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Erakıncı G. Donörlerde parazitlere karşı oluşan antikorların aranması. İzmir: Ege Üniversitesi Sağlık Bilimleri Enstitüsü. 1997.
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Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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Results (800-1000 word)
Discussion and conclusion (> 1200 word)
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Whole text should not exceed 4500 words except for resources and English summary.

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MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE

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CONTENTS

EDITORIAL

The first year ends

We left behind a year with happiness of achieving our goals. We hope to meet, in the next issues along with…

In this issue, there are four original articles and a case report. The articles are branches nursing, cardiology, parasitology, microbiology, general surgery, internal medicine 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 article was reviewing the factors affecting critical thinking and empathic disposition, the other was based the cultivation of *acanthamoeba*. Endoscopy results in nonspecific abdominal pain have been the subject of our journal. In addition, correlation of platelet to lymphocyte ratio of metabolic syndrome was discussed. On the other hand, the case report is about odontogenic myxoma located in the mandible.

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

Middle Black Sea Journal of Health Science December 2015; 1(3): 1-8

ORIGINAL ARTICLE

The Factors Affecting Critical Thinking and Empathic Disposition of Nursing Students

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Abstract

Objective: The aim of this study was to determine the factors affecting critical thinking and empathic disposition of nursing students and the relationships between the empathy and critical thinking disposition in nursing education.

Methods: The study was carried out on undergraduate students at nursing school during 2011-2012 academic years. The sample consisted of 276 students who had agreed to participate in the research, 30.7% of them were the first year students (freshman), 27.4 % were the second year students (sophomore), 20.2% were the third year students (junior) and 20.9% were the fourth year students (senior) of nursing program. California Critical Thinking Disposition Inventory (CCTDI) designed by Kökdemir and Empathic Tendency Scale invented by Dökmen were used to assess the research data. Furthermore, an "Individual Information Form" questioning the grades, genders and parental education levels of the nursing students was prepared and applied by the researcher.

Results: The mean score for the Empathic Tendency Scale was 53.25±6.57, the mean score for the Critical Thinking Disposition Scale was 209.95±25.26. Correlation analysis revealed significant relationships between the students' scores of Empathic Tendency Scale and total score of Critical Thinking Disposition Scale ($r=0.186$ $p<0.05$). Significant differences were observed in total scores for the Critical Thinking Disposition Scale, Empathic Tendency Scale and Analytical Thinking Sub-scale of student groups ($p<0.01$). The differences were especially distinctive in fourth year students of nursing program. However, there was no relationship between the nursing students' critical thinking disposition sub-scales and empathic tendencies ($p>0.05$). It was detected that nursing students' critical thinking ability was not affected by their genders and parental education levels.

Conclusion: A significant relationship was observed between the Empathic Tendency and general Critical Thinking Disposition and Analytical Thinking skills. The differences were also observed between the class levels in terms of Empathic Tendency and Critical Thinking Disposition and it was also observed that nursing students' critical thinking disposition scores were considerably low. These findings suggest that these skills can be enhanced with education. Current findings revealed that emphasis should be placed on practical works to develop students' ability of empathy and critical thinking disposition in class levels.

Key words: Critical Thinking Disposition, Empathic Tendency, Nursing student

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Introduction

In recent years, one of the issues mostly discussed is not what people should think, it is rather how to think. The development of critical thinking (CT) skills has long been recognized as a prior issue in higher education. Critical thinking is described by the American Philosophical Association (APA) in the Landmark Delphi Study as purposeful and self-regulatory judgment which results in interpretation, analysis, evaluation and

inference (Facione, 1990).

Critical thinking is a crucial skill for nurses and other healthcare professionals and it is essential to effectively manage complex care situations in fast-paced environments that demand increasing accountability. The processes of clinical decision-making and problem-solving require advanced critical thinking skills (Carter et al., 2015).

The dynamic changes in socio-cultural and political form of society are also effective on healthcare system. Such dynamism increases the responsibilities of healthcare workers and nurses, requires the development of multidimensional care and critical thinking skills (Riddle, 2007; Dil and Öz, 2015). Various status and powers have been given from society to nursing profession with this process of change. It has been the cause of several expectations from the nursing profession such as people with dedication, loyal and unreliable, do the right thing, full-do, take responsibility for the decision, making the right decisions, self-direction. Besides, it is expected to have acquired modern professional qualifications of nurses to meet the community's expectations (Riddle, 2007; Senita, 2008). On the other hand, using problem solving strategies and strong knowledge of decision making ability are expected from the nurse. Understanding this process completely, correctly and use in patient care are based on the development of professional skills such as critical thinking skills (Birol, 2004). All national and international nursing organizations accept the critical thinking skills as basic elements of nursing practice and describe critical thinking skills as a universal behavior (Kataoka-Yahiro and Saylor, 1994).

When faced with uncertainty due to lack of adequate information and limited time to decide with this uncertainty, shortcuts are used and these shortcuts require decision-making ability and critical thinking skills (Emir, 2012). Also Kataoka-Yahiro and Saylor (1994) developed a model of critical thinking in making clinical decisions necessary for safe and effective nursing care. The purpose of the model was to give leadership to take clinical decisions when clinical experience of nurse begins in order to provide safe and effective nursing care and it is comprised of five dimensions (Perry and Potter 1997, Potter and Perry 2003). According to this model, five dimensions of critical thinking is as follows: Essential Nursing Career Information, Nursing Experience, Critical Thinking Competency, General Qualifications (Specific Qualifications in Clinical Environment-Specific Qualifications for Nursing), Attitudes to Critical Thinking (Confidence – Independent Thinking – The Equality – Responsibility – Take Risks – Discipline – Low Volunteering – Wholeness – Perseverance-Wonder – Creativity), Critical Thinking Standards (Intellectual Standards: Open –Right – Honest – Specific – Exact-Important –Associated with the Subject – Significant – Enough – Consistent – Rational – General – Appropriate; Professional Standards: Ethical Criteria for Decision-making in nursing, Evaluation Criteria – Professional Liability (Perry and Potter 1997, Potter and Perry, 2003).

The nurse needs to synthesize and analyze all the knowledge and information while providing health care to individuals with impaired health. Multi-dimensional care understanding is forcing nurses to consider critically and flexible. Nurses with these features can establish cause-effect relationships. Professional career, autonomy and power affect the nurses positively (Banning, 2006; Riddell, 2007). The quality of nursing care depends on integration of critical thinking skills with implementation environment. National League for Nursing (1991) emphasized that the critical thinking was essential in nursing education, the critical thinking skills were important professional qualifications for nursing school graduates (Adams et al., 1999).

Empathy is also one of the basic qualities needed in order to identify patient and problems. Empathic tendency is a feature that can be developed through training (Hodges, 1991). Acquiring and maintain positive health behaviors, based on basic interpersonal empathy is significantly effective (Akgöz and Karavuş, 2005). Nursing is a profession that based on human relations. The effectiveness of the care process depends on the ability of nurses to communicate effectively with others. Nurses need to understand the experiences of individuals through both verbal and non-verbal communication, both should be able to express themselves correctly; the person must be able to communicate effectively with care as a caregiver. Also help-based relationship is the most fundamental component of empathy (Tutuk and Doğan, 2002). In the literature; it is observed that there were many studies on the nursing students' empathic skills. Most of these studies

show that education was effective on development of emphatic skills (Tutuk ve Doğan, 2002; Mete and Gerçek, 2005; Ançel, 2006; Avcı et al., 2013).

The objectives of nursing education at universities are; to ensure students' creativity, critical thinking skills and the continuation of the self-development. At the end of the educational process, the prospective nurse is expected to solve the patient's health problems; able to use scientific method of problem solving and possess the sufficient knowledge, attitudes and skills (Kang et al., 2009). Nursing training in the university level aims to solve the health problems of the patients by using the knowledge, attitude and skills of the individuals, yet it may be positive or stressful for the individual (Money, 2007; Kang et al., 2009). To teach students about professional values in nursing education, it is important to improve communication and helping skills and there is a direct relationship with empathic tendency of the nursing profession (Avcı et al., 2013). To what extent the empathic skills of nursing students can be developed is not known and to what extent they can show success and academic achievement is not also well known. The relationships between these variables and critical thinking are also poorly understood. Studies that are examining dispositions and critical thinking skills of nurses are very limited in Turkey. Nurses' critical thinking dispositions were reported to be in low- and medium-levels in a previous study (Dirimmeşe, 2006). The studies that are examining the relationships between the critical thinking disposition and empathy are also quite limited. The aim of this study was to identify critical thinking disposition and empathic tendency levels of nursing students and to examine the factors influencing these parameters.

Materials and Methods

Design

A descriptive design was adopted.

Setting and Sample

Sample was recruited from Ordu University, Department of Health in 2011-2012 academic year with 1st, 2nd, 3rd, and 4th year students who are attending the courses. The population of the study consisted of 315 students. The sample of the study who agreed to participate in the study comprised of 276 students.

Data Collection, Measurements

Data for the study was collected by using a questionnaire form, the California Critical Thinking Tendency Scale (CCTDI) designed by Kökdemir (2003) and Empathic Tendency Scale invented by Dökmen (1998).

CCDTI was developed by Facione and validity-reliability works were performed in accordance with Kökdemir (2003).

This scale consists of six sub-scales and a total of 51 items. The CCTDI scores less than 240 are assessed as low and the scores over 300 are assessed as high (Kanbay et al., 2011). Total internal consistency coefficient of the original scale (Cronbach's alpha) was 0.90 and the internal consistency coefficients of the subscales (Cronbach's alpha) varied between 0.72 - 0.82.

Empathic Tendency Scale was developed by Dökmen (1988). The scale is rated in 1-5 likert scale and consisted of 20 questions. This scale was developed to measure the component of empathy and the potentials of empathy in everyday life (Duru, 2002). Total internal consistency coefficient of the original scale (Cronbach's alpha) was 0.82 and the internal consistency coefficients of the subscales (Cronbach's alpha) were around 0.72.

