholecystectomy, biliary tract injury, biliary fistula

INTRODUCTION

Biliary tract injuries are complications that may develop following surgical procedures involving the biliary tract. The most common causes include laparoscopic cholecystectomies (LC), open cholecystectomies (OC), hepatic hydatid cysts and operations, exploration of the bile duct, biliary malignancy surgeries and several etiologic causes for operations, abdominal trauma, and cholelithiasis.

LC has become widespread after 1990s and has become the first line treatment for symptomatic cholelithiasis today.[1] It has significant advantages over OC, but it is associated with increase in bile duct leakage, and OC is performed in selected cases.[2] The incidence of biliary tract injuries in LC is between 0.3 and 0.7% and is associated with important mortality and morbidity.[3,4] Although biliary stenosis is common in cases with OC, the total bile duct injuries are reported as 0.1-0.2%.[5] The most important factors in the optimal treatment are early detection of injury and the severity of damage, clinical condition of the patient, and experience and facilities of the physicians.

Endoscopic retrograde cholangiopancreatography (ERCP) is one of the most important methods in the diagnosis and treatment of bile leakage and stenosis. The aim of our study is to determine the effectiveness and safety of ERCP in the diagnosis and treatment of post-operative bile leakage and stenosis and to share our experience.

METHODS

Patients who underwent ERCP due to post-surgical bile injuries at University Hospital between January 2017 and February 2020 were included in the study. This study was approved by the ethics committee of the Hitit University Faculty of Medicine with the number 2020/202. Demographic data, etiologies, clinical conditions, endoscopic treatment methods, and results of the patients were extracted from hospital computer system.

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ORCID iDs of the authors: T.D. 0000-0002-6279-1018; H.K. 0000-0002-2197-7473; E.S.A. 0000-0002-0439-0590; B.Y. 0000-0001-6563-8142.

Corresponding Author: Tolga Duzenli **E-mail**: tolgaduzenli@yahoo.com

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Routine laboratory tests including complete blood tests, liver tests, and imaging (ultrasonography, computed tomography, and magnetic resonance imaging) findings were examined. The procedures were performed by Fujinon ED-530 XT duodenoscope device under sedo-analgesia performed by an anesthesiologist. After ERCP imaging, sphincterotomy, balloon or basket extraction, catheter or balloon dilatation, and stent replacement were performed if indicated. Data were recorded from patients' ERCP reports.

Injury types were determined by the Amsterdam and Strasberg classification systems.[6,7] According to Amsterdam classification, Type A is leakage from the cystic duct, Type B is leakage from the major bile ducts, Type C is bile duct strictures without concomitant biliary strictures, and Type D is complete transection of the biliary duct.[6] The Strasberg classification is as follows: Type A: bile leakage from cystic duct or liver bed without further injury; Type B: partial occlusion of the biliary tree, most frequently of an aberrant right hepatic duct; Type C: bile leak from duct which is not communicating with the common bile duct; Type D: lateral injury of the biliary ducts without loss of continuity; Type E: circumferential injury of biliary tree with a loss of continuity; Type E1: the stricture is located more than 2 cm from bile duct confluence (BDC); Type E2: the stricture is located less than 2 cm from BDC; Type E3: the stricture is located at BDC; Type E4: stricture is involving both right and left bile ducts; and Type E5: all bile ducts are completely occluded.[7]

Endoscopic treatment success criteria were improvement of the symptoms, signs and laboratory values, and decreased biliary drainage. Patients who needed surgery or PTK after the procedure were considered as unsuccessful endoscopic treatment.

Complications of ERCP were identified according to Cotton criteria, which are bleeding, perforation, pancreatitis, cholangitis, cardiopulmonary complications, anesthesia complications, and other complications.[8] The time period between surgery and ERCP was defined as early (<10 days) and late (≥10 days).

Statistical analyses were performed using the Statistical Package for the Social Sciences version 26.0 (IBM SPSS Corp.; Armonk, NY, USA). Descriptive statistics (frequency, percentage, mean, median, and standard deviation) of the study group were determined. Nonparametric tests were used for comparing groups, and Chi-square test was used to compare categorical data. $P < .05$ was considered statistically significant.

Main Points

- ERCP is a safe and effective method for post-operative biliary tract injuries that should be considered before percutaneous procedures and reoperation.
- ERCP provides definitive diagnosis in almost all biliary tract injuries and treats successfully in most of them whether surgery to ERCP time is early (first 10 days) or late (10-30 days).
- Biliary stenting effectively recovers biliary leakage, and stent removal should be performed by repeat ERCP to check the common bile duct for stones or mud, instead of solely stent removal.

RESULTS

A total of 30 patients (16 females and 14 males) with a mean age of 56.6 ± 17.4 years (range: 25-86) were included in our study. Common bile duct was selectively cannulated in the first or second procedure in all of the patients (cannulation success rate 100%, n = 30). Among the patients, 26 patients had bile leakage or stenosis after cholecystectomy, and four had biliary injury after hepatic hydatid cyst surgery. The most common symptoms and signs in these cases were abdominal pain (n = 24, 80%), bile leakage from the percutaneous drainage catheter (n = 21, 70%), jaundice, or hyperbilirubinemia (n = 6, 20%). ERCP was performed in the early period in 18 patients (60%).

ERCP was successful and sufficient in 25 patients (83.3%), but four (13.3%) patients needed surgery and one patient (3.3%) underwent percutaneous transhepatic cholangiography (PTC) after ERCP. The characteristics of the patients are presented in Table 1.

The 26 patients with post-cholecystectomy complication are summarized in Table 2 and Figure 1 according to Amsterdam and Strasberg classifications. Three patients with Amsterdam Type C/Strasberg Type E3, one patient with Amsterdam Type B/Strasberg Type D, and one patient with Amsterdam Type A/Strasberg Type A could not be treated successfully with ERCP.

Endoscopic sphincterotomy + biliary stent with plastic biliary stents (80%) was used as the main treatment method, and six patients (20%) had received endoscopic sphincterotomy without stenting. In two of these six patients, stenting was not required because leakage was very minimal. In the other four patients, stent could not be inserted because the proximal of stenosis/leakage could not be observed. These four patients had to have additive intervention (one patient had PTC and three patients had surgery).

Among the 24 patients who had biliary stenting, repeated ERCP was performed for the 22 patients whose condition was post-operative biliary leakage (one of the remaining two had repeated surgery despite the stent and the other one had post-operative biliary stricture). While 81.8% (n = 18/22) of these 22 patients had normal imaging findings in control ERCP, 18.2% (n = 4) patients had stones or mud in the common bile duct. We observed that biliary leakage was recovered in all patients (100%, n = 22/22). The remaining patient needed surgery despite ERCP and biliary stenting.

Four patients (13.3%) had cystobiliary fistula after hydatid cyst surgery. These patients were treated with ERCP by sphincterotomy and biliary plastic stent insertion. Treatment success was 100% in patients with cystobiliary fistula.

Laboratory parameters before ERCP are presented in Table 3. Laboratory results were compared according to sex and ERCP time (early or late), and no significant difference was observed between the groups. Our median time to ERCP was 6.5 days,

Table 1. Demographic and Clinical Characteristics of Patients Undergoing ERCP due to Postoperative Bile Duct Injury

		n (total = 30)	%
Age			
	Between 25 and 86 (median: 58.5)		
Sex			
	Female	16	53.3
	Male	14	46.7
Surgery type			
	Laparoscopic cholecystectomy	19	63.3
	Open cholecystectomy	4	13.3
	Hydatid cyst	4	13.3
	Conversion of laparoscopic to open cholecystectomy	3	10
Bile leakage symptoms			
	Abdominal pain	24	80
	Postoperative biliary drainage	21	70
	Jaundice or hyperbilirubinemia	6	20
Treatment type			
	ERCP	25	83.3
	ERCP+PTC	1	3.3
	ERCP + surgery	4	13.3
ERCP time			
	Postop 2-30 days (median: 6.5 days)		
	Early (first 10 days)	18	60
	Late (after 10 days)	12	40
Endoscopic treatments			
	Endoscopic sphincterotomy	6	20
	Endoscopic sphincterotomy + biliary stent	24	80
Post ERCP complication			
	No	28	93.3
	Yes (post-ERCP pancreatitis)	2	6.7
Was ERCP sufficient?			
	No	5	16.7
	Yes	25	83.3
The number of ERCP procedure			
	1	8	26.7
	2	18	60
	3	4	13.3

Table 1. (Continued)

		n (total = 30)	%
ERCP diagnosis			
	Post-cholecystectomy stenosis	4	13.3
	Biliary leakage	26	86.7
Prognosis			
	Exitus	1	3.3
	Healed	29	96.7

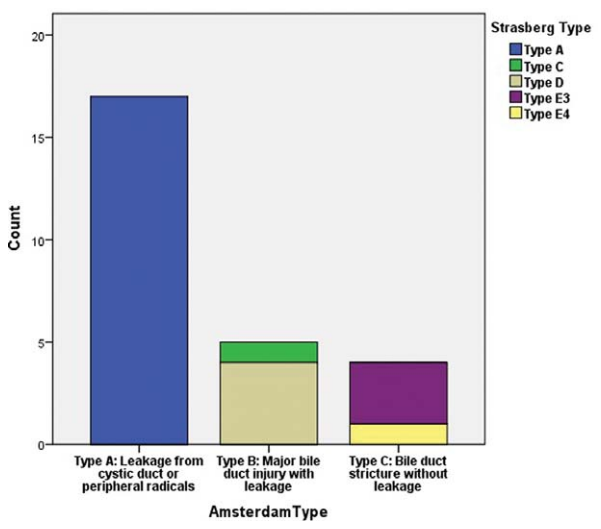
ERCP, endoscopic retrograde cholangio pancreatography; PTC, percutaneous transhepatic cholangiography.

Table 2. Distribution of Patients with Post-cholecystectomy Cile Duct Injury According to the Amsterdam and Strasberg Classications

		n = 26	%
Amsterdam type*			
	Type A	17	56.7
	Type B	5	16.7
	Type C	4	13.3
Strasberg type†			
	Type A	17	56.7
	Type C	1	3.3
	Type D	4	13.3
	Type E3	3	10.0
	Type E4	1	3.3

*Amsterdam classification: Type A: leakage from cystic duct or peripheral radicals; Type B: major bile duct injury with leakage; Type C: bile duct stricture without leakage; Type D: complete transection or excision of common bile duct.
†Strasberg classification: Type A: bile leak from cystic duct stump or minor biliary radical in gallbladder fossa; Type B: occluded right posterior sectoral duct; Type C: bile leak from divided right posterior sectoral duct; Type D: bile leak from main bile duct without major tissue loss; Type E1: transected main bile duct with a stricture more than 2 cm from the hilus; Type E2: transected main bile duct with a stricture less than 2 cm from the hilus; Type E3: stricture of the hilus with right and left ducts in communication; Type E4: stricture of the hilus with separation of right and left ducts; Type E5: stricture of the main bile duct and the right posterior sectoral duct.

Figure 1. Graphic for the patients with post-cholecystectomy bile duct injury according to the Amsterdam and Strasberg classifications.



and there was no difference between early or late ERCPs in terms of effectiveness and safety.

Post-ERCP pancreatitis was observed in two patients (6.7%). One of them had hydatid cyst and the other had LC surgery. Both were female and underwent ERCP in late period (post-operative 30 days). Mild post-ERCP pancreatitis developed in both patients, and improvement was observed with conservative treatment.

Mortality occurred in one patient (3.3%). This patient was a 76-year-old male with an LC, who underwent ERCP, sphincterotomy, and biliary stent insertion for Amsterdam Type A and Strasberg Type A bile leakage. Despite the benefits of endoscopic treatment and improvement of clinical/laboratory parameters, he died 11 days after the procedure due to hospital infection and sepsis.

DISCUSSION

Biliary tract injuries that may occur after biliary tract operations are complications that are sometimes difficult to treat, and they require a multidisciplinary approach including surgeons, gastroenterologists, and radiologists.

In addition to the role of endoscopic approach in diagnosis, its efficacy and safety in the treatment of post-operative bile duct

Table 3. Laboratory Assays of Patients Who Underwent ERCP (Before the Procedure)

Pre-ERCP test	Minimum	Maximum	Mean ± std. deviation
WBC (10^3/mm^3)	4.930	19.560	9,379 ± 3,200
Hemoglobin (g/dL)	8.1	14.6	11.27 ± 1.63
PLT (10^3/mm^3)	131	684	281.8 ± 148.7
AST (U/L)	14	212	53.9 ± 49.3
ALT (U/L)	6	214	49.0 ± 54.0
Total bilirubin (mg/dL)	0.2	12.8	1.86 ± 3.2
Direct bilirubin (mg/dL)	0.1	7.7	0.878 ± 1.904
Urea (mg/dL)	12	54	29.07 ± 12.61
Creatinine (mg/dL)	0.3	1.5	0.75 ± 0.30
Sodium (mequiv./L)	131	153	137.5 ± 4.4
Potassium (mmol/L)	3.2	5.1	3.98 ± 0.49

WBC, white blood cell count; PLT, platelet count; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

injuries are now widely accepted.[9] Even the sphincterotomy alone is useful in some cases via reducing the pressure in the biliary tract and accelerating the closure of the leakage site. But the main suggested and accepted treatment is sphincterotomy with biliary plastic stent insertion if possible.[10–12]

Our study demonstrated that ERCP is associated with high treatment success, minimal invasion, and low complication rates in patients with post-operative bile duct injuries. Our high treatment success rates support the efficacy and safety of endoscopic therapy in accordance with the existing literature.[10,13–15] The general approach is to insert a biliary stent following ES, which reduces the transpapillary pressure gradient and gives the chance of the recovery of leakage.[10–12] However, the types and properties of stents to be applied are not currently standardized. Katsinelos et al. inserted seven French and 10 French diameter biliary plastic stents in patients with post-operative biliary leakage and showed a similar clinical improvement and treatment success rates in patients. Similarly, Kaffes et al.[10] stated in their study that the stent diameter has no effect on treatment results. In addition, the use of fully covered self-expandable metal stents has increased for post-operative bile injuries in recent years, especially in refractory cases, and successful treatment has been reported with these stents.[16–18]

When we examine our patients according to the Amsterdam and Strasberg classifications, we observed that the cystic duct is the most common site for leakage, and the second common site is aberrant branch of the right hepatic duct, which is compatible with the literature.[2] The literature states that a proportion of patients are injured during surgery and are repaired intraoperatively.[19] However, since our study consisted of patients who underwent ERCP, we do not have any data of the patients diagnosed intraoperatively.

No significant difference in laboratory data was found between patients who underwent ERCP in the early and late periods, the reason for that might be the low number of patients in this study. In addition to that, it is known that not only laboratory changes but also symptoms and signs play a significant role in the early period of post-operative bile duct injuries.[15]

The study performed by Fasoulas et al.[15] reported that "surgery to ERCP" period was long, and the main reason for this might be that the physicians were not aware enough in the post-operative early period. In addition to that, mild symptoms, non-specific clinical presentations, and laboratory assays that do not worsen rapidly may play a role.[2] Another study showed that referring a patient with bile duct injury after LC after 4 days to a specialist center had experienced more post-treatment complications, more invasive procedures, and longer hospitalization as compared to the patients who referred before 4 days.[20] Our median time for ERCP was 6.5 days, which was similar to the literature. On the other hand, the present study showed that ERCP was safe and effective whether "surgery to ERCP" time was early or late.

In our study, four of 26 patients who underwent post-cholecystectomy ERCP had stenosis. Three of them could not be treated with ERCP, and additive intervention was required (surgery or PTC). The last one is still followed up with stent changes with 3-4 months intervals. Parlak et al.[21] investigated patients with post-cholecystectomy stenosis and reported that increasing the number of stent insertion in recurrent ERCP procedures has favorable effects on long-term stenosis treatment.

Most of the patients in our study needed two procedures of ERCP. The main reason for this is to remove the biliary plastic stents and to check the leakage status of the biliary tract. It is controversial whether the second ERCP is essential in patients with formerly ERCP and biliary plastic stenting for biliary leakage. Studies showed that pathological findings like refractory leakage, stones, and mud can be seen in 22-28% of the patients at the control ERCP in patients with biliary stenting.[22,23] On the other hand, another study showed that only one of 64 patients in control ERCP had bile duct stones, and that endoscopic stent removal alone without cholangiography could be more optimal for control ERCP.[24] Our data showed that 81.8% of our patients who underwent stent insertion on ERCP for biliary leakage had normal imaging on control cholangiography; however, 18.2% had bile duct stones. We observed that biliary leakage was recovered in all of our patients. Because of the high percent of stones or mud in common bile duct, we consider that cholangiography with biliary stent removal at the control ERCP should be routinely performed. However, large-scale prospective randomized studies are needed to support this suggestion. A current approach is to use biodegradable stents for post-operative bile duct leakage, which provides fewer ERCPs. In the study performed by Siiki et al., biliary plastic stents were inserted in 24 patients with post-operative complications, and biodegradable biliary stents were inserted in eight patients; no difference was detected in treatment efficacy between them.[25] Because biodegradable biliary stents are expensive and not commonly used, we have used biliary plastic stents in all of our ERCP procedures. However, in the near future, we consider that the use of this kind of stents will increase for post-operative bile duct injury.

The major limitation of our study was its retrospective design and low number of patients. But since our hospital is the reference center of the region, patients generally continued the follow-up at our hospital, which strengthens our data.

CONCLUSION

ERCP is a safe and effective method that might be considered before percutaneous procedures or surgery, providing definitive diagnosis in almost all biliary tract injuries and treating successfully in most of them. Biliary stenting effectively recovers biliary leakage, and stent removal by repeat ERCP should be performed to check the common bile duct for stones or mud, instead of solely stent removal. The management of post-operative biliary injuries must be done by an effective cooperation of the radiologists, surgeons, and gastroenterologists.

Ethics Committee Approval: This study was approved by the ethics committee of the Hitit University Faculty of Medicine with the number 2020/202.

Informed Consent: N/A

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Original Article

Evaluation of Effectiveness and Safety of Everolimus Eluting Stent System (XIENCE V) in the Treatment of Coronary Artery Lesions

Ugur Nadir Karakulak[1], Ergun Baris Kaya[1], Mehmet Levent Sahiner[1], Necla Ozer[1], Hikmet Yorgun[1], Ali Oto[2], Kudret Aytemir[1]

[1]Hacettepe University Faculty of Medicine Cardiology Department, Ankara, Turkey

[2]Ankara Memorial Hospital, Cardiology Department, Ankara, Turkey

ABSTRACT

Objective: Drug eluting stents have become an important component in percutaneous treatment of patients with symptomatic coronary artery disease. The population in the previous drug eluting stent studies has very risk profile, and therefore not reflects real-world information. Moreover, there are limited data to evaluate risk factors and predictors of intended outcomes. In this study, everolimus eluting stent (XIENCE V) implantations and follow-up results in patients with coronary artery disease were evaluated.

Methods: A total of 833 patients who underwent everolimus eluting stent deployment for coronary artery lesions were enrolled. Baseline demographic, clinic and angiographic data, procedure-related complications, and outcomes during follow-up were studied.

Results: As primary endpoints, all-cause mortality was 1.3% and target lesion failure was 2.3%. As secondary outcomes, cardiac death, target lesion revascularization, target vessel revascularization, myocardial infarction, and stent thrombosis were seen 0.84, 0.8%, 2.2%, 0.6%, and 0.24%, respectively. Premature discontinuation of dual antiplatelet therapy and presentation with acute coronary syndrome were strong predictors for all-cause and cardiac mortality.

Conclusion: Despite heterogeneity and high-risk profile of our patients, procedural-related complications and primary and secondary outcomes were very low with high clinical device and procedure success. These results demonstrate effectiveness and safety of the XIENCE V everolimus eluting stent in a highly complex, real-world patient population.

