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The aim of the journal is to publish original articles with highest clinical and scientific quality at the international level. Middle Black Sea Journal of Health Science also publishes reviews covering fundamental innovations in health education, editorial articles, case reports and original images.

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Chapter in Edited Book

Hornbeck P. Assay for antibody production. Colign JE, Kruisbeek AM, Marguiles DH, editors. *Current Protocols in Immunology*. New York: Greene Publishing Associates; 1991. p. 105-32.

Book with a Single Author

Fleiss JL. *Statistical Methods for Rates and Proportions*. Second Edition. New York: John Wiley and Sons; 1981.

Editor(s) as Author

Balows A, Mousier WJ, Herramaflfl KL, editors. *Manual of Clinical Microbiology*. Fifth Edition. Washington DC: IRL Press. 1990.

Conference Paper

Entrala E, Mascaro C. New structural findings in *Cryptosporidium parvum* oocysts. Eighth International Congress of Parasitology (ICOPA VIII); October, 10-14; Izmir-Turkey: 1994. p. 1250-75

Thesis

Erakıncı G. Donörlerde parazitlere karşı oluşan antikorların aranması. İzmir: Ege Üniversitesi Sağlık Bilimleri Enstitüsü. 1997.

Article in Electronic Format

Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: <http://www.cdc.gov/ncidod1EID/cid.htm>.

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a) Original research: Prospective, retrospective and all kinds of experimental studies

Structure

English title, author names and institutions.

Abstract (average 200-400 word)

Introduction (200-500 word)

Methods (800 -1000 word)

Results (800-1000 word)

Discussion and conclusion (> 1200 word)

References (most 30)

Whole text should not exceed 4500 words except for resources and English summary.

b) Short papers: Prospective, retrospective and all kinds of experimental studies

Structure

English title, author names and institutions.

Abstract (average 200-400 word)

Introduction (150-300 word)

Methods (most 600 word)

Results (most 600 word)

Discussion and conclusion (most 800 word)

References (most 20)

Whole text should not exceed 2700 words except for resources and English summary.

c) Case Report: They are rarely seen articles which differs in diagnosis and treatment. They should be supported by enough photographs and diagrams.

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English title, author names and institutions.

Abstract (average 100-300 word)

Introduction (150-300 word)

Case report (most 600 word)

Discussion and conclusion (most 1000 word)

References (most 20)

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Structure

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Abstract (average 200-400 word)

Introduction (200-500 word)

The compilation text also including appropriate sub-headings (2000-3500 word),

Conclusion (50-150 word)

References (most 35)

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e) Letter to the Editor

English title, author names and institutions.

Abstract (average 100-300 word)

There is no need to open sub part in the letter text, it must be written as to include the main text (most 550 word) and results (50-150 word).

Discussion and conclusion (average 200 words)

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Whole text should not exceed 1200 words except for resources and English summary.

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Structure

Abstract (average 200-400 word)

Surgical technique

Conclusion (50-150 word)

References (most 15)

g) Differential Diagnosis: Are the case reports which have current value. Includes reviews for similar diseases.

Structure

Abstract (average 100-150 word)

Topics related to the subject.

Conclusion (50-150 word)

References (3-5 inter)

h) Original Images: Rarely seen annotated medical images and photographs in the literature.

Structure

300 words of text and original images about the subject

References (3-5 inter)

i) What is Your Diagnosis?: Are the articles prepared as in questions and answers about rarely seen diseases which differ in the diagnosis and treatment .

Structure

Topics related to the subject.

References (3-5 inter)

i) Questions and Answers: Are the texts written in form of questions and answers about scientific educative –instructive medical issues.

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About the Second Issue

We are excited about being together with the second issue of our journal which started broadcasting life in April 2015.

In this issue, there are two original articles, a review, a case report and a letter to the editor. The articles are branches nursing, cardiology, parasitology and dentistry. In the selection process of the studies presented in the journal, great attention has been shown to broadcast in the different health areas.

While one of the original articles was reviewing the sexual health in college students, the other was based on the epidemiology of hydatid disease in animals in Ordu for the first time. In the review, free-living amoeba has been the subject of our journal. In addition, the case report is about dentigerous and enriched with the literature review. On the other hand, bifid uvula and cardiac manifestations association was discussed in the letter to the editor.

In our journal publications process, I extend my thanks to our authors, article assessment referees, our editorial board members and our technical team for their support.

See you soon...

PhD. Asst. Prof. Ülkü KARAMAN

Director in Charge

The Reasons of University Students for Selecting Sexual Health Course

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Abstract

Objective: The research was conducted to determine the reasons of university students taking "Sexual Health" course for choosing it.

Methods: The study was conducted at Karadeniz Technical University, Trabzon, Turkey. The research population consisted of all 64 sophomore students (10 male, 54 female) studying at Karadeniz Technical University, Faculty of Health Sciences Department of Nursing and taking "Sexual Health" lesson as an elective course. Developed by researchers, "Feedback Form on the Sexual Health Course" was used as a data collection tool. Data were analyzed using the number and percentage values.

Results: The findings indicate that the majority of the students had insufficient knowledge about sexual health (93.8%), wanted to get information about everything related to sexual health (35.9%), chose the course to be informed about sexuality and reproductive health (45.4%), hadn't received sexual health training before (58.7%), wanted education on sexual health (100%), wanted sexual health course to be 'compulsory' (96.9%) and should be given at secondary level (50.0%) and generalized across the country (96.9%).

Conclusion: The research findings were discussed in the light of relevant literature emphasizing the importance of sexual health course for university students and referring to the requirement of the comprehensive sexual health education.

Key words: Sexual health course, university students, sexual health education, nursing.

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*This research submitted as a oral presentation to the 13th National Congress of Nursing Students, 1-3 May, 2014, Trabzon, Turkey.

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Introduction

The period of youth is a period in which an individual gain social maturity, reach a level where he/she can make his/her own decisions, can act independently and establish a healthy relationship with the opposite sex (CETAD, 2006). During this period, since they cannot think over the results of the events thoroughly young people can easily exhibit risk taking behaviors (HÜKSAM, 2002), therefore increasingly face with risks such as sexual intercourse at an early age, early marriage, sexually transmitted diseases (STDs), unwanted or early pregnancy, and childbirth complications and remain vulnerable.

Also during this period, the lack of efficient distribution of information regarding health needs of young people lead them to tend towards risky behaviors (Alpua, 2006). Yet in the General Assembly of World Sexual Health Union in Sydney in 2007 and in Development plans, the requirement of young people's being informed about sexuality and health issues and providing universal access to information in order not to face any possible problems that might adversely affect their overall well-being are clearly indicated (Bozdemir and Özcan, 2011).

However, implementations show that sex education is not included in the education system and has very limited space in other courses (Çalışandemir et al., 2008; Çok and Kutlu, 2010). Existing health facilities are not sufficient enough for young people in terms of sexual health information, consultancy and service delivery (Nalbant and Bulut, 2001). Moreover, the fact that their families have not received formal education about sexual education, feel shy to talk about it and perceive sexuality as a taboo causes inability to talk about sexual issues comfortably (Gölbaşı, 2005; Pınar et al., 2009; Erbil et al., 2010; Bozdemir and Özcan, 2011). However, during the period of youth, also known as gender identity development period, young people are known to be in need of getting accurate and reliable information about sexual health (Biri et al., 2007). Because, the young develop their values related to sexuality and make decisions regarding their sexual behavior in the process of becoming adults (Civil and Yıldız, 2010). That is why training in sexual matters will contribute individuals to organize their sexual life consciously, develop positive attitudes and values towards sexuality in growth and maturing process and make smarter choices in sexual behavior (Güler and Yöntem, 2007). Studies suggest that young people have a low level of knowledge about sexual health, generally get information about sexuality from their friends and the internet, parents cannot give enough information because of feeling uncomfortable to talk about sexual matters and they have not received any training on sexual health issues and want to receive information (Gökengin et al., 2003; Hazır -Bıkmaz and Güler, 2007; Çetin et al., 2008; Siyez, 2009; Topkaya and Sümer, 2010, Civil and Yıldız, 2010) To protect nursing students' own sexual health and enable them to provide guidance on this issue for healthy or sick individuals in their professional life will be possible with the scientific knowledge and skills

they have acquired from reliable sources. Based on this need, it was aimed to determine and evaluate the reasons of nursing students at Faculty of Health Sciences Department, Karadeniz Technical University for choosing the sexual health course.

Materials and Methods

Type of research

This study is a descriptive research.

Population and sample

The population of the research consisted of all sophomore students (64) studying at the Faculty of Health Sciences Department of Nursing, Karadeniz Technical University in the spring semester, 2013-2014 academic year and taking "Sexual Health" course as an elective lesson. Election was not made in the study sample, all the students [10 male (15.6%), 54 females (84.4%)] were reached.

Data Collection Tool

As the data collection tool 'Feedback Form on Sexual Health Course' developed by the researchers through literature review was used. A group of 10 students taking Sexual Health course was applied a preliminary study at the end of the lesson in order to control the clarity of the form. Preliminary application forms were finalized after the necessary arrangements by asking expert opinion in the field. In the form, there are a total of 30 questions consisting of closed and open-ended questions to assess students' feedback about the course.