Data Analysis

Data analysis was performed by using "SPSS 15.0" and data were assessed through frequency, Pearson Correlation Test, variance, Kruskall-wallis tests.

Results

About 73.3% of participants were female, 30.7% were studying at the first year, 27.4 % in the second, 20.2% in the third and 20.9% in the fourth year. Considering the academic achievement of nursing students, general point average (GPA) was 2.57. About 43.5% of participant students had a GPA of between 2.00 - 2.50 and 42.8% had a GPA of between 2.50 - 2.99.

Mothers of 65%, and fathers of 41.9% of the participant students were primary school graduates and mothers of 90.6% of students were homemakers. The monthly family income of 50% of nursing students was between 0 and 1500 TL and 63.7% of families had 0-3 children (Table 1).

Table 1. Distribution of The Demographic Characteristics of Students

Demographic Characteristics	n	%
The Mother's Educational Status		
Illiterate	27	9.8
Primary School	180	65.2
Secondary and High School	53	19.2
University	16	5.8
The Father's Educational Status		
Illiterate	1	0.4
Primary School	117	42.4
Secondary and High School	112	40.6
University	46	16.7
Family Income		
0-1500 TL	138	50
1501-2500 TL	102	37
2501-3500 TL	29	10.5
>3501 TL	7	2.5
Number of Children in Family		
0-3	176	63.7
3-6	51	31.5
>7	13	4.7
The GPA of students		
<1.99	13	4.7
2.00-2.50	120	43.5
2.51-2.99	118	42.8
3.00-3.50	22	8.0
3.51-3.99	3	1.1

Almost 92.3% of the nursing students' critical thinking disposition levels were low and 7.7% were moderate. Total average CCTDI score of entire students was 209.95±25.26. The average score for analytic thinking subscale was 49.93±8.95; the average score for self-confidence subscale was 28.26±6.03; the average score for open-mindedness subscale was 48.09 ±8.88; the average score for systematic thinking subscale was 25.59±4.71; the average score for truth-seeking subscale was 24.77±5.81; the average score for inquisitiveness subscale was 33.62±7.07 and finally the average score for empathy was 53.25±14.80 (Table 2).

The average score for total critical thinking disposition was 198.70±21.72 for under the age of 20 and 210.05±26.84 for ages between 20-31 years. There was a highly significant relationship between age and critical thinking disposition total score and empathic tendency ($p<0.001$) (Table 3).

The average score for the total critical thinking disposition was 195.85±17.76 for the first year students; 200.92±24.39 for the second year students; 203.42±25.19 for the third year students and 220.53±26.31 for the fourth year students. There was again a highly significant relationship between classes and critical thinking disposition total score and empathic tendency ($p<0.001$) (Table 3).

Table 2. The Average Score for Critical Thinking Disposition Level and Empathy of Students

	Median	The average	Standard Deviation
Critical Thinking Disposition Total Score	204	209.95	25.26
Analytic Thinking Sub-scale	51	49.93	8.95
Open-minded Thinking Sub-scale	48	48.09	8.88
Self-Confident Thinking Sub-scale	28	28.26	6.03
Truth-Seeking Thinking Sub-scale	25	24.77	5.81
Systematic Thinking Sub-scale	26	25.59	4.71
Inquisitive Thinking Sub-scale	34	33.62	7.07
Empathy	58	53.25	14.80

The average score for empathy was 46.52±14.56 for the first year students; 52.81±13.70 for the second year students; 51.50±15.42 for the third year students and 65.39±6.57 for the fourth year students ($p<0.001$) (Table 3).

There was a positive relationship between the emphatic tendency and critical thinking disposition total score ($r=0.186$ $p<0.05$) (Table 4).

In addition to there was also positive relationship between the class level and empathy ($r=0.393$ $p< 0.01$), CCTDI ($r=0.317$ $p< 0.01$).

Discussion

Nurses must often consider multiple options together and give quick decisions. Critical thinking is a vital issue for them. Thus, nursing education directed to develop critical thinking skills of students.

In the present study, 92.3% of the nursing students' critical thinking disposition levels were low and 7.7% were moderate. CCTDI total average score of entire student was 209±25.26. The present value is lower than the values reported in earlier studies. In previous studies, the average CCTDI total score was reported as 296.02±4.30 (Shin et al, 2006); 284.93±25.58 (Suliman&Halabi, 2007); 264.7±24.01 (Ip et al, 2000). Despite different values in Turkey, the level of critical thinking disposition of nursing students was mostly reported as moderate (Kaya 1997; Dil&Coşkun 2001; Dil&Öz 2005; Dirimeşe 2006). Such findings were

Table 3. Relations between Demographic Variables, Empathy and Critical Thinking Disposition

		Total Score of CCTDI		p	Empathy		p
		Average	SD		Average	SD	
Age	<20	198.70	21.72	p<0.001	49.33	14.65	p<0.001
	21-30	210.05	26.84		57.17	13.94	
Gender	Male	205.37	26.48		53.81	14.57	p>0.05
	Female	203.83	21.89	p<0.001	51.69	15.42	
Class Level	First year	195.85	17.76	p<0.001	46.52	14.56	p<0.001
	Second year	200.92	24.39		52.81	13.70	
	Third Year	203.42	25.19		51.50	15.42	
	Fourth year	220.53	26.31		65.39	6.57	
Monthly Income	<500TL	195.93	23.96	p>0.05	46.80	13.61	p<0.05
	501-1500TL	204.62	23.60		53.64	15.28	
	1501-2500TL	204.09	25.09		54.27	14.35	
	2501-3500TL	216.92	28.94		50.48	14.97	
	>3500TL	195.57	30.61		62.57	9.62	
Father's Occupation	Officer	204.65	27.22	p>0.05	54.88	14.12	p<0.05
	Worker	206.68	22.72		54.16	15.34	
	Self-employ.	206.42	24.74		53.78	15.34	
	Artisan	205.45	26.73		51.26	13.93	
	Retired	197.35	25.92		47.15	14.35	
	Farmer	186	14.14		39.50	14.84	

Table 4. Results of Correlation Analysis

	Critical Thinking Disposition Total Score	Analytic Thinking Sub-scale	Open-minded Thinking Sub-scale	Self-Confident Thinking Sub-scale	Truth-Seeking Thinking Sub-scale	Systematic Thinking Sub-scale	Inquisitive Thinking Sub-scale
Empathic Tendency Scale	r=0.186 p<0.05	r=0.000 p>0.05	r=0.057 p>0.05	r=0.017 p>0.05	r=0.098 p>0.05	r=0.088 p>0.05	r=0.039 p>0.05

different from the current findings. The students of Foreign Languages Department had the greatest GPAs and they were followed by psychology, nursing, history, and education and business majors.

A large number of students had low critical thinking level in present study.

However, high critical thinking disposition levels were reported in some earlier studies (Profetto-Mc Grath, 2003; Dirimeşe, 2006). These findings are in contrast with the finding. In a study carried out by Bulut et al. (2009), critical thinking level of 90.7% of nursing students were reported as low (211.03 ± 22.72).

There was a positive relationship between the class level, age and critical thinking total score (p<0.001) and there was a negative relationship between the class level and analytic thinking subscale (p<0.01). There was also a positive relationship between the class level and systematic thinking subscale (p< 0.05).

Sulliman (2006) examined the critical thinking skills and disposition based on class levels and investigated if there is a difference in learning styles. The first year (n = 80) and the second year (n= 50) nursing students participated in that study and significant differences were observed between the critical thinking disposition, abilities and learning styles of nursing students. Kawashima and Petrini (2004) compared nursing students with the nurses working at hospitals in terms of critical thinking dispositions and indicated that critical thinking disposition levels of the nurses were lower than the student groups.

Bulut et al. (2009) reported significant differences between the class level and the mean scores for critical thinking disposition level (t=6.779, p=0.000). Öztürk and Ulusoy (2008) indicated increasing critical thinking skills in the

second, third and fourth years and the values reached to the highest levels in graduate students. Brooks and Sepherd (1990) in the US reported lower critical thinking levels for undergraduate nursing students. Shin (1998) investigated critical thinking of undergraduate and graduate nursing students and observed significant differences between critical thinking levels of nursing students at different level of education. Ip et al. (2000) assessed the critical thinking trends of 122 undergraduate nursing students in Hong Kong and reported significant differences in critical thinking scores of the first-year students. Walsh and Hardy (1999) compared the critical thinking scores of university students at different majors. Adams et al. (1999) indicated increasing critical thinking levels with the increasing years of experience in nursing profession. Banning (2006) found that only two out of 37 nurses were able to use of critical thinking in clinical decision making but others were quite limited in using critical thinking abilities. These findings are similar to current findings.

In present study, a significant relationship was observed between the analytic thinking an academic achievement and gender of nursing students ($p<0.05$). No significant relationship was observed between the critical thinking total score and academic achievement trends ($p>0.05$) and there was a significant relationship between the analytic thinking sub-scale, inquisitive thinking and academic achievement ($p<0.05$).

Emir (2012) reported that critical thinking dispositions of the students didn't differ according to their academic achievements. These findings are in line with the current data. Fisher (1995) mentioned in their books that the critical thinking and dispositions increase the academic success of individuals. Stewart and Dempsey (2005) implemented case studies to develop critical thinking dispositions and skills of nursing students and identified an increase in academic achievement scores with critical thinking abilities. Ellermann et al. (2006) in a study with nursing students in 2004, used the concept maps in the course of teaching and observed increased academic achievement by the end of the period. These results are similar to current findings.