Keywords: Drug eluting stent, everolimus, coronary artery disease

INTRODUCTION

Drug-eluting stents (DESs) are widely used in the treatment of coronary artery disease (CAD).[1] These stents are coated with antiproliferative drugs, which keep the growth of peripheral intimal and smooth muscle tissue under control and elute drugs for a certain time period.[2] Choice of drug and elution kinetics affects clinical outcomes. Everolimus-eluting stent (EES) systems consist of chrome-cobalt skeleton with thin struts coated with highly biocompatible synthetic fluoropolymers.[3] Many studies have demonstrated better angiographic results with EESs than other DESs.[4,5]

The aim of this study was to determine effectiveness and reliability of stents, especially new-generation stents, which are essential tool of interventional cardiology. Examination of endpoints of myocardial infarction (MI) and stent thrombosis, which can emerge during clinical follow-up of patients in whom stent is implanted, was conducted, and mortality data and relevant effective and/or predictive factors were analyzed.

METHODS

Study Design and Study Population

This single-center, observational, retrospective study was conducted with a total of 1,052 patients aged >18 years who had EES implanted. Patients with symptomatic CAD and signs suggestive of myocardial ischemia on myocardial perfusion scan performed following the positive exercise stress test or those with ≥70% coronary artery lesion detected in conventional angiography performed following the detection of hemodynamically significant stenosis on computed tomography (CT) angiogram were enrolled. Information about the patients was retrieved from hospital files, medical records, laboratory results, and computerized database of angiography laboratory. In addition to basic demographic characteristics of age and gender, accompanying diseases were recorded. Patients who had contraindication for dual antiplatelet treatment (DAPT) consisting of acetylsalicylic acid and clopidogrel, and patients with high probability of undergoing surgical intervention within 6 months

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ORCID iDs of the authors: U.N.K. 0000-0001-9146-8765; E.B.K. 0000-0002-4424-3704; M.L.S. 0000-0002-0985-3144; N.O. 0000-0001-7914-0169; H.Y. 0000-0001-7673-229X; A.O. 0000-0002-0501-882X; K.A. 0000-0001-9279-8424.

Corresponding Author: Ugur Nadir Karakulak **E-mail:** ukarakulak@gmail.com

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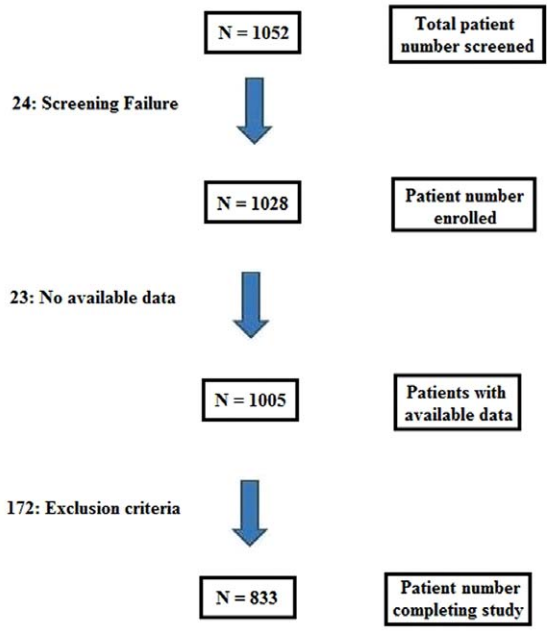
Figure 1. Flow chart of the study design.



were excluded.[6] Patients who had malignancy with life expectancy of <1 year were also excluded. Eventually, 219 patients were excluded, and the study was carried out with 833 patients (Figure 1). The present study was conducted in accordance with the Declaration of Helsinki, and ethics committee approval was received for this study from the ethics committee of Hacettepe University (2013:16969557-1200).

Interventional Procedure, Medical Treatment, and Follow-Up

Everolimus-eluting Xience V stent (Abbott Vascular, Inc., Temecula, CA, USA), with a cobalt-chrome stent platform coated with everolimus was used. In order to avoid vascular overlapping or shortening, severe lesion (vascular luminal stenosis >70%) angiogram with at least two views was displayed. Graft was implanted on lesion according to previously determined nominal pressure recommended by manufacturer and standard coronary angioplasty procedure. Decision to dilate before or after procedure was determined based on physician's preference.

Main Points

- Everolimus eluting stents are clinically effective, reliable, and safe treatment of coronary artery lesions in a patient population with a very heterogeneous clinical spectrum.
- Premature discontinuation of dual antiplatelet therapy and clinical presentation with ACS are associated with unfavorable outcomes including all-cause and cardiac mortality.
- Although male gender is a predominant risk factor for atherosclerosis and clinical cardiovascular disease, female gender was demonstrated to be a risk factor for all-cause and cardiac mortality. In addition, many cardiovascular risk factors were more common in female patients.

Prior to procedure, when performed under elective conditions, any previously prescribed acetylsalicylic acid and/or clopidogrel treatment was administered at daily dose. For patient who had not received acetylsalicylic acid and/or clopidogrel earlier, loading oral dose of acetylsalicylic acid (300 mg) and clopidogrel (600 mg) was administered. During angiography, intravenous bolus dose of heparin (70-100 U/kg) was delivered. Patients with acute coronary syndrome (ACS) were also given loading dose of acetylsalicylic acid (300 mg) and clopidogrel (600 mg) and intravenous bolus dose of 70-100 U/kg heparin during angiography.

After the procedure, patients were monitored continuously in the coronary intensive care unit, and electrocardiographic examination and blood pressure measurements were performed. Patients were followed-up at 1st, 6th, and 12th month after stent implantation, and then at yearly intervals. Need to maintain DAPT for at least 12 months was explained to all patients while in the hospital.[6] Compliance with DAPT was questioned during follow-up visits, and importance of this treatment was stressed by the physician.

Information about patient survival was retrieved from national Death Reporting System (www.obs.gov.tr) with a national ID number of the patient and patient hospital file number.

Endpoints and Definitions

All definitions and endpoints are based on definitions formulated by Academic Research Consortium.[7] Primary endpoints were all-cause mortality and target lesion failure (TLF) as a composite endpoint incorporating MI, target lesion revascularization (TLR), and cardiac mortality. Secondary endpoints were cardiac mortality, MI, TLR, target vessel revascularization (TVR), and stent thrombosis.

Statistical Analysis

Statistical evaluation was performed using the Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA) and MedCalc 11.4.2 (MedCalc Software, Ostend, Belgium) software. Normal distribution was assessed using Kolmogorov–Smirnov test. Numerical variables with normal and non-normal distribution were displayed using mean ± standard deviation and median, respectively. Categorical variables were indicated as numbers and percentages. For intergroup comparisons of numerical variables with normal distribution, the t-test was used, and for those with non-normal distribution, independent samples t-test and Mann–Whitney U test were applied. Chi-square test and Fisher's exact chi-square test were used to compare pairwise categorical data. To determine the effects of risk factors associated with all-cause and cardiac mortality, the multivariate Cox regression analysis was used to identify independent predictors following the univariate Cox regression analysis. Kaplan–Meier analysis was employed to demonstrate the correlation between endpoints and risk factors during monitoring period. $P < .05$ was accepted as level of significance with 95% confidence interval and 5% standard error.

RESULTS

Baseline Demographic Characteristics

Baseline demographic characteristics of the patients are provided in Table 1. A total of 833 patients (male: n = 621, 74.5%;

Table 1. Baseline Demographic Characteristics of All Study Populations (n = 833)

Age (year)	62.1 ± 10.3
Gender	
Female	212 (25.5%)
Male	621 (74.5%)
Hypertension	522 (62.7%)
Diabetes mellitus	256 (30.7%)
Hyperlipidemia	361 (43.3%)
Family history of coronary artery disease	186 (22.3%)
Smoking habitus	
Non smoker	624 (74.9%)
Ex smoker	58 (7.0%)
Active smoker	151 (18.1%)
Peripheral artery disease	44 (5.3%)
Cerebrovascular disease	25 (3.0%)
Systolic dysfunction	123 (14.8%)
Atrial brillation	60 (7.2%)
Chronic kidney disease	22 (2.6%)
Chronic obstructive pulmonary disease	42 (5.0%)
Malignancy	45 (5.4%)

female: n = 212, 25.5%) were enrolled. Mean age of the patients was 62.1 ± 10.3 years. Female patients were older than male patients (66.1 ± 10.2 vs. 60.8 ± 9.9; $P = .001$). Cardiovascular risk factors diabetes mellitus (DM) (41.0% vs. 27.2%; $P = .001$) and hypertension (HT) (75.9% vs. 58.1%; $P = 0.001$) were significantly more common in women, while the incidence of other accompanying diseases was similar in both groups. The percentage of active and previous smokers was higher among male population compared with female patients (29.3% vs. 6.6%; $P = .001$).

Information about the history of CAD and presenting symptoms is shown in Table 2. Nearly half (48.5%) of the patients had no history of CAD. A great majority (84.9%) of the stent-implanted patients underwent coronary angiography under elective conditions, while 126 patients (15.1%) presented to the clinic with ACS.

Table 2. Information Regarding History of Coronary Artery Disease and Presenting Symptoms of All Study Populations (n = 833)

Previous coronary artery disease	
None	404 (48.5%)
Atherosclerotic heart disease	64 (7.7%)
Percutaneous coronary intervention	211 (25.3%)
Coronary artery bypass operation	154 (18.5%)
Presentation	
Elective admission, positive results of	707 (84.9%)
Ischemic symptoms	476 (57.1%)
Treadmill testing	70 (8.4%)
Coronary computed tomography angiography	129 (15.5%)
Myocardial perfusion scintigraphy	32 (3.8%)
Acute coronary syndrome	126 (15.1%)

Characteristics of Stents and Lesions

A total of 1,134 stents were implanted. The number of EES for each individual was as follows: 1 (n = 587; 70.5%), 2 (n = 199; 23.9%), 3 (n = 39; 4.7%), and 4 (n = 8; 1.0%). Stents were available in 6 diameters, ranging from 2.25 mm to 4.00 mm, and the mean diameter was 2.86 ± 0.38 mm. Stent with a diameter of 2.75 mm was implanted most frequently (28.2%). Stents of 2.50 mm and 3.00 mm in diameter were implanted in 22.9% and 21.6% of the patients, respectively. The mean stent length was 20.4 ± 6.2 mm (range: 8-38 mm). The 18-mm stent was most commonly implanted (31.5%), while the 15-mm and 23-mm stents were used in 19.8% and 18.8% of the patients, respectively.

Characteristics and localization of 1,134 lesion are shown in Table 3. The mean length and diameter of lesion were 18.0 ± 5.9 mm and 2.65 ± 0.35 mm, respectively. Stent implantation was performed for 13 different native or grafted vessels in this study. Most frequently, the left anterior descending artery (45.7%) was the target, followed by right coronary artery (27.6%) and circumflex artery (16.6%). A total of 15 bifurcation lesions and seven ostial lesions were found.

Endpoints and Follow-Up

Endpoints and other events seen during the follow-up period are shown in Table 4. A median follow-up period of the study was 16 months (range: 1-70 months). Among the 833 patients, all-cause and cardiac mortality were seen in 11 (1.3%) and seven (0.84%) patients, respectively. As a primary endpoint, TLF was seen in 19 (2.3%) patients. As a secondary endpoint, TLR (n = 7; 0.8%), TVR (n = 18; 2.2%; total stenosis rate: 3.0%), MI (n = 5; 0.6%), and stent thrombosis (n = 2; 0.24%) were observed in indicated number of patients. None of the patients were transferred to department of cardiovascular surgery during short- or long-term monitoring period.

Table 3. Characteristics and Localization of Coronary Lesions (n = 1,134)

Lesion length (mm)	18.0 ± 5.9
Lesion diameter (mm)	2.65 ± 0.35
Lesion localization	
Left anterior descending artery	518 (45.7%)
Circumex artery	188 (16.6%)
Right coronary artery	313 (27.6%)
Obtuse marginalis	41 (3.6%)
Intermediate artery	11 (1%)
Diagonal branch	29 (2.6%)
Left main coronary artery	8 (0.7%)
Saphenous—right coronary artery graft	11 (1%)
Saphenous—obtuse marginalis graft	4 (0.4%)
Radial—obtuse marginalis graft	1 (0.1%)
Saphenous—left anterior descending artery graft	4 (0.4%)
Saphenous—diagonal graft	4 (0.4%)
Left internal mammary artery graft	2 (0.2%)
Bifurcation lesion	15 (1.3%)
Osteal lesion	7 (0.6%)

Table 4. Study Endpoints (n = 833)

Mortality	
All cause mortality	11 (1.3%)
Cardiac death	7 (0.84%)
Target lesion failure	19 (2.3%)
Target lesion revascularization	7 (0.8%)
Target vessel revascularization	18 (2.2%)
Myocardial infarction	5 (0.6%)
Stent thrombosis	2 (0.24%)
Premature discontinuation of dual antiplatelet therapy	11 (1.3%)
Hospitalization	
Heart failure acute decompensation	17 (2%)
Atrial brillation	12 (1.4%)
Device therapy	
Pacemaker	15 (1.8%)
Implantable cardioverter debrillator	11 (1.3%)
Cardiac resynchronization therapy	8 (1%)

The patients were also classified according to severity and timing of stent thrombosis. One case each of acute and subacute stent thrombosis was seen. Stent thrombosis was observed in 0.17% of 1,134 stents implanted. During the follow-up period, 11 (1.3%) patients discontinued DAPT prematurely.

All-Cause Mortality

Eleven (1.3%) deaths were seen in total of 833 patients. Seven patients died due to cardiac causes, and four cases were all-cause mortality (Table 5). Mean age of patients who died was higher than that of those who survived (75.7 ± 15.2 vs. 62.0 ± 10.1; $P = .013$). In women, who generally have lower risk of CAD, all-cause mortality was more common and finding was significant when compared with male patients (2.8% vs. 0.8%; $P = .036$). History of CVD was more frequently observed in deceased patients relative to those who survived (18.2% vs. 2.8%; $P = .003$). The presence of CKD was more often seen in patients who deceased (18.2% vs. 2.4%; $P = .001$). In 45.5% of the deceased patients, the EF ≤ 50% was observed, while the corresponding percentage was only 14.4% in those who survived ($P = .014$). Average EF of the deceased and surviving patients was estimated at 49% and 60%, respectively. Admission symptoms also differed markedly. Nearly half (45.5%) of those who deceased, but only 14.7% of surviving patients, presented with clinical manifestations of ACS ($P = .010$). Premature discontinuation of DAPT was more frequently and markedly detected among patients who deceased (36.4% vs. 0.9%; $P = .001$). History of other known risk factors, such as DM and CAD, was not found to be correlated with mortality rate ($P > .05$). AF was more frequently detected among patients who deceased without any statistically significant intergroup difference (18.2% vs. 7.1%) ($P = .185$).

In multivariate Cox regression analysis that is used to analyze the effects of significant risk factors detected in univariate regression analysis, increase in age (1.1-fold; 95% CI, 1.064-1.219; $P = .001$), premature discontinuation of DAPT (48-fold; 95% CI, 11.019-216.429; $P = .001$) (Figure 2), and admission with clinical manifestations of ACS (4.6-fold; 95% CI, 1.077-19.914; $P = .039$) (Figure 3) increased all-cause mortality rate as indicated in parentheses.

Cardiac Mortality

Among the 833 patients, cardiac mortality was seen in only seven (0.8%) patients (Table 5). Mean age of the patients who exited due to cardiac causes was significantly higher than that of those who survived (80.5 ± 13.7 vs. 62.0 ± 10.1; $P = .001$). Cardiac mortality was also more commonly seen among female patients. Cardiac mortality was seen in five (2.3%) female and two (0.32%) male patients ($P = .014$). A total of 71.4% of cardiac mortality was in female patients, who constituted only 25.5% of all study population.

Peripheral artery diseases (PADs) and CVD were more frequently detected in patients who died of cardiac causes when

Table 5. Clinical Characteristics of the Patients in Terms of Mortality (n = 833)

	Alive (n = 822)	All cause death (n = 11)	Cardiac death (n = 7)	P*	P†
Age	62.0 ± 10.1	75.7 ± 15.2	80.5 ± 13.7	0.013	0.001
Gender, male	616 (74.9%)	5 (45.5%)	2 (28.6%)	0.036	0.014
Hypertension	512 (62.3%)	10 (90.9%)	6 (85.7%)	0.061	0.266
Diabetes mellitus	254 (30.9%)	2 (18.2%)	1 (14.3%)	0.518	0.683
Hyperlipidemia	357 (43.4%)	4 (36.4%)	3 (42.9%)	0.765	0.976
Family history of CAD	185 (22.5%)	1 (9.1%)	1 (14.3%)	0.472	0.604
Smoking habitus				0.655	0.719
Nonsmoker	615 (74.8%)	9 (81.8%)	6 (85.7%)		
Exsmoker	58 (7.1%)	-	-		
Active smoker	149 (18.1%)	2 (18.2%)	1 (14.3%)		
Peripheral artery disease	42 (5.1%)	2 (18.2%)	2 (28.6%)	0.111	0.006
Cerebrovascular disease	23 (2.8%)	2 (18.2%)	2 (28.6%)	0.003	0.001
Systolic dysfunction	118 (14.4%)	5 (45.5%)	2 (28.6%)	0.014	0.269
Atrial brillation	58 (7.1%)	2 (18.2%)	-	0.182	0.466
Chronic kidney disease	20 (2.4%)	2 (18.2%)	1 (14.3%)	0.001	0.047
COPD	41 (5%)	1 (9.1%)	1 (14.3%)	0.436	0.306
Malignancy	43 (5.2%)	2 (18.2%)	-	0.115	0.534
Previous CAD				0.461	0.326
None	396 (48.2%)	8 (72.7%)	5 (71.4%)		
Atherosclerotic	64 (7.8%)	-	-		
PCI	210 (25.5%)	1 (9.1%)	-		
CABG	152 (18.5%)	2 (18.2%)	2 (28.6%)		
Presentation				0.016	0.038
Elective	701 (85.3%)	6 (54.5%)	4 (57.1%)		
ACS	121 (14.7%)	5 (45.5%)	3 (42.9%)		
Premature discontinuation of DAPT	7 (0.9%)	4 (36.4%)	4 (57.1%)	0.001	0.001
Implanted stent count				0.875	0.926
1	579 (70.4%)	8 (72.7%)	5 (71.4%)		
2	196 (23.8%)	3 (27.3%)	2 (28.6%)		
3	39 (4.7%)	-	-		
4	8 (1%)	-	-		

ACS, acute coronary syndrome; CABG, coronary artery bypass graft; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; DAPT, dual antiplatelet therapy; PCI, percutaneous coronary intervention.
*P value between alive and all cause death.
†P value between alive and cardiac death.

Figure 2. Kaplan-Meier estimated survival analysis for all-cause mortality in terms of premature discontinuation of dual antiplatelet therapy.