Feedback Form on Sexual Health Lesson

In developing the form, we benefit from the following publications: Hacettepe University Women's Problems Research and Application Center, the project of investigating the factors affecting the adolescents and youth's sexual / reproductive health in Turkey, HÜKSAM (2002), the World Health Organization-WHO (2011), Youth and health risks report, Sexual Education Treatment and Research Association-CETAD (2006), the national and advocacy project in the field of sexual and reproductive health through local media, Acknowledgement File 1 and CETAD (2007), "Youth and Sexuality" file, the issue that young people talk least but are concerned most: Sexuality. Furthermore, other studies' data collection tools the aims of which overlap with this study were also taken into account. For face and content validity, three experts' opinions were taken and the form was finalized in accordance with their feedback.

Data Collection

Research data were collected after obtaining written permission from the administration of the Faculty and verbal consent from the students. Before handing out the forms, the aim of the study was explained to students in the classroom by the researchers and voluntary students completed the form 15-20 minutes.

Ethical statement

Before the study could begin, permission was obtained from the institution, and the participants were then invited to participate in the study. They were also informed by the researchers and through an approval protocol that they would not be paid for their participation. The researchers guaranteed participants that their identities and answers would be kept confidential. The study conformed to the principles of the Declaration of Helsinki.

Data Analysis

Data were analyzed using SPSS 17.0 software package. The frequencies and percentages of the data obtained from 'Feedback Form on the Sexual Health Course' developed in order to determine students' views on the sexual health course were calculated.

Research Limitations

The fact that the results only represent the institution where the study was conducted and the views of the students in nursing department taking sexual health course was accepted as the limitation of the study.

Results

84.4 % of the students involved in our study were female, 15.6% were male, mean age: 20.0 ± 1.13 years, 89.1 % had nuclear family, 59.4% graduated from high school, 56.2% spent most of their life in Black sea region. The fathers (35.9%) and the mothers (68.8%) of the students are high school and primary school graduates respectively.

When Table 1 is examined, it is seen that students' median age of receiving first information on sexual health issues is $14:00 \pm 1.85$ (Min: 10.00 Max: 18:00). 81.3% learned information about sexual health from their friends, 93.8 % do not have sufficient information about sexual health and 35.9% want to learn everything related to sexual health (Table 1).

Table 1. Students' sexual health information resources and information needs

	Number	%
The median age of receiving first information about sexual health issues.	14.00±1.85 (Min: 10.00, Max: 18.00)	
Information resources on sexual health*		
Friend	52	81.3
Media	47	73.4
Mother	23	35.9
Sister	8	12.5
Family elder	4	6.3
Films	3	4.7
Brother	2	3.1
Father	2	3.1
Do you have sufficient information about sexual health?		
Yes	4	6.2
No	60	93.8
Sexual health issues that you want to get information on		
About everything	23	35.9
About healthy sexual relationships	20	31.3
About sexually transmitted diseases	9	14.1
About what sexuality means	3	4.7
About family planning	2	3.1
About maternal and child health	2	3.1
I have no idea	5	7.8

*More than one answer was given and percentages were calculated based on n

When Table 2 is examined, it is seen that 58.7% of the students did not receive education about sexual health, all the students (100.0%) want education on sexual health, 50.0% think sexual health education should be given starting from the secondary school, 96.9% state that sexual health education should be compulsory, 46.9% express that it would be more appropriate to give the education separately to girls and boys and 96.9% point out that sexual health education should be widely launched at schools (Table 2).

Table 2. Students' views regarding sexual health education

Views	Number	%
Have you received information on sexual health?		
Yes	27	41.3
No	37	58.7
Should education be given on sexual health?		
Yes	64	100.0
No	-	-
When should education be given on sexual health?		
As of primary school	13	20.3
As of secondary school	32	50.0
At high School	9	14.1
Before getting married	3	4.7
At university	4	6.3
It does not matter	3	4.7
Should sexual health course be compulsory or elective?		
Compulsory	62	96.9
Elective	2	3.1
Should sexual health course be given to girls and boys together or separately?		
Separately	30	46.9
Together	25	39.1
Elective	9	14.1
Should sexual health course start at school?		
Yes	62	96.9
No	2	3.1

Table 3. Reasons of students for selecting sexual health course

Reasons of Selecting Sexual Health Course*	Number	%
To have knowledge about sexuality and reproductive health	29	45.4
To correct the wrong things that I know to be true	18	28.1
To inform the people around me	15	23.4
Because it is a compulsory elective course	11	17.2
To create infrastructure for obstetrics course	11	17.2
To protect my sexual health and apply to my life	7	10.9
To be able to answer the questions my friends and people around me ask	4	6.3
To be able speak up and correct the wrong things spoken secretly	2	3.1
To learn pregnancy prevention methods	2	3.1
Because I wonder sexual health issues	2	3.1
To learn how to troubleshoot issues related to sexuality	1	1.6
The status of sexual health course to meet expectations		
Yes	60	93.8
No	4	6.2

*More than one answer was given and percentages were calculated based on n.

When Table 3 is examined, it is understood that 45.4% of students chose sexual health course "to have information about sexual and reproductive health" and 93.8% expressed that it met their expectations (Table 3).

Discussion

This research was conducted to assess the reasons of sophomore students studying at Nursing Department for choosing "Sexual Health" course. In general, research findings indicate that students' main sources of information about sexual health are their friends and media. Moreover, fathers, brothers and films were expressed as minimum information sources referred by students. Similarly in Esgin's (2000) study, it is stated that 82% of young people can speak about sexuality most comfortably with their friends from their own gender. In the other studies carried out in Turkey it is observed that basic information sources of young people about the issue are friends, books, television, the internet, newspapers and families respectively (Karabulutlu and Kılıç, 2011; Atan Ünsal et al., 2012; Kumcağız et al., 2013). Since sexuality issues are perceived as "shame and sin" especially in developing countries like Turkey, young people can speak about such issues more freely with friends because families carry on their traditional attitude asserting that sexuality is "intimate" and prefer not to talk about it with their children. Thus, young people consult the sources outside school to meet their information needs regarding sexual and reproductive health and are often informed incorrectly (HÜKSAM, 2002; Çalışandemir et al., 2008).

In our study it was found out that students do not have sufficient information about sexual health and want to learn everything related to it. Studies suggest that young people do not have enough information especially about sexuality, reproductive physiology, pregnancy prevention or CYBH but are willing to get information and education on these issues (HÜKSAM, 2002; CETAD, 2006). In their study Kadioğlu et al. (2008) investigated students' views on sexuality and sexual education suggests that 61.0 % of them found the information about the sexuality slightly enough. Blum and Nelson (2004) indicated that two-thirds of female and male students had no sufficient information about sexuality.

The research findings on information needs reveal that students want to improve themselves more on sexuality and sexual health issues.

In our study, the ratio of the students stating they had not received sexual health education so far and considered sexual health education should start widely at secondary schools is quite high and this was assessed as a notable finding. Today, information on sexuality and reproduction are taught as compulsory or elective courses in university curriculums starting from primary schools in many Western countries. In Turkey, it is emphasized that primary school years is a crucial period for gaining knowledge, skills and attitudes about sexuality by indicating that the highest enrollment rate (92%) is during primary school period (Bıkmaz and Güler, 2007). But today in our country sexual health education is still not included in the formal education system (HÜKSAM, 2002; Çok and Kutlu, 2010). It is known that, on the basis of this, there lies a concern about sexual health education would lead young people to wrong sexual intercourse and in the early stages (Nicolle et al., 1999; Gürsoy and Gençalp, 2010). However, research shows that these concerns are needless. According to Yıldırımkaaya, in a survey of with 317 thousand people in 41 countries around the world, 98% of participants stated young people should receive sexual education at schools before the age of 16 (Gürsoy and Gençalp, 2010).

The research findings by CETAD (2006) with 1537 people in 20 cities in seven regions reveal that 75% of the population is willing to give sexual education at schools. Likewise, according to a study conducted at Marmara University campus with 3665 students, 76.5% of students stated that they should be given sexual health education at schools (Aydoğan, 2001). The study carried out with 519 teachers working at primary schools in Adana showed that the ratio of the participants supporting sexual education course at schools was 97.5 %. (Akbaş, 2000). These findings are consistent with the other research findings (Pinar et al., 2009; Dağ et al., 2012; Kumcağız et al., 2013). Students must be supported on sexual health by giving them education on these issues so that they can access accurate information on sexual matters, make right decisions primarily about themselves and behave professionally away from wrong judgments (Tokuç et al., 2011).

The most effective, cheapest and accessible way of this is to integrate sexual health education into mainstream school curriculum (Gürsoy and Gençalp, 2010). Another remarkable finding of this study is the students' view about the appropriateness of giving sexual health education to girls and boys separately. Considering the study regarding sex education at the national level, it is seen that in the continuation of a project initiated by Ministry of Education, Health Administration Department in 1994, in the first pilot schools girls and boys began to be taught "sexual education" course in separate classes, but this practice did not remain in force for a long time (Çalışandemir et al., 2008). In a study carried out by CETAD (2006) in seven regions, 20 cities with 1537 people, the question "Should sex education be given to boys and girls together or separately?" was replied as separately by 65.9% of the participants and together by 34.1%. Unlike our study Kumcağız et al. (2013) found out that 38.3% of the counselors wanted sexual health lessons in small groups consisting of girls and boys and likewise 38.3% of them wanted sexual health lessons in big groups consisting of girls and boys. These findings suggest that on nursing students' sexual attitudes and behaviors, ongoing effects of conventional structure fueled with gender discrimination and male-female differences still exist.