In present study, there was a relationship between the class level, age and empathy ($p< 0.01$). The average score of empathy was 53.25±14.80 for nursing students. The average score of empathy was 46.52±14.56 for the first year students, 52.81±13.70 for the second year students, 51.50±15.42 for the third year students and 65.39±6.57 for the fourth year students ($p<0.001$). There was a positive relationship between the Critical Thinking Disposition Inventory (CCTDI) total score and Empathic Tendency Scale ($p<0.05$). Tutuk et al. (2002) reported the average score of empathic tendency of nursing students as 69.55. In the same study, according to the empathic tendency average scores, a significant difference was not observed in emphatic tendency of different age groups. Although the mean scores of empathy were higher than the present study, data were quite similar to current ones.

Akgöz and Karavuş (2005) reported the average score of empathic skills of working midwifes as 128.2. Öz (1998) indicated the empathic tendency average score of nursing students as 70.25. Dizer & İyigün (2009) reported the highest empathic propensity score as 72.4 ± 9.3 for 20-25 years age group and 72 ± 8.6 for the ages 31 years and older, but there was no relationship between the empathic tendency and age groups ($p>0.05$). According to current results, empathic tendency average scores of nursing students was found to be the lowest in the first year, the highest in the fourth year and increasing empathic tendencies were observed with increasing educational levels. Such results were due to lessons (interpersonal relations, communications) learned in nursing education and close contact with more patients. Empathic tendency of nurses increase with the increasing years of experience (Öz, 1998).

Conclusion

Critical thinking and empathic tendency are two significant tools used in patient care. Present results revealed that average critical thinking dispositions scores of nursing students were low. There was a relationship between the critical thinking dispositions total score and empathic tendencies. Age, gender and class level affected critical thinking disposition level of nursing students. Class level, age, monthly income and father's occupation affected the empathy. Academic achievement did not affect the critical thinking level. Increasing critical thinking disposition scores were observed with increasing class levels. It is believed that such an increase was because of increased age and level professional knowledge.

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ORIGINAL ARTICLE

Same Day Upper and Lower Endoscopy Results in Patients Presenting with Nonspecific Abdominal Pain

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Abstract

Objective: The aim of this study was to evaluate the upper gastrointestinal endoscopy and colonoscopy results in patients who presented with abdominal pain and received a diagnosis of nonspecific abdominal pain following the examination and tests.

Methods: We included a total of 52 patients who presented at the emergency service between 01.01.2011 and 01.01.2012 with symptoms of abdominal pain and received a diagnosis of nonspecific abdominal pain following normal examination, routine blood and urine tests and ultrasound analysis. All patients underwent upper gastrointestinal endoscopy and colonoscopy on the same day. The results were evaluated and diagnoses made according to the endoscopy and pathology findings.

Results: There were 23 (44.2%) males and 29 (55.8%) females. The mean age was 54.5 ± 15.3 (23-86) years. A pathology was present on upper endoscopy in 47 (90.4%) patients and lower endoscopy in 27 (51.9%) patients. Comparison of the pathology rates for upper endoscopy and colonoscopy showed a significantly higher rate for upper endoscopy. The most common findings were chronic gastritis with upper endoscopy 13 males (25%) and 17 (32.7%) females, hemorrhoids with colonoscopy 10 males (19.2%) and 12 (23.1%) females. A pathology was present on both examinations in 25 (48%) patients. Cancer was found in 7 (13.5%) patients (6 gastric, 1 colon cancer). *Helicobacter pylori* was (+) in 53.8% of the cases.

Conclusion: Same day upper and lower endoscopy in patients with nonspecific abdominal pain provided important results. However, we feel upper endoscopy should have priority when it is not possible to perform both investigations.

Key words: Abdominal pain, endoscopy, gastrit,

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Introduction

Non-specific abdominal pain (NSAP) can be defined as a clinical picture where no cause can be found on examination or with investigations in patients who present to the hospital with abdominal pain and where the abdominal pain symptom gradually disappears by itself. Non-specific abdominal pain can be an indicator of disorders that may require surgery or conservative treatment (Graff and Robinson, 2001).

It has been reported that abdominal pain is present in about 5-10% of the patients who present

to the emergency service and 35-40% of this group is diagnosed as non-specific abdominal pain that cannot be linked to any disease (Graff and Robinson, 2001; Lameris et al., 2007). The number of studies on NSAP is limited. The incidence of malignancy has been reported to be high with esophagogastroduodenoscopic and colonoscopic investigations in non-specific abdominal pain, especially in patients above the age of 50 (Graff and Robinson, 2001; Lameris et al., 2007). The approach to these patients, the analyses required and the follow-up durations could not be determined for sure as a result of these studies. We aimed to compare the results of same-day esophagogastroduodenoscopic and colonoscopic investigations in patients diagnosed with NSAP in our study.

Materials and Methods

The data of this descriptive study were obtained by investigating the files of 52 patients who had presented to the general surgery outpatients department with non-specific abdominal pain between 01 January 2011 and 01 January 2012. Local Ethics Committee approval (Kafkas University local Ethics Committee-80576354-050-99) was received for the study. The patient history, physical examination findings, laboratory values (biochemical, complete blood count and urine), standing direct abdominal X-ray, and whole abdominal ultrasound results were available in the charts of the 52 patients studied. The signed information and consent form of the patients who had undergone upper GIS endoscopy and colonoscopy were also available in the charts. The patients were diagnosed by using upper GIS endoscopy and colonoscopy. No complication related to the procedure or any other procedures were reported in the charts.

Results

There were 23 (44.2%) males and 29 (55.8%) females. The mean age was 54.5 ± 15.3 years. Pathological findings were found with upper endoscopic investigation in 47 (90.4%) and lower endoscopic investigation in 27 (51.9%) patients. Pathological findings were present during both investigations in 25 patients. The most common finding was hemorrhoid with colonoscopy 19 (36.5% / 7 males and 12 females) and chronic gastritis with upper GIS endoscopy 34 (65.4% / 13 males and 21 females). We had 7 patients (13.5%) were diagnosed with cancer (6 with upper GIS endoscopy and 1 with colonoscopy). Only 3 patients had no pathology on either investigation. The *Helicobacter pylori* rate was 53.8%. The results of colonoscopic and upper GIS endoscopic investigations are presented in table 1-2.

Table 1 Upper GIS Endoscopic İnvestigation Results (Order Based on Percentage)

Diagnosis	Frequency-Prevalence (n)	(%)
Normal	5	9.6
Gastritis	19	36.5
Cancer	6	11.5
Esophagitis	2	3.8
Hiatal hernia	1	1.9
Gastritis+ Hiatal Hernia	9	17.3
Gastritis + Polyp	1	1.9
Gastritis + Esophagitis	2	3.8
Esophagitis + Hiatal Hernia	3	5.7
Hiatal Hernia + Ulcer	1	1.9
Gastritis + Hiatal Hernia + Bulbitis	2	3.8
Gastritis + Esophagitis + Hiatal Hernia	1	1.9
Total	52	9.6

Table 2: Colonoscopic Investigation Results (Order Based on Percentage)

Diagnosis	Prevalence	%
Normal	25	48.1
Diverticulosis	7	13.5
Polyp in colon, pseudopolyps + Hemorrhoids	12	23.1
Cancer	1	1.9
Diverticulosis + Polyp in colon, pseudopolyps + Hemorrhoids	2	3.8
Polyp in colon, pseudopolyps + Hemorrhoids	2	3.8
Hemorrhoids + anal fissure	2	3.8
Diverticulosis + Polyp in colon + Hemorrhoids	1	1.9
Total	52	100.0

Discussion

Patients who present to the emergency services and surgical outpatients with non-specific abdominal pain can create difficulties in terms of diagnosis and treatment. Patients diagnosed with NSAP constitute 30%-40% of the patients who present to these departments (Bavunoğlu and Şirin 2005; Özgüç et al., 2008; Stefanidis et al., 2009).

The symptoms that can be found in patients presenting with non-specific abdominal pain are loss of appetite, nausea and vomiting. This patient group has been reported to consist of 60% females and 40% males (Aygencel et al., 2009). The female patient rate in our study was higher, similar to the literature. The most common reasons of abdominal pain are acute appendicitis, mesenteric lymphadenopathy, and nonspecific abdominal pain in young people and bowel obstruction, diverticular and hepatobiliary diseases in the elderly (Luken et al., 1990; Luken et al., 1993; Ağalar et al., 1999; Bavunoğlu and Şirin, 2005). Young adults make up the majority of the cases with non-specific abdominal pain. The NSAP rate was reported to be 32% under the age of 50 and 10% above the age of 50 by Kraemer et al. (2000). A rate of 60% was found in patients above the age of 50 in our study, in contrast to that reported in the literature. We believe that the reason is that young patients with decreasing symptoms refuse same-day upper GIS endoscopy and colonoscopy.

The symptoms and findings as well as laboratory evaluations, imaging methods and endoscopic methods are important in the diagnosis of these patients. Ultrasonography may help diagnose certain conditions in patients presenting with abdominal pain. However, it mostly provides negative signs in patients with abdominal pain of unknown cause. While some centers regard ultrasonography as sufficient for the diagnosis of the cause of the pain in patients with NSAP, other centers recommend abdominal tomography. However, it may not be possible to make a diagnosis even with other imaging methods. Abdominal computed tomography and laparoscopy are recommended routinely with the patients monitored 24 hours after discharge to diagnose patients with NSAP in certain studies (Özgüç et al., 2008). The radiation dose and cost of CT and the cost and risks of laparoscopy are the disadvantages of the two methods. We preferred to perform ultrasonography in our patients due to radiation of CT in our study. We used endoscopic methods as an alternative method to CT to avoid radiation and to laparoscopy to avoid an invasive procedure in patients with normal ultrasonography. Upper gastrointestinal endoscopy is recommended for patients with odynophagia, chest pain, vomiting, weight loss, epigastric burning symptoms and iron deficiency anemia in addition to abdominal pain, and colonoscopy is recommended for patients with anemia, hematochezia, chronic diarrhea, weight loss, and change in intestinal habits in addition to abdominal pain (Van Mook et al., 2001; Varadarajulu et al., 2005; Stray and Weberg, 2006; Harris et al., 2007). Major pathology was found in more than 50% of the patients evaluated with upper GIS endoscopy due to symptoms of abdominal pain, odynophagia, weight loss, and vomiting by Varadarajulu et al. (2005) and the authors recommended upper GIS endoscopy in patients with odynophagia, burning, and weight loss, especially when the patient is an elderly male. The most common etiology in children presenting with abdominal pain was reflux esophagitis with upper GIS endoscopy in the study conducted by Thakkar et al. (2007). It was emphasized in the multi-center study of Harris et al. (2007) that colonoscopy is required less commonly in patients with non-specific symptoms such as abdominal pain and diarrhea and that colonoscopy is important in the diagnosis of colorectal cancer if iron deficiency anemia and positive occult blood in the stool are found in elderly patients.