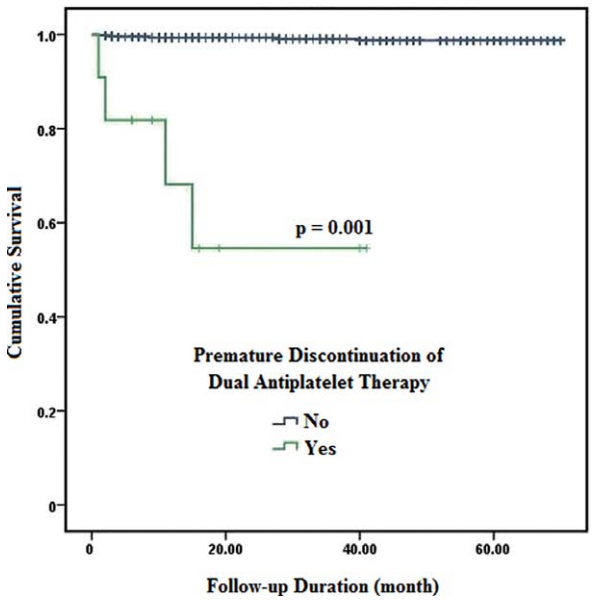
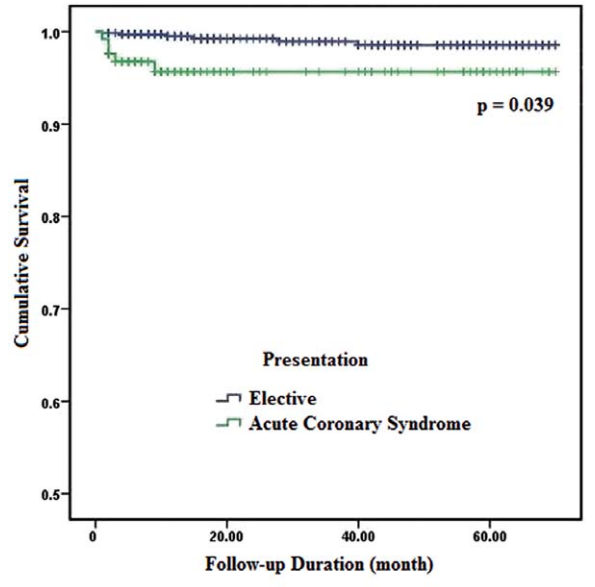
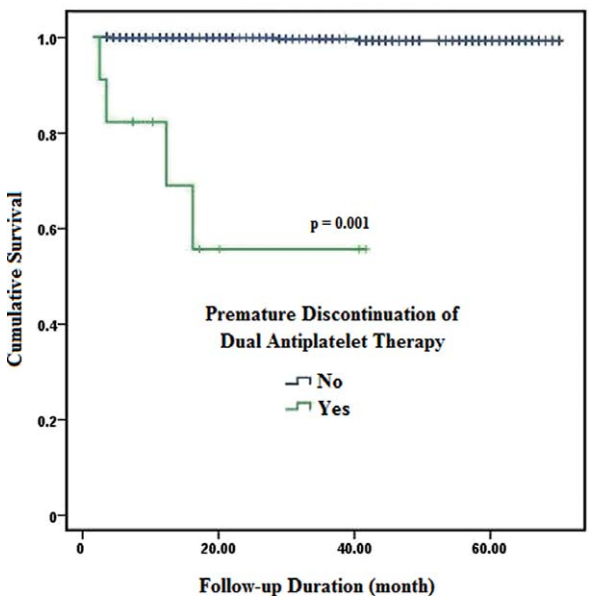


Figure 3. Kaplan-Meier estimated survival analysis for all-cause mortality in terms of clinical presentation of the patients.



compared with those survived (PAD: 28.6% vs. 5.1%, $P = .006$; CVD: 28.6% vs. 2.8%; $P = .001$). Similar to all-cause mortality, the presence of CKD and admission symptoms were significantly different between patient groups. CKD was seen in 14.3% of the patients who deceased of cardiac causes and in 2.4% of patients who survived ($P = .047$). Of the patients who deceased, 45.5% were admitted to the hospital with clinical manifestations of ACS ($P = .038$), while the figure was only 14.7% of those who survived. Premature discontinuation of DAPT was markedly higher in patients who deceased of cardiac causes (57.1% vs. 0.9%; $P = .001$).

Figure 4. Kaplan-Meier estimated survival analysis for cardiac mortality in terms of premature discontinuation of dual antiplatelet therapy.



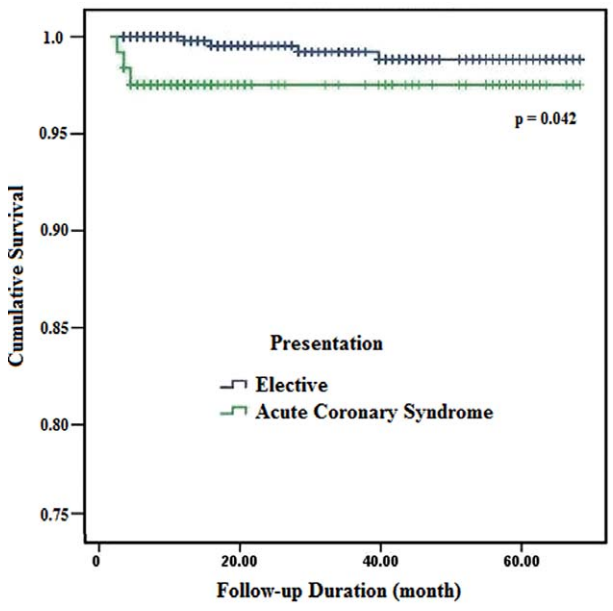
In multivariate analysis of risk factors with a significant affect on cardiac mortality as determined in univariate Cox regression analysis, age (95% CI, 1.104-1.631; $P = .003$), premature discontinuation of DAPT (95% CI, 27.409-13391.68; $P = .001$) (Figure 4), and ACS at admission (95% CI, 1.145-1270.092; $P = .042$) (Figure 5) were found to be predictors of cardiac mortality.

DISCUSSION

Effectiveness and reliability of EES were analyzed in the context of prevalence of seven endpoints, risk factors and predictors of all-cause and cardiac mortality, and multiple clinical and angiographic variables. This study included relatively large number of patients and applied only a few exclusion criteria. Patients and lesions with of varied characteristics were evaluated, providing us with a real-life information.

As an outcome, this real-life stent implantation study has revealed that EES is clinically effective and reliable in the treatment of wide spectrum of coronary artery lesions. During the median 16-month follow-up period, incidence rate of primary endpoints of all-cause mortality and TLF was found to be 1.3% and 2.2%, respectively. Incidence of secondary endpoints of stent thrombosis, TLR, TVR, and MI was 0.24%, 0.8%, 2.2%, and 0.6%, respectively. At this point, it is important to remember

Figure 5. Kaplan-Meier estimated survival analysis for cardiac mortality in terms of clinical presentation of the patients.



that study population had relatively large number of patients with extremely heterogeneous distribution. It included patients with high-risk profile and did not impose strict exclusion criteria. EES implantation proved to be highly effective procedure with a relatively low incidence of adverse events.

Everolimus eluting stents have been subject of multiple studies, including large-scale clinical studies such as A Clinical Evaluation of the XIENCE V and Everolimus Eluting Coronary Stent System in the Treatment of Patients with De Novo Native Coronary Artery Lesions (SPIRIT) II study. EESs have been compared with paclitaxel-eluting stents (PESs) and evaluated separately in studies. SPIRIT II study's 5-year outcomes were published in 2013,[8] SPIRIT III study's 1- and 5-year outcomes were released in 2008 and 2013, respectively,[9,10] and SPIRIT IV 2-year outcomes were published in 2011.[11] Strict clinical and angiographic restrictions were implemented in all three comparisons of EESs and PESs, and the implantation of at most two stents per patient was permitted. In addition to patients with unstable angina pectoris, cases with acute MI, CVD, and CKD (serum creatinine level >2.5 mg/dL or those in need of dialysis) were excluded. Similarly, in the EXecutive RCT: evaluating XIENCE V in a multi-vessel disease (EXECUTIVE) trial (2013), which also compared EESs and PESs, patients who experienced MI within 72 hours and those with the history of CKD or CVD were excluded from the study.[12]

Patients with ostial, bifurcation, left main coronary artery (LMCA), and totally occlusive lesions were not included in SPIRIT studies. Stent diameter was 2.5-4.25 mm in SPIRIT II and 2.5-3.75 mm for SPIRIT III and IV studies. In EXECUTIVE study, patients with LMCA and saphenous vein graft lesions were excluded, and the effectiveness of EES was tested in patients with multiple (2 or 3) vessel disease, including bifurcation lesions. In our study, dimension of the stents was not restricted, and EESs of six diameters (2.25-4.0 mm) and eight lengths (8-38 mm) were implanted. The present study used 33- and 38-mm stents, which were not used in aforementioned studies, in 5.6% (n = 63) of lesions.

Several other large-scale comparative clinical studies on other DESs have been conducted.[13] Furthermore, clinical studies that include test of effectiveness, reliability, and safety of EESs are available.[14] Almost every year, new clinical studies are being conducted on EESs and DESs.[15] Retrospective study of Hermiller et al.[16] and prospective study of Latib et al.[17] were designed as single arm (only EES) trials. The SPIRIT V study tried to overcome restrictions in patient selection implemented in previous SPIRIT studies. Diabetic patients were included, and when needed, more than 1 stent was used for individual patient. Lesions were classified and recorded based on the American College of Cardiology/American Heart Association (ACC/AHA) criteria.[18] Number of lesions and stents were not restricted, and a high-risk group was evaluated separately from low-risk group. Similar to XIENCE V USA,[19] RESOLUTE III All Comers,[20] A Trial of Everolimus-Eluting Stents and Paclitaxel-Eluting Stents for Coronary Revascularization in Daily Practice,[4] and Xience Stent Evaluated at Rotterdam Cardiology Hospital[21] studies, in our study, high-risk patients and lesions were analyzed in the context of whole population.

Inclusion of clinically high-risk patients in DES study is important, as they reflect real-life conditions. In this study, CKD patients constituted 2.6% of total population, and the presence of CKD confronts us as risk factor for all-cause and cardiac mortality. Approach of our center to such patients involves hospitalization of the patient prior to procedure, consultation with nephrology department, cessation of nephrotoxic drugs under the surveillance of health professionals of our service, use of small quantity of contrast material during procedure, and performing procedure on day of dialysis session for dialyzed patients. In the XIENCE V USA study, renal failure, which was detected in nearly 11% of their patients, was found to be predictive factor for stent thrombosis, cardiac mortality, MI, and TLF.

History of CVD is another clinical risk factor. Incidence was 3.0% in total study population. As revealed in many studies, it is strong risk factor for both all-cause and cardiac mortality. The presence of CVD, which was exclusion criterion in SPIRIT study, was found to be a risk factor for cardiac mortality and MI in only XIENCE V USA study. In addition to CVD, another "forgotten" risk factor, PAD, was analyzed in this study and stood out as risk factor for cardiac mortality.

Diabetes mellitus, which is considered to be equivalent to CAD and comes to the forefront as a risk factor at every stage, was detected in 30.7% of present study patients. Though similar incidence of DM was observed in other DES studies, no significant correlation with any endpoint was detected. HT was detected in 62.7% of the patients. Median age of our study population (62.1 years) is comparable to those reported in Photoablation Using the Turbo-Booster and Excimer Laser for In-

Stent Restenosis Treatment (PATENT), and PATENT-2 studies, which investigated the prevalence of HT in Turkish population, concluded in 2005 and 2012, respectively. In both of these studies, the prevalence of HT among individuals aged between 60 and 69 years was 70.0% and 67.9%, respectively.[22,23]

DESs decrease in-stent restenosis by inhibiting neointimal proliferation with anti-proliferative agents they contain. On the other hand, DESs increase risk of thrombosis by delaying endothelization. Therefore, for all DESs, DAPT has vital importance.[24,25] In our study, patient compliance with DAPT was extremely high. The importance of DAPT is emphasized by both physicians and nurses during hospital stay, at discharge, and every polyclinic visit at our center. Results of this study determined premature withdrawal from DAPT to be independent risk factor for all-cause and cardiac mortality. Kaplan–Meier curve of correlation between these endpoints and premature withdrawal from DAPT demonstrated that most of these cases occurred within first few years of follow-up.

CAD is the most common cause of death among men and women. Though in risk scoring for atherosclerosis and clinical cardiovascular disease, male gender is a predominant risk factor, advanced-age female patients are exposed to risks of cardiovascular disease and death as frequently as male patients, if not more. Even though female patients constituted one-quarter of study patient population, half of all-cause and cardiac mortality rates were encountered among female patients. At first glance, it seems to be a contradictory condition, but several studies have yielded comparable results.[26,27] In our study, strong risk factors for CAD, such as DM and HT, were most common among female patients as in other studies.[28] However, female patients were significantly older than male patients. As expected, history of smoking and CAD were most common among men. Higher incidence of CAD among men and increased cardiovascular mortality rate among women are still debatable. Is female gender a risk factor by itself? Or does advanced age in group of female patients and more frequently seen HT and DM among them affect endpoints? A study conducted by Lansky et al.[27] followed-up EESs implanted in 469 male and 200 female patients for 1 year. Although no difference between groups was found regarding all-cause and cardiac mortality rates, LVR and TLR were more frequently seen among female patients.[27] In their study, female patients were relatively older, and DM and HT were most frequently observed. Also, in the XIENCE V USA study, female gender was found in multivariate analysis to be a stronger predictor for cardiac mortality, MI, and TLF. In our study, female gender was also demonstrated to be a risk factor for all-cause and cardiac mortality.

Study Limitations

Despite relatively large number of patients included in our study, this is a single-centered, retrospective study. Median follow-up period was relatively short for the evaluation of stent effectiveness and safety. Due to its design, only Xience V EES was evaluated, and comparative evaluation with other stent types or control group was not performed. Use of or withdrawal from DAPT was determined based on patient's personal statement, which might not reflect real data. Since endpoints were seen in small number of cases, extreme care should be used in the interpretation of risk and predictive factors of statistical analysis.

Dimensions and severity of lesion were not determined using quantitative measurements, but assessments were made based on observational decision of the principal investigator. Many characteristics of lesion, including calcification, ACC/AHA classification, thrombotic load, exit angle, and plaque morphology, were not studied. Intravascular ultrasound employed to determine both lesion characteristics and effectiveness of stent implantation was not performed in this study. In our center, lesions are not classified and intravascular ultrasound is not used routinely. Therefore, we deemed it unnecessary to use assessments and tests not routinely employed in our clinic for this study.

CONCLUSION

Incidence rates of all endpoints, including all-cause mortality and cardiac mortality, were low in EES implanted patients. For all-cause mortality and cardiac mortality; advanced age, ACS at admission, and premature discontinuation of DAPT were predominant predictive risk factors. EES has utmost effectiveness and reliability in the management of coronary artery stenosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Hacettepe University (2013:16969557-1200).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - N.O., A.O., K.A., H.Y.; Design - N.O., A.O., K.A.; Supervision - E.B.K., M.L.S., N.O., A.O., K.A.; Resources - X.X., X.X.; Materials - E.B.K., M.L.S., H.Y.; Data Collection and/or Processing - U.N.K., E.B.K., M.L.S., H.Y.; Analysis and/or Interpretation - U.N.K., H.Y.; Literature Search - U.N.K., E.B.K., M.L.S., H.Y.; Writing Manuscript - U.N.K.; Critical Review - N.O., A.O., K.A.

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Original Article

Knowledge and Use of Traditional Medicinal Animals in the Arba Minch Zuriya District, Gamo Zone, Southern Ethiopia

Mulugeta Kebebew[1], Erchafo Mohamed[2], V.B. Meyer-Rochow[3,4]

[1]Department of Biology, College of Natural Sciences, Arba Minch University, Ethiopia

[2]Department of Biology, College of Natural Sciences, Wachamo University, Ethiopia

[3]Department of Plant Medicals, Agricultural Science and Technology Institute, Andong National University, Andong, Republic of Korea

[4]Department of Ecology and Genetics, University of Oulu, Finland

ABSTRACT

Objective: To collect ethnozoological data in connection with medicinal animals and their products used by the inhabitants of the Arba Minch Zuriya region of Ethiopia and to put on record information on traditional treatments of diseases and disorders.

Methods: The survey was conducted during the months of February to May 2018. Data were gathered through semi-structured surveys and depended on group discussions with 90 people, of which 17 were key and the remainder general informants.

Results: Altogether 20 animal species comprising 12 mammals, one bird, three reptiles, two insects, and two fish were used in 30 distinctive ways to treat disorders of the eye and skin ailments such as anaemia and malaria as well as various other disorders and injuries. Twenty percent of all health problems involved the skin. Bovidae were the most important medicinal animals with a use of 14%, and bile was the most widely employed animal product.

Conclusion: Traditional therapies involving animal species are still being practiced in rural areas of Ethiopia and this knowledge is of importance and should not get lost. However, Overexploitation and overhunting pose a serious threat to the therapeutic species. For the conservation and management of these species, the local residents' cooperation and understanding are needed.

Keywords: Therapeutic animals, ethnozoology, indigenous knowledge, traditional medicine

INTRODUCTION

Throughout the world, humans and animals have interacted since time immemorial. Animals were feared, hunted, consumed, and used in various ways such as to treat diseases or as part of festivities and subjects in myths and beliefs.[1–4] Animals have played a larger than average scope of roles in virtual all human endeavours and their influence on religion, workmanship, music, dance, literature and other distinctive social articulations of humankind is undisputed. Specifically, focusing on the therapeutic uses of animals and their products in Ethiopia, information has been passed down orally from generation to generation. This knowledge needs to be seen as a major component of the Ethiopian human social legacy, but if unrecorded it is in danger of being lost.[5–7]

Recognizing the natural assets that wild and domestic therapeutic animal species represent, people living in Ethiopia and other developing countries have embarked on safeguarding the resource to render it sustainable.[5,8,9] According to data published by the WHO,[10] nearly 80% of the world's population live in developing countries where they largely depend on customary medication for treating sicknesses and ailments of humans and animals. There is not only an interest in the developing world, but also now a worldwide interest in documenting traditional healing methods from different parts of the globe involving animals and their products. Ethiopia is rich in ethnic communities whose languages, cultures, and traditions differ and whose members live in different parts of the country. What unites them all is that they have been using traditional methods of medication for generations, but that despite the long history of their ethnobotanical and ethnozoological knowledge, few attempts have been made in the past to record this knowledge.

To provide an inventory of the traditionally used medicinal animals for certain areas of Ethiopia, those of the Kafta-Humera District, Northern Ethiopia, were recorded by Giday et al.[5] Thirty traditional healers of that district reported that 16 species of animals (44% of which were domestic species) were used to treat 18 different human ailments. The parts and products of the animals that were used therapeutically included bile, milk, blood, pancreas, urine, hair, and fecal matter. Among the Amaro

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ORCID iDs of the authors: M.K. 0000-0002-9185-9325; V.M. 0000-0003-2616-0099; V.B.M.R. 0000-0003-1531-9244.

Corresponding Author: Victor Benno Meyer-Rochow **E-mail:** meyrow@gmail.com

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Woreda residents of Southern Ethiopia, investigated by Dereje and Meseret,[6] 90 respondents declared that 21 species of animals (14 mammals, four birds, and three reptiles) were used to prepare remedies for 46 ailments and that animal flesh (33.8%) had the highest use, followed by fat (11.5%), bone (8.6%), and blood (8.6%). Python, warthog, crested porcupine, and bushpig were of great relative importance (RI), but only 61.7% of the respondents did and 38.3% did not use traditional medicines, which in the majority of cases were administered orally.

Data based on information from 36 purposively selected respondents formed the basis of an ethnozoological study among the indigenous people of Metema Woreda in Northwest Ethiopia.[7] This study revealed that 51 species were used to treat around 36 different kinds of ailments. Although the majority of the animals used were mammals (27 species), the remainder contained birds (nine species), arthropods (seven species), reptiles (six species), and fish and annelids (one species each). The therapeutic use of rare or protected species, eg, cheetah, gazelle, elephant, monkeys, etc., was of some concern and highlighted the need to have more details on the traditional uses that indigenous people put native species to.

This present study was initiated to collect additional information on the uses of traditional medicinal practices from tribal areas within the Arba Minch Zuriya, Gamo Gofa Zone, Southern Ethiopia. Since until now no ethnotherapeutic uses had been reported from this rather remote region of Ethiopia, all our observations can be regarded as new. The main aim of this study was to enrich the country's database of medicinal animals and to provide additional information on the indigenous knowledge of ethnic communities in Ethiopia on how to use the species therapeutically without endangering their continued survival in the future.