In our study, students said to have chosen sexual health course "to have information about sexual and reproductive health" and "to correct the wrong things they know to be correct". Similarly in a study examining the views of prospective psychology counselor on sexual health education by Kumcağız et al. (2013), 59.1% of students said to have chosen sexual health course 'to obtain correct information'. Özcebe et al. (2007) determined that one out of four women and two out of four men among 2963 people aged 15-24 years old had no information about women's ovulation and they had insufficient knowledge about reproductive organ. Pinar et al. (2009) suggest that 81.9% of university students wanted to be given information about sexual health. To educate young people about sexual health will enable them act maturely and responsibly, make right choices and protect and improve sexual health in the community.

Conclusion

It is understood that as in the other studies with university students, a large part of the undergraduate nursing program students also consult non-school resources about sexual matters, had insufficient knowledge, and chose the lesson to obtain information about sexual health reproduction and it met their expectations. The findings of this study suggest that a comprehensive sexual education in nursing education will play an important role in increasing students' proficiency and knowledge on this issue and meeting their information needs.

Informed Consent: Verbal informed consent was obtained from students who participated in this study

Peer-review: Externally peer-reviewed.

Author Contributions: Concept KYÇ; Design KYÇ; Supervision KYÇ, SA; Materials KYÇ, AP; Data Collection and/or Processing KYÇ, AP; Analysis and/or Interpretation KYÇ; Literature Review KYÇ, SA, RE; Writing KYÇ; Critical Review KYÇ,SA,RE.

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Cystic Echinococcosis of Cattle of Sheep in Ordu

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Abstract

Objective: Cystic Echinococcosis (CE) a parasitic disease that affects both human and animal health, shows a broad distribution as well. Parasites are frequently seen in humans and animals living in less developed countries. It is difficult to diagnose in intermediate hosts since the clinical findings are not evident enough. Early diagnosis increases the success rate in the treatment of the disease. The risk factors of the CE in humans can be listed as working in a rural area, keeping a dog, lack of information about the disease, age, gender and potable water supply. Surgical and chemotherapeutic treatment costs of the CE patients, their hospital expenses, deaths, loss of income caused by the fact that they are not able to work when they are ill and during the recovery period create a heavy burden on the economies of the countries. When economic and social losses regarding the cases which cannot be treated since they are not diagnosed are taken into account, control of the disease becomes highly significant. Furthermore, loss of carcass value in sheep, goats and cattle, loss of infected livers and implicit losses in yield cause serious economic loss.

Methods: According to the data obtained, no study has been conducted in Ordu province concerning the epidemiology of Hydatid cyst. The aim of this study is to make a retrospective evaluation on the data of Cystic echinococcosis found in sheep and cattle slaughtered between the years 2008-2014 in the abattoir affiliated to the Municipality of Ordu, a province which also has some stray dog population.

Results: The data for this study have been obtained from the abattoir records. In the abattoir affiliated to the Municipality of Ordu 26117 cattle and 1791 sheep were slaughtered between 2008-2014. As a result of the analysis performed, 1149 cattle and 114 sheep were infected with cystic echinococcosis.

Conclusion: Presence of cattle and sheep with hydatid cyst in this study supports the idea that there are infected dogs in this region. This situation can be explained by the fact that the parasite can complete its evolution through the animals slaughtered in an uncontrolled manner. The study has also shown us that the control activities should be conducted in accordance with the epidemiology of the parasite and the results obtained.

Key words: Cystic echinococcosis, *Echinococcus granulosus*, Ordu

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Introduction

Hydatid cyst is a zoonosis found in intermediate hosts and caused by the metacestode form of the tapeworm *Echinococcus granulosus*. The adult form of the parasite resides in small intestines of the carnivore such as dogs and foxes; and the larval form resides in internal organs of the humans and animals such as sheep, goats, cattle and pigs which are intermediate hosts. The disease can localise mainly in the liver and lungs and almost in all other

organs such as kidneys, spleen, brain, bones and heart. Animal movements and its capability of adapting to various types of hosts, the host, environmental and social factors are influential in the spread of *Echinococcus granulosus*. The larva of the parasite called the hydatid cyst mostly grows in internal organs, livers and lungs of the farm animals. The cysts containing protoscolex are called fertile cysts. Older sheep have a higher number of fertile cysts. Adult tapeworms grow from the protoscolex released in the small intestines of the dogs eating offal containing fertile cysts. In the cities which have less stray dogs, the dogs generally cannot reach to internal organs of the sheep (Oku et al., 2004). However, in rural areas, the dogs become infected by eating lungs and livers of the older sheep slaughtered. Humans become infected when they receive the eggs of the tapeworm from contaminated environment or by close contact with infected dogs. Therefore, CE is mostly found in people who live in unproductive rural areas with sheep and dogs kept for protecting and herding these animals (Oku et al., 2004; Craig et al., 2007). Surgical and chemotherapeutic treatment costs of the CE patients, their hospital expenses, deaths, loss of income caused by the fact that they are not able to work when they are ill and during the recovery period create a heavy burden on the economies of the countries. When economic and social losses in the cases which cannot be treated since they are not diagnosed are taken into account, control of the disease becomes highly significant. Furthermore, loss of carcass value in sheep, goats and cattle, loss of infected livers and implicit loss in yield lead to serious economic loss (Perry and Randolph, 1999; Torgerson, 2003; Budke et al., 2005; Sariözkan and Yalçın, 2009). *E. granulosus* can be seen in almost all continents in the world. The incidence rate of the parasite has been identified higher in some regions of South America, Australia, Africa and Eurasia. There are also some regions where the infection is defined as endemic or sporadic. Furthermore, it has been reported that the parasite has never been found in Greenland and Iceland (Akyol, 2004; Kilimcioğlu and Ok, 2004).

This disease, which closely concern human and animal health in Turkey, is seen in Eastern, North Eastern and Central Anatolia and rarely in the western region where the people,

who migrated from our eastern region. According to the studies carried out with regard to the epidemiology of the parasite, in the neighbouring countries of Turkey, the incidence rate is 0.7-20/100000 in humans, 5.8-82% in cattle, 30.6-80% in sheep, 24% in goats and 5-8.4% in pigs (İnan et al., 2001; Kachani et al., 2001; Kurdovar et al., 2001; Saeed, 2001; Soturaki et al., 2001). In Turkey it is reported that the incidence rate is approximately 0.87-6/100000 in humans, 25.9% in cattle, 30.6% in sheep, 12.7% in goats and 1.4% in horses (Özçelik, 2001; Gıcık et al., 2004).

According to the data obtained, no study has been conducted in Ordu province concerning the epidemiology of Hydatid cyst. The aim of the study is to make a retrospective evaluation on the data of Cystic echinococcosis found in sheep and cattle slaughtered between the years 2008-2014 in the abattoir affiliated to the Municipality of Ordu, a province which also has some stray dog population.

Materials and Methods

This study is made a retrospective evaluation on the data of Cystic echinococcosis found in sheep and cattle slaughtered between the years in January 2008-July 2014 in the abattoir affiliated to the Municipality of Ordu, a province. Age of the animals' determination could not be made due to the cutting of retrospective assessment.

Results

The study revealed that 26117 cattle and 1791 sheep were slaughtered from 2008 to 2014.

The animals were brought for slaughter to Ordu Municipality Abattoir mainly from Samsun, Amasya, Tokat, Sivas and Giresun; and rarely from Adana, Kars and Ağrı provinces. As a results of the evaluation performed 4.4% (1149/26117) cattle and 6.36% (114/1791) of the sheep were found to be positive.

No study was conducted in order to find out if the cysts were fertile or not.

The slaughter rates of the cattle and sheep by years are given in the table.

Discussion

Cystic echinococcosis, which develops as a result of the infestation of *Echinococcus granulosus* larva that are connected to the tapeworm family,

is a parasitic zoonosis threatening human and animal health in the world as well as in Turkey. While this infection frequently seen particularly in rural area

Table: Distribution of the slaughtered cattle and sheep by years

Year	The number of slaughtered animals	
	Cattle	Sheep
2008	6051	566
2009	5098	469
2010	2520	233
2011	2004	76
2012	4233	188
2013	4125	108
2014	2086	151
Total	26117	1791

populations causes a disease prognosis that can be quite serious and sometimes lethal in humans, it also causes economic loss because of the structural and functional disorders developing in various organs and tissues of the animals (Balkaya and Şimşek, 2010).

Turkey is among the countries where this disease is endemic. According to the studies conducted throughout Turkey, it is reported that *E. granulosus* is seen with a rate of 0.94-54.5% (Zeybek and Tokay, 1990; Ayçiçek, 1998) in dogs and the rate for cystic echinococcosis is 4.5-56.5% in cattle (Erkut and Kahyaoğlu, 1996; Poyraz et al., 1990; Avcıoğlu et al., 2010). In this study, hydatid cyst is found in 4.39% of the cattle and in 6.36% of the sheep slaughtered in the abattoir affiliated to the Municipality of Ordu.

In a retrospective study conducted in Hatay province between the years 2003-2007 with the aim to identify the prevalence of hydatid cyst, 1.158 (%3.23) cystic echinococcosis cases defined in 35.812 cattle slaughtered in the abattoir affiliated to the Municipality of Antakya were analyzed. In the light of the information gathered, it is reported that the prevalence was 5.448 (%5.8) in 2003, 8.800 (%2.7) in 2004, 9.539 (%2.6) in 2005, 7.875 (%2.6) in 2006, and 3.850 (%3.2) in 2007 (Hakverdi et al., 2008).