We found pathology in 47 patients who presented with NSAP through the esophagogastroduodenoscopic investigation and in 27 patients through the colonoscopic investigation. We also made a diagnosis that could cause abdominal pain in 47 patients with esophagogastroduodenoscopy and 25 patients with colonoscopy. We were able to diagnose more patients with esophagogastroduodenoscopy than with colonoscopy, similar to other studies (Van Mook et al., 2001; Varadarajulu et al., 2005; Stray and Weberg, 2006; Harris et al., 2007; Thakkar et al., 2007). The patients were treated in accordance with the diagnoses. No other pathology was found with 1 year of follow-up. The NSAP group of course consists of patients with abdominal pain where the cause cannot be found by imaging methods and the symptoms mostly decrease by themselves with observation. Whether the diagnoses made with endoscopy in this patient group are definite is controversial. However, endoscopic methods may still be appropriate to detect and simultaneously treat pathologies such as polyp, ulcer, and cancer. One must not forget that gastrointestinal malignancies can develop later on in 10% of NSAP patients over the age of 50 as reported by de Dombal (1988). Endoscopic methods should therefore not be ignored for the early diagnosis of cancer in patients with NSAP.

The biggest deficiency of our study was the small number of patients.

We believe that endoscopic investigations of NSAP patients in studies conducted with large patient series can at least enlighten us regarding their importance in the early diagnosis of the disorders that may create a basis of cancer.

Conclusion

In conclusion, same-day upper and lower gastrointestinal endoscopic investigations in NSAP patients seem to be important in terms of aiding the diagnosis. We believe that upper GIS endoscopy should be preferred if it not possible to perform both investigations.

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

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ORIGINAL ARTICLE

The Cultivation of *Acanthamoeba* Using Different Axenic and Monoxenic Media

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Abstract

Objective: *Acanthamoeba* species are the ubiquities free-living amoebae and can infect humans, causing diseases such as keratitis and encephalitis. *Acanthamoeba* species are often grown on non-nutrient agar spread with *Escherichia coli* or peptone-yeast extract-glucose. We investigated the amount of growth of *Acanthamoeba* in different axenic and monoxenic media.

Methods: The non-nutrient agar with *Pseudomonas aeuroginosa*, *Enterobacter aerogenes*, *Staphylococcus aeurous*, *Escherichia coli*, and *Klebsiella pneumoniae* were used as monoxenic media. The encystation, mycological peptone-maltose, peptone yeast extract glucose, roswell park memorial institute 1640 and trypticase beef hemoglobine media were used as axenic media.

Results: We compared the growth of *Acanthamoeba* species in different axenic and monoxenic media in this study. In relation to the growth rate, the non-nutrient agar with *Pseudomonas aeuroginosa* had the highest values achieved among monoxenic media and roswell park memorial institute 1640 media was the highest value among axenic media.

Conclusion: In view of the results, we can affirm that these monoxenic media are adequate to grow of *Acanthamoeba* species. In addition, a classic and basic medium that supports the growth of *Acanthamoeba* species consists of peptone yeast extract glucose. However, the roswell park memorial institute 1640 media was an excellent commercially available media for the growth of *Acanthamoeba* and it was able to keep *Acanthamoeba* by long periods of time.

Key words: *Acanthamoeba*, axenic, monoxenic, media

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Introduction

Acanthamoeba species are the ubiquitous free-living amoebae that cause keratitis, encephalitis, cutaneous and other lesions (Penland and Wilhelmus, 1997; Khan, 2006). The life cycle takes place in two stages are trophozoite (active form) and cyst (inactive form) (Heredero et al., 2012). Their survival depends on their morphological and physiological adaptation to varying and potentially lethal environmental conditions (Heredero et al., 2012). *Acanthamoeba* strains can show different pathogenic ability

(Niyyati et al., 2013). Research on the pathogenic potential of *Acanthamoeba* is complicated by the changes induced by prolong axenix laboratory culture, such as the down regulation of its virulence and encystment capacity and its altered sensitivity to drugs (Verissimo et al., 2013).

Acanthamoeba able to tolerate a range of growth conditions and appear to be prepared to go from bacterized to axenic media without adaptation or selection (Schuster, 2002). A classic and basic media that supports the growth of *Acanthamoeba* consists of axenic and monoxenic media (Penland and Wilhelmus, 1997). Bacteria-free media have been developed for axenic media and include peptone yeast extract glucose (Penland and Wilhelmus, 1997; Heredero et al., 2012). The peptone yeast extract glucose media is the most commonly used for the growth of *Acanthamoeba* (Ahmed, 2009; Axelsson et al., 2009). Acanthamoebic trails have been detected on blood agar, non-nutrient agar and *Acanthamoeba* has been shown to preferentially phagocytize erytrocytes of different animal species (Penland and Wilhelmus, 1998). It has been reported that non-nutrient agar is very rapidly, inexpensive and easy to handle experimentally for growth of *Acanthamoeba* (Axelsson et al., 2009; Borin et al., 2013).

In this study, it was evaluated the efficiency of axenic and monoxenic media in its growth, with the aim of selecting one which allowed better growth, was easier to prepare, and was able to keep *Acanthamoeba* by long periods of time.

Materials and Methods

Acanthamoeba strains *and sources*

Strains of *Acanthamoeba* isolated in the Department of Parasitology, Faculty of Medicine, Cukurova University were used to compare recover on various media. It was shown that GenBank accession number, species, genotype and isolation sources of *Acanthamoeba* strains in table 1.

Table 1 GenBank accession number, species, genotype and isolation sources of *Acanthamoeba* strains.

Acession No	Species	Genotype	Isolation sources
KJ446982	*A. castellanii*	T4	Adana-water and soil samples
KJ446976	*A. castellanii*	T4	Kutahya-water and soil samples
KJ446979	*A. griffinii*	T3	Adana-water and soil samples
KJ446980	*A. griffinii*	T3	Kutahya-water and soil samples
KJ446983	*A. jacobsi*	T15	Adana-corneal scraping
KJ446981	*A. jacobsi*	T15	Kutahya-water and soil samples

Axenic media

Encystation media (EM): EM media consisted of the following ingredients; 1 g KCl, 0.25 g Tris, 8 g $MgSO_4$, 0.75 g $CaCl_2$, 1 g $NaHCO_3$, and 100 mL distilled water. Also, 0.5 mg/mL penicillin (Sigma-Aldrich, Chemical, France), and 0.5 mg/mL streptomycin (Sigma-Aldrich, Chemical, France) were added (Riviere et al., 2006).

Mycological peptone-maltose media (MPM): MPM media was prepared by mixing 10 g mycological peptone and 5 g maltose, 100 mL distilled water, and supplemented with 0.5 mg/mL penicillin (Sigma-Aldrich, Chemical, France), 0.5 mg/mL streptomycin (Sigma-Aldrich, Chemical, France) (Henriquez et al., 2009).

Peptone yeast extract glucose media (PYG): 2 g peptone, 1 g yeast extract, and 1 g glucose, pH 7.2 in 100 mL distilled water and autoclaved at 121ºC for 15 min. In addition, 0.5 mg/mL penicillin (Sigma-Aldrich, Chemical, France), and 0.5 mg/mL streptomycin (Sigma-Aldrich, Chemical, France) were added (Khan et al., 2002).

Roswell Park Memorial Institute Media 1640 (RPMI): The culture flask containing 15 mL RPMI 1640 media (Sigma-Aldrich, Chemical, France) was supplemented with 12% heat-inactivated calf serum (FCS) (Sigma-Aldrich, Chemical, France), 0.5 mg/mL penicillin (Sigma-Aldrich, Chemical, France), 0.5 mg/mL streptomycin (Sigma-Aldrich, Chemical, France), (Sharief et al., 2008).

Trypticase beef hemoglobine media (TBH): The media was prepared by dissolving 1 g trypticase, 1 g beef extract, 1 g yeast extract, 1 g peptone, 1 g liver extract, 0.5 g glucose, 0.8 g NaCl, 0.5 g L-Proline, 0.13 g $NaHCO_3$, 0.26 g Na_2HPO_4, 0.65 g KH_2PO_4, 0.2 g $FeNH_4$, 1 g bovine hemoglobine of 100 mL distilled water. Finally, 0.5 mg/mL penicillin (Sigma-Aldrich, Chemical, France), and 0.5 mg/mL streptomycin (Sigma-Aldrich, Chemical, France) solution were added (Limoncu et al., 2004).

Monoxenic media

The non-nutrient agar (Becton Dickinson Microbiology Systems, Cockeysville, Maryland) was used for growth of *Acanthamoeba*. *Escherichia coli* (*E. coli*) (ATCC 25922), *Enterobacter aerogenes (E. aerogenes)* (ATCC13048), *Klebsiella pneumoniae (K. pneumoniae)* (ATCC 13883), *Pseudomonas aeruginosa (P. aeruginosa)* (ATCC 27853), and *Staphylococcus aeureus (S. aeureus)* (ATCC 25923) were used for monoxenic media. The agar plates were prepared by adding 0.5 mL of the standardized bacterial suspension and *Acanthamoeba* strains.