METHODS

Description of the Study Area

Arba Minch is the capital of the Arba Minch Zuria district (Figure 1), around 437 km from Addis Abeba, the capital of Ethiopia. Arba Minch's longitude and latitude are 06°2'N and 37°33'E, respectively, and its altitude ranges from 1200 to 1285 masl. The temperature of the district ranges between 17 and 30°C. Precipitation is bimodal and amounts to 900 mm annually. The wet season covers the months March, April, and May, and September, October, and November but may extend up to December, January, and February.[11] According to the latest data available, ie, the 2007 census, the district had a population of 165,680 of which 82,774 were male and 82,906 were female.

Main Points

- Traditional therapies involving animals and their products are still being used in rural parts of Ethiopia.
- Altogether 13 species of the therapeutic animals of the region (65%), involving mainly mammals and reptiles, were obtained from the wild, and seven (35%) represented domestic animal species. With a relative importance index of RI = 0.912, the fox Vulpes vulpes turned out to be the most versatile species.
- Thirty different kinds of preparation methods to treat disorders were recorded. The most important routes of administration were oral, dermal, and nasal.
- Overexploitation and overhunting pose a threat to the therapeutic species. For the conservation and management of these species, the local residents' cooperation and understanding are needed.

Reconnaissance Survey and Study Site Selection

The surveillance was conducted from March 2017 to April 2018. Prior to the study, authorization to carry out the investigation was obtained from the local governing body. The approved methodology was followed, and the consent of the interviewed members was acquired. A decision on the choice of the study site depended on prior information that had been gathered from community pioneers, proficient seniors, and a number of customary healers of the area.

Informant Selection

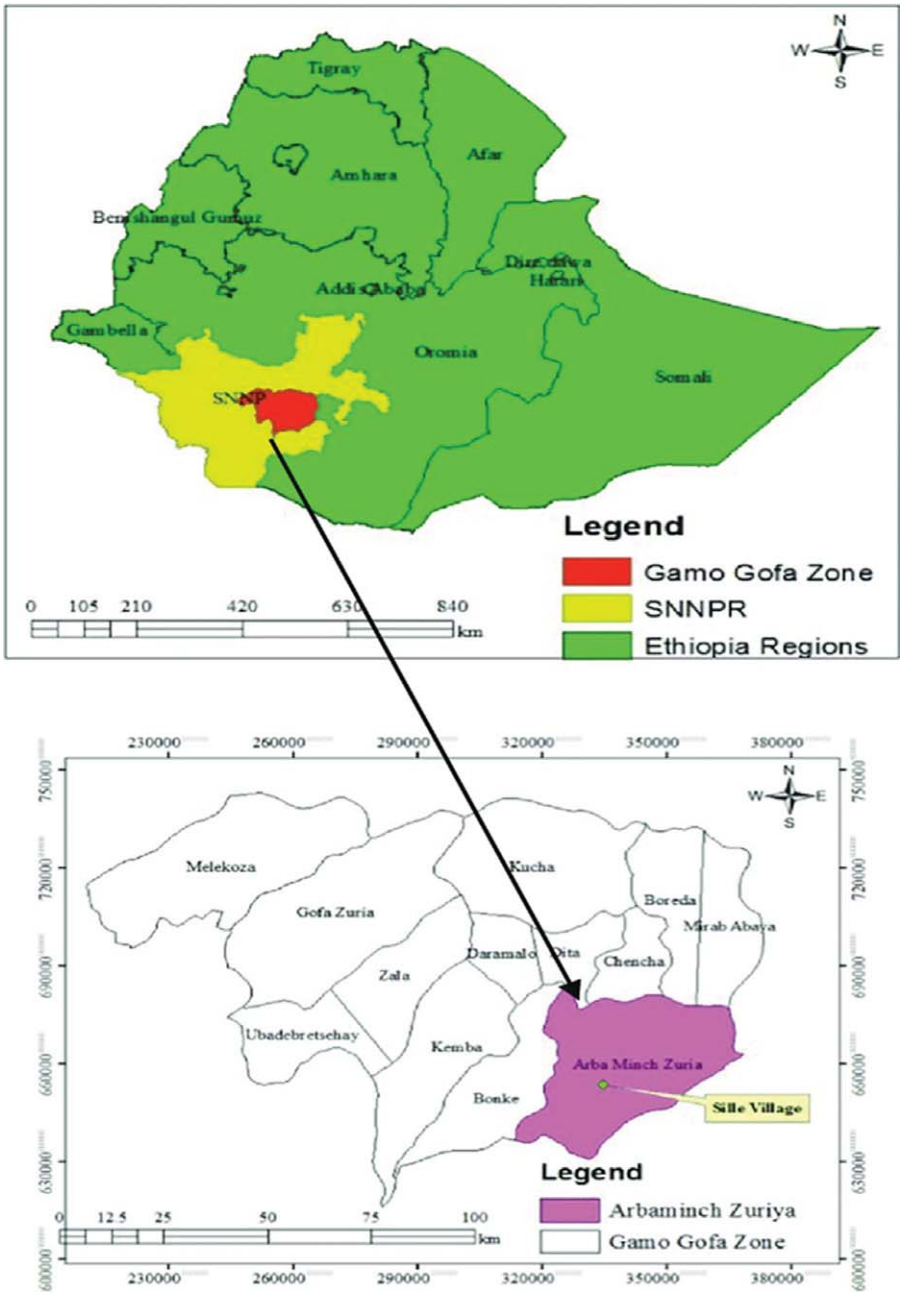
Ninety informants (45 males and 45 females) between 30 and 85 years of age were contacted during this research. Among these, 17 (nine males and eight females) were key informants, while the remainder of 73 were general informants. Random and purposive sampling strategies were employed to choose respective general informants and traditional healers. Affiliated pioneers and respected old individuals assisted with identifying the key informants. The general informants were randomly chosen during field and house visits. All interviews were administered after obtaining voluntary consent of every informant and assuring them that the data collected were used only for academic purposes.

Data Collection and Identification

Following standard methods,[5–7,12] ethnozoological data assortment was accomplished from February to April 2018 by living in close contact with the community in the study region. Semistructured interviews, guided field walks, direct observations, and focus group discussions with key informants and other knowledgeable community members were carried out. The responses of our informants were copied down. The semistructured interviews contained a checklist of questions focusing on the vernacular names of medicinal animals, their habitats, parts of the animals or their products used, medication preparation methods, materials utilized during preparation, condition of preparation, additives/ingredients used during preparation and administration, dosage administrated, and route of administration. Moreover, reaction to the medication (assuming there was any) was likewise included.

Observations with informants on field walks took place to see and watch in their habitats those animals the informants referred to and produced voucher specimens of.[5] Besides focus group discussions with conventional healers, conversations with local knowledgeable people and key informants were held to obtain additional data and to check the reliability of their statements. On some occasions, the preparation methods of the therapeutic animals were meant to remain secret and were,

Figure 1. Map of Ethiopia and the study region



therefore, excluded from the discussion. Most field observations were directed with only one informant at a time in order to safeguard the secret information; this was what the healers specifically requested. Specimens were gathered on site, and together with the information of the local name and photographs, the dead skin, hair, fur, and other characteristic materials were taken to Arba Minch University (AMU) for inspection. Formal identification of the therapeutic animals was conducted by zoological experts of AMU, comparing the collected material with internet images and published animal keys. Molecular analyses of the collected therapeutic animals were not possible and not deemed necessary.

Ethics Statement and Consent to Participate

This investigation was approved by the Committee for Ethical Research of the Department of Biology, Arba Minch University (AMU/021/2017). Prior to collecting the data, in each case of an interview, we obtained oral informed consent to proceed. All informants were given detailed information on the objectives

Table 1. Household Characteristics of the Respondents (n = 90)

	Sex		Age group (in years)					Educational status				
Variable	M	F	20–30	31–40	41–50	51–60	>60	Illiterate	1–4 grade	5–8 grade	9–10 grade	11–12 grade
Frequency	45	45	18	37	27	14	4	21	33	32	13	1
Percentage	50	50	18	37	27	14	4	21	33	32	13	1

Table 2. Proportion of Animal Species Used in Traditional Medical Treatments

No.	Animal species	Number of species	Percentage
1	Mammals	12	60
2	Birds	1	5
3	Reptiles	3	15
4	Fish	2	10
5	Insects/arthropods	2	10

of the research and understood that it was not carried out for business purposes but for scholastic reasons. All participants gave verbal informed consent to participate in this study; they were allowed to withdraw their information at any point of time. The informants completely accepted the idea and objectives of the study and consented to have their names and personal information published if required.

Data Analysis

The ethnozoological data were analyzed using appropriate statistical tools such as Microsoft Office Excel Spreadsheet®. Excel was used to calculate sums and percentages and to tabulate and draw graphs. Descriptive statistics, for example, percentage and statistical distribution, were employed for analyzing animal habitat, animal part(s) or product(s) used, methods of preparation medication, dosages administrated, and route of administration. Data were presented in graphs and tables, and they were interpreted and discussed. The fidelity level quantifies the importance of a species for a given purpose. It refers to the share of informants claiming the employment of a specific animal species for the identical major purpose was calculated for the foremost frequently reported disease or ailments as FL (%) = (Np/N) × 100, where Np is the number of informants that claim a use of the animal species to treat a specific disease and N is the total number of informants that use the species as a medication to treat a given disease.[13]

The RI value is employed to quantify the variety of medicinal applications; it was computed for every claimed medicinal animal. The formula used was as follow: R = NP+NBS, where RI stands for relative importance and NP is the computed value obtained by dividing the quantity of properties (specific ailments treated) recognized in connection with a species divided by the overall number of properties attributed to the foremost versatile species (species with the best number of properties). The number of body systems (NBS) value is obtained by dividing the quantity of the body systems (ailment categories) treated by a given species by the overall number of body system treated by the foremost versatile species.[14]

RESULTS AND DISCUSSION

Socio-demographic Characteristics of the Respondents

Data on the socio-demographic attributes of the respondents with respect to age, sex, and educational status are introduced in Table 1. Of the 90 informants interviewed in the study region, 45 were male and 45 female. The majority of the informants (37%) were in the age group of 31-40, followed by 27% aged 41-50; just 18% of the respondents were in the 20-30-year-old group and 18% were over 50 years of age. Regarding their educational backgrounds, most of the respondents (33%) had completed the first cycle of primary education (1-4 grades), but only 13% had completed secondary high school (9-10 grades).

Traditional Medicinal Animals Used by Peoples of the Study Area

Nineteen species of the therapeutic animals were gathered and archived from the study region (Table 2). Mammals registered the highest percentage in both number of animal parts and animal products (60%), trailed by reptiles (15%). In addition, 10% each of the therapeutically used species were identified as fishes and insects, and the sole representative of the birds, the domestic chicken, accounted for 5% of the medicinally utilized fauna-based groups of animals (Table 2). This outcome demonstrates that the interviewed people of the Arba Minch Zuria district have therapeutic uses for only a relatively small number but taxonomically wide range of diverse species of animals to treat diseases and bodily dysfunctions. The presence and usage of such few therapeutic animals by people in the study area suggests that the people of the region may increasingly use

nontraditional medication and drugs. However, conventional medicines are still considered useful and important, especially for the poor who have little access to modern medicines and do not have the money to pay for expensive new drugs. A similar number of diverse taxonomic groups of therapeutic animals and related ethnomedicinal knowledge has been reported from some other regions of Ethiopia.[5–7,12,14]

Habitats and Abundance of Medicinal Animals

Altogether 13 species of the therapeutic animals of the region (65%) were obtained from the wild and seven (35%) represented domestic animal species. This shows that the traditional healers rely more on the wild than the domestic species and additionally hints at the possibility that some wild animal species of the study region could be overused. This observation in concert with the perception obtained in interviews affirmed that customary healers generally have less interest in employing domesticated species for their treatments of specific illnesses. Species used primarily as food (like the domestic animals) appear to be less appreciated as a source to treat sick people with. This finding agrees with observations on the therapeutic inventories of other tribes where wild medicinal animals also dominated[5–7,12] and could be driven to some extent by the consumers' preferences for drugs and potions from wild rather than domestic species.

Animal Parts/Products Used as Traditional Medicine

Results of this segment of the survey showed that various parts of the medicinal species were utilized therapeutically by the local practitioners to prepare potions and remedies. Of the 20 kinds of therapeutic material to treat various health problems, meat (18%), bile (10%), and feces/excrement (10%), followed by blood (8%), teeth, bone, and milk (all 6%) of specific animals, were most commonly used (Table 3). The risk of destroying the medicinal animal resource is especially high in connection with meat and bile as these items are used for a wide range of illnesses and usually require the killing of an animal. Collecting therapeutic bile, meat, and teeth has consequences for the survival of an animal, but collecting feces, feathers, eggs, and honey usually has only a minor effect on the survival of an individual animal especially when contrasted with meat, bile, teeth, and bones (or bone fragments). Elsewhere in Ethiopia, meat, bile, teeth, and feces were often also the most commonly used animal parts used to treat medical issues,[5–7,12] although for the West Gojjam Zone of Javittenan, North Achefer, and Bahir Dar Zuria districts' honey and meat had the highest use followed by purified butter, milk, liver, and cheese.[15]

Mode of Remedy Preparation

Local healers employ a great variety of methods to prepare traditional medicines. They frequently prepare some food to ingest (e.g. like a soup to which are added a variety of ingredients). However, direct uses dominate (68.97%), and soups with (6.90%) and without ingredients (6.90%) are the three main methods of producing a potent medicine (Table 4). Preparation and application methods vary, based on the types of disease to be treated, the actual site of the ailment, and the animal (or animal part) involved. A minority of preparations are made from mixtures of different animal species with water and a variety of different additives like honey, sugar, butter, salt, and milk. These added substances have different functions, e.g., to reduce the toxicity, to enhance the flavor, to lessen the chance of vomiting, and to avert diarrhea, and this could be the reason for the observation that one and the same animal part or product can be used to treat sometimes quite different organs or illnesses.[16] Specific herbal remedy preparations with their possible synergistic benefits may be prescribed.[17] Information was also provided that the medicine could be blended with regular food and beverages, so that it would either change in taste or could be taken without being noticed. Although in some cultures the placenta has a therapeutic role to play,[18] e.g., sheep placenta in traditional obstetrics in Nigeria,[19] no such uses were revealed by our informants, but it needs to be reiterated that the healers were very particular about certain treatments and animal uses that were considered too secret to be revealed.

Routes of Administration

Various routes of administering the medicinal animal preparations are known to the people of the area. The most important routes of administration are oral, dermal, and nasal. Dermal application in the form of a lotion, cream, or plaster is the dominant route (50.00%), followed by oral application (46.43%) (Figure 2). Oral and dermal routes of administration permit quick interactions of the prepared medicines with pathogens or the inflamed tissue and enhance the medicines' curative power. They have been shown to be the most widely used forms of administration also in other parts of Ethiopia.[5–7,11] Nasal administration involves inhaling fumes and/or introduction of the therapeutic agent into the nose. It is the least common method of treatment.

Relative Importance of Species and Fidelity Level

The RI of a species refers to the relative use of it in the preparation of the remedies. Material from the fox (*Vulpes vulpes*) is used to prepare remedies for four ailments, making the fox the most versatile species with an RI of 0.912. The two next most therapeutically important species are the cow (*Bos taurus*) and the chicken (*Gallus gallus*) with an RI of 0.881 each. These animals are followed by sheep (*Ovis aries*) (RI = 0.728), bats (RI = 0.681), goat (*Capra aegagrus*) (RI = 0.681), and *Papio anubis* monkeys (RI = 0.681) (Table 5). As an insect, the honey bee's score on the basis of its multipurpose product honey would be as high as 0.912, but considerably lower if we focused on the direct therapeutic use of its larvae alone.

The FL is determined to identify the most frequently treated malady or ailment category as mentioned by the informants. Skin-related diseases, i.e., various forms of dermatoses, vision-related problems, and malaria were the three main categories, scoring respective FL values of 0.5-4.00, 0.88-1.00, and 0.71-0.94. Species with an FL of 1.00 are the pig (*Sus scrofa)*, lacertilian lizards, the African warthog (*Phacochoerus africanus*), the goat (*Capra aegagrus hircus*), and snakes. The possibility exists to use different remedies for similar ailments[20] as has been shown for other regions elsewhere in the world, especially given irregular accessibility to particular species.[21] Alves and Alves[22] suggest that various species of therapeutic animals may have comparable restorative properties and that pharmacological scrutiny could possibly confirm their effectiveness as zoo-therapeutic cures.

Table 3. Therapeutic Species and Their Uses in Treating Specic Disorders in Human Subjects

Name	Scientific name	Taxon	Habitat	Condition	Main part(s) used	Mode of preparation and method of administration	Organ or illness treated
Monkey	*Papio anubis*	Cercopithecidae	Domestic	Dry/dry	Fecal matter	Dried fecal matter is fumigated	Sleeping sickness
					Hind, skin	Hind or skin applied to heal	Broken/ displaced bone, burn wound
Cow	*Bos taurus*	Bovidae	Domestic	Fresh	Bile	Bile massaging around closed eye	Eye/vision
				Fresh	Bile	Drinking the raw bile	Malaria
				Fresh	Gut content	Massaging gut content around male genitals to stimulate the organ	Male sex organ
				Fresh	Horns	Crushed horn taken with injera (traditional Ethiopian atbread)	Malaria
				Fresh	Leg	Leg cuts with ingredients turned into a healthy soup	Wrist fracture
				Fresh	Liver	Eaten directly	Anemia
				Fresh	Spleen	Eaten directly	Anemia, malaria, trachoma
				Fermented	Yoghurt	Eaten or drunk	Gastritis
Goat	*Capra hircus*	Caprinae	Domestic	Fresh	Oviduct	Mixed with butter and anointed to the head	Eye: vision disorder
				Fresh/dry	Fecal matter	Dried, powdered, mixed with water, and smeared over the head	Fighting dandruff
				Fresh	Milk	Directly given to drink	Eye problems, pain, headache, measles, TB, snake bite, vomiting, rheumatism
Sheep	*Ovis aries*	Caprinae	Domestic	Fresh	Bile	Drinking fresh bile	Malaria
					Blood	Drinking fresh blood	Anemia
					Milk	Drinking directly	Malaria

Table 3. (Continued)

Name	Scientific name	Taxon	Habitat	Condition	Main part(s) used	Mode of preparation and method of administration	Organ or illness treated
Pig	*Sus scrofa domesticus*	Suidae	Wild	Fresh	Meat	Consuming the meat	Rheumatism and headache
					Blood	Anointing the infected part	Skin infections
Warthog	*Phacochoerus* spp.	Suidae	Wild	Dry	Teeth	Heating the teeth and apply	Swellings, warts, toothache, and rheumatism
				Fresh	Blood	Drinking directly	Malaria, asthma, and rheumatism
Hyena	*Crocuta crocuta*	Hyaenidae	Wild	Dry	Skin	Tying skin to the neck	Protection from "Evil eye" and "Bad Spirits"
				Dry	Bone	Tying around the neck	Epilepsy and "Bad spirits"
Fox	*Vulpes vulpes*	Canidae	Wild	Fresh	Bile	Putting bile once/day on the nose for one week	Heart-related problems
				Dry	Teeth	Tying teeth around the neck	Throat problems
				Fresh	Pancreas	Tying pancreas to the arm	Spleen problems
				Fresh	Blood	Smearing it on the scalp	For hair growth
				Fresh/dry	Brain tissue and meat	Directly consumed	Epilepsy, mental disorders
Cat	*Felis catus*	Felidae	Domestic	Dry	Teeth	Rubbing teeth on the head	Skull glands
				Dry	Skin	Tying it around part of body	Spiritual problems
Groundhog	*Marmota monax*	Sciuridae	Wild	Fresh	Meat	Pounded meat to be consumed	Fattening of body
Rabbit	*Oryctolagus cuniculus*	Leporidae	Wild	Dry	Meat	Rubbing dried meat over the injury	Skin problems
				Dry	Fur	Fur is burnt; ash mixed with butter and creamed onto the burnt body part	Burns
Fruit bat	sp.	Pteropodidae	Wild	Dry	Bones	Inhaling fumes from fumigated bones	Mental illness
				Dry	Meat	Consuming the dried meat	Mental disorder and hepatitis

