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Erakıncı G. Searching for antibodies against parasites in donors. İzmir: Ege University Health Sciences Institute. 1997.
Article in Electronic Format
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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Abstract (average 200-400 word)
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MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE

AUGUST 2019 **VOLUME 5** **ISSUE 2**

CONTENTS

EDITORIAL

The fifth year…

This year there are seven articles in our secaond issue, fifteen original.

The articles' branches are Parasitology, Moleculer biology, Orthopedic, gynecology, Microbiology, Biochemistry, Dentistry, Neurosurgery, Physiology and Internal Medicine. While the one original in article was reviewing incidence of Metabolic Syndrome in Subclinical Hypothyroid patients, the another was about antimicrobial activity of silver nanoparticles synthesized with extract of tomato plant against bacterial and fungal pathogens. In addition, others report effects of autologous platelet-rich plasma on endometrium thickness and pregnancy rates during intrauterine insemination. The moreover presented the clinical predictive value of the neutrophil to lymphocyte ratio as a biomarker in lumbar disc herniation. There are also a lot of research that I think will interest you in this issue.

Many thanks to the authors, referees and editorial board who contributed to this issue hope to meet again in different and new issues you have pleasure while reading…

PhD, Assoc. Prof. Ülkü KARAMAN

Editor

Middle Black Sea Journal of Health Science August 2019; 5(2):47-53

RESEARCH ARTICLE

The incidence of Metabolic Syndrome in Subclinical Hypothyroid patients

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Abstract

Objevtive: The aim of this study was to evaluate the incidence of metabolic syndrome, a risk factor for chronic diseases such as cardiovascular diseases, diabetes mellitus and stroke, in patients with subclinical hypothyroidism.

Methods: A total of 108 patients with subclinical hypothyroidism followed in the outpatient clinic of Ordu University Faculty of Medicine Training and Research Hospital between 2015-2018 were included in the study. Height, waist circumference, weight, blood pressure, High Density lipoprotein (HDL) cholesterol and triglyceride levels and fasting blood glucose levels were recorded from the archive records of the patients. Body Mass Index (BMI) was calculated. Metabolic Syndrome (MetS) diagnoses were made according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines.

Results: Of the 108 patients included in the study, 74 (68.5%) were female and 34 (31.5%) were male. The mean age of the female patients was 38.3±12.5 years and the mean age of the male patients was 40.5±11.7 years. In this study, the prevalence of MetS was 42.6% (47.3% in females and 30.8% in males) in patients with subclinical hypothyroidism. MetS incidence was 1.5 times higher in female patients than in male patients. Among all patients, the most common MetS component was low HDL cholesterol (54.6%) and abdominal obesity (52.8%).

Conclusion: The incidence of MetS was higher in female patients with subclinical hypothyroidism than in male patients. In addition, close follow-up of patients with subclinical hypothyroidism in terms of Metabolic Syndrome, which is a risk factor for many chronic diseases such as diabetes mellitus, cardiovascular diseases and stroke, will benefit in reducing the mortality of patients.

Key words: Subclinical hypothyroid, metabolic syndrome, incidence.

Suggested Citation: Dugeroglu H. The Incidence of Metabolic Syndrome in Subclinical Hypothyroid Patients. Middle Black Sea Journal of Health Science, 2019; 5(2): 47-53.

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Introduction

Metabolic Syndrome (MetS), as in the whole world, is a definition that includes important risk factors for diseases with high mortality rates such as diabetes mellitus, cardiovascular disease and stroke in our country (Balkau et al., 2007). Today, the diagnosis of MetS is based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines, which is accepted by many researchers (National Cholesterol Education Program (NCEP) Expert Panel on Detection, 2002). According to NCEP ATP III criteria, low HDL cholesterol level, high

triglyceride level, increased plasma glucose level, increased waist circumference and increased blood pressure are diagnosed by the presence of three and / or more of the five criteria (National Cholesterol Education Program (NCEP) Expert Panel on Detection, 2002).