In a study aiming at defining the seasonal prevalence of cystic echinococcosis, 203 (5.3%) out of 3846 cattle slaughtered in the abattoir of Kars Municipality throughout one year had cystic echinococcosis. According to this study, it was observed that the infection had the highest prevalence in spring (37.4%) and it was less frequently seen in autumn (15.8%) (Demir and Mor, 2011).

Balkaya and Şimşek (2010) investigated 2088 cattle slaughtered at the Combined Facility of Erzurum Meat and Fish Authority and in a private abattoir in order to define the epidemiology of fascioliasis and hydatidosis in Erzurum. All the internal organs, liver and lungs in particular, of the slaughtered animals were analyzed and hydatid cysts were found in 717 (34.3%) cattle. Hydatid cysts were observed only in the lungs in 520 animals (72.5%), only in the liver in 83 animals (11.6%), both in the lungs and in the liver in 109 animals (15.2%), in the spleen in 4 animals (0.6%) and in the heart in 1 animal (0.1%).

In another study conducted in Sivas, on the other hand, 765 cattle slaughtered in three different abattoirs in April and May were investigated and 273 (35.7%) were defined positive. 66 (8.6%) of the animals, which were positive, had hydatid cysts only in the liver and 103 had only in the lungs (Acıöz et al., 2008). When the rarely localised hydatid cyst cases which are reported in humans and animals are examined according to the data obtained from the studies carried out on humans and animals, it seems as if the number of cases were higher in humans. This situation can be attributed to the factors such as the diagnosis opportunities being more limited for animals compared to humans and not being able to perform a detailed examination after slaughter (Avcıoğlu et al., 2010).

Conclusion

No study has been conducted in this region before concerning the epidemiology of the parasite. It is thought that this study can be a reference for future epidemiological studies. The presence of cattle and sheep with hydatid cysts according to the study supports the idea that there are infected dogs in this region. This situation can be explained by the fact that the parasite can complete its evolution through the animals slaughtered in an uncontrolled.

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Risks and Threats Comes with Global Warming: Pathogenic Free Living Amoeba

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Abstract

Free living amoebae like *Naegleria*, *Acanthamoeba*, *Balamuthia* and *Sappinia* are known appearing opportunistic and also fatal protozoa in humans and other animals. They are widely distributed in soil and water in the world. They cause “Primer Amoebic Meningoencephalitis” the host immune response to these protist pathogens differs from each other to evidence by the postmortem laboratory findings from the affected patients.

This review was performed with a search in Medline, PubMed, Science Direct, Ovid, and Scopus literatures by the search terms of “pathogenic free-living amoeba infections”.

Analysis of a detailed review and literature shown that *Naegleria fowleri*, *Acanthamoeba* and *Balamuthia* and also *Sappinia sp.* infections are causing extensive brain damage to the host immune response. In human infection due to related to brain, skin, lung and eyes have increased significantly during the last years. They have different effects on epidemiology, immunology, pathology, and clinical features of the infections produced. This particular review planned to raise awareness about free-living amoeba, which found in a patient who applied to ESOGU Hospital Neurology Clinic because of suddenly unconsciousness and coma and diagnosed with *Naegleria fowleri*.

Clinicians should be aware of PAM infections and include in differential diagnosis of meningoencephalitis. PAM should be suspected in young adults and children with acute neurological symptoms as described below and recent exposure to fresh water. Preventive and control measures include public health education and awareness among medical practitioners and adequate control of public water supplies.

Key words: Pathogenic free-living amoebae, global warning, *Naegleria*, *Acanthamoeba*, *Balamuthia*, *Sappinia*

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Introduction

Acanthamoeba, *Naegleria*, *Balamuthia mandrillaris* and *Sappinia spp.* genus are members of Free-living Amoebae, (FLA), which are aerobic, eukaryotic and protists species as well. They occur worldwide and can potential cause of opportunistic infections in humans and other animals (Visvesvara et al., 2006; Visvesvara et al., 2007; Visvesvara et al., 2013). They could be found in soil, dust, air, seawater, drinking water, swimming pools, sewage, eyewash solutions, contact lens, dental treatment units, and dialysis units.

Due to the fact that these amoebae have the ability to exist as free-living organisms in nature and only occasionally invade a host and live as parasites within host tissue, they have also been called amphizoic amoeba. (Trabelsi et al., 2012; Szenasi et al., 1998).

Taxonomy of FLA was revised several times, last data from genomic sequencing studies and based on morphologic, biochemical and molecular approaches by zoologist. This four genus FLA has been classified to two super groups; *Acanthamoeba* and *Balamuthia* classified under the super group Amoebozoa. *Acanthamoebidae*, *N. fowleri* classified under the super group Excavata: *Heterolobosia*. *Sappinia* and *Vahlkampfiidae* belong to super group Amoebozoa: *Flabellinea*: *Thecamoebidae* (Adl et al., 2005).

All of four amoebae are known so far to cause infections of the central nervous system (CNS). Several species of *Acanthamoeba* (i.e. *A. castellanii*, *A. culbertsoni*, *A. hatchetti*, *A. polyphaga*, *A. rhyodes*), the only known species of *Balamuthia*, *B. mandrillaris*, two species of *Sappinia* genus, *S. diploidea* and *S. pedata*, and only one species of *Naegleria*, *N. fowleri*, are known to cause disease in humans and other animals (Khan, 2006; Visvesvara et al., 2007).

A shared appearance of these species is the very large central nuclear endosome, quite different from *E. histolytica*, where differentiation may be necessary in tissue sections. Under dry conditions, trophozoites form became resistant cysts that permit survival and also airborne dispersal; cysts can resist chlorination. Many of species are thermophilic and are cause of "humidifier fever" and also extrinsic allergic alveolitis, fever, dyspnea, cough. Some bacteria, like *Legionella* and *Parachlamydia*, *Acanthamoebae* may live symbiotically within these amoebae, persisting within the phagosome, being resistant to lysosomal enzymes. Three genera of free-living amoebae cause human infections, and a few cases caused by *Sappinia* sp. (Trabelsi et al., 2012).

***Naegleria* species**

Naegleria is an amoeboflagellate with two trophozoite forms. It is a thermophilic amoeba that grows well in tropical and subtropical climates. Among the 30 species of *Naegleria*, only one species *N. fowleri* was known human pathogen and caused a disease called Primary Amebic Meningoencephalitis (PAM). This infection characterized by an acute fulminant meningoencephalitis leading to death 3-7 days after exposure. Victims are healthy, young individuals with a history of recent water-related sport activities. This amoeba moves rapidly with a single

pseudopodium, it can transform into a non-feeding flagellate in hypotonic media, and these free-swimming forms facilitate dispersal. Cysts are thin walled and spherical. The portal of entry is the olfactory neuroepithelium. The pathologic changes are an acute hemorrhagic necrotizing meningoencephalitis with modest purulent exudate, mainly at the base of the brain, brain-stem and cerebellum. Trophozoites can be seen within the CNS lesions located mainly around blood vessels. (Sugita et al., 1999; Diaz et al., 2010; Wiwanitkit 2004; Saylor et al., 2015; Lawande et al., 1980).

N. fowleri is thermophilic amoeba, which able to grown at 45 °C temperature thermal water, fresh water lakes, swimming pools, but cannot survive seawater. Additionally, it was isolated from the healthy asymptomatic children nasal mucosa. *Naegleria* has includes amoeboid trophozoite (10-25µm) and resistant cyst form (8-20µm). The trophozoite is an active form that changes size and shape; it feeds bacteria and other organic matter. Amoeboid form transforms into two flagellate forms that are usually two flagella into the distilled water, but it will convert amoeboid form when conditions are favorable. Cysts are usually spherical single walled with fine pores. Only amoeboid form is found in tissues. *N. fowleri* cultured non-nutrient agar plates coated bacteria and at 45°C like *Acanthamoeba*, and also axenic media (Schuster et al., 2004; Martinez, 1993).

***Acanthamoeba* species**

Acanthamoeba is the most common amoebae and found in soil; fresh, brackish, and sea water; sewage; swimming pools; contact lens equipment; medicinal pools; dental treatment units; dialysis machines, heating, ventilating, and air conditioning systems; mammalian cell cultures; vegetables; human nostrils and throats; and human and animal brain, skin, and lung tissues. It has no flagellate form. The small pseudopodia are multiple, thin, and spike-like; they are called acanthopodia. Cysts are thick walled, angulated, and floating; their dispersal may be wind borne. Several species are pathogenic but morphological classification is unsatisfactory; rRNA sequences differentiate into 15 genotypes. *Acanthamoeba* is sometimes isolated from throat or nasal swabs or from stool specimens. It causes species of human infections by *A. castellanii*, *A. culbertsoni*, *A. catchetti*, *A. healyi*, *A. divionensis* and *A. polyphaga*. Other species are nonpathogenic and thermophilic (Schuster et al., 2004).

Acanthamoeba has two forms: a vegetative or trophozoite (8-10µm) and dormant cyst (8-29µm). Trophozoites has a single nucleus and a fine acanthopodia of the body. Cyst has been known to survive in vitro greater than 20 years. Laboratory cultivation is realized on non-nutrient agar medium seeded with Gram negative bacteria which are preferably not encapsulated and pigmented (*Escherichia coli* or *Enterobacter aerogenes*) to allow for growth of trophozoite forms incubated 30°C. They can also axenic medium and mammalian cell cultures.