Table 3. (Continued)

Name	Scientific name	Taxon	Habitat	Condition	Main part(s) used	Mode of preparation and method of administration	Organ or illness treated
Chicken	*Gallus gallus*	Phasianidae	Domestic	Fresh	Whole body	Cooked and eaten	Wound and injury
				Dry	Excrement	Combining excrement with mud and applied to infected skin	Skin problems
				Fresh	Eggs	Drinking raw egg yolk	Heart failure
				Fresh	Liver and fat	Consumed raw	Pneumonia and swellings
Lizard	*Lacerta* spp.	Tropiduridae	Wild	Dry	Fecal matter	Pounded fecal matter, dried and with secret ingredients applied to swellings	Skin problems
				Fresh	Whole body	Making a drink by adding water and salt	Cough and anemia
Python	*Python* sp.	Pythonidae	Wild	Dry	Bone	Crushing the bone, tying and banding	Rabies and swellings
				Fresh/dry	Meat	Consumption and for anointing	Rabies, foot crack, and ear disorder
				Fresh	Fat	Applied to infected area	Ear disease, wound
Snakes	*Bitis harenna*	Ophiidae	Wild	Dry	Brain, skin	Used fresh to rub over the eye	Eye: vision disorder
Nile perch	*Lates niloticus*	Percidae	Wild	Fresh	Bile	Drinking fresh bile directly	Eye disorder
				Fresh/dry	Meat	Consumed directly	Rheumatism
Catfish	*Bagrus docmak*	Bagridae	Wild	Fresh/dry	Meat	Consumed directly	Rheumatism
Honey bee	*Apis mellifera*	Apidae	Domestic	Fresh	Honey	Eating or drinking the honey	Swarts, asthma, respiratory, throat pain, diarrhea, cough, TB, mumps, and heart failure
				Fresh	Larvae	Consuming directly	Stomach disorder
Stingless bee	*Meliponula* spp.	Apidae	Wild	Fresh	Honey	Eating or drinking the honey	Stomachache, eye disorders, and coughs

Table 4. Mode of Preparation of Traditional Medicines in the Study Area

No.	Mode of preparation	No. of preparation	Percentage
1	Direct use	20	68.97
2	Soup	2	6.90
3	Ingredients added	2	6.90
4	Crushing	1	3.45
5	Heating	1	3.45
6	Binding	1	3.45
7	Drying	1	3.45
8	Pounding	1	3.45

Figure 2. Major methods (routes) of traditional medicine administration in the study area

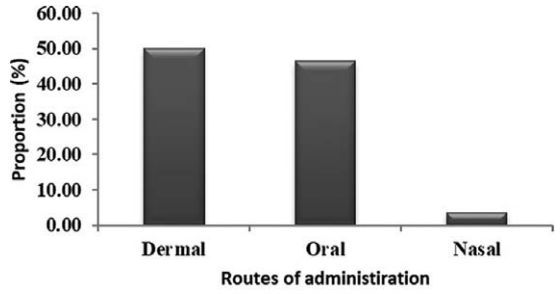


Table 5. Relative Importance (RI) of a Species

Common name	Scientific name	Relative importance (RI)
Fox	*Vulpes vulpes*	0.912
Cow	*Bos taurus*	0.881
Chicken	*Gallus gallus*	0.881
Sheep	*Ovis aries*	0.728
Flying fox	*Cynopterus sphinx*	0.681
Goat	*Capra aegagrus hircus*	0.681
Monkey	*Papio anubis*	0.681

Risk of Zoonotic Disease

Zoonosis alludes to illnesses, known zoonotic diseases, which are passed from animals to people. Animals can transmit harmful pathogens like viruses, bacteria, fungi, and parasites and consequently make people ill and ingestion of bile, for instance, as reported by Costa-Neto and Motta,[23] can transmit *Salmonella* spp., and lead to chronic diarrhea and even toxic shock. Zoonotic diseases may be mild or severe but may spread in the human population and lead to epidemics. Therefore, information on animal diseases (diseases transmitted between animals and humans) was sought.

We found that few respondents knew about the risk of zoonotic diseases and then only those that could read and write. This shows that the literate informants are likely to be less at risk of being contaminated with animal disease than those informants who are illiterate (uneducated). Although using therapeutic animals in treating diseases is generally considered a risk-free affair by consumers, zoonotic diseases are, nevertheless, increasing according to some of the illiterate (uneducated) respondents.[5,24] Thus, it seems necessary to warn of the dangers and the possibility to contract some of the more widespread zoonotic diseases like tuberculosis or rabies from animals, an aspect of considerable importance when dealing with animals and their tissues as remedies for human illnesses.

Threats to Medicinal Animals

In the Arba Minch Zuria district, the biggest threats for the future of the use of animals and their products in traditional remedies were according to our informants in the order of importance: (1) habitat loss and degradation, (2) overexploitation, (3) exotic species, (4) climate change, and (5) pollution. Ethnobotanical and ethnozoological research from elsewhere in Ethiopia had revealed very similar results.[5,25] Realistically, the incentive to conserve therapeutic animals within the area was seen to be very poor. However, a start is the awareness of the role of preservation in maintaining a sustainable use of the therapeutic animals in the Arba Minch and that needs to be supported in the region. It should be possible to find ways to halt the decline of the number of therapeutic animal species of the region by providing funds as well as land for domesticating therapeutic animals and helping the Traditional Healers Association in their activities with professional guidance.

CONCLUSION

The results of the present study are new and have shown that people of the Arba Minch Zuria district traditionally use a variety of therapeutic animals. As elsewhere in Ethiopia, specific animal-based medicines are prepared by Arba Minch Zuria healers using wild and domestic species of not only primarily mammals but also reptiles and birds. Most practitioners obtain their animals and their products from the wild by hunting, which could affect the distribution and abundance of the untamed species. An accurate estimation of the effect is not possible until a systematic ecological inventory of the study area has become available. It was noted that the men of the region possessed a greater knowledge of the traditionally used medicinal animals than the females. The proximity to Nech Sar National park created access to interact with diverse wild animal species. Relatively small numbers of the urban and rural inhabitants within the study area rely only on traditional medicine, which suggests that the fashionable health service plays a significant role in filling the gap between traditional and modern medical care. However, as Meyer-Rochow[16] points out, what we must not do is to belittle or outright dismiss without any evidence a treatment method that for centuries has been an accepted way to confront a disease simply because it seems to be based on superstition or subscribes to the tenet of "let likes be cured by likes."

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Arba Minch University (AMU/021/2017).

Informed Consent: An informed consent was obtained from all informants, and approval to carry out the study was given by the Ethics Committee of Arba Minch University.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.M.. M.K.; Design - E.M, M.K.; Supervision - M.K.; Data Collection and/or Processing - M.K.; Analysis and/or Interpretation - V.B.M.-R.; Literature Search - V.B.M.-R.; Writing Manuscript - M.K., V.B.M.-R.; Critical Review - E.M, M.K., V.B.M.-R.

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Original Article

ERG Channels Contribute to the Excitability of Pyramidal Neurons in Hippocampal CA1

Caner Yildirim[1], Ziya Çakir[2], Ramazan Bal[1]

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

[2]Vocational Higher School Of Health Services, Gaziosmanpaşa University, Tokat, Turkey

ABSTRACT

Objective: Ether-a-go-go-related genes (ERG; Kv11) include three different erg channels, namely, Kv11.1, Kv11.2, and Kv11.3 or ERG1, ERG2, and ERG3. The aim of this study is to investigate the effects of ERG channel blockers on the biophysical properties of pyramidal cells in the hippocampus CA1 area.

Methods: The characterization of ERG currents was obtained using the whole cell configuration of the patch clamp technique. In the current clamp, we used selective ERG channel blockers (E-4031 (10 μM), dofetilide (1 μM), ergtoxin (200 nM), and terfenadine (10 μM)), which significantly incremented the input resistance of the pyramidal neurons ($P < .05$). All other ERG channel blockers except dofetilide have significantly increased neuronal excitability of hippocampal CA1 pyramidal neurons ($P < .05$). They also increased the action potential (AP) firing rate of cells in response to a square current pulse ($P < .05$).

Results: In the voltage clamp, the biophysical characteristic of ERG channels was determined by the measurement of tail currents. The E-4031-isolated current was observed at nearly −65 mV. The voltage-depended activation and inactivation curve of ERG channels was fitted with Boltzmann function, resulting in the $V_{1/2}$ value of −48.95 mV, the slope factor of 4.54 mV and the $V_{1/2}$ value of −77.35 mV, the slope factor of 10.58 mV, respectively. The exponential function is used to determine deactivation kinetics of ERG channels. It was observed that the rate of deactivation increased when the membrane potential was more hyperpolarized.

Conclusion: In conclusion, both current and voltage clamp studies showed that ERG channels contribute to the modulation of excitability and frequency of AP in pyramidal neurons of the hippocampus in mice.

Keywords: ERG channels, excitability, hippocampus, neurophysiology, electrophysiology

INTRODUCTION

The "ether-a-go-go-related gene" (ERG) potassium channels were first discovered in drosophila.[1] Despite that it has been studied in many tissues, the physiological roles of ERG channels are best demonstrated in cardiac myocytes cells. In these cells, ERG currents contribute to repolarization phase of the AP and the plateau formation.[2] Moreover, ERG channels are studied in neuronal cells, including medial vestibular nuclei neurons,[3] medial nucleus of the trapezoid body,[4] cerebellar Purkinje neurons,[5] and cochlear nucleus neurons.[6]

ERG channels are known to have unusual biophysical properties. Due to the structural properties of these channels, the inactivation kinetics are much faster than the activation kinetics so that the greatest conductance occurs during repolarization.[4] Furthermore, one of the reasons for the inactivation kinetics to be faster than activation in the ERG channels is that the selectivity filter is formed by the carbonyl atoms of the GFG sequence. Most of the other voltage-gated potassium channels are formed by carbonyl atoms of the GYG sequence.[7,8] ERG channels have a role as strong inward rectifiers that conduct outward currents in hyperpolarized membrane potentials. However, unlike conventional inward rectifier channels, they must be initially stimulated to be activated by depolarization,[9] whereas the voltage-gated Kv channels are typically activated by depolarization of the membrane.[10,11]

The hippocampus is a highly complex structure that is responsible for consolidating memory in the human brain. The dendrites of pyramidal neurons have many voltage-gated ion channels, some of which are present at very high concentrations.[12] Also in the hippocampus, it is one of the most stained structures by anti-ERG antibodies. One of the densely stained cell types is pyramidal cells located in the CA1 and CA3 regions.[13] While ERG2 channels are reported not to be expressed in pyramidal neurons in the hippocampus, ERG1 and ERG3 channels are reported to be expressed extensively.[14,15] While ERG channel expressions are heavily labeled in the hippocampus area, there are little data on the properties of biophysical currents. In the present study, we investigated the biophysical properties of ERG channels in the pyramidal neurons and their contribution to passive and active membrane properties such as resting membrane potential, input resistance, number of AP, and excitability.

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ORCID iDs of the authors: C.Y. 0000-0003-0091-9925; R.B. 0000-0003-3829-8669.

Corresponding Author: Caner Yildirim **E-mail**: caneryildirim27@gmail.com

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METHODS

Preparation of Hippocampal Slices

Hippocampal slices were prepared from BALB/c strain mice. Animals were treated in compliance with the guidelines of Gaziantep University Animal Experiments Local Ethics Committee. We have started experiments after obtaining ethical approval from Gaziantep University Local Animal Use Committee (Gaziantep, Turkey; approval date and number: June 25, 2017/39). Immediately after decapitation, the head of the animal was submersed and oxygenated with artificial cerebrospinal fluid (aCSF), and then the brain was quickly removed from the head. After removal, the hippocampus was placed on the teflon block through the superglue. Slices of the hippocampus were cut at a thickness of 300-350 μm using a vibratome (Frederick Haer and Co, Newbrunswick, ME, Canada). The slices were transferred to the recording chamber for recording. In the recording chamber, the slices were perfused with aCSF at a rate of 4 mL/min, and the temperature of the aCSF was kept at constant of 33°C with a feedback-controlled heater (Warner Instruments, Hamden, CT, USA).[16–21]

Solutions and Chemicals

The pipette solution is composed of 110 mM of potassium gluconate, 14 mM of phosphocreatine (Tris salt), 4.5 mM of $MgCl_2$, 9 mM of EGTA, 9 mM of HEPES, 4 mM of ATP (Na-salt), and 0.3 mM of GTP (tris salt). pH of the pipette solution was adjusted to 7.4 with KOH.[6]

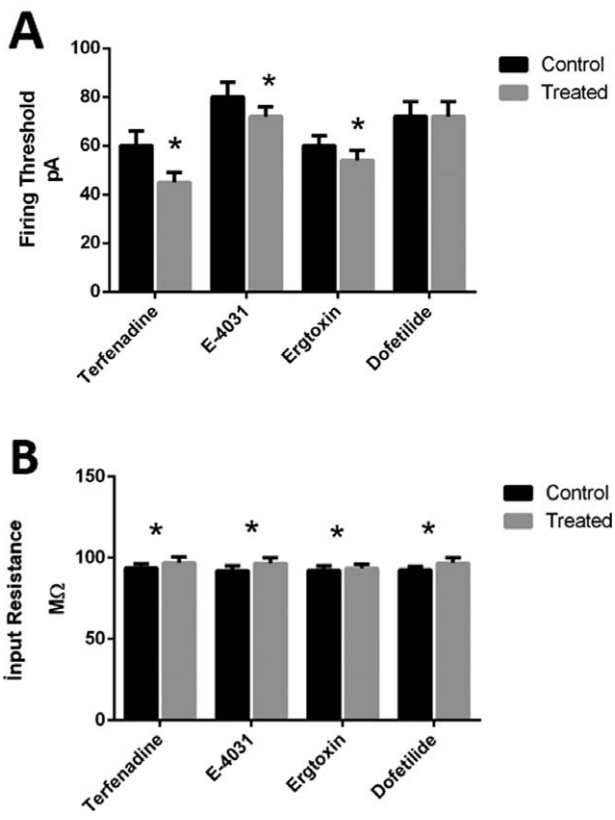
Two different extracellular perfusion solutions were used in this study. The aCSF was used as the extracellular solution, which contains 138 mM of NaCl (Merck), 10 mM of HEPES (Merck), 10 mM of glucose (Merck), 4.2 mM of KCl (Merck), 2.4 mM of $CaCl_2$ (Merck), and 1.3 mM of $MgSO_4$ (Sigma Aldrich). pH of the solution was adjusted to 7.40 using NaOH.[23]

To isolate ERG currents in voltage clamp experiments, we used "high potassium-containing aCSF." To make the amplitude of the ERG channel currents more visible, the K^+ concentration of the solution was increased to 40 mM.[5] Also, this solution was prepared as calcium free to avoid the activation of Ca^{2+}-dependent K^+ channels. To keep the osmolarity of the solution constant at 295-305 mOsm/L, the NaCl concentration in the solution was reduced. Thus, "high potassium aCSF" was prepared with the following constituents: 102 mM of NaCl, 40 mM of KCl, 10 mM of glucose, 10 mM of HEPES, and 3.7 mM of $MgSO_4$.

Main Points

- In this study, the characterization of ERG channels was performed using the parch clamp technique.
- ERG channel antagonists were found to increase excitability in pyramidal neurons in the CA1 region.
- Biophysical properties of ERG channels were determined by voltage clamp studies.
- As a result, it was determined that ERG channels contribute to cell excitability in pyramidal neurons in the CA1 region.

Figure 1. (A) When ERG channel blockers were applied to the pyramidal neurons, they easily stimulate according to their control situations (except dofetilide). The application of dofetilide did not change cell excitability. (B) All the ERG channel blockers increased the input resistances of the cells as statistically significant (*$P < .05$).



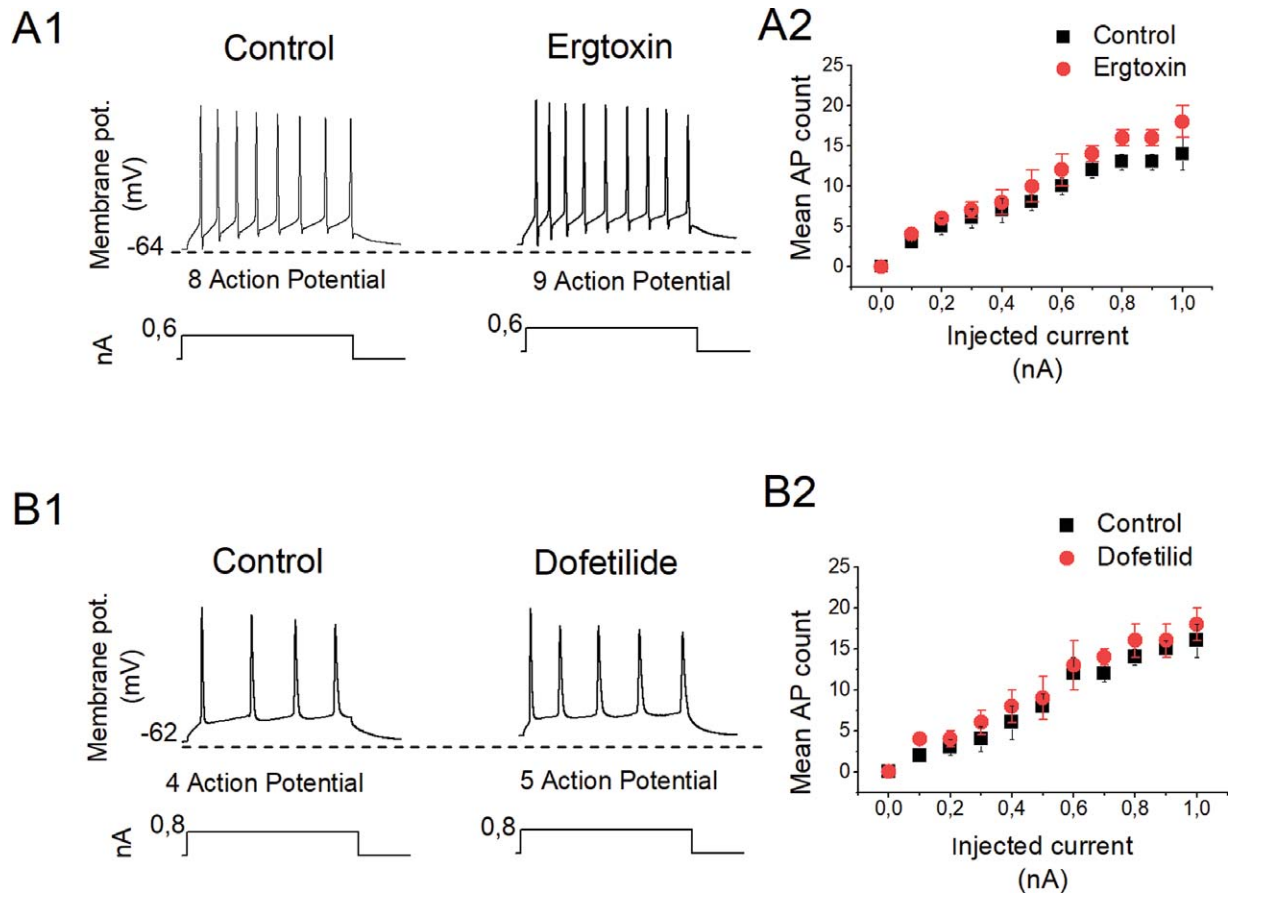
To inhibit the ERG channel in the current clamp experiments, ergtoxin (200 nM; Alemon lab, Israel), E-4031 (10 μM; Alemon Lab, Israel), terfenadine (10 μM; Sigma Aldrich), and dofetilide (1 μM; Alemon Lab, Israel) were used. DMSO was used to dissolve E-4031 and terfenadine. The final concentration of DMSO never exceeded 0.1%. In the voltage clamp studies, 10 μM of E-4031 1-[2-(6-methyl-2-pyridyl) ethyl]-4-(4-methylsulfonylaminobenzoyl) piperidine (Alemon Lab, Israel) was used to isolate the E-4031 isolated current.