In many literature studies, the prevalence of MetS is seen between 7-46.5% in women and 8-24.2% in men in the adult population (Ford et al., 2002; Gupta et al., 2003). However, many parameters such as age, gender, dietary habits, physical activity level, diet and genetic structure affect the prevalence of MetS. MetS is also a proinflammatory and prothrombotic state (Reaven, 1988). In one study, it is thought that the most important reason for MetS development is decreased insulin sensitivity and increased insulin level (Grundy, 2006). In another study, it has been observed that patients with MetS have increased mortality by reasons such as diabetes, cardiovascular diseases and stroke (Balkau et al., 2007).

Nowadays, especially in the excess or lack of thyroid hormones, there are significant changes in the waist circumference, weight and Body Mass Index (BMI) of individuals (Lonn et al., 1998). In addition, this poses a risk for many important diseases such as cardiovascular diseases, diabetes and stroke (Kllein, 1989). Increased vascular resistance in hypothyroid patients may cause blood pressure development (Morkin et al., 1983). Although the effect of TSH on adipose tissue in obese patients is not yet fully understood, In the study of Karakurt et al. (2009) the relationship between thyroid function and obesity was investigated and as a result, they stated that pituitary gland contributed to obesity independently of sT3 and sT4 by TSH.

The incidence of subclinical hypothyroidism increases with age. Especially, it is more common in women (Hollowell et al., 2002). In a study, while it is seen in 2.9% of men, it is seen in 11.6% of women. In addition, thyroid autoantibodies are positive in most patients because of the etiologic cause of Hashimato disease, which is the underlying cause of many patients with subclinical hypothyroidism (Parle et al., 1991). Because the diagnosis of subclinical hypothyroidism is clinically insignificant, it can often be difficult to diagnose. TSH measurement is helpful in the diagnosis of subclinical hypothyroidism with high sensitivity. With advanced age, the thyroid gland is atrophied. In these individuals, the level of sT4 decreases by 50-60%, but with the decrease in sT4 consumption, the level of sT4 can be normal. Therefore, TSH level may be moderately high in patients. Patients are diagnosed with subclinical hypothyroidism with these laboratory values (Parle et al., 1991).

Both subclinical hypothyroidism and MetS are considered as atherogenic risk factors (Monzani et al., 2006). In both cases, it is important to increase the tendency for hypertension, dyslipidemia and hypercoagulation in patients (Foldes et al., 2004). The aim of this study was to evaluate the prevalence of Metabolic Syndrome, a risk factor for chronic diseases such as diabetes mellitus, cardiovascular diseases and stroke, in patients with subclinical hypothyroidism.

Methods

The records of 108 patients with subclinical hypothyroidism who were followed-up and treated at the Internal Medicine Department of Ordu University Faculty of Medicine between 2015-2018 were evaluated retrospectively. Free T4 (fT4) (N:0.8-1.8 ng/dL) and free T3 (fT3) (N:2.0-4.4 ng/dL) levels in the normal range, TSH level between 4.2-10 ng/dL all patients older than 18 years of age with a diagnosis of subclinical hypothyroidism were included.

Age, gender, waist circumference, height, weight, blood pressure, HDL cholesterol and triglyceride levels and fasting blood glucose levels were recorded. Body mass index (BMI) was calculated. BMI was calculated by dividing the patient's weight (kilograms) by the square of height (meters). According to World Health Organization (WHO) criteria for the classification of BMI <18.5 kg/m^2, underweight; 18.5-24.9 kg/m^2, normal; 25-29.9 kg/m^2, overweight; 30-39,9 kg/m^2, obese; ≥40 kg/m^2 was determined as morbid obese. In addition, the obesity frequency was defined as obesity in accordance with the WHO criteria with a BMI ≥ 30 kg/m^2. The waist circumference was measured from the plane passing between the lower costa and the spina iliaka anterior superior, while the patient was standing (World Health Organization (WHO), 1998). Blood pressure measurements were made using the sphygmomanometer from both arms in sitting position after resting for at least 15 minutes. After 8-12 hours fasting, HDL cholesterol and triglyceride values and fasting plasma glucose levels were evaluated. MetS diagnoses were made

according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines (Table 1). Patients who had three and/or more positive criteria for the five criteria in the NCEP ATP III diagnostic guideline were accepted as MetS.

Table 1. National Cholesterol Education Program Adult Treatment Panel III Criteria for Metabolic Syndrome (NCEP ATP III).