Incubation and temperature tolerance: The axenic media was autoclaved at 121ºC for 15 min and the pH values of axenic media were 7.2. After cooling, 5 ml of the extract was filtered in each of the 25 cm^3 flasks. The axenic and monoxenic media were incubated at 27ºC under standard atmospheric conditions and examined ever day under an inverted microscope (Leica DM3000, Houston, Texas) with a 10X ocular lens and a 20X objective. The cysts and trophozoites of *Acanthamoeba* from two days media were harvested and counted with a Thoma hemocytometer (Hirschmann Laborgerae, Eberstadt, Germany).

Results

The reproduction of *Acanthamoeba* was increased on 2th day in all axenic media. Furthermore, the growth of value of the RPMI 1640 media was higher than the other media. In TBH, EM, MPM, PYG the number of *Acanthamoeba* were high between the days 6th and 8th day. The number of *Acanthamoeba* in axenic media except the RPMI 1640 media was lower after the 10th day (Table 2, Figure 1). When looking at sustain of growth rates of long time, the best growth rates were achieved with RPMI 1640 media in first experience.

The results of non-nutrient agar spread with different bacteria were compared. The number of *Acanthamoeba* were obtained different level in diagnosis of *Acanthamoeba* from highest to lowest in the order of *P. aeruginosa* > *E. aerogenes* > *S. aeureus* > *E. coli* > *K. pneumoniae*. The highest values achieved between the 4th and 8th day in non-nutrient agar with all bacteria in relation to the growth rate. After 8th day, reproduction of *Acanthamoeba* was decreased day by day. The rank order of non-nutrient agar with bacteria for the recovery of *Acanthamoeba* is presented in Table 3 and Figure 2.

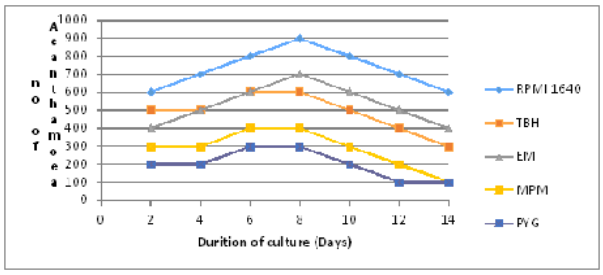

Figure 1 The reproduction of *Acanthamoeba* in different axenic media; Roswell park memorial institute (RPMI-1640), Trypticase beef hemoglobin media (TBH), Encystation media (EM), Mycological peptone-maltose (MPM), and Peptone yeast extract glucose (PYG) axenic media. The figure was shown comparing the number of *Acanthamoeba* different media for each time

Table 2 Reproduction of *Acanthamoeba* in Roswell park memorial institute (RPMI-1640), Trypticase beef hemoglobin media (TBH), Encystation media (EM), Mycological peptone-maltose (MPM), and Peptone yeast extract glucose (PYG) axenic media.

Medium	Number of *Acanthamoeba* reproduced on day						
	2	4	6	8	10	12	14
RPMI 1640	6 X 10^2	7 X 10^2	8 X 10^2	9 X 10^2	8 X 10^2	7 X 10^2	6 X 10^2
TBH	5 X 10^2	5 X 10^2	6 X 10^2	6 X 10^2	5 X 10^2	4 X 10^2	3 X 10^2
EM	4 X 10^2	5 X 10^2	6 X 10^2	7 X 10^2	6 X 10^2	5 X 10^2	4 X 10^2
MPM	3 X 10^2	3 X 10^2	4 X 10^2	4 X 10^2	3 X 10^2	2 X 10^2	1 X 10^2
PYG	2 X 10^2	2 X 10^2	3 X 10^2	3 X 10^2	2 X 10^2	1 X 10^2	1 X 10^2

*The initial inoculation for all of strains is 2 X 10^2 and reproduction of all strains is similar in axenic media.

Table3 Reproduction of *Acanthamoeba* in non-nutrient with *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterobacter aerogenes* (*E. aerogenes*), *Staphylococcus aeureus* (*S. aeureus*), *Escherichia coli (E. coli*) and *Klebsiella pneumoniae* (*K. pneumonia*) monoxenic media.

Media	Number of *Acanthamoeba* reproduced on day						
	2	4	6	8	10	12	14
P.aeruginosa	5 X 10^2	6 X 10^2	7 X 10^2	7 X 10^2	6X 10^2	5 X 10^2	4 X 10^2
E.aerogenes	4 X 10^2	5 X 10^2	6 X 10^2	6 X 10^2	5 X 10^2	4 X 10^2	3 X 10^2
S.aureu	3 X 10^2	4 X 10^2	5 X 10^2	5 X 10^2	4 X 10^2	3 X 10^2	2 X 10^2
E.coli	2 X 10^2	3 X 10^2	4 X 10^2	4 X 10^2	3 X 10^2	2 X 10^2	1 X 10^2
K.pneumoniae	2 X 10^2	2 X 10^2	3 X 10^2	3 X 10^2	2 X 10^2	1 X 10^2	1 X 10^2

*The initial inoculation for all of strains is 2 X10^2 and reproduction of all strains is similar in monoxenic media.

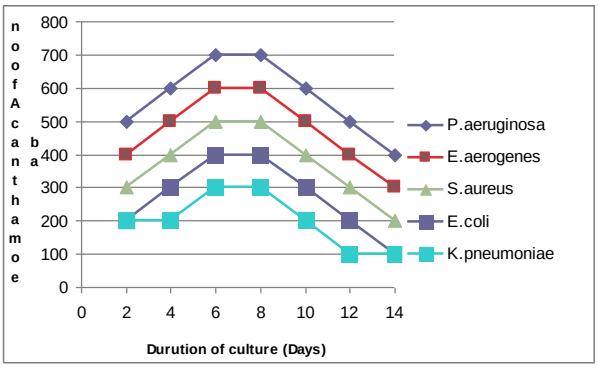

Figure 2 The reproduction of *Acanthamoeba* in non-nutrient with *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterobacter aerogenes* (*E. aerogenes*), *Staphylococcus aeureus* (*S. aeureus*), *Escherichia coli (E. coli*) and *Klebsiella pneumoniae* (*K. pneumonia*) monoxenic media. The figure was performed comparing the values of different media for each time.

Discussion

The cultivation of *Acanthamoeba* is a useful approach for yielding an amount of parasites appropriate for various diagnostic purposes, for improving our knowledge of host parasite relationships and for determining of molecular, biological and immunological characteristics of the parasite (Borin et al., 2013; Verissimo et al., 2013; Jain et al., 2015;). Although, a great number of studies reported that the present *Acanthamoeba* in worldwide, we could find very few studies providing satisfactory information about the media of *Acanthamoeba*. Therefore, we used different media and compared these media for *Acanthamoeba* in this study.

To various degrees, *Acanthamoeba* can be readily established in axenic media from initially bacterized cultures by providing an enriched nutrient media with antibiotics added to kill off contaminating bacteria (Schuster, 2002). The basic nutrient media that is used for *Acanthamoeba* typically contains peptone, yeast extract, and glucose, in concentrations generally higher than those used for growth in bacterized cultures (Schuster, 2002). The PYG media is recommended for growth of *Acanthamoeba* parasite (Peretz et al. 2015). The RPMI 1640 and TBH media the most widely were used media for *Leishmania* parasite (Limoncu et al., 1997; Sharief et al., 2008). As far as we know, these media have not been used before to growth *Acanthamoeba*. However, we used these media and in view of the results these media were effective in the growth of *Acanthamoeba*. When large amounts of *Acanthamoeba* in short periods of time will be required, it would be convenient to use RPMI 1640 media.

Acanthamoeba can be readily cultivated on either non-nutrient agar or agar media containing low concentrations of nutrients in the presence of living or killed bacteria. In general, the bacteria of choice include non-mucoid strains of *K. pneumoniae*, *E. aerognes*, and *E. coli* (Schuster, 2002). We used non-nutrient agar with living *P. aeruginosa*, *E. aerogenes*, *S. aurous*, *E. coli* and *K. pneumoniae* and we found that *P. aeuroginosa* most effective of growth *Acanthamoeba*. Non-nutrient agar with an overlay of live *E. coli* is recommended for the recovery of *Acanthamoeba* but is not readily available (Penland and Wilhelmus, 1997). Although the non-nutrient agar with *E. coli*, the species widely recommended for use in the isolation of *Acanthamoeba*, was significantly lower than those on media with all other live bacterial species except *K. pneumoniae* in this study (Penland and Wilhelmus, 1998).

In cultivations carried out to produce of *Acanthamoeba* species outside natural media in the best possible way, care should be taken to make the media compatible with nutritional and environmental conditions of the natural media. We found that RPMI 1640 media is the most rapid and easy for growth of *Acanthamoeba*, it should be employ as the standard method for growth *Acanthamoeba*.

Conclusion

Acanthamoeba species are able to growth different conditions and different media. The studies about growth of *Acanthamoeba* are important for developing vaccine and drug for *Acanthamoeba* infections. Our results provide further evidence for the effect of prolonged axenic and monoxenic media on the growth of *Acanthamoeba*. As a result of this study, the RPMI 1640 media and non-nutrient agar with *K. pneumoniae* can be used in studies about vaccine, drug and pathogeniticity of *Acanthamoeba*. However, future studies should be carried out to further explore this issue.

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ORIGINAL ARTICLE

Correlation of Platelet to Lymphocyte Ratio with Presence and Severity of Metabolic Syndrome

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Abstract

Objective: The aim of this study is to evaluate the relation between the components of Metabolic syndrome (MS) and the inflammation and the platelet-to-lymphocyte ratio (PLR) recognized as a novel marker of pro-thrombotic state.