Acanthamoeba can cause Granulomatous amoebic encephalitis (GAE) cutaneous and nasopharyngeal infections in immunocompromised patients a painful keratitis generally in contact-lens users. *Acanthamoeba* infections are rarely encountered in humans and animals, but showed that *Acanthamoeba* antibodies more than 80% of normal human populations (Chappell et al., 2001). Cutaneous acanthamoebiasis is cause rarely opportunistic infections in immunocompromised patients, especially HIV infected. Characteristic lesions are nodules, fistulas, papules and skin ulcerations contains with cyst and trophozoites. Lesions are usually face, trunk and extremities and spread blood stream of other tissues (Visvesvera et al., 2007; Patel et al., 2010).

Acanthamoeba keratitis (AK) is usually developed immunocompetent persons a corneal infection. The main risk factors are use of extended contact lens trauma. The lesion is typically only one eye involved. In recently, AK cases have been increasing in developing countries - correlating to contact lens wearers (85%) (Visvesvera et al., 2007; Patel et al., 2010; Tu et al., 2010).

Balamuthia mandrillaris

Balamuthia is closely related to *Acanthamoeba* and not a leptomyxid amoeba; it shows little directional movement and has an irregular or branched shape. Cysts are thick walled and spherical. Human infections formerly attributed to *Hartmannella sp.* are now all thought to be due to *Balamuthia mandrillaris*, a species described in 1993 from a mandrill baboon that died of meningoencephalitis in San Diego zoo. *Balamuthia* can only be cultured on tissue culture monolayers. About 100 cases have been reported worldwide, but many are from Latin America. *B. mandrillaris* like several species of *Acanthamoeba* are pathogenic "opportunistic" free-living amoebas

which cause Granulomatous Amebic Encephalitis (GAE) in humans and animals. GAE is an infection, usually seen in debilitated, malnourished individuals, in patients undergoing immunosuppressive therapy for organ transplants and in AIDS. The granulomatous component is negligible, particularly in immunocompromised individuals. Pathologically these amoebas produce patchy, chronic or sub-acute granulomatous encephalitis with the presence of trophozoites and cysts. The portal of entry is probably through the respiratory tract or an ulceration of the skin reaching the CNS by hematogenous spread. *B. mandrillaris* does not feed on bacteria and cultured non-nutrient agar. These amoeba growth in also tissue cultures. Its life cycle comprises trophic amoeboid stage (50-60µm) and cyst (12-30µm) with triple layer wall no pores. They may be found in brain, eyes, skin, lungs and other organs (Visvesvara et al., 2007; Saylor et al., 2015).

Sappinia species

Sappinia genus have comprises of *S. pedata* and *S. diploidea* name of two distinct species. They widely found of mammalian feces, soil, freshwater, forest liter, elk, bison, cattle and lizard rectum. It has two form trophozoite (40-80 µm and cyst 18-25 µm). Cysts are double walled and have nuclear pores and it can survive passage through the stomach. *Sappinia sp.* has not been shown to be lethal in human and experimental animals and never implicated pathology. Only one reported of encephalitis immunocompetant young man who survived. This cases caused by *Sappinia* may developed from earlier sinus infection and developed unconscious, seizure and also solitary mason sectioning showed necrotizing hemorrhagic inflammation of containing trophozoite form especially blood vessels. The patient's outcome after surgical excision of necrotic lesion, and successful treated of azitromycin, pentamidine, Itraconazole. A more phylogenetic study is needed to indicated that species this genus (Gelman et al., 2003; Visvesvera et al., 2007).

Free-living amoebae; Epidemiology, Pathology and Clinical Finding

FLA infected patients are usually give a history of swimming or diving in warm fresh water or spa water between 2 and 14 days before the illness began. Common-source outbreaks occur during warm summer months in temperate countries. This waterborne disease was discovered in Australia in

the 1965. Since then, it has been reported from about 15 other countries in Africa, Asia, Europe and North and South America (Heggie, 2010; Khan, 2006; Szenasi et al., 1998; Trabelsi et al., 2012; Visvesvara et al., 2006).

Primer Amoebic Meningoencephalitis (PAM)

PAM is caused by *Naegleria fowleri*, it follows intranasal infection during swimming in warm, contaminated freshwater. Most victims have been children or young adults and the disease is almost invariably fatal (WHO Library "Emerging issues in water and infectious diseases series, Visvesvara et al., 2007; Heggie, 2010). Recent studies shown that in the USA were identified, Australia was the only country where *N. fowleri* has been associated with public water supplies. In USA recently, the causal agent of a PAM case in the US was diagnosed as *Paravahlkampfia francinae* a new species of the free-living amoeba genus *Paravahlkampfia* that was isolated from the cerebrospinal fluid of a patient with headache, sore throat, and vomiting, presenting typical symptoms of PAM caused by *N. fowleri*. Thus awareness of novel emerging amoebae as causative agents of PAM should also be considered (Visvesvara et al., 2009; Kristenssons, 2013). Amoebic trophozoites pass the cribriform plate from the nasal mucosa to the olfactory bulbs and subarachnoid space. In postmortem examination of the brain shows cerebral softening and damage to the olfactory bulbs; cysts are never formed in the tissues. The earliest symptoms are sudden headaches, high temperature, and nuchal rigidity, followed by nausea, vomiting, irritability and restlessness. Nuchal rigidity usually occurs with positive Kernig and Brudzinski signs. Photophobia may occur late in the clinical course, followed by neurological abnormalities, including lethargy, seizures, confusion, coma, diplopia or bizarre behavior, leading to death within a week. Cranial nerve palsies (third, fourth, and sixth cranial nerves) may indicate brain edema and herniation. Intracranial pressure is usually raised to 600mm H₂O or higher. Cardiac rhythm abnormalities and myocardial necrosis have been found in some cases. Death is usually increased intracranial pressure with brain herniation, leading to cardiopulmonary arrest and pulmonary edema. Rarely, chronic and progressive infection of the CNS may involve the lungs (da Rocha-Azevedo et al., 2009). AGE is usually associated with an underlying debilitating disease

or immune suppressed individuals including HIV-AIDS patients and diabetics. More than 200 cases have been documented since the first human case was reported in 1995. But, some of them are missed clinically and discovered at autopsy or in preserved pathological material. Specific antisera enables amoebae to be recognized by immunofluorescence staining (Martinez et al., 1980; Visvesvara et al., 2006; Visvesvara et al., 2007; Khan, 2006; da Rocha-Azevedo et al., 2009). Immunocompetent patients are usually young adults and children. At first, nasal symptoms and headache are soon followed by fever, neck rigidity, coma, and, later, convulsions; mostly it is fatal within a few days. CSF is usually turbid and bloody with high protein, low glucose and neutrophils. Amoebae must be urgently looked for wet specimens using phase-contrast microscopy. Unless amoebae are seen, bacterial meningitis will be suspected; on Gram staining amoebae appear as indistinct smudges. Fixed preparations stained with iron haematoxylin will show full details of nuclear structure. Confirmation is by culture at 37°C using a gram negative bacterial lawn on non-nutrient agar (Visvesvara et al., 2007).

Amoebic Keratitis (AK)

Infections are usually due to *Acanthamoeba sp.* and also associated with contact lens users. Risk factors include poor hygiene when handling lenses and their cases, use of chlorine-based disinfectants, swimming or washing eyes while wearing lenses, after gardening and too prolonged use of plastic or unwashed lenses. Corneal lesions are painful and present as painless and progressive ulcers leading eventually to perforation. Frequently confused with microbial keratitis, including *Pseudomonas*, *Staphylococcus* and *Herpes simplex*. Amoebae are found in corneal scrapings or histologically in corneal tissue, but can be missed unless stained with iron haematoxylin or immunofluorescence. PCR is an available method now. Fresh material is using for cultures from a bacterial lawn on non-nutrient agar, should be at 30°C. They usually (90%) of cases are due to genotype T4 (Khan, 2006; Visvesvara et al., 2007; Martín-Navarro et al., 2008; da RochaAzevedo et al., 2009; Verani et al., 2009; Tu and Joslin, 2010; Patel et al., 2010).

Granulomatous Amoebic Encephalitis (GAE)

Infection causes due to *Acanthamoeba* and *Balamuthia* and the main route of the lower respiratory tract followed by haematogenous spread to the brain. Other routes of entry are the skin, the nasopharynx, the lungs and the stomach. Primary lesions have been described at all these sites. Many species of *Acanthamoeba* can cause GAE, also known as *Acanthamoeba* Granulomatous Encephalitis (AGE). AGE is a rare, chronic, progressive infection of the CNS that may involve the lungs (da Rocha-Azevedo et al., 2009). That is usually associated with an underlying debilitating disease or immune suppressed individuals including HIV-AIDS patients, diabetics (Khan, 2006; Visvesvara et al., 2007; da Rocha-Azevedo et al., 2009).

B. mandrillaris are soil contamination of skin and craniofacial wounds is an important risk factor. Causes of immunocompromise include malignancy, collagen disorder, alcoholism, diabetes mellitus, AIDS, and steroid or immunosuppressant therapy. Recently two patients with *B. mandrillaris* infection have been described both of whom had received a kidney graft from the same donor. However, in

Peru, most of the patients infected with *B. mandrillaris* have no obvious cause for immunosuppression. Amoebic lesions resemble chronic bacterial brain abscesses or localized sub-acute hemorrhagic necrosis; involvement of the meninges is common and also present with headache and meningism, others with evidence of a focal brain lesion. Unless these amoebae are found in wet tissue preparations or cerebrospinal fluid, the diagnosis will be usually based on histology, often at autopsy. Cysts may be seen in tissue but trophozoites may be missed unless stained with iron haematoxylin or immunofluorescence using specific antisera. Cultural diagnosis at 37°C from fresh biopsies or cerebrospinal fluid is sometimes possible. PCR methods are becoming available (Deetz et al., 2003; Schuster et al., 2006).