To prevent synaptic activity, synaptic blockers were added to aCSF. A 10 μM APV-5, 5 μM DNQX, and 1 μM strychnine were added to block glutamatergic NMDA, AMPA, and glycinergic receptors, respectively. A 1 μM tetrodotoxin (Alexis Biochemicals, USA) was added to block voltage-dependent sodium channels; 1 mM CsCl was added to block mixed cation channels; and 1 mM tetraethylammonium (Fluka) and 1 mM 4-aminopyridine (4-AP) (Sigma Aldrich) were added to inhibit delayed rectifier K^+ current and transient outward K^+ current, respectively.[6]

Table 1. Passive and Active Membrane Properties of CA1 Pyramidal Neurons

Input resistance (MΩ) (mean ± SE)	Capacitance (pF) (mean ± SE)	Resting membrane potential (mV) (mean ± SE)	Time constant (ms) (mean ± SE)	AP amplitude (mV) (mean ± SE)
84.6 ± 4.7 (n = 80)	-63.7 ± 2.4 (n = 80)	-64.4 ± 3.4 (n = 80)	22.6 ± 3.8 (n = 80)	74 ± 6.2 (n = 80)

Figure 2. Effect of ERG channel blockers, (A1) ergtoxin, (B1) dofetilide, on the number of APs induced by square current pulses of 150 ms in pyramidal neurons. (A1) Application of ergtoxin increased the number of APs induced from 8 to 9 at the given current amplitude. (B1) Application of dofetilide increased the number of APs from 4 to 5. (C1) Application of ergtoxin increased the number of APs from 8 to 9. (D1) Application of dofetilide increased the number of APs from 4 to 5. (A2 and B2) The increase in the number of APs upon the application of ergtoxin (1 μM) and dofetilide (10 μM) was plotted as a function of varying current amplitudes, respectively.

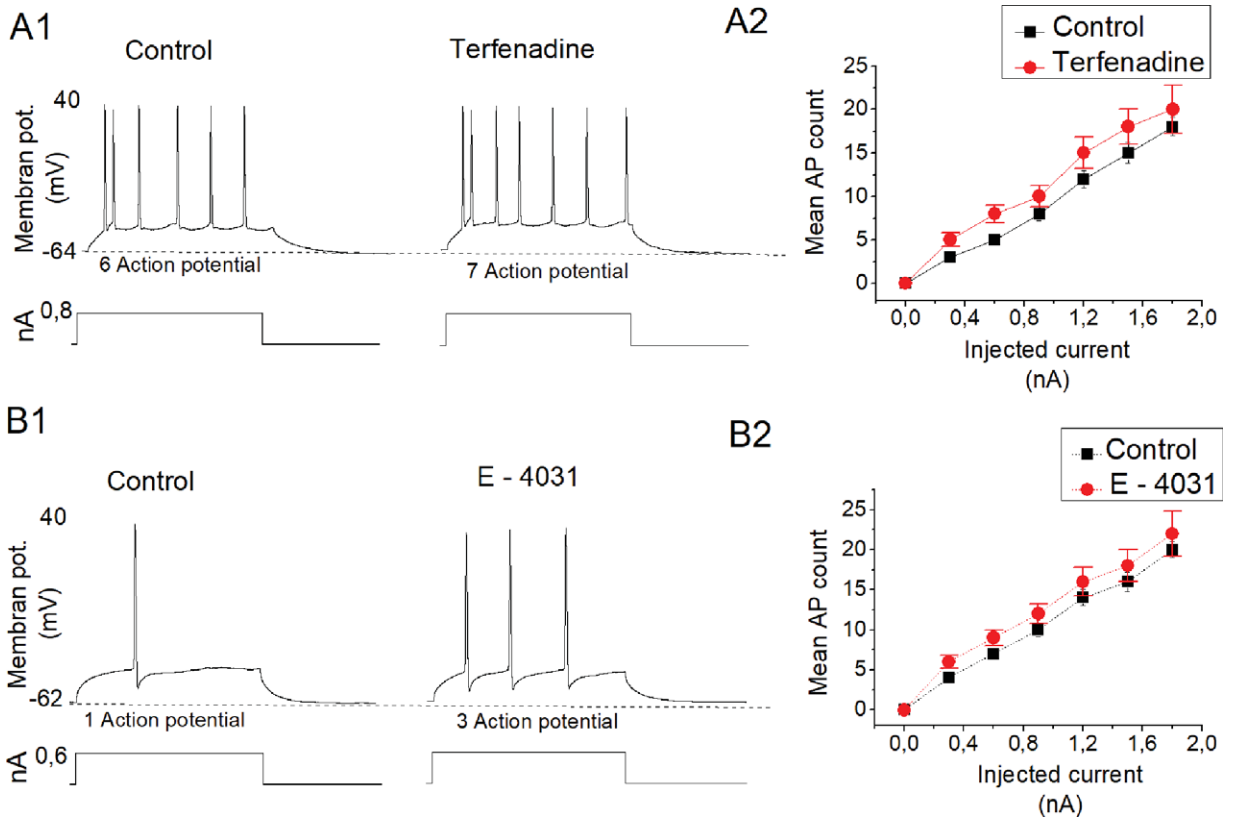


Intracellular Recordings and Data Acquisition

Intracellular recordings were obtained with the whole-cell configuration using the patch clamp technique. Patch pipettes were obtained using borosilicate glass capillaries (GC150F-10, Harvard Apparatus; Flaming/Brown micropipette puller, Model P-97, Sutter Instrument). We only used pipettes with a resistance between 5 and 7 MΩ. Series resistance ranges from 6 to 13 MΩ (compensated by 70%, 10 μs lag), which was between Rinput and Rinput/10. All the records were obtained by the whole-cell configuration using an Axopatch-200B amplifier, and then transferred to a computer equipped with an A/D converter and the Digidata 1440 series (Axon Instruments, Foster City, CA, USA), and the current and voltage recordings were sampled at 10–40 kHz. Data were analyzed offline. The junction potential was measured to be –12 mV, and therefore, all recordings were compensated for –12 mV.[23]

Analysis of the voltage-dependent activation, inactivation, and deactivation kinetics was performed using OriginPro 2018 (64-bit; SR1 b9.5.1.195), and the voltage-dependent steady-state

Figure 3. Effect of ERG channel blockers, (A1) terfenadine and (B1) E-4031, on the number of APs induced by square current pulses of 150 ms in pyramidal neurons. (A1) Application of terfenadine increased the number of APs induced from 6 to 7 at the given current amplitude. (B1) Application of E-4031 increased the number of APs from 1 to 3. (C1) (A2 and B2) The increase in the number of APs upon the application of terfenadine (10 μM) and E-4031 (10 μM) was plotted as a function of varying current amplitudes, respectively.



activation and inactivation curves were fitted with the Boltzmann function (equation 1):

$$I(v) = 1/(1 + \exp(V1/2 - Vm)/k) \quad (1)$$

where I(v) is the normalized current, Vm is the test potential, $V_{0.5}$ is the potential for half-maximal activation, and k is a slope factor measuring the apparent gating charge.

The exponential function is used to determine deactivation kinetics of ERG channels. To determine the rate of deactivation, the decay phase of tail currents was fit with either a single or double exponential of the forms.

Exponential function (equations 2 and 3):

$$I(t) = A \exp(t/\tau) + C \quad (2)$$

or

$$I(\tau) = A \text{ fast } \exp(t/\tau \text{ fast}) + A \text{ slow } \exp(t/\tau \text{slow}) + C \quad (3)$$

Equations 2 and 3 are single and double exponential functions, respectively, where τ refers to the time constant of deactivation, A is the amplitude of each component, and C is a constant.

Finally, the graphics was drawn using OriginLab (OriginPro 2018 (64-bit; SR1 b9.5.1.195) and Graph Pad Prism 8.

Statistical Evaluation of the Data

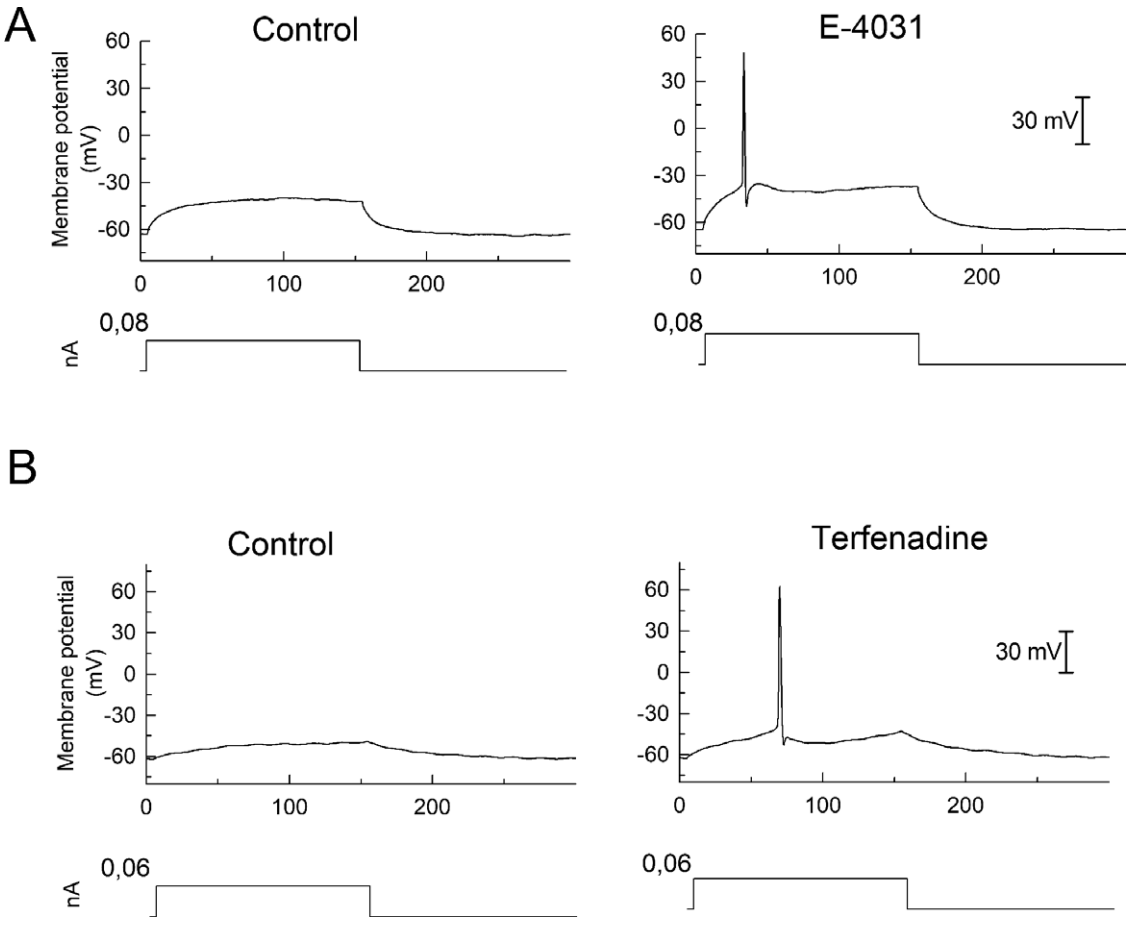
Statistical evaluation was done using the Statistical Package for Social Sciences Version 23.0 (IBM SPSS Corp.; Armonk, NY, USA) package program. Descriptive statistics for numerical variables are presented as mean ± S.E.M. (n = number of cells). Student t test was performed to determine the statistical difference. The threshold for the statistical significance was set at $P < .05$.

RESULTS

Current Clamp Experiments

The data presented in this study consist of electrophysiological recordings obtained from 80 pyramidal neurons in the CA1

Figure 4. Effects of terfenadine and E-4031 on excitability of pyramidal neuron. (A) While an 80-pA current did not cause the neuron to reach threshold for AP induction, the same amplitude of current (80 pA) induces APs after the application of E-4031. (B) The 60-pA current did not induce any AP, but it caused the neuron to fire APs after the application of terfenadine.



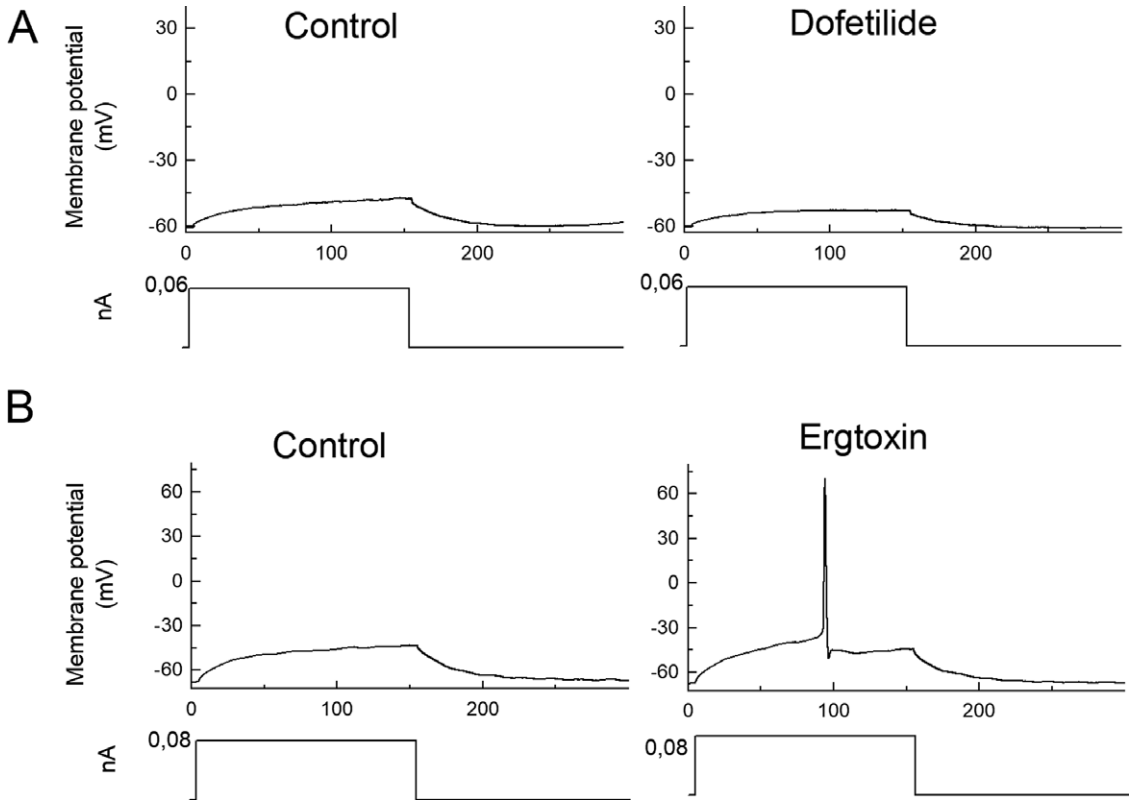
region in the hippocampus of mice. The identification of the cells was done as previously described.[25] The input resistance, capacitance, time constant, amplitude of the AP, and the resting membrane potential of the cells were measured, and these values are shown in Table 1.

ERG channels may be selectively inhibited by E-4031, dofetilide, terfenadine, and ergtoxin.[2,6] All the ERG channel blockers in pyramidal neurons caused slight depolarization of the membrane potentials. However, this effect was not significant ($P > .05$).

The application of all the ERG channel blockers increased the input resistance of pyramidal neurons (terfenadine, from 93.7 ± 2.6 MΩ to 96.9 ± 3.7 MΩ (n = 10); E-4031, from 92.1 ± 2.8 MΩ to 96.5 ± 3.4 MΩ (n = 10); dofetilide, from 92.4 ± 2.4 MΩ to 96.8 ± 3.1 MΩ (n = 10); and ergtoxin, from 92.2 ± 2.8 MΩ to 93.6 ± 1.4 MΩ (n = 10)). The increase in the input resistance was found to be significant in all the cases ($P < .05$) (Figure 1B).

The application of ERG channel blockers increased the number of APs in cells induced by the injection of square current pulses (Figures 2 and 3). The application of terfenadine increased the number of APs induced by square current pulses in every cell tested. The number of APs was plotted as a function of current amplitude injected in Figures 2 and 3. However, the amplitude of the threshold current for the induction of AP in different pyramidal cells tested varied too much. For example, a current amplitude of 0.6 nA was not large enough for one neuron to fire an AP, whereas the same current amplitude was enough to fire 10 APs for another neuron. Therefore, the error bars of the mean values increased due to large variations in the responses of neurons to currents injected, and statistical significance did not appear in the relationship as shown in Figure A2. For that reason, the statistical comparisons before and after the blockers were based on the recordings of 6-7 AP during 150 ms current pulses, for which different current amplitudes were required in different neurons. The application of ergtoxin (1 μM), dofetilide (10 μM), terfenadine (10 μM), and E-4031 (10 μM) increased the number of APs by 21.6 ± 6.48% (n = 8), 16 ± 3.48% (n = 10), 18.6 ± 6.48% (n = 10), and 12 ± 1.48% (n = 10), respectively.

Figure 5. Effects of dofetilide and ergtoxin on excitability of pyramidal neuron. (A) Dofetilide did not reduce the inhibitory effect of ERG channels on cell excitability and did not affect cell excitability. (B) While an 80-pA square current pulse did not cause the neuron to reach threshold for AP induction, the same amplitude of square current pulse (80 pA) induces APs after the application of ergtoxin.



ERG channel blockers are known to increase the excitability of cells. It was tested whether these blockers showed the same effect in pyramidal cells. For example, when a 60 pA current was injected to the cell, the cell did not fire APs, but when the ERG channel blockers were applied to the cell, it was observed that the cells fired the APs at the same current injection. It should be noted that because of the different size, dendritic branching and synaptic input numbers of the pyramidal cells, in order to fire the AP, the amplitudes of the currents to reach the threshold to the cells were different. All other ERG channel blockers except dofetilide significantly increased the excitability of the cells ($P < .05$). When the ERG currents in the cells were blocked according to the control situations, it was seen that they reached their threshold value more easily for firing (Figure 4 and Figure 5).