Method: 70 patients with MS criteria were included in the study. 70 healthy people with matching age and gender characteristics were included in the study as the control group. The patients were divided into three groups with regard to the number of MS characteristics: Group 1 (the patients with three MS criteria), group 2 (the patients with four MS criteria), and group 3 (the patients with five MS criteria). PLR was calculated in regard to the complete blood count.

Results: The patients with MS had significantly higher PLR level compared to those without MS. However, PLR level were not associated with severity of MS. A moderate positive correlation was revealed between the severity of MS and PLR ($r=0.419$, $p<0.001$) and a strong positive correlation between the severity of MS and hs-CRP ($r=0.562$, $p<0.001$). Also, a positive correlation was detected between hs-CRP and PLR in our patient population ($r=0.281$, $p=0.002$). In order to specify the level of PLR to predict MS, ROC curve analysis was performed. The cut-off level for PLR with optimal sensitivity and specificity was calculated as 0,132 (Area under curve [AUC] =0.744 [0.655-0.833], $p<0.001$). For that level, the sensitivity was 73% and the specificity was 68, 4%.

Conclusion: This is the first study which determines the fact that there is a relation between MS criteria, the inflammation and PLR, which is a new marker of the pro-thrombotic state. There is also a positive correlation between PLR and hs-CRP in these patients. PLR may be beneficial in predicting the adverse cardiovascular cases in the patients with MS.

Key words: Metabolic Syndrome, platelet/lymphocyte ratio, inflammation

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Introduction

Metabolic syndrome (MS) is a case basically beginning with insulin resistance (IR), containing a combination of the systemic disorders such as abdominal obesity, glucose intolerance or diabetes mellitus, dyslipidemia, hypertension and coronary artery disease (CAD), and accompanied by vascular inflammation and pro-thrombotic tendency. The prevalence of metabolic syndrome increases with the increased physical inactivity and the central obesity in our society (Bloomgarden, 2003). MS includes more than one risk factor in terms of cardiovascular diseases. IR, which is one

of the basic pathological mechanisms composing MS, is related to especially proinflammatory and pro-thrombotic state (Calles-Escandon et al., 1998 and Hori et al., 2005). Pro-thrombotic and proinflammatory tendency has an important role in the exacerbation of the atherosclerotic process and the formation of serious clinical states.

The studies carried out demonstrate the relation between the components of MS and the tendency to proinflammatory state. A simple indicator of proinflammatory state in the patients with MS is the detection of the increased C - reactive protein (CRP) in these patients. It is additionally known that the levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) increase in these patients (Yudkin et al., 1999). Inflammation is known to have a role in the onset and the progress of atherosclerotic illnesses (Hansson et al., 2005). Platelet-to-lymphocyte ratio (PLR) has recently been identified as a biological indicator of the balance between inflammation and thrombosis especially in the patients with malignancy (Smith et al., 2008; Wang et al., 2013). There are evidences about the negative consequences of the increase in PLR in the cardiovascular diseases (Azab et al., 2012; Acar et al., 2013). The aim of this study is to assess the relationship between MS, its components and PLR, which has recently been recognized as an inflammation and pro-thrombotic indicator.

Materials and Methods

In a retrospective longitudinal study, our study population consisted of non-selected 194 patients, who visited our clinic from January to August. The exclusion criteria were determined as infection (n:4), chronic systemic inflammatory diseases (n:5) and the patients using drug affecting the number of leukocytes (steroid, chemotherapeutic etc.) (n:3). Also, the patients with renal failure (n:6), liver failure (n:3), secondary hypertension (n:16) and known coronary artery disease (n:9), heart failure (n:7), and severe heart valve diseases (n:4) were excluded from the study. Finally, the study population consisted of 140 patients (n:70 patients who had metabolic syndrome, n:70 age- and sex-matched healthy subjects). All participants gave an informed consent and the study was approved by the local ethics committee.

For the diagnosis of MS, National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP ATP 3) were used (Adult Treatment Panel III, 2001) The presence of 3 or more criteria below was accepted as MS.

1- Hypertension: Existing antihypertensive therapy or blood pressure ≥130/85 mm/Hg

2- Dyslipidemia: Plasma triglyceride (TG) level ≥150 mg/dL (≥1.7 mmol/L)

3- Low HDL cholesterol level: In women <50 mg/dL (<1.3 mmol/L), in men <40 mg/dl (<1.0 mmol/L)

4- Abdominal Obesity: Waist circumference (WC) in men >102 cm, WC in women >88 cm

5- Glucose: Fasting blood glucose ≥110 mg/dL (≥5.6 mmol/L) Type 2 DM or impaired glucose tolerance test.

The patients were divided into three groups with regard to the number of MS characteristics: Group 1 (the patients with three MS criteria), group 2 (the patients with four MS criteria), and group 3 (the patients with five MS criteria).

Blood Pressure (BP) was measured after at least 15-minute rest in sitting position. The mean of all three measurements with five-minute intervals were considered as BP. High sensitive C-Reactive protein (hs-CRP), total cholesterol, TG, HDL, LDL levels, urea, creatinine and plasma glucose were measured in the venous blood samples obtained in the morning after eight-hour fasting. The complete blood count was studied in our hematology unit with Beckman-Coulter Gen-S system device (Beckman-Coulter Inc., USA). The weight, height, and WC were measured while fasting and standing up by the standard measuring tools. The narrowest diameter between costal arch and anterior superior iliac spine was measured for WC. Body mass index (BMI) (kg/m^2) and body surface area (BSA) (m^2) were calculated using the formulas "weight (kg)/height $(m)^2$" and "BSA (m^2)= 0.007184 x Height $(cm)^{0.725}$ x Weight $(kg)^{0.425}$" respectively.

Statistical analysis:

Independent sample *t* test or Mann-Whitney *U* test were used for continuous variables, and chi-square test for categorical variables. One-way analysis of variance (ANOVA) or Kruskal-Wallis tests were used to compare more than two groups. Correlations were assessed using Spearman's test. Receiver operating characteristic (ROC) curve analysis was used to determine the optimum cut-off level of PLR to predict the metabolic syndrome. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Version 15.0 (SPSS Inc., Chicago, Illinois). Continuous variables

were defined as mean ± standard deviation, and categorical variables were given as percentages. Any *P* value <0.05 was considered as statistically significant.

Results

70 patients with MS and 70 people with matching age and gender as the control group were included in the study. The characteristics of two groups were summarized in Table 1. As showed in Table 2, while the number of neutrophils, white blood cells (WBC) and lymphocyte were higher in the patients with MS than the control group, the platelet count and the hemoglobin level were found similar.

Table-1: Differences between clinical and laboratory parameters of the groups with and without metabolic syndrome

	MS (–)	MS (+)	P value
Age (years)	47,0±13,7	48,3±9,51	0,542
WC	85,75±10,9	97,8±10	<0,001
BMI (kg/m^2)	24,5±3,92	29,2±5,51	<0,001
FBG (mmol/L)	5,0±0,77	6,2±1,48	<0,001
TG (mmol/L)	1,46±0,56	2,38±1,12	<0,001
HDL (mmol/L)	1,23±0,20	0,95±0,22	<0,001
LDL (mmol/L)	2,7±0,84	3,00±0,59	0,028
SBP (mmHg)	129±15,7	134±18,8	0,086
DBP (mmHg)	81,1±10,45	85,3±9,72	0,024

MS: Metabolic syndrome, **WC:** Waist Circumference, **BMI:** Body Mass Index, **FBG:** Fasting Blood Glucose, **TG:** Triglycerides, **DL:** High Density lipoprotein, **LDL:** Low Density lipoprotein, **SBP:** Systolic Blood Pressur**e, DBP:** Diastolic Blood Pressure

Table-2: The hematologic parameters of the groups with and without metabolic syndrome

	MS (–)	MS (+)	P value
WBC	6560±983	7150±1442	0,010
Hemoglobin (g/dL)	13,5±0,5	13,5±0,50	0,562
PLR	0,12±0,04	0,016±0,05	<0,001
Platelet count (×1000)	282,6±66,3	288,1±67,7	0,653
Lymphocyte Count	2431±551	1823±501	<0,001
Neutrophil Count	3984±817	5182±1171	<0,001
Hs-CRP	0,65±0,33	1,12±0,49	<0,001

MS: Metabolic Syndrome, **WBC:** White Blood Cells, **PLR:** Platelet to Lymphocyte Ratio, **Hs-CRP:** High sensitive-C reactive protein

Also, hs-CRP level was significantly higher in MS group than the control group (1.15±0.52 vs 0.64±0.34, p<0.001). (Figure-1).

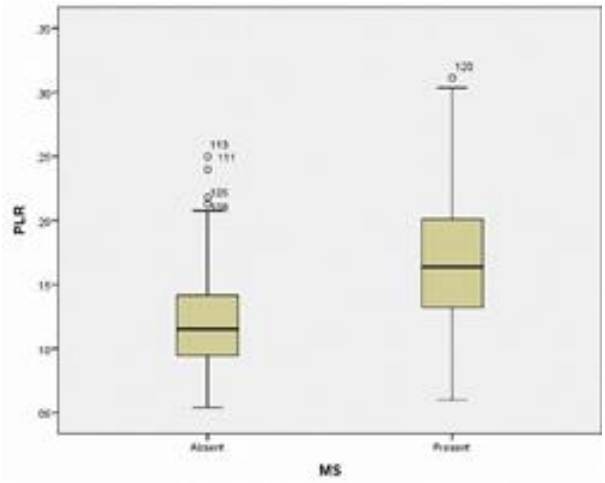

Figure-1: PLR values according to MS

The patients with MS had significantly higher PLR level compared to those without MS. However, PLR level were not associated with severity of MS (Figure 2).