Risk Factor	
Abdominal obesity (Waist circumference)	
Male	>102 cm
Female	>88 cm
HDL Cholesterol	
Male	<40 mg/dL
Female	<50 mg/dL
Triglycerides	>150 mg/dL
Arterial blood pressure	≥ 130/85 mmHg
Fasting plasma glucose	≥ 100 mg/dL

Exclusion Criteria: Patients with incomplete and/or inadequate archive records, patients with thyroidectomy, patients with overt hypothyroidism, pregnant women, patients with chronic kidney disease and chronic liver disease, cardiovascular disease (such as hypertension, heart failure, coronary artery disease) and patients receiving treatment for diabetes mellitus, patients with malignancy, patients with cerebrovascular disease, patients with endocrine diseases that may cause obesity, and patients with MetS previously were excluded from the study.

Statistical Analysis: Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp. Armonk, NY). In the evaluation of the data; number (n), percentage (%), mean and standard deviation were used for descriptive statistics. Data were analyzed by Kolmogorov-Smirnow test. Student-t test was used to compare the groups. Pearson and Spearman tests were used for correlation analysis. P value less than 0.05 was considered significant.

Results

The study included 108 patients with subclinical hypothyroidism. 74 patients were female (68.5%) and 34 (31.5%) were male. The mean age of the patients was 42.5±10.5 years (mean age of female patients was 38.3±12.5 years, and the mean age of male patients was 40.5±11.7 years). The clinical and laboratory features of the patients with subclinical hypothyroidism were divided into two groups according to their gender and groups were compared in terms of BMI and MetS criteria (Table 2). Accordingly, there was a significant difference between male and female groups in height, weight, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, triglyceride level and fasting plasma glucose levels (p=0.007; p=0.032; p=<0.001; p=0.023; p=<0.001; p=0.021; p=<0.001; p=<0.001).

Table 2. Clinical and laboratory characteristics of patients diagnosed with subclinical hypothyroidism according to gender, comparison of values according to gender.

Variables	All patients (n:108)	Female patients (n:74)	Male patients (n:34)	P value
	Mean±SD	Mean±SD	Mean±SD	
Age (years)	42,5±10,5	38,3±12,5	39,5±11,7	0,341
Height (cm)	158±5,3	162±4,2	168±3,6	0,007
Weight (kg)	75,5±15,1	80,7±14,7	76,1±13,8	0,032
BMI (kg/m^2)	31,5±6,7	33,8±7,9	29,6±8,5	<0,001
Obesity (BMI> 30 kg/m^2), (%)	53,8	53,6	52,3	0,211
Waist circumference (cm)	98,6±16,9	101,5±16,7	93,2±13,2	0,023
Systolic blood pressure (mmHg)	121,3±18,2	128,8±17,1	114,2±17,4	<0,001
Diastolic blood pressure (mmHg)	75,3±11,3	78,8±14,7	73,2±16,2	0,021
HDL cholesterol level (mg/dL)	49,7±14,2	48,5±10,8	48,4±12,7	0,642
Triglyceride level (mg/dL)	125,2±12,8	158,6±16,8	134,1±13,2	<0,001
Fasting plasma glucose (mg/dL)	98,4±19,7	104,2±14,6	97,6±12,9	<0,001
MetS incidence (%)	42,6	47,3	30,8	<0,001

n: Number,%: Percent, Mean ± SD: Mean ± standard deviation, BMI: Body mass index, HDL: High Density lipoprotein, MetS: Metabolic syndrome

Table 3. Comparison of the frequency and gender of the metabolic syndrome components in male and female patients with subclinical hypothyroidism.

Variables	All patients (n:108)	Female patients (n:74)	Male patients (n:34)	P value
Abdominal obezite, (%)	52,8	54,6	51,3	0,021
Low HDL cholesterol, (%)	54,6	57,3	52,2	<0,001
Increased Triglyceride, (%)	36,8	38,7	37,2	0,342
Increased Fasting plasma glucose, (%)	18,7	24,7	6,8	<0,001
Increased Arterial blood pressure, (%)	39,1	43,6	23,7	<0,001
Metabolic Syndrome, (%)	42,6	47,3	30,8	<0,001

n: Number, %: Percent, HDL: High Density lipoprotein.