In addition to the number of affected individuals is increasing worldwide. Moreover, both *Acanthamoeba* and *Balamuthia* cause infections of the lungs and skin (Khan, 2006; Maciver, 2007; Visvesvara et al., 2007; da Rocha-Azevedo et al., 2009). Recently, *B. mandrillaris*, has been discovered to cause a fatal encephalitis in humans (Martin et al., 2014). This encephalitis is

known as *Balamuthia* amoebic encephalitis (BAE). There are worrying features of BAE that are emerging, even compared to AGE and PAM. PAM is restricted to bodies of warm freshwater, such as swimming pools and lakes, and so can be avoided after its presence has been identified. AGE is mostly a disease of the immunocompromised, and so affects a small subpopulation of individuals who could conceivably be monitored for early signs of AGE; for example, by inspection of cerebrospinal fluid (CSF) (Deetz et al., 2003; Maciver, 2007). The unpredictable nature of the disease may mean that BAE is even less likely to be diagnosed in time for medical intervention and, like AGE and PAM, it is essential for BAE to be diagnosed early if it is to be treated successfully (Deetz et al., 2003; Maciver, 2007). Additionally, *Sappinia diploidea* and *Sappinia pedata* species, also belonging to the free-living amoeba group, that normally live in soil contaminated with feces of elk, bison, and cattle, have been identified as causing encephalitis in an otherwise healthy individuals (Gelman et al., 2001; Qvarstrom et al., 2009; Walochnik et al., 2009). In a retrospective study shown that, there are at least 13,939 cases of acute encephalitis diagnosed between 1990 and 1999 in California, among these cases, 0.1% was attributed to Naegleria, 0.63% to other protozoans, and 34.7% were from unspecified causes. (Maciver et al., 2007)

Treatment of Free Living Amoeba

Therapy and prognosis of FLA infections is problematic because of the lack of clear-cut symptoms, the lack of a good reliable diagnostic test, and the fact that diagnosis is often made postmortem. However, several patients with GAE caused by *Acanthamoeba spp.* as well as some with *Acanthamoeba* cutaneous infection without CNS involvement have been successfully treated with a combination of pentamidine isethionate, sulfadiazine, flucytosine, and fluconazole or itraconazole. Topical applications of chlorhexidine gluconate and ketoconazole cream in addition to antimicrobials have resulted in therapeutic success for cutaneous *Acanthamoeba* infection, but in many cases, had to be discontinued because of undesirable side effects of the medications (Visvesvara et al., 2007).

A combination of factors of late diagnosis, suboptimal efficacy of antimicrobial therapy, and problems inherent to the immunocompromised host, makes for a poor prognosis for GAE patients. Treatment of *Acanthamoeba* keratitis has been

fairly successful. A variety of drugs have been used, including chlorhexidine, polyhexamethylene biguanide, propamidine isethionate, dibromopropamidine isethionate, neomycin, paromomycin, polymyxin B, clotrimazole, ketoconazole, miconazole, and itraconazole (Visvesvara et al., 2007; da RochaAzevedo, 2009).

A few patients have survived to *N. fowleri* caused by PAM. Lately used of aggressively treated with intravenous and intrathecal Amphotericin B, intravenous and miconazole, also oral rifampin. It was believed that Amphotericin B and miconazole had a synergistic effect but that rifampin was without effect on the amoebae. Based on in vitro testing and in vivo mouse studies, amphotericin B was reported to be more effective against *N. fowleri* than amphotericin B methyl ester, a water-soluble form of the drug (Visvesvara et al., 2007; da Rocha-Azevedo et al., 2009). Additionally, Azithromycin has been shown to be effective a macrolide antimicrobial against both in vitro and in vivo and also sensitive to voriconazole. However; other macrolides (erythromycin, clarithromycin) are less effective this parasite (Visvesvara et al., 2007; da Rocha-Azevedo et al., 2009). Another drug, miltefosine, an alkylphospholipid, has also been shown to have amoebicidal potential. Significantly, medical cure has been achieved with the application of either polyhexamethylene biguanide (PHMB) or chlorhexidine gluconate with or without Brolene. When medical treatment failed, a combination of debridement and penetrating keratoplasty has been used with good results in some cases (Schuster et al., 2006).

Balamuthia amoebic encephalitis (BAE) is a rare, sub-acute to chronic disease that is characterized by hemorrhagic necrotizing lesions or brain abscess (Bravo and Sanchez, 2003). They have been treated empirically with steroids as well as with antibacterial, antifungal and antiviral agents with almost no effect upon the course of the infection. Anti-inflammatory steroids that were administered may have actually facilitated spread of the infection by suppressing the inflammatory response (Deetz et al., 2003; Martínez et al., 2010; Bravo et al., 2011).

In vitro studies have shown that pentamidine and propamidine isethionates were amoebastatic but not amoebicidal. Recent information based on in vitro data has shown that miltefosine was able to lyse the amoebae. Voriconazole, however, had

virtually no effect on *Balamuthia* (Maciver., 2007; Visvesvara et al., 2007).

Recently, novel therapeutic approaches of the application of siRNA in *Acanthamoeba* species (Lorenzo-Morales et al., 2005; 2008; 2010) has opened a novel approach for the progress of future therapies based on siRNAs alone or in combination with chemical compounds.

Conclusion

FLA infections are emerging diseases that gained recently much attention. They are ubiquitous and widely organisms in the worldwide. In the future they will be increasingly dangerous because of thermophilic and polymorphic structure, easy adaptation and resistible cyst wall. In patients who have meningitis symptoms, FLA is an important agent to be considered. In conclusion, there is a vital necessity of simple and rapid methods of diagnosis for these fatal amoebae that live in fresh water sources.

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CASE REPORT

Dentigerous Cyst Associated with an Ectopic Tooth in the Subcondylar Area: A Case Report and Literature Review

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Abstract

Dentigerous cysts associated with an ectopic third molar in the subcondylar area are fairly rare, and information is limited about its causes and characteristics. This article reports a case of dentigerous cysts associated with an ectopic third molar in the subcondylar and reviews the literature on the clinical signs and symptoms and different treatment methods of this condition.

We report a case dentigerous cyst associated with an ectopic mandibular third molar in the subcondylar area. 14 cases of dentigerous cysts associated with an ectopic third molar in the subcondylar area reported in the English-language literature over the past 30 years, identified from Medline databases are also reviewed.

We found a mean age at diagnosis of 48.1 years and a higher prevalence in women. Facial pain and swelling on the preauricular region or ipsilateral side of the mandible were the most common symptoms. Among the 14 case reports identified, 7 were by an extraoral approach and 7 were by an intraoral approach. The most commonly used extra-oral approaches are retromandibular.

The etiology of ectopic mandibular third molars has not yet been completely clarified. They must be removed if they cause symptoms or are associated with cystic pathology. The treatment of third molars in the condylar region is divided into conservative and, in most cases, surgical removal by intra or extra-oral route. The surgical approach must be carefully planned according to the location and position of ectopic third molars.

Key words: Dentigerous, subcondylar, ectopic tooth.

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Introduction

Ectopic teeth are those that are impacted in unusual positions or have been displaced and are at a distance from their normal anatomic location (Shivashankara et al., 2012). The reason as to why third molars are ectopically placed is still unclear. However, ectopic eruption may result owing to one of the following 3 processes: developmental disturbance, iatrogenic activity, or pathologic process, such as a tumor or a cyst (Buyukkurt et al., 2010; Shivashankara et al., 2012; Lambade et al., 2013).

Dentigerous cysts, also called follicular cyst, are the most common developmental odontogenic cysts, and second most common cystic lesions of the jaw next to radicular cysts (Kocer et al., 2002; McCrea, 2009). They account for approximately 24 % of all true cysts in the jaw (McCrea, 2009). These cysts are caused by the expansion of dental follicles, which result from accumulation of fluid between the tooth crown and epithelial components (Edamatsu et al., 2005). The mandible is likely to be the primarily affected site, as cysts are located in the mandible in 75 % of the cases (Ustuner et al., 2003). These cysts most commonly arise from mandibular third molars, followed by maxillary third molars and maxillary canines (Kocer et al., 2002; Buyukkurt et al., 2010). Clinical examinations reveal a missing tooth or teeth and an asymptomatic swelling that sometimes results in facial asymmetry and possible pathologic fracture (Kocer et al., 2002; McCrea, 2009). Dentigerous cysts surrounding impacted teeth often displace these teeth into ectopic positions (McCrea, 2009; Buyukkurt et al., 2010). The degree of displacement may be dramatic. It has been reported that mandibular third molars may be moved to the ramus, condylar, or coronoid regions or to the inferior cortex of the mandible (McCrea, 2009; Kim, 2011).

The standard treatment for a dentigerous cyst is enucleation and extraction of the tooth involved (Martinez-Perez and Varela-Morales, 2001; Kocer et al., 2002; Buyukkurt et al., 2010; Bowman et al., 2014). In large cysts, an initial marsupialization to diminish the size of the osseous defect followed by enucleation and tooth extraction has been advocated (Martinez-Perez and Varela-Morales, 2001).

However, treatment of ectopic third molars associated with dentigerous cysts in the condylar region are recommended to prevent the morbidity caused by infection of a cyst, the malfunction of the temporomandibular joint, and the risk of fracture in an area where the bone is thin (Salmeron et al., 2008; Kim, 2011).