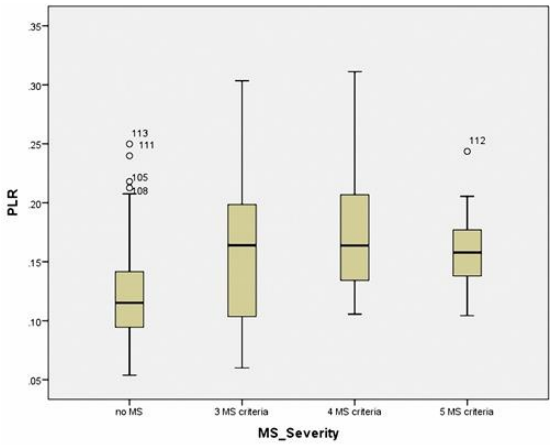

Figure-2: PLR values according to MS severity

A moderate positive correlation was revealed between the severity of MS and PLR (r=0.419, p<0.001) and a strong positive correlation between the severity of MS and hs-CRP (r=0.562, p<0.001) was found (Figure 3 and 4).

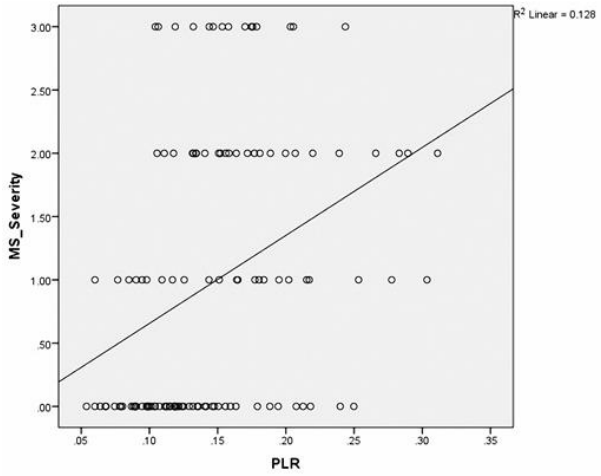

Figure-3: Correlations of MS severty with PRL

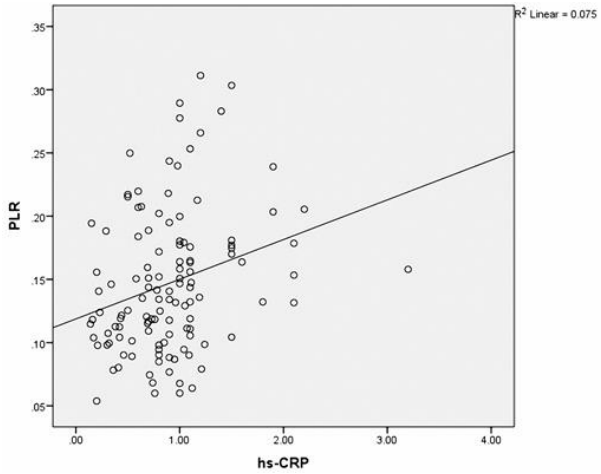

Figure-4: Correlations of PRL with hs-CRP

Also, a positive correlation between hs-CRP and PLR was detected in our patient population (r=0.281, p=0.002). In the subjects without MS, PLR was detected to be significantly lower compared to those with MS meeting three, four, and five criteria (0,12±0,04 *vs* 0,16±0.06, 0,18±0.06, and 0,16±0.04). However, there were no differences between the patients meeting three, four and five MS criteria (0,16±0.06, 0,18±0.06, and 0,16±0.04, three vs four p:0,169, three vs five p:0,963, four vs five p:0.241).

In order to specify the level of PLR to predict MS, ROC curve analysis was performed. The cut-off level for PLR with optimal sensitivity and specificity was calculated as 0,132 (Area under curve [AUC] =0.744 [0.655-0.833], p<0.001). For that level, the sensitivity was 73% and the specificity was 68,4% (Figure 5).

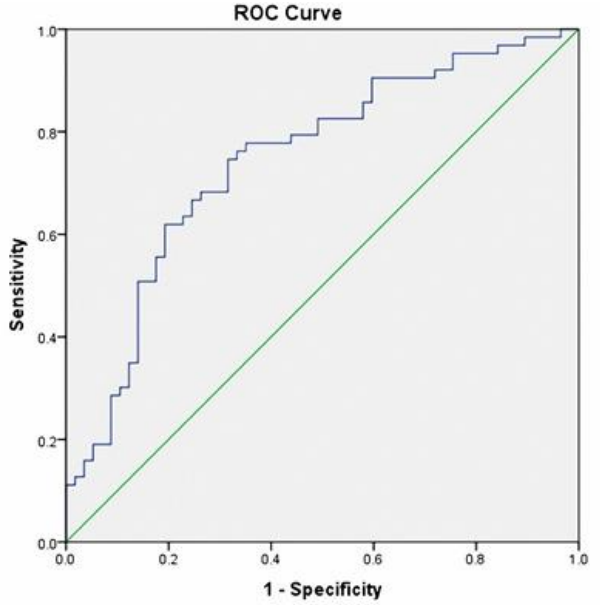

Figure-5: Receiver–operating characteristic (ROC) curve analysis plot to determine the cutoff value of PRL in MS

Discussion

As a summary of the results of this study; firstly, an important correlation between the inflammation and PLR, which was recognized as an indicator pf pro-thrombotic progress, was detected. Secondly, there was no correlation between the increase in PLR and the increase in the number of MS criteria. Thirdly, a significant relation between PLR and hs-CRP level associated with inflammation in the patients with MS was detected. The data obtained in this study about the relation between PLR and MS is the first findings in the Literature.

MS arises from the combination of the metabolic sourced risk factors increasing the development of atherosclerosis, and is a metabolic disorder based on IR (Moreno et al., 2004). The classical components of MS are the increased BP, atherogenic dyslipidemia, glucose intolerance, central obesity, vascular inflammation and pro-thrombotic state. These components demonstrate the treatment goals in addition to the diagnosis of metabolic (Bloomgarden, 2003). The relation of almost all of MS components with the systemic inflammation was presented (Fröhlich et al., 2000). The simplest indicator of the increased proinflammatory state in the patients with MS is the increased C-reactive protein level. Moreover, as the number of MS components increases, the amount of C-reactive protein increases, too. The increased C-reactive protein amount is related to the increased cardiovascular cases (Ridker et al.,

2003). This causes to think that both diabetes mellitus and atherosclerosis may be related to the inflammation (Haffner et al., 2006).

In the case of obesity, proinflammatory and proatherogenic cytokines, one of the criteria composing MS, such as IL-6, TNF-α, resistin, visfatin, the omentum, leptin, plasminogen activator inhibitor -1(PAI-1) and many bioactive adipokinine are released from the increased adipose fat tissue (Mazurek et al., 2003; Baker et al., 2006; Fain et al., 2008). IL-6 level, one of these inflammatory cytokines increases in parallel with the fat tissue, and IL-6 also induces the production of C-reactive protein. The numerous inflammatory cytokines released from the adipose tissue such as TNF-α and IL-6 induce the inflammatory activity, an important part of the atherosclerotic process (Chan et al., 2005). PLR is an important inflammatory marker found in the recently performed studies; and it was detected to be related to the major negative cardiovascular cases (Azab et al., 2012).

The hyperglycemic state consisted as a result of IR leads to the abnormalities in the coagulation system, and causes to the pro-thrombotic environment at the result of the platelet dysfunction and the generation of thrombin (Desouza et al., 1999). One of the best indicators of the pro-thrombotic state in the patients with MS is the increased PAI-1 and the fibrinogen level in the patients with obesity. The fibrinogen reflects the inflammatory activity as an acute phase reactant, and as well as is recognized as one of the new risk factors (Ross et al., 1999). This increases the tendency to the generation of thrombus leading to the development of acute coronary cases. It is a fact known for a long-term that the platelet activation and the aggregation have a role in the pathophysiology of acute coronary syndromes (Vaughan et al., 2002). For example; there is a correlation between the mean platelet volume (MPV) and the functional status of platelets. In many studies, it was showed that MPV was higher in the patients with CAD and acute coronary syndrome in proportion to the normal individuals (Kishk et al., 1985; Bath et al., 1996). Moreover, MPV was detected to be an independent indicator of reperfusion and mortality (Huczek et al., 2005). Therefore, the platelet activation has an important role in the onset and the progress of atherosclerosis (Tsiara et al., 2003). On the other side, there are studies suggesting that the low number of lymphocytes composing PLR in peripheral blood is related to the major cardiac negative cases (Ommen et al., 1997; Acanfora et al., 2001). Thus, PLR is recognized as a new marker compounding both two hematological parameters, and has been detected to be related to some major negative cardiovascular cases in a certain number of recent studies. It was also detected that the high level of PLR was an indicator of long-term mortality in non-ST elevation myocardial infarction (Azab et al., 2012).

This is the first study about this subject performed in the patients with MS although there are previous studies determining the relationship between PLR and the negative cardiovascular cases. The high PLR in the patients with MS may be the beginning and the predictor of the increased procoagulant and proinflammatory state, and may be the preview of the adverse cardiovascular events in these patients.

Limitations

The most important limitation of the study is the low number of the patients included in the study. The second one is to make the assessment supposing that each component of MS has the same effect. It could be more beneficial to evaluate the individual differences of the components of MS and to analyze their relations with PLR one by one. But, this could not be performed as the patient number was insufficient for the subgroup analysis.

Conclusion

This study is the confirmation of the existence of the increased inflammation in the patients with MS by PLR which is a new marker. This study is important from the clinical view as it suggests that PLR may be used in predicting the adverse cardiovascular cases in the patients with MS and the beginning of the cardiovascular disease development.

Informed Consent: Verbal informed consent was obtained from patients who participated in this study
Peer-review: Externally peer-reviewed.
Author Contributions: Concept YK.; Design AK.; Supervision AK.; Materials YK,AK; Data Collection and/or Processing YK, OB, ZYG, AB.; Analysis and/or Interpretation ZYG; Literature Review MKÇ OB; Writing OB; Critical Review YK
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study hasn't received any financial support.