The incidence of Metabolic Syndrome and Metabolic Syndrome components were evaluated for male and female patients with subclinical hypothyroidism. Accordingly, the incidence of MetS was 42.6% (47.3% in females and 30.8% in males) in patients with subclinical hypothyroidism. MetS incidence was 1.5 times higher in female patients than in male patients. In addition, according to Metabolic Syndrome components, abdominal obesity, triglyceride elevation, fasting plasma glucose elevation and high blood pressure were higher in the female patient group ($p = 0.021$; $p < 0.001$; $p < 0.001$; $p < 0.001$; 0.001) (Table 3). Among all patients, the most common MetS component was low HDL cholesterol (54.6%) and abdominal obesity (52.8%). This ranking was also valid for female and male groups (The frequency of HDL cholesterol in female group was 57.3%, abdominal obesity frequency was 54.6%, HDL cholesterol in male group was 52.2%, abdominal obesity frequency was 51.3%) (Table 3).

Discussion

Metabolic Syndrome, hyperglycemia, elevated blood pressure, dyslipidemia, visceral obesity, insulin resistance as well as hyperuricemia, microalbuminuria, atherothrombosis tendency and vascular inflammation with many features such as cardiovascular, metabolic and renal complications are among the most common and most important causes (Oguz, 2006). In general, a patient should be investigated for the underlying etiologic cause after the diagnosis of MetS. The most important reasons of MetS are sedentary life and obesity (Rennie et al., 2003). In addition, thyroid function should be evaluated in these patients. In a study, it was reported that the incidence of MetS increased in patients with normal free T4 levels and diagnosed as subclinical hypothyroidism (Roos et al., 2007). Also, in this study, it was stated that high TSH level caused insulin resistance in patients and played a role in the development of MetS. In our study, the incidence of MetS in patients with subclinical hypothyroidism was 42.6%. In another study, it was found that subclinical hypothyroidism caused glucose intolerance in patients, increased tendency to hypertension, decreased HDL level and increased LDL level (Tamer et al., 2005). In our study, the majority of patients had low HDL levels (54.6%), hypertension (39.1%) and increased glucose (18.7%).

Turkey Metabolic Syndrome Research Group (METSAR) published a report, the average incidence of the MetS in Turkey, 33.9% (39.6% for women and 28.0% among males) has announced (Kozan et al., 2007). According to the data of Turkish Adult Adults with Heart Disease and Risk Factors (TEKHARF), the prevalence of MetS in adults 30 years and older was 32.8% (38.6% in women and 27% in men) (Onat and Sansoy, 2002). Akbulut et al. (2011) found that the incidence of MetS was 30.2% in premenopausal women and 47.6% in postmenopausal women according to the NCEP ATP III criteria. Kitiş et al. (2010) found the incidence of MetS to be 31.9%. However, the incidence of MetS was higher in different literature studies. For example, Varlıbas et al. (2006) found that the frequency of MetS was 67.2% in patients who were followed up due to ischemic cerebrovascular disease in the neurology department, while Baltali et al. (2004) found the prevalence of MetS as 44.8% after coronary by-pass. In our study, we found the incidence of MetS in patients with subclinical hypothyroidism as 42.6% (47.3% in women and 30.8% in men). However, in previous studies, we believe that MetS itself is an increased atherothrombotic and proinflammatory tendency because of the high

prevalence of MetS. However, in our study, we believe that subclinical hypothyroidism has prepared itself for the development of MetS and that it is clinically associated with MetS. In a study carried out in Japan in 2006 and NCEP ATP III criteria were applied, the incidence of MetS was determined as 14.7% in women and 26.9% in men (Miyatake et al., 2006). According to the results of this study, the reason why our study rates are high may be due to the different nutritional habits of the countries, ethnicity, genetic factors and lifestyle characteristics.