Voltage Clamp Experiments

Voltage dependence activation

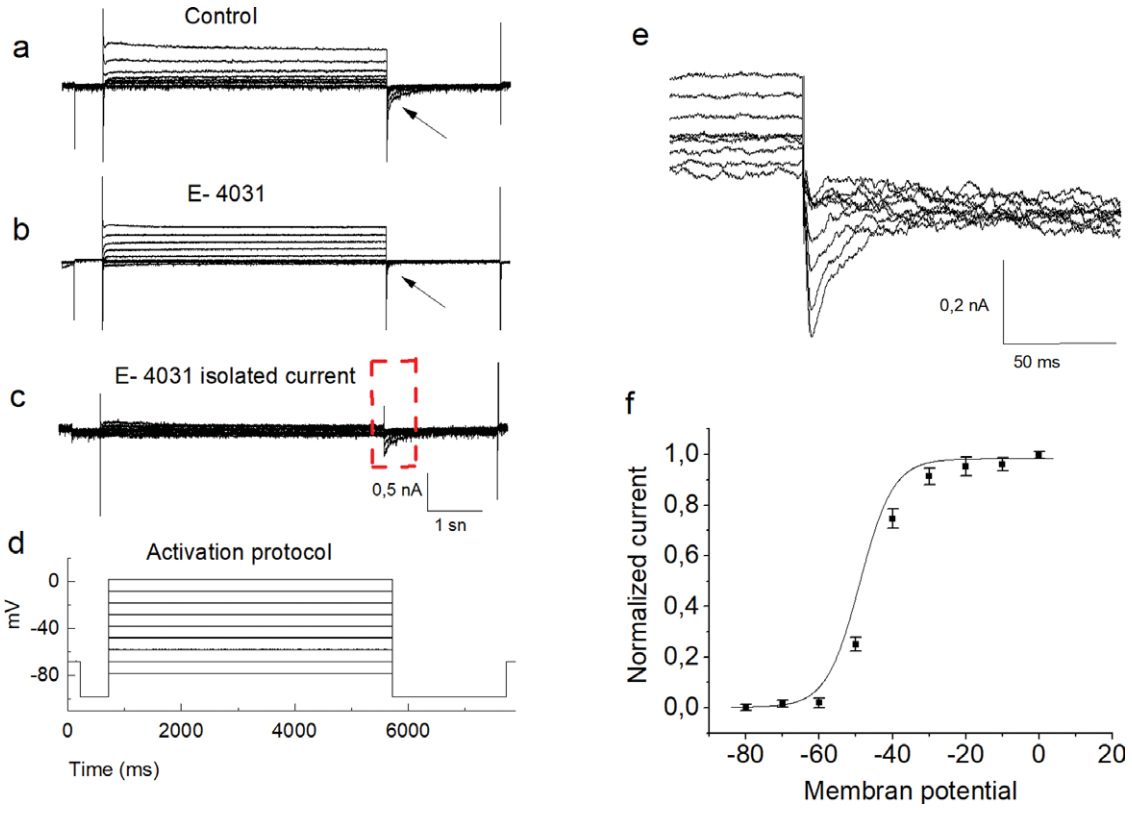
In voltage clamp studies, tail currents were obtained using a voltage protocol given in Figure 6D. According to this protocol, the holding potential of neurons was adjusted to –70 mV after that the membrane potential was stepped to a range of potential from –120 mV to 0 mV in 10 mV increments for 5 seconds and then step to –110 mV. The tail currents were observed at around –100 mV.

The activation curve of the ERG channels was obtained by plotting the amplitude of the normalized tail currents. The E-4031 isolated current was observed at nearly –65 mV, and the amplitude of the tail current increased gradually with more depolarized membrane potential. The curve was fitted with the Boltzmann function and gave a V1/2 value of –48.95 mV and a slope factor of 4.54 mV (Figure 6).

Voltage dependence of inactivation

To study the voltage-dependent steady-state inactivation of ERG channels, the voltage was stepped to 0 mV for 4 seconds to activate ERG channels completely[25] and then was stepped to a range of potential between –120 and 0 mV in 15 mV steps for 2 seconds to allow quick recovery from inactivation (Figure 7D). It should be noted that ERG channels are expected to deactivate at membrane voltages where ERG channels are recovered from inactivation.[2,4,5,8,25,26] Therefore, the decay phase of the tail currents was thought to represent deactivation of ERG channels, which were fitted by an exponential function. The amplitudes of the tail currents were extrapolated at the

Figure 6. Voltage-dependent activation of E-4031 isolated ERG current in pyramidal neurons. The ERG current studied with the voltage protocol shown in (D). ERG currents were isolated by a pharmacological approach. After eliminating all other known ionic currents, the current traces recorded were thought to include ERG current as well as the unblocked components of the other currents and the leak currents. The application of a specific ERG channel antagonist, E-4031, was supposed to block the current through ERG channels. E-4031-isolated current (shown in C) was obtained by subtracting the current traces in the presence of E-4031 (shown in B) from the current traces recorded in control conditions (shown in A). (C) The subtracted traces, E-4031 isolated currents. An enlarged scale of the tail current traces (shown in red rectangular area in panel C) is shown in (E). (F) Activation curve of the ERG channels. Each data point represents mean value from eight pyramidal neurons.



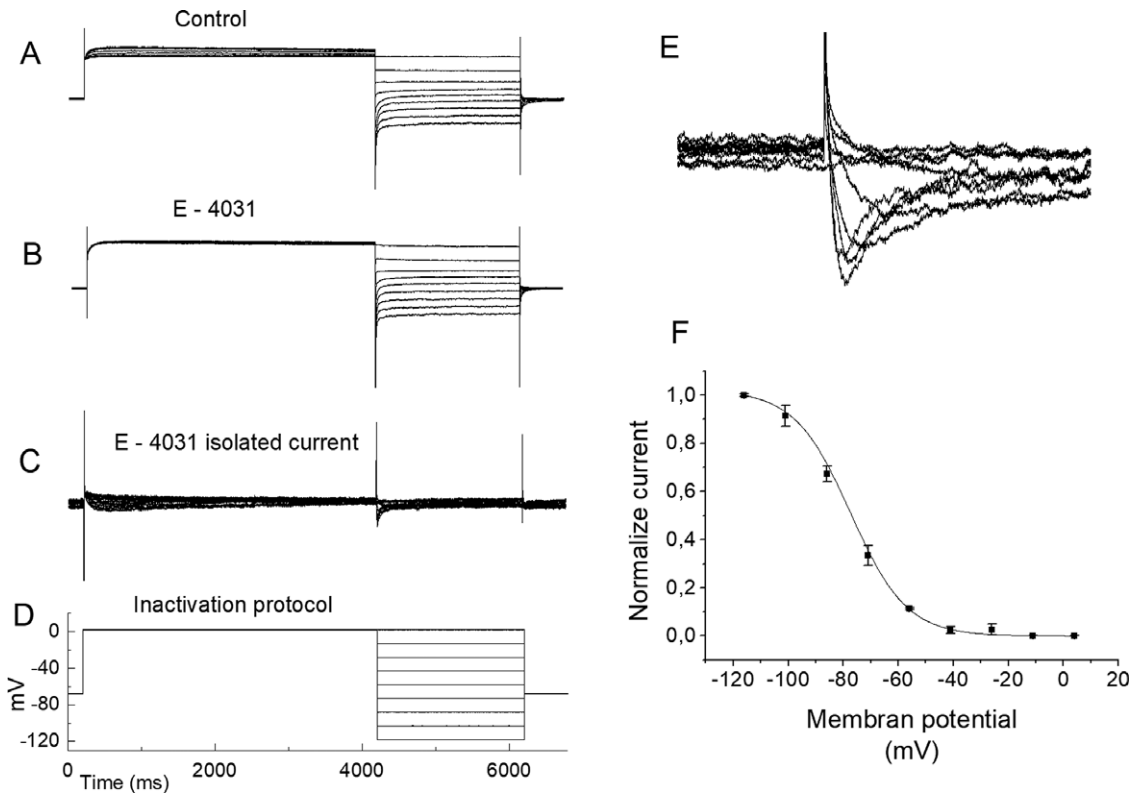
beginning of the hyperpolarizing voltage steps, namely, the end of the depolarizing voltage step during the exponential fitting procedure. The steady-state inactivation curve for ERG channels was constructed by plotting amplitude of the normalized tail currents as a function of voltage steps. The curve was fitted with the Boltzmann function, leading to a V1/2 value of –77.35 mV and a slope factor of 10.58 mV (Figure 7). The same voltage protocol was used to study deactivation kinetics. The traces induced by voltage steps between –120 and –100 mV were fitted best with double exponential, while the traces induced by voltage steps that were more depolarized than –100 mV were fitted best with a single exponential. The rate of deactivation increases as the membrane potential becomes more hyperpolarized (the time constant was 22 ms at –120 mV and 108 ms at –90 mV) (data not shown).

DISCUSSION

In the present study, we found that (1) all ERG channel antagonists caused an increase in the input resistance of the pyramidal neurons. (2) The application of ERG channel blockers increases the number of APs induced by DC current pulses. (3) An increase in the excitability of cells was observed when the ERG channels were blocked in pyramidal neurons. APs were induced by less amount of current injection after the application of ERG channel blockers (except dofetilide) compared to control. (4) The activation curve for ERG channels had a V1/2 value of –48.95 mV and a slope factor of 4.54 mV, the tail current first appeared at around –65 mV, and the amplitude of the tail current increased gradually with more depolarized membrane potential.

In the current study, according to the Boltzmann fitting of the steady-state activation curve, we obtained a V1/2 value of –48.95 mV and a slope factor of 4.54 mV. These data are comparable to those of others[4,5,25] and our previous study.[6] The value of V1/2 in the activation curve of Purkinje cells was found to be –50.7 mV[27] and V1/2 was –56 and –58 mV in neurons of medial nucleus of trapezoid body (MNTB) in 12- and 25-day-old mice, respectively.[4] In the pyramidal neurons, values of voltage dependence of ERG channels steady-state inactivation (–77.35 mV) were comparable to those in the Purkinje neurons (–70.6 mV)

Figure 7. Voltage dependence kinetics of E-4031-isolated ERG current in pyramidal neurons. The E-4031 isolated current was isolated by subtracting the currents recorded in the presence of the E-4031 (B) from the control currents shown in (A). The inactivation voltage protocol is given in (D). (E) An enlarged scale of the tail current traces (shown in red rectangular area in (C)) is shown. E-4031-isolated current clearly shows the hooks. (F) Inactivation curve of the ERG channels. Each data point represents the mean value from six pyramidal neurons.



and MNTB neurons (V1/2 of –72 mV).[4,25] According to the activation curve, the tail current first appeared at around –65 mV, and the amplitude of the tail currents became larger at more depolarized potential, namely, the threshold of activation of ERG channels in pyramidal neurons was found to be close to resting membrane potential of the neurons. According to the steady-state inactivation curve, roughly 20% of ERG channels was ready to activate from the resting potentials of –64.4 ± 3.4 mV. These suggest that 20% of ERG channels activates following depolarization at resting potentials, and more depolarized potentials and deactivation tail currents seem to insert its effect on excitability.

The blockage of ERG currents resulted in an increased number of APs. There are other reports demonstrating that the blockage of ERG currents increases neuronal excitability in cerebellar Purkinje neurons, embryonic serotonergic neurones, mouse spinal cord, medial vestibular nucleus neurones, mouse auditory brainstem neurons, neonatal mouse Purkinje cells, and ventral cochlear nucleus neurons in mice.[3–6,9,22,25,27,28]

The increase in the number of APs after the application of ERG channel blockers during current stimulation could be accounted for only partially by the increase in input resistance, since with a larger input resistance, the same amplitude of the current results in larger depolarizations, and therefore more number of APs. Namely, the increase in input resistance decreases the amount of excitatory current necessary to depolarize the cell. The increase in the number of APs is also likely to be partially due to the block of potassium ion efflux through ERG channels, which has a depressing effect on excitability because greatest conductance occurs during repolarization with a hyperpolarized membrane potentials upon activation by depolarization. Block of the conductance by ERG channel blockers eliminates the hyperpolarizing effect ERG current, flow of which normally occurs during repolarization upon depolarization otherwise. The absence of the ERG current seems to be the major factor for the changes in the number of APs during current stimulation.

According to the Fano, the role of ERG channels is probably the modulation of excitability and transmitter release in rat hippocampal slices.[14] In this respect, any pathologies in ERG channel expressions or ERG channels mutations may lead to excessive excitability of neurons by removing this inhibitor effects. In accord with this statement, Chiesa et al.[29] speculated that one

of the underlying causes of hyperexcitability and hippocampal epileptic activity might be associated with ERG channel mutations or insufficient expressions of the ERG channel genes.

CONCLUSION

In conclusion, the findings suggest that ERG channels may have an inhibitoric effect on neuronal excitability. Therefore, the elimination of the naive inhibitory effect of ERG channels with its specific blockers would increase the frequency of the AP in response to current pulses and may cause hyperexcitability.

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Review

Applications of Photobiomodulation Therapy in Oral Medicine—A Review

Mohamed Faizal Asan, G Subhas Babu, Renita Lorina Castelino, Kumuda Rao, Vaibhav Pandita

Department of Oral Medicine and Radiology, Nitte (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Mangalore, India

ABSTRACT

Applications of photobiomodulation (PBM) in dentistry have been of great interest in the recent times. It can both stimulate and suppress biological effects. The property of PBM contributes to the analgesic, anti-inflammatory, and wound healing effects. Photobiomodulation therapy (PBMT) has a wide variety of clinical applications that include wound healing, prevention of cellular death, promotion of repair mechanisms, reduction of inflammation, pain relief, etc. Hence, it is being used effectively in the field of oral medicine and has shown promising results in the management of oral mucosal lesions, orofacial pain, and other orofacial ailments without much significant adverse effects. The purpose of this review is to discuss the applications of PBMT in the field of oral medicine.

Keywords: Lasers, mucositis, orofacial pain, photobiomodulation therapy, temporomandibular disease

INTRODUCTION

Laser (light amplification by stimulated emission of radiation) was first produced in the year 1960 by Theodore H. Maiman. Later in the late 1960s, Endre Mester developed a laser for therapeutic purposes, and he described the use of laser biostimulation for wound healing.[1] They can both stimulate and suppress biological effects; hence, it was known to produce effects of photobiomodulation (PBM). The property of PBM contributes to the analgesic, anti-inflammatory, and wound healing effects.[2,3] This noninvasive and nonthermal therapeutic properties of PBM have been used in the management of various neuromuscular, painful musculoskeletal, and trauma-related conditions.[4] The application of photobiomodulation therapy (PBMT) in dentistry has been of great interest in recent times. The purpose of this review is to discuss the applications of PBMT in the field of oral medicine.

PHOTOBIOMODULATION THERAPY

PBMT utilizes nonionizing forms of light sources, such as light-emitting diodes (LEDs), lasers, and broadband light to produce photochemical and photophysical reactions in various tissues.[5] There are evidences in the literature for PBM using monochromatic lasers, quasimonochromatic LED lights, noncoherent, and polychromatic light sources also.[6]

Laser devices used for PBM are smaller, compatible, handheld devices and are much safer when compared to the surgical lasers. PBM is based on the property of Arndt Schultz law, according to which, smaller doses have the ability to stimulate a biological response, doses at medium range can impede, and massive doses can destroy.[4] The technical specifications and considerations of these lasers differ from the surgical lasers[7,8] (Table 1). Though the therapeutic window of these lasers is broad, it is essential to calculate the required amount of dose (i.e., the energy density) before any therapeutic application.[9] The energy density can be calculated by dividing the energy of the laser with the area of the irradiated region. The calculation of required energy density depends on factors like thickness, type, area of the tissue, and pigmentation in the region being irradiated and the wavelength used. Laser waves can be transmitted more freely through fat and mucosa than muscle.[7] The PBM devices that are used commonly for various therapeutic applications in the field of oral medicine are semiconductive diode lasers. Aluminum–gallium–indium–phosphide lasers (AlGaInP) and gallium–aluminum–arsenide lasers (GaAlAs) were the most commonly used lasers for PBM in the field of oral medicine. Surgical lasers can also be used in low-energy output level for the purpose of PBM.[10]

MECHANISM OF ACTION

PBMT has a wide variety of clinical applications, which include wound healing, prevention of cellular death, promotion of repair mechanisms, reduction of inflammation, pain relief, etc. When a PBM light source is targeted on a specific site of the body, it causes the penetration of light energy into the body cells. Based on the optical properties of the tissue being

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ORCID iDs of the authors: M.F.A. 0000-0001-9747-1914; S.B.G. 0000-0001-9383-7886; R.L.C. 0000-0002-8696-549X; K.R. 0000-0002-6214-1381; V.P. 0000-0003-3163-5300.

Corresponding Author: Subhas Babu G **E-mail**: goginenisb@yahoo.co.in

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Table 1. Technical Parameters and Considerations for Photobiomodulation Therapy

Parameter	Specification
Wavelength	600–1000 nm
Waveform	Continuous, pulsed, modulated
Spectrum	Red to infrared region
Power density	Calculated by laser output power (mW)/beam area (cm^2) 1 mW/cm^2 to 50 mW/cm^2 (average range)
Energy (expressed in Joule as J)	Calculated by mW × seconds
Dose calculation (J/cm sq units)	Calculated by energy ÷ irradiated area
Output power	1 mW up to 500 mW
Treatment interval (average)	Two to three treatments per week for several weeks depending on the nature of the application and chronicity of the disorder

irradiated, the absorption and scattering of the light in the tissue differs. Hemoglobin and melanin have high absorption ability to absorb light of wavelength shorter than 600 nm. In contrast, water absorbs light significantly at a higher wavelength of about 1150 nm.[11] Light energy gets absorbed by cytochrome c oxidase and antenna pigments, which passes it to the mitochondria. In mitochondria, the electromagnetic energy will be utilized to produce Adenosine Triphosphate (ATP). Increased ATP production stimulates the fibroblasts and promotes collagen formation. The energy produced at the site of irradiation stimulates local microcirculation, thereby promoting the process of wound healing.[4]

It also causes dissociation of nitric oxide from cytochrome c oxidase, thereby shifting the cell redox potential toward oxidation.[12] Furthermore, it causes the activation of intracellular signaling pathways and induction of transcriptional changes such as nuclear factor κB. This prevents apoptosis, cell death, promotes growth factor production, antioxidant response, and stimulates repair[13] (Figure 1).

The analgesic effect of PBMT may be due to their ability to inhibit Aδ and C fibers. They also cause inhibition of the release of mediators from injured tissues and decrease the concentration of pain mediators. They are also known to increase the acetylcholine esterase activity, thereby reducing the concentration of acetylcholine.[11]

Main Points

- Brief overview of photobiomodulation and their mechanism of action.
- Therapeutic applications of photobiomodulation in oral medicine.
- Efficiency and advantages of photobiomodulation in the management of orofacial ailments.