In the present paper, we report the case of dentigerous cysts associated with an ectopic tooth in the subcondylar area and review the literature reports of this condition over the past 30 years. A search of Medline from 1984 to 2014 using the key words “dentigerous cyst,” “ectopic tooth,” “subcondylar,” and “ectopic third molar and mandibular condyle” was conducted.

Case

A 52-year-old woman was referred due to pain, swelling, and episodic purulent intraoral secretion through the retromolar region for about 3 months. She had no other visual complaints. On general examination, the patient was apparently healthy with no significant past medical history. Physical intraoral examination revealed a swelling overlying the left condylar area with purulent secretion and an absence of the left mandibular third molar. The gingival and alveolar mucosa covering the lesion was normal. Extra-oral examination did not reveal any swelling or symptoms.

Panoramic radiography revealed a large, well-defined radiolucency surrounding the crown of a deeply impacted left mandibular third molar at the subcondylar area (Fig 1) and Computed Tomography (CT) confirmed a well-defined unilocular radiolucent lesion which is related to an unerupted mandibular molar and close anatomical relationship between the molar roots, cyst, and the mandibular canal and also confirmed lingual cortex destruction through on the left ramus (Fig 2a,2b). Based on clinical and radiological findings dentigerous cyst, radicular cyst, fibrous dysplasia, and ameloblastoma were suspected.



Figure 1: The panoramic radiography showing dentigerous cyst associated with an ectopic third molar in the subcondylar region.

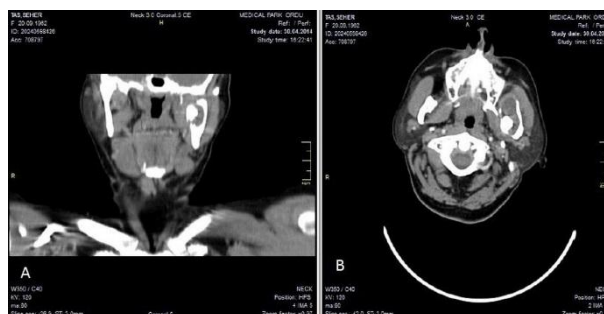


Figure 2: (A) Coronal section of CT scan showing the downward crown position of the ectopic third molar with the proximity to the internal cortical bone of the mandible. (B) Axial section of CT scan showing the ectopic third molar.

Surgical removal of the cyst under general anesthesia was planned. After informing the patient of all possible complications that can occur during and after the surgery, a signed consent form was obtained from the patient. After endotracheal intubation, local anesthesia was injected (Ultracaine-DS Forte, Aventis/ Turkey) and an intraoral incision was performed from the molar area to the ramus and retromolar area and the mucoperiosteal flap was raised. The cyst capsule was dissected carefully from the inferior alveolar nerve and the ectopic impacted third molar was removed (Fig 3). After placing a drain, primary mucosal closure was achieved.

The patient was prescribed postoperative antibiotics (Amoxicillin /Clavulanic Acid and Ornidazole), analgesic (Paracetamol), and mouthwash (Chlorhexidine Gluconate/ Benzylamine Hydrochloride) for five days and advised to remain on a soft and liquid diet for four weeks.



Figure 3. Intraoperative photograph.

The specimens were sent out for histopathologic examination. The pathology report confirmed the diagnosis was a dentigerous cyst, showing a thin, fibrous wall lined by two to three layers of flat epithelial cells resembling reduced enamel epithelium. The connective tissue showed slight inflammatory infiltrate (Fig 4).

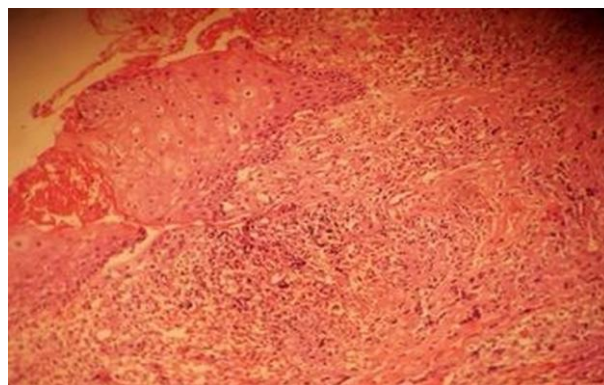


Figure 4. Histopathologically, cyst was lined by keratinized squamous epithelium.

On the second day following surgery, the drain was taken out and on the seventh day the sutures were removed. The patient was non-symptomatic and there was no postoperative condylar fracture or paresthesia (Fig 5). The patient is checked in regularly visits.



Figure 5: Postoperative panoramic radiography

Discussion

Ectopic teeth are those that are impacted in unusual positions or that have been displaced and are at a distance from their normal anatomic location (Iglesias-Martin et al., 2012; Lambade et al., 2013) and have been reported in the mandibular condyle, coronoid process, orbit, palate, nasal cavity, floor of mouth, and maxillary sinus (Buyukkurt et al., 2010; Pace et al., 2010; Shivashankara et al., 2012; Lambade et al., 2013). The etiology of an ectopic tooth has not yet been completely clarified. However, an ectopic eruption may result due to either developmental disturbances, iatrogenic activity, or pathological process such as dentigerous cysts (Pace et al., 2010; Buyukkurt et al., 2010; Shivashankara et al., 2012; Iglesias-Martin et al., 2012). Dentigerous cysts are the second most common odontogenic cysts after radicular cysts, accounting for approximately 24 % of all true cysts of the jaw and are the most common developmental cyst of the

jaw (Kocer et al., 2002; McCrea, 2009). The third molar is the tooth most frequently involved and the posterior area of the mandible is the most common site of dentigerous cysts (Kocer et al., 2002; Ustuner et al., 2003). However, dentigerous cysts associated with an ectopic tooth in the subcondylar region are very rare and, as far as we could determine, only 14 cases had been reported since 1984 (Table I).

Dentigerous cysts are benign odontogenic lesions arising from the completed crown of impacted, embedded, or unerupted teeth (Martinez-Perez and Varela-Morales, 2001; Kocer et al., 2002). They are more prevalent in males and are most common in the second or third decade of life, and are rarely seen during childhood (Ustuner et al., 2003). However, among the 14 cases reported in the present study (Table 1), we found a higher prevalence in women, including 8 cases. The mean age was 48.1 years. The age of these patients ranged from 30 to 68 years old.

Diagnosis is based on clinical findings together with imaging tests, mainly panoramic radiograph and CT. Pace et al. reported that a panoramic radiography together with a clinical examination is usually sufficient for diagnosis of an ectopic third molar (Pace et al., 2010). However, CT scans are sometimes required to determine a more precise anatomical position and its relation to adjacent structures. On radiographic examinations, dentigerous cysts appear as unilocular lucent cysts of varying sizes, with well-defined sclerotic borders, associated with the crown of an impacted tooth (Ustuner et al., 2003; McCrea, 2009;).

Dentigerous cysts are typically asymptomatic. These lesions progress slowly and are usually painless, but may cause facial swelling and delayed tooth eruption (Kocer et al., 2002; Ustuner et al., 2003; Buyukkurt et al., 2010). Among the 14 case reports summarized in Table I, the most common signs and symptoms associated with ectopic teeth are facial pain (Medici et al., 2001; Tumer et al., 2002; Suarez-Cunqueiro et al., 2003; Wang et al., 2008; Salmeron et al., 2008; Gadre and Waknis, 2010; Iglesias-Martin et al., 2012), facial swelling on the preauricular region or ipsilateral side of the mandible (Tumer et al., 2002; Suarez-Cunqueiro et al., 2003; Salmeron et al., 2008; Pace et al., 2010; Gadre and Waknis, 2010; Scott et al., 2012; Iglesias-Martin et al., 2012; Bowman et al., 2014) and trismus (Wassouf et al., 2003; Salmeron et al., 2008; Iglesias-Martin et al., 2012), difficulty in mastication (Bux and Lisco, 1994;

Medici et al., 2001). In 3 of the 14 summarized patients, intraoral or extraoral drainage of purulent material have been reported (Salmeron et al., 2008; Pace et al., 2010; Gadre and Waknis, 2010). Enucleation has been the standard treatment for dentigerous cysts, along with extraction of the associated tooth (Buyukkurt et al., 2010). However, the surgical removal of an ectopic mandibular third molar with acute inflammation or cystic lesions is recommended to prevent further complications, such as diffuse osteolysis, condylar process deformity or weakening of the bone predisposing to fracture (Medici et al., 2001; Gadre and Waknis, 2010; Kim, 2011).