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CASE REPORT

Odontogenic Myxoma Located in The Mandible: A Case Report

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Abstract
Odontogenic myxomas are benign but locally invasive tumor originating from primordial mesenchymal tooth forming tissues which do not metastasise. They can be found in both the maxilla and mandible, usually associated with a tooth germ. It occurs mostly in the second or third decades of life and affects mainly the posterior mandible. Radiographically it appears as a unilocular or multilocular radiolucency with irregular margins. They are usually asymptomatic, with the potential to attain great size without noticeable signs and symptoms. Nevertheless, in the current literature, several symptoms have been associated with odontogenic myxomas: tooth displacement and mobility, malocclusion, facial asymmetry, delayed eruption of teeth, disturbance of speech and mastication,pain, and mandibular nerve paresthesia. They are rare tumors and account for 3.3-15.7% of all odontogenic tumors in adults.
In this case report, a case of Odontogenic myxoma (OM) localized in the mandible was presented.
Key words: Enucleation, mandible, odontogenic myxoma.

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*In this case report, a case of Odontogenic myxoma localized in the mandible was presented.

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Introduction

Odontogenic myxoma (OM) is a benign, locally invasive and aggressive, non-metastasizing neoplasm of the jaw bones (Leiser et al., 2009). It is a slow growing tumor consisting of an accumulation of mucoid ground substance with little collagen, the amount of which determines whether it can be called a myxofibroma (Simon et al., 2004). Histopathologically these benign neoplasms were classified by the World Health Organization as benign odontogenic neoplasms of ecto-mesenchymal origin consisting of rounded and angular cells embedded in an abundant myxoid stroma with few collagen fibrils probably originating from either the dental papilla follicle or periodontal ligament (Mayrink et al., 2013).

OMs are one of the most common lesions of jaw bones, ranging from about 3% to 8% of odontogenic tumors and cysts (Keszler et al., 1995). Most of the OMs reported were young adults affected mostly in their second and third decade of life with marked female predilection (Manne et al., 2012). OM is generally depicted as slow growing tumor with the potential to attain considerable size without noticeable signs and symptoms. The molar and ramus regions of the mandible are most frequently involved, whereas the premolar–first molar region is the site of predilection in the maxilla (Noffke et al., 2007). The possible surgical management can vary from simple curettage and peripheral ostectomy up to segmental resection (Leiser et al., 2009; Manne et al., 2012; Mayrink et al., 2013).

Case

A 33 year-old man presented to Ordu Faculty of Dentistry, Department of Oral and Maxillofacial Surgery for routine dental examination. Clinical examination revealed no facial asymmetry extra orally on inspection. On palpation, there was no swelling or tenderness present. The family history was non-contributory and there was no trauma history or any developmental abnormalities. In intraoral examination there was no buccal and lingual cortical plate expansion. Panoramic radiograph was obtained for radiographical examination. In the panoramic radiograph there was an uniocular radiolucencies at left mandible; from canine to mesial root of first molar **(Fig 1).**

Figure 1: Preoperative OPG view showing uniocular radiolucent lesion involving mandibula.

Differential diagnosis of dentigerous cyst, odontogenic keratocysts, unicystic calcifying epithelial odontogenic tumor and ameloblastoma was considered. We decided to treat the lesion with associated non-vital teeth root resection and enucleation. 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. Under local anesthesia a mucoperiosteal flap was raised from the lower left mandible from canine to first molar. Bone was removed with a bur and adequate coolant.

The lesion was removed and premolars were resected from apical. The lesion was sent to pathological analysis **(Fig 2-A)**. Pathological report showed that the lesion was odontogenic myxoma **(Fig 2-B, C, D).**

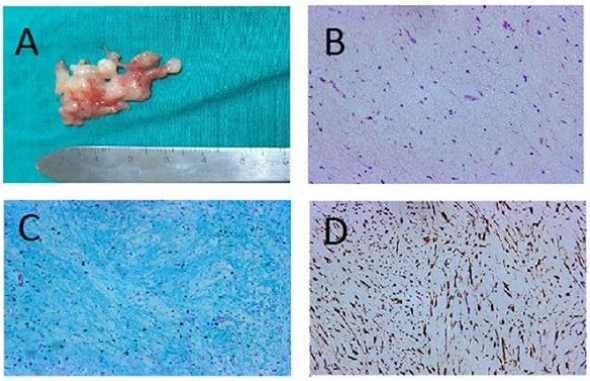

Figure 2: **A)** The lesion was removed. **B)** The histology is essentially identical to that seen in H&E. **C)** Myxoid stroma stained with alcalin blue. **D)** The cells are immunoreactive with antibody against vimentin.

The surgical wound was closed primarily with silk sutures. Antibiotic therapy (amoxicillin+clavulanate, 1250mg/day), and an analgesic (paracetamol) were prescribed after surgery. The patient was reassessed 7 days after surgery. The sutures were removed with satisfactory wound healing**.** The postoperative course was uneventful and the patient was asymptomatic in the 15 months period of follow-up **(Fig 3).**

Figure 3: Postoperative OPG view 15 months after surgery.

Discussion

OM is regarded as a locally invasive tumor that does not metastasize and presents slow and asymptomatic expansion, sometimes resulting in perforation of the cortical borders of the affected

bone (Zainine et al., 2013). In many cases, these lesions are diagnosed accidentally by a routine dental checkup appearing as a "soap bubble " (Kawase-Koga et al., 2014). Clinically, myxomas are benign, insidious lesions,often largely asymptomatic. Thus they most commonly appear as a painless swelling which can attain a large size before diagnosis (Halfpenny et al., 2000) Other symptoms tend to manifest later, as a consequence of expansion in relation to adjacent structures. These include pain or, especially in the maxilla, involvement of surrounding soft tissues (Halfpenny et al., 2000; Boffano et al., 2011; Zainine et al., 2013).

Radiographically, OMs frequently appear as unilocular or multilocular radiolucencies with well-defined margins and fine bony septa. The lesion sizes are correlated with their locularity (Kaffe et al., 1997; Morihiro Higo, 2015). Lesions >40 mm tend to be multiloculated, and smaller lesions tend to be uniloculated (Kaffe et al., 1997; Morihiro Higo, 2015). The presentation often is describedas a honeycomb, soap-bubble, tennis-racket, or ground-glass pattern (Morihiro Higo, 2015). Differential diagnosis like ameloblastoma, ameloblastic fibroma, odontogenic fibroma, central hemangioma, or odontogenic keratocyst along with odontogenic myxoma could be listed as initial diagnostic hypothesis based on the clinical and radiological findings (Manne et al., 2012). Computed tomography was more likely to display a cortex and its perforation, tooth displacement and root resorption were more reliably observed on conventional radiography (MacDonald-Jankowski et al., 2004).

OM is associated with a high rate of recurrence, due to its gelatinous nature and the absence of a capsule; prolonged clinical and radiological surveillance is therefore mandatory. The lesion is not radiosensitive and studies have shown no long-term cure with radiotherapy alone (Landa et al., 2002)

Radical surgery, excision, or enucleation followed by curettage of the surrounding bony tissue have all been advocated as treatment options (Lo Muzio et al., 1996; Li et al., 2006; Rocha et al., 2009). Complete surgical removal by conservative treatment can be difficult, because, unlike most benign neoplasms, the myxoma is not encapsulated and its myxomatous tissue infiltrates the surrounding bony tissue without its immediate destruction (Lo Muzio et al., 1996). Conservative treatments like enucleation and curettage have several advantages over more radical treatments, such as segmental or block resection, and hemimandibulectomy with reconstruction surgery (Kawase-Koga et al., 2014). Conservative treatments are substantially less invasive, can be achieved by means of an intraoral surgical approach, preserve function and aesthetics, have a shorter hospitalization time, and are more cost-effective (Rocha et al., 2009; Kawase-Koga et al., 2014). Nonetheless, the risk of recurrence after more conservative surgery is higher as the myxoma is not encapsulated and its myxomatous tissue infiltrates the surrounding bony tissue without causing immediate destruction (Colburn and Epker, 1975; Lo Muzio et al., 1996; Li et al., 2006; Rocha et al., 2009; Boffano et al., 2011; Kawase-Koga et al., 2014). Few authors in the literature in the last 20 years with a consistent number of patients report on surgical treatment, follow-up, and recurrence of their study populations. According to Sloootweg's et al. (1986) 15 patients the maxilla was involved in 4 cases whereas the tumor was situated in the mandible in 11 cases. Of the 9 patients who underwent conservative treatment, one exhibited recurrent tumor. Six patients were treated by resection including uninvolved adjacent tissue; none of them has so far exhibited recurrence (Slootweg and Wittkampf, 1986). Li's identified that in their 5 cases treated conservatively by enucleation, the remaining 20 cases were treated by relatively radical procedures, including block/segmental resection and partial or total maxillectomy or mandibulectomy. Follow-up data were available on 22 patients and only 1 patient initially treated by enucleation had a recurrence (Li et al., 2006). According to Boffano et al. (2011) 3 patients of the 10 patient treated by enucleation and curettage. Instead, in the remaining 7 patients, segmental resection and immediate reconstruction were decided. At follow-up, no patient showed recurrence of the lesion (Boffano et al., 2011). In the mandible it should be possible to extirpate carefully all macroscopically visible turnout tissue by enucleation followed by curettage of the surrounding bone (Slootweg and Wittkampf, 1986). Perhaps a conservative approach may be used for smaller lesions to preserve function, reserving more radical surgery for recurrences and larger lesions (Lo Muzio et al., 1996; Kansy et al., 2012; Kawase-Koga et al., 2014).

Conclusion

Our protocol is to perform conservative surgery by enucleation and curettage when lesions were

smaller than 3 cm, whereas a segmental resection with immediate reconstruction is preferred in patients affected by bigger tumors. Long-term follow-up is required, in particular when conservative surgery is preferred.

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