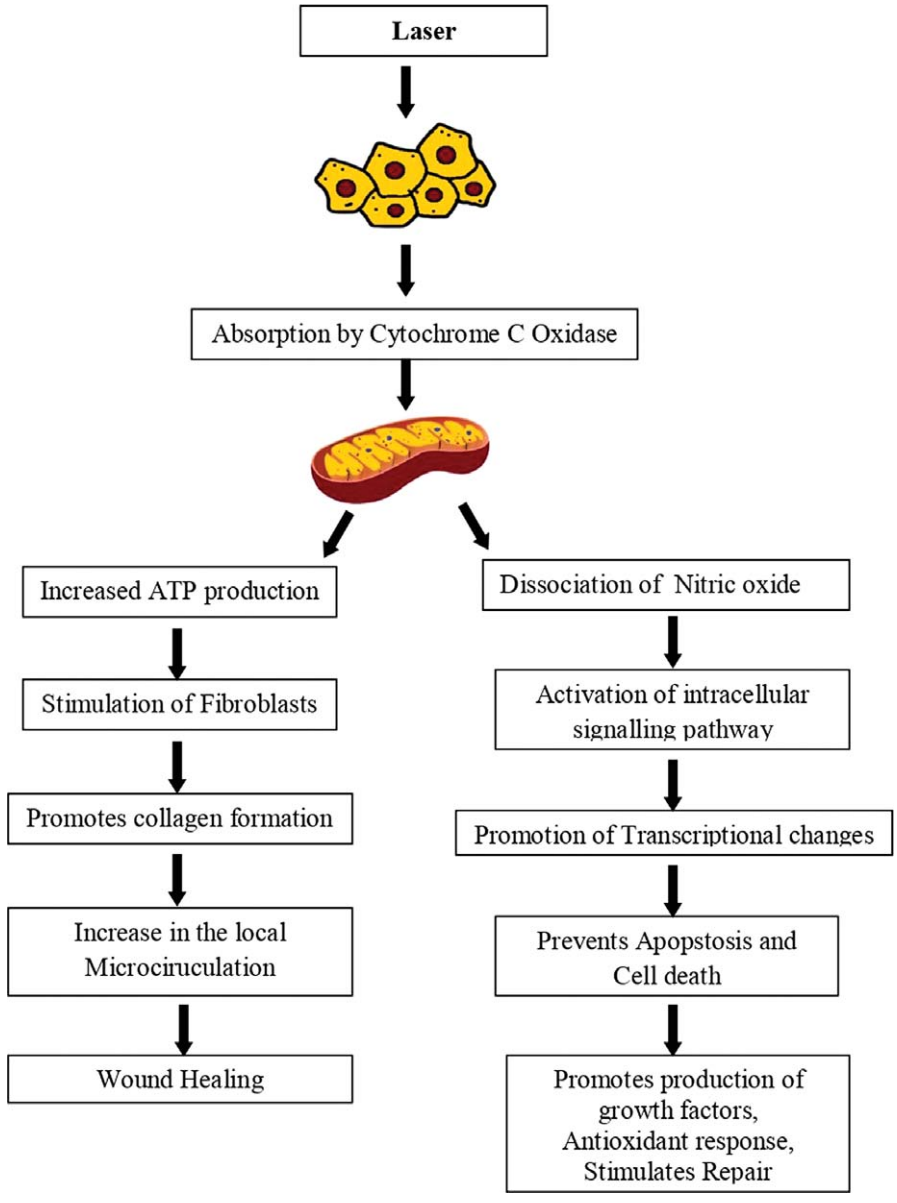
PHOTOBIOMODULATION IN ORO FACIAL AILMENTS

There are several applications of PBMT in orofacial ailments, which have been reported in the existing literature. But the dosage, methodology, and the light source used for PBM widely differ among various studies.[14] Considering their clinical usage in the field of oral medicine, the PBMT has a wide variety of applications ranging from the management of orofacial pain to the treatment of oral mucosal lesions.

Orofacial Pain

Orofacial pain can be defined as pain that is localized to the region above the neck, in front of the ears, below the oribitomeatal line, and also within the oral cavity. It includes both the pain of odontogenic origin and temporomandibular joint disorders.[15] The etiology of orofacial pain is extensive and diverse. PBMT has been used as an effective treatment option for orofacial pain. The analgesic effect of PBMT is gradual, and it requires multiple sessions with different wavelengths.[16] A systematic review by Chow et al.[17] has reported that photobiomodulating lasers can slow down the conduction and reduce the amplitude of action potentials. There have been many studies in the literature evaluating the use of PBMT for the treatment of orofacial pain. Most commonly used therapeutic wavelength for PBM in orofacial pain is 780-830 nm using GaAlAs lasers.[18] They have provided significant relief in orofacial pain. PBMT causes neural changes such as inhibition of action potential generation, inhibition of signal conduction in primary afferent neurons, and inhibition of conduction of C-fibers within the first few minutes of irradiation which causes an immediate analgesic effect.[19] In case of acute inflammatory conditions, it can aid in an early resolution of the inflammation. But most cases of orofacial pain are chronic in origin and require treatment for a longer period of time varying from two to three times per week for 3-4

Figure 1. Mechanism of action of photobiomodulation therapy in stimulation of wound healing.



weeks.[20] Patients suffering from pain might experience a mild increase in the pain immediately after the therapy. This reflects the actual improvement of the patient's condition, and the pain level usually decreases within a day.[21]

Oral Mucositis

The inflammation of the oral mucosal membrane is termed as oral mucositis. This is commonly seen in patients suffering from head and neck cancer as a side effect of chemo and radiotherapy. PBMT is being used in the preventive and therapeutic management of oral mucositis. Studies have shown lasers of 632-970 nm to be effective in treating cases of oral mucositis, achieving about 62% risk reduction and prevention of severe mucositis within a week in patients receiving chemo and radiotherapy.[22] Therapeutic wavelengths that are used in the management of oral mucositis include 670-830 nm using GaAlAs, 630-680 nm using InGAlP, and 632.8 nm using He–Ne lasers, whereas the prophylactic wavelengths include 660 nm of InGaAlP and 630 nm of GaAlAs lasers.[10] A recent study by Marin-Conde et al.[23] has demonstrated PBMT in patients undergoing chemo and radiotherapy is effective in reducing the incidence and severity of oral mucositis, without any significant side effects.

Alteration in the taste perception and dysphagia are also major side effects in patients undergoing radiotherapy for head and neck cancers (HNC). One session of PBMT with a diode laser operating at 635 nm with a dose of 3 J/cm^2 used in contact mode to irradiate different areas of the tongue has shown

significant improvement in taste perception. Similarly, cancer therapy-induced dysphagia has also been successfully managed by irradiation of various intraoral and extraoral sites. The intraoral sites that were irradiated include soft palate and oropharynx, while the extraoral irradiated sites include the midline of the neck, lateral part of the neck, ventrolateral parts of the pharynx, and larynx.[24]

North American Association for photobiomodulation therapy does not recommend PBMT directly over an active tumor to prevent the risk of transformation and stimulation of active cancer cells.[25] But recent studies on the safety of PBMT in HNC patients have proved that PBMT does not affect the overall survival, time to local recurrences, and disease-free survival of patients treated with radiotherapy with/without chemotherapy.[26]

Burning Mouth Syndrome

Burning Mouth Syndrome (BMS) has a complex etiology with diverse clinical presentation and possesses a challenge in its management. PBMT has been used as treatment option in BMS. PBMT for a period of 4-10 weeks targeting various sites like labial mucosa, tongue, and buccal mucosa has shown significant improvement in the symptoms.[27] Diode lasers comprising gallium–aluminum–arsenide (GaAlAs) in a wavelength of 830 nm, indium–gallium–aluminum–phosphide (InGaAlP) in a wavelength of 685 nm, and energy of about 2-5 J per point have been used for irradiating sites, where the pain is experienced and proved to be beneficial to the patients.[28] PBMT has shown to reduce the levels of salivary proinflammatory cytokines (tumor necrosis factor-α and interleukin-6), which can be directly related to the improvement of the condition. The reduction in pain perception may be attributed to the inhibitory action of PBMT on the neural impulse conduction and inhibition of pain mediators.[29]

Temporomandibular Disorders

Temporomandibular disorders (TMDs) can be due to arthrogenic, myogenic causes, or both. The use of PBMT for TMD is based on the type and location of the pain. In case of pain due to arthrogenic causes, irradiation is limited only to the temporomandibular joint area, while in patients with pain due to myogenic causes, specific points in the masticatory muscles are irradiated. Studies in PBMT for TMD differ in their site of application and parameters of the light source. The therapy requires multiple sessions at regular intervals for a specific period of time. Rodrigues et al.[30] have shown successful management of temporomandibular joint disorders using GaAlAs diode laser in a wavelength of 780 nm and a spot size of 0.04 cm^2 applied in contact mode. They followed different treatment protocols for myogenic and arthrogenic TMD. In case of TMD due to myogenic origin, PBM of 10 mW, 5 J/cm^2, and 0.2 J for 2 seconds per point was recommended, while for patients affected by joint problems, PBMT of 70 mW, 105 J/cm^2, 60 seconds on five points on the joint area, and 4.2 J per point was recommended. Treatment that consisted of two sessions per week for a period of 4 weeks showed significant relief among the patients. Sayed et al.[31] recommended a treatment protocol based on six sessions of PBMT (three times per week for 2 weeks) with gallium arsenide laser at 904 nm, 0.6 W, 60 seconds, and 4 J/cm^2 in the trigger points, and the patients showed significant improvement in the symptoms with increased mouth-opening ability. The disadvantage of PBMT for the management of TMD is that it requires multiple visits at different intervals and varied duration depending on the clinical presentation of the patient. Recently, Del Vecchio et al. conducted a study to overcome this limitation of multiple visits by using a laser device that emits a beam with a wavelength of 808 nm, at 5 J/min, 250 mW, and 15 kHz for 8 minutes, for irradiating the area of pain. The duration of treatment was twice daily for 1 week. The first application of laser was made by the clinician, and the patients were educated about the application and safety of the device so as to enable the patients to perform the other successive applications by themselves at home. This method showed improvement only in some patients.[32] The shortcoming of this method may be attributed to the difference in the technique of application by the different patients.

Oral Mucosal Lesions

Recurrent aphthous stomatitis (RAS) is a very common and painful ulcerative lesion of the oral cavity. PBMT has been successfully employed as a treatment modality for the management of recurrent aphthous ulcers. PBMT with a diode laser of 940 nm used in noncontact mode for 30-45 seconds with a pause for 10-20 seconds and a total of about 2 minutes in a single session has shown faster reduction of pain and healing of ulcers. Apart from providing instant pain relief with a rapid decrease in the size of the lesion, they have also shown to prevent recurrence even after 1 year of follow-up. Since no medications were required, adverse effects of using medications could be prevented. Hence, PBMT is a safe and clinically effective therapeutic modality for treating RAS.[33]

Oral lichen planus is a chronic inflammatory mucocutaneous disorder with varied forms of appearance. Various studies have reported the use of diode lasers operating in a wavelength range of 630-970 nm, duration of each session ranging from few seconds to 8 minutes, and 2-3 sessions per week may be required for complete healing of the lesion.[34] PBMT with red diode lasers has proved to reduce the pain level and sign scores in all the patients. Ultraviolet and helium–neon lasers were also used in the management of oral lichen planus; the drawback of using an ultra violet-B excimer laser is that it is potentially a carcinogen and may lead to side effects like erythema and soreness.[35] Red diode lasers are safer to use without causing any significant side effects. Hence, it is a good alternative for conventional corticosteroid therapy in treating of oral lichen planus.

Herpes labialis is a common viral illness affecting the orofacial region caused by herpes simplex virus-1. Though the primary conventional therapy includes symptomatic treatment and antivirals, PBMT has also been used effectively in the management of herpes labialis in all age groups, including pediatric patients.[36] Stona et al.[36] have shown successful management of recurrent herpes labialis in a pediatric patient with the use of an infrared diode laser operating in a wavelength of 780 nm for 80 seconds, targeting at four points over the herpetic lesion in a dose of 5 J/cm^2 for one point given for 3 consecutive days, which has shown complete relief of symptoms and scabbing

with no reported recurrence. Several studies with PBMT using lasers operating in a wavelength range between 632.8 nm and 870 nm, power of 5-80 mW, and 2.04-48 J/cm^2 have also proved to be successful in the management of the recurrent herpetic lesions.[37]

Vesiculobullous Disorders

PBMT has been employed in the management of oral lesions of vesiculobullous disorders like pemphigus and pemphigoid. Pemphigus vulgaris is a blistering disease that can affect the skin and mucous membranes. Though corticosteroids are the first line of therapy, there have been reports using PBMT in the management of oral lesions in pemphigus. PBMT with a wavelength of 660-780 nm targeted at the lesions in a dose of 8 J/cm^2 per point has shown good improvement in the symptoms and has caused regression of the lesions.[38] Mucous membrane pemphigoid (MMP) represents a group of chronic inflammatory, subepithelial blistering disease that can manifest as oral, ocular, skin, genital, nasopharyngeal, esophageal, and laryngeal lesions. Cafaro et al.[39] have successfully demonstrated PBMT in the management of MMP with a 980 nm GaAlAs diode laser in a dose of 4 J/cm^2, used in noncontact mode. All the mucosal lesions and perilesional tissues up to 0.5 cm need to be irradiated twice weekly, until the complete resolution of lesions.

Paresthesia

Paresthesia in the orofacial region may be due to a wide variety of causes. Paresthesia of the inferior alveolar nerve is the most common to occur after any surgical procedure of the mandible. PBMT has been used in the management of paresthesia both extra orally and intraorally depending on the region and the nerve affected. PBMT with lasers operating in a wavelength of about 820-830 nm targeting the affected area with an energy dose of about 4-6 J/cm^2 has been used for the management of post-operative paresthesia. PBMT for the management of paresthesia requires multiple sessions and has been proved to be effective in improving the neurosensory recovery.[40,41]

CONCLUSION

PBMT has been proved to be an effective treatment modality in the management of several orofacial disorders by enabling enhanced wound healing, reducing inflammation, and pain. There is a good number of evidence in the literature on the clinical efficacy of PBM in various medical and dental applications. Further newer applications of PBMT in dentistry and standardization of the wavelength, dosage, and treatment duration for different disorders should be explored.

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Short Communication

Detection of Human Herpesvirus-6 in Cerebrospinal Fluid of Patients with Meningococcal Meningitis—Report of Two Cases from Gaziantep, Turkey

Tekin Karslıgil, Yasemin Zer, Mehmet Erinmez

Department of Medical Microbiology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

ABSTRACT

Meningitis is a very severe and life-threatening clinical table. For rapid diagnosis of meningitis, DNAs or RNAs of the possible pathogens are investigated using syndromic panel-based testing, and it is very commonly used for early guidance to therapy in recent years. Depending on the specificity and sensitivity of the tests, it is possible to detect more than one pathogen. In this research, cerebrospinal fluid of two patients with early diagnosis of meningitis based on their clinical findings were tested using BioFire FilmArray Multiplex PCR (Biomerieux, France). *Neisseria meningitidis* and human herpesvirus-6 were codetected. For further evaluation of clinical meaning of this codetections, this case report is presented.

Keywords: Meningitis, cerebrospinal fluid, meningococcemia

INTRODUCTION

Molecular syndromic panel-based tests provide opportunity to use only one test for the detection of most common pathogens of infectious diseases. The advantages of these multiplex PCR tests are rapidness and being able detect viral, bacterial, and fungal pathogens at the same time.[1] The BioFire FilmArray Meningitis/Encephalitis (ME) panel is used for the detection of bacteria, viruses, and yeast in cerebrospinal fluid (CSF) specimens: 14 pathogens composed of six bacterial, seven viral, and one fungal pathogens in 1 hour time. The FilmArray ME panel test is a rapid and trustworthy diagnostic tool for the management of meningitis and can simply be applied in routine diagnostic workflows. Coordinated evaluation of test results and expected clinical findings requires qualified users and the awareness of probable false-negative or false-positive results.[2] Leber et al.[3] found overall sensitivity and specificity of Biofire FilmArray as 95% and 99.2%, respectively. Human herpesvirus-6 (*HHV-6*) is included in the FilmArray ME panel; however, the overall sensitivity and specificity of the panel is high; HHV-6 exhibits latency and probable chromosomal integration. Hence, cautious analysis of *HHV-6* detection in FilmArray ME panel is required. The clinical diagnosis should usually not be made by molecular detection of *HHV-6* in CSF alone.[4,5]

Neisseria meningitidis is a Gram-negative diplococcus, which is a causative agent for meningoccal meningitis and meningococcemia.[6] HHV-6 has become progressively acknowledged as an emergent central nervous system pathogen. HHV-6 has been confirmed to be neurotropic and is a causative agent for a number of neurologic conditions like multiple sclerosis, post-transplant limbic encephalitis, mesial temporal sclerosis, and encephalitis/meningitis in immunocompetent patients.[7] CSF samples of two patients who had meningitis symptoms, admitted to our clinical microbiology laboratory between December 2019 and January 2020 from Gaziantep University Faculty of Medicine Pediatrics Clinic for bacterial investigation and meningitis/encephalitis multiplex PCR search panel. This panel gives results in 90 minutes duration and used especially in emergency cases.

First case (LC): A 1-year-old female patient attended our hospital with fever, malaise, sleepiness, and refusing to eat complaints. At the first examination by a pediatrician, neck stiffness was seen. Blood tests, lumbar puncture, and computerized tomography (CT) were done for differential diagnosis. In blood tests, hemoglobin was 9.1 g/dL (11.1-14.7 g/dL) and CRP (C-reactive protein) was 90.6 mg/L (0-5 mg/L). CT imaging results showed normal findings. CSF samples taken by lumbar puncture were sent to biochemistry and microbiology laboratories. CSF biochemistry results revealed the micrototal protein in CSF was 84 mg/dL (15-45 mg/dL) and the glucose level in CSF was 61 mg/dL (45-80 mg/dL). In our microbiology laboratory, tuberculosis PCR, brucella agglutination, meningitis/encephalitis multiplex PCR search panel, CSF Gram stain, and bacterial culture investigations of CSF sample were done. Tuberculosis PCR and CSF brucella agglutinations test results were negative. There was no bacteria seen, and 10 white blood cells per high-power field (WBCs/HPF) were seen in CSF Gram stain. Three

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ORCID iDs of the authors: T.K. 000-0001-7672-3625; Y.Z. 000-0002-9078-9900; M.E. 0000-0002-3570-3510.

Corresponding Author: Mehmet Erinmez **E-mail**: mehmeterinmez92@hotmail.com

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different agar plates, blood agar, chocolate agar, and eosin–methylene blue agar (EMB), inoculated with CSF sample. After a 24-hour incubation, there was a bacterial growth, and the Gram stain from the colonies showed Gram negative diplococci and identified using Vitek 2 automated system (Biomerieux, France) as *N. meningitidis.* Meningitis/encephalitis multiplex PCR search panel (BiofireFilmarray, Biomerieux, France) results showed the sample was HHV-6 and *N. meningitides* positive.

Second case (NS): A 16-year-old female patient attended our hospital with a fever, stomach pain, vomiting, dizziness, and skin rash complaints. At the first examination by a pediatrician, there was no neck stiffness, and Kernig and Brudinzski signs, but skin findings were relatable to meningococcemia. Blood tests, lumbar puncture, and CT were done for differential diagnosis. In blood tests, platelets were 12 $\times$ 10^3/μL (158-374 10^3/μL), prothrombin time (PT) was >200 seconds (12-16 seconds), activated partial thromboplastin time (aPTT) was >500 seconds (26-37.2 seconds), and CRP was 160.4 mg/L (0-5 mg/L). CT imaging results showed normal findings. CSF samples taken by lumbar puncture were sent to biochemistry and microbiology laboratories. CSF biochemistry results revealed the micrototal protein in CSF was 72 mg/dL (15-45 mg/dL) and the glucose level in CSF was 54 mg/dL (45-80 mg/dL). In our microbiology laboratory, tuberculosis PCR, brucella agglutination, meningitis/encephalitis multiplex PCR search panel, CSF Gram stain, and bacterial culture investigations were done with CSF. Tuberculosis PCR and CSF brucella agglutination test results were negative. There was no bacteria seen, and 25 WBCs/HPF were seen at CSF Gram stain. Three different agar plates, blood agar, chocolate agar, and EMB, inoculated with CSF sample. After a 24-hour incubation, there was a bacterial growth, and the Gram stain from the colonies showed Gram negative diplococci and identified using Vitek 2 automated system (Biomerieux, France) as *N. meningitidis.* Meningitis/encephalitis multiplex PCR search panel (BiofireFilmarray) results showed the sample was HHV-6 and *N. meningitidis* positive.

CSF samples from both patients were investigated with in-house PCR method, and both samples were HHV-6 negative.

CONCLUSION

Larger studies may be required to comprehend disadvantages and advantages of multiplex PCR methods for investigating meningoencephalitis. Also, our cases create a question: Is there a need to confirm all codetections by other methods? In previous studies, Du et al.[8] found five codetections among 25 positive specimens in their research using BioFire FilmArray ME panel, and also Leber et al.[3] found five codetections among 136 positive specimens in their research using BioFire FilmArray ME panel. Detection of HHV-6 and *N. meningitides* together by multiplex PCR method needs to be evaluated by larger studies to understand possible relationship in meningitis cases between these infectious agents. Also, there is a need to understand if HHV-6 has an effect on clinical prognosis of meningococcal meningitis patients.

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