In the treatment of ectopic third molars in the condylar region, several approaches have been used, including an intra-oral approach (Medici et al., 2001; Wassouf et al., 2003; Wang et al., 2008; Gadre and Waknis, 2010; Scott et al., 2012;) an endoscopic intraoral approach (Suarez-Cunqueiro et al., 2003) and an extra-oral approach (Bux and Lisco, 1994; Tumer et al., 2002; Salmeron et al., 2008; Pace et al., 2010; Iglesias-Martin et al., 2012; Bowman et al., 2014). Among the 14 cases summarized in Table I, 7 (Bux and Lisco, 1994; Tumer et al., 2002; Salmeron et al., 2008; Pace et al., 2010; Iglesias-Martin et al., 2012; Bowman et al., 2014) were by an extraoral approach and 7 (Medici et al., 2001; Wassouf et al., 2003; Wang et al., 2008; Gadre and Waknis, 2010; Scott et al., 2012;) were by an intraoral approach. The most commonly used extra-oral approaches are retromandibular (Bux and Lisco, 1994; Pace et al., 2010; Iglesias-Martin et al., 2012) and pre-auricular (Tumer et al., 2002; Salmeron et al., 2008). These external approaches have the advantage of good surgical exposure, especially to the body and ascending ramus, to the condylar region, but may result in complications such as cutaneous scar formation, damage of temporomandibular joint components, facial nerve injury in the case of pre-auricular access, or damage of the marginal mandibular branch of the seventh cranial nerve (Medici et al., 2001; Wang et al., 2008; Sanghera and Jones, 2013). However, Bowman et al. reported that the transmasseteric antero-parotid Approach (TMAP) reduced the surgical morbidity anticipated with alternative approaches, as it has a low risk of iatrogenic injury to the facial nerve and also reduces the risk of injury to the lingual nerve (Bowman et al., 2014). Although it provides a smaller surgical site, the intra-oral approach helps in avoiding a visible

facial scar and injury to the facial nerve (Wang et al., 2008; Iglesias-Martin et al., 2012; Sanghera and Jones, 2013). The intraoral approach is, therefore, preferable in order to avoid visible scars and facial nerve injury, while the others are secondary options if necessary (Wassouf et al., 2003; Gadre and Waknis, 2010). Therefore, we also chose this method for our case.

Table 1. Literature review of the dentigerous cyst associated with an ectopic third molar in the subcondylar region in subjects.

Author/year	Age	Gender	Side	Symptom	Treatment
Bux and Lisco, 1994	66	Female	Right	Trismus Fever	Retromandibular Approach
Medici et al, 2001	41	Female	Right	Difficulty in Mastication Pain and Swelling in the Preauricular Region	Intraoral Approach
Tuner et al, 2002	47	Male	Right	Facial Pain Facial Swelling	Preauricular Approach
Wassouf et al, 2003	49	Female	Left	Submandibular and Masseter Space Abscess	Intraoral Approach
Suarez-Cunqueiro et al, 2003	45	Male	Right	Facial Pain Facial Swelling	Endoscopic Approach
Salmeron et al, 2008	53	Female	Right	Limitation of Mouth Opening Facial Pain	Endaural Approach
Salmeron et al, 2008	41	Male	Left	Hard Swelling with Pain Episodic Purulent Intraoral Secretion	Preauricular Approach
Wang et al, 2008	31	Female	Right	Facial Pain Facial Swelling	Intraoral Approach
Gadre and Waknis, 2009	30	Female	Right	Facial Pain Facial Swelling	Intraoral Approach
Gadre and Waknis, 2009	40	Male	Left	Facial Pain Facial Swelling Purulent Discharge	Intraoral Approach
Pace et al, 2010	53	Male	Right	Skin Swelling Discharged From Skin Lesion	Retromandibular Approach
Martin et al, 2012	53	Female	Left	Intense Pain And Swelling in the Preauricular Region Trismus Approach	Retromandibular Approach
Bowman et al, 2014	56	Male	Right	Preauricular Swelling	Transmasseteric antero-parotid Approach
Scott et al, 2012	68	Female	Left	Tender Swelling in the Preauricular Region	Intraoral

In order to treat dentigerous cysts associated with ectopic third molars in mandibular condyle, the Endoscopic approach should be used when is not approached due to the limited surgical field and the poor visualization in inaccessible anatomical regions. The use of this method has considerable advantages, such as good illumination, clear and magnified visualization of the operating field and as a result, a more conservative surgery with precise dissection (Suarez-Cunqueiro et al., 2003). In certain situations endoscopic removal is possible; however it is not preferred in some cases because of the difficulty that would be encountered in sectioning and removing the tooth and ensuring complete removal of all cystic material (Pace et al., 2010).

Conclusion

A dentigerous cyst associated with an ectopic third molar in the subcondylar area is fairly rare. In the literature, 14 cases had been reported since 1984. However, the true incidence is still unknown. The incidence seems to be higher in women and fourth decades, as in other studies. Ectopic third molars must be removed if they cause symptoms or are associated with cystic pathology. The treatment of third molars in the condylar

region is divided into conservative and, in most cases, surgical removal by intra or extra-oral route. The surgical approach must be carefully planned according to the location and position of ectopic third molars.

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept MMO, FA; Design-MMO; Supervision MMO, FA; Data Collection and/or Processing MMO, HY; Analysis and/or Interpretation MMO, NS; Literature Review MMO; Writing MMO, HY; Critical Review MMO

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Coexistence of Bifid Uvula and Cardiac Manifestations in a Patient: Is it a Syndrome or Coincidence?

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Abstract

Loeys-Dietz syndrome (LDS) is a rare life-threatening condition, with autosomal-dominant inheritance, which is caused by heterozygous mutations in the genes encoding transforming growth factor beta receptors 1 and 2. It is characterized by a triad of tortuous arteries and aortic aneurysms, cleft palate or bifid uvula, and hypertelorism. Loeys-Dietz syndrome is a recently-described connective tissue disorder with features similar to those of Marfan syndrome, and the vascular type of Ehlers-Danlos syndrome. In Loeys-Dietz syndrome, the aortic aneurysms are prone to rupture at a smaller size than other aneurysms, putting children with Loeys-Dietz at great risk for dying if the aneurysm is not identified and treated early. Here we report 54-year-old-man who had atrial septal defect, aortic root dilatation and bifid uvula, which may be a variant of LDS.

Key Words: loeys-dietz syndrome, bifid uvula, atrial septal defect

To the editor

A 53 year-old man was admitted to the cardiology department with exertional dyspnea and atypical chest pain for 2 days. The patient reported no previous cardiac history except hypertension and diabetes mellitus. He had been used Ramipril 5 mg orally once a day for 7 years and metformin 500 mg orally twice a day for 5 years. On physical examination his blood pressure was 140/85 mm/hg and heart rate was 72 bpm. In detail examination we detected the bifid uvula which was operated before. His father was operated due to bifid uvula in his family history (Figure).



Figure 1: Bifid uvula which was operated before.

A 3/6 systolic murmur was heard in the pulmonary area on heart auscultation. The electrocardiography was sinus rhythm and there was no ischemic st and t wave abnormalities.

Transthoracic echocardiography (TTE) showed dilated aortic root with 3.2 cm, mild aortic valve regurgitation and a normal size of ascendan aorta. TTE also demonstrated dilated right chambers and an abnormal turbulent flow along the interatrial septum that associated with an atrial septal defect. Transesophageal echocardiography showed the defect diameter of atrial septal defect was 1.4 cm. There was also

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mild aortic regurgitation with bicuspid aortic valves. On computed tomography ascending, descending, thoracic and abdominal aorta were in normal size. After the patient's consent the atrial septal defect closed with device procedure. The patient was discharged on medical treatment with 6 months acetylsalicylic acid and mentioned above hypertension and diabetes medication. In the postoperative follow-up the patient was asymptomatic and did not have any complication and was discharged in good condition.

The classic triad of Loeys–Dietz syndrome involves arterial tortuosity and aneurysms, hypertelorism, and bifid uvula or cleft palate (Loeys BL et al., 2006). Loeys–Dietz syndrome (LDS) resembles to Marfan syndrome, however it tends to be more aggressive. LDS have some difference from Marfan syndrome such as lens dislocation etc. Also aortic root surgery should be considered with milder aortic root dilation (40 mm for Loeys–Dietz, versus 50 mm for Marfan) due to increased risk for aortic dissection and rupture with smaller aortic diameters (Williams JA et al., 2007). In initial reports, LDS patients were classified into two types depending on severity of craniofacial features (type 1) or cutaneous features (type 2) (Loeys BL et al., 2006). Some genetic mutations seen in LDS. Patients with mutations in TGFBR1, TGFBR2, SMAD3, or TGFB2 show more widespread and/or aggressive vascular disease when compared with Marfan syndrome or thoracic aortic aneurysm and dissection, irrespective of the severity of systemic features, a mutation in any of these genes in combination with documented aneurysm or dissection should be sufficient for the diagnosis of LDS. This will alert clinicians caring for these patients to the need for specialized patient counseling and management. At the same time the evidence based expansion of the clinical spectrum in LDS including patients with minimal or no dysmorphic features should be considered by clinicians. Such reasoning and practices have proven productive in the diagnosis and care of patients with Marfan and vascular Ehlers–Danlos syndromes (MacCarrick et al., 2014).

In our knowledge coexistence of only bifid uvula and cardiac manifestations (atrial septal defect, bicuspid aorta, aortic root dilatation) have not been reported in literature before. However, these pathologies can be found together in Loeys–Dietz syndrome (LDS), which is an

autosomal-dominant connective tissue disorder first characterized by aortic aneurysms and generalized arterial tortuosity, hypertelorism, and bifid uvula or cleft palate. In addition cardiac malformations such as atrial septal defect, bicuspid aorta etc. can be seen in this syndrome (Loeys BL et al., 2005) As mentioned above, our patient had no aortic/ arterial abnormalities and hypertelorism. Coexistence of bifid uvula and cardiac manifestations are may be a variant of LDS or incidental.

Informed Consent: Written informed consent was obtained from the patient who participated in this case.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – ZYG; Design- TI; Supervision- OB; Data Collection and/or Processing- AK; Analysis and/or Interpretation - ZYG; Literature Review -OB; Writing -ZYG; Critical Review -EA

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