Obesity, a component of metabolic syndrome, is an important risk factor for both cardiovascular diseases and many metabolic diseases. Demir et al. (2010) found that waist/hip ratio and BMI were higher in patients followed with MetS diagnosis and found that BMI was positively correlated with systolic blood pressure and diastolic blood pressure. In addition, in some studies, the risk of death in patients with BMI> 30 kg/m^2 was reported to be high (Onat et al., 2002; Bloomgarden, 2003). Also, in many studies, obesity has been reported to be included in changeable risk factors within MetS components (Bloomgarden, 2003; Misra and Khurana, 2009; Demir et al., 2010). In our study, the most common MetS component in patients with subclinical hypothyroidism was abdominal obesity (52.8%). In a study by Tagliaferi et al. (2001), TSH levels of obese patients were significantly higher than the control group. They reported that TSH levels were normal in proportion to the weight they had given after their diet due to obesity. However, they reported that levothyroxine treatment for obese patients with high TSH levels did not alter the weight status and lipid profiles of the patients. In the light of all these findings, it is emphasized that elevated TSH level will be a cause of obesity etiology (Tagliaferi et al., 2001). Karakurt et al. (2009) in the study of the obese, TSH level was higher than the control group. However, no significant relationship was found between sT3-sT4 and obesity. As a result of this and similar studies, it is concluded that TSH is a risk factor independent of thyroid function in obesity (Bengel et al., 2003; Karakurt et al., 2009).

In addition, in some studies, subclinical hypothyroidism was not associated with MetS, but the rate of subclinical hypothyroidism was higher in the patients followed with the diagnosis of MetS than in the control group (Takashima et al., 2007). In a study by Shanta et al. (2009), they found the incidence of MetS in males with subclinical hypothyroidism in the Indian population, especially in female patients (21.9% versus 6.6%). In our study, the incidence of MetS in female patients with hypothyroidism was 1.5 times higher than in male patients (47.3% in females and 30.8% in males). Some literature studies recommend levothyroxine treatment for patients diagnosed with subclinical hypothyroidism and MetS components. Although Levothyroxine treatment has been shown to improve MetS components, this approach has not yet been accepted by the authorities (Fadeyev et al., 2006; Villar et al., 2007). However, there is a need for studies that require extensive follow-up of the patients with levodyroxine treatment, which shows whether there is an improvement in MetS parameters in the long term.

Conclusion

In conclusion, the incidence of MetS was higher in women with subclinical hypothyroidism than in men. In addition, close follow-up of patients with subclinical hypothyroidism in terms of Metabolic Syndrome, which is a risk factor for many chronic diseases such as cardiovascular diseases, diabetes mellitus and stroke, will benefit in reducing the mortality of patients.

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RESEARCH ARTICLE

Clinical Outcome of Arthroscopic Treatment of Anterior Ankle Pathologies

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Abstract

Objective: The ankles that are the main organs cause standing over the earth can be damaged by different reasons. The ankles become painful with the problems like impingement syndrome that is soft tissue hipertrofies, osteochondral defects that is cartilage damages, traumatic or degenerative arthropaties, intraarticular tumoral tissues and some problems like that. These painful ankles can be treated with conservative methods. If it is impossible to treat painful ankles with conservative way it can be treated by minimal invasive ankle arthroscopy that is cheaper and much more satisfied, of treatment.

Methods: We aim to evaluate the results and effectiveness of arthroscopic treatment of the patient who has the forefoot disorders. Also, we hope to compare the advantages, difficulties and probable complications of the arthroscopic treatment according to conservative therapy and to investigate the factors that can effect the clinical results. The 40 patients who were practiced and operated with ankle arthroscopy who was followed at least one year were compared statistically with pre-op and post-op condition. By this way the effectiveness of arthroscopy for the patients who have anterior ankle problems was determined and the main factors that effect the results were investigated. According to the result of statistical analyses preop and postop AOFAS and NPS score avarages were found clear different to each other.

Results: The ankle arthroscopy that is practised by experienced surgeons with an appropriate procedure after a good clinical practise and a proper indication causes a high patient satisfaction and it causes less and temporary complications. Because of all these reasons we believe that the ankle arthroscopy can be practised by the appropriate surgeon to the appropriate patient population.

Conclusion: We believe that the outcomes of ankle arthroscopy performed properly by experienced hands after determining the patient population who were accurately diagnosed by careful clinical examination and necessary imaging methods are greatly satisfactory, and the acceptability is high thanks to complications being less and usually transient.

Key words: Ankle arthroscopy, ankle pathologies, anterolateral impingement, osteochondral defect, ankle arthrosis

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Introduction

In Turkey, it is known that the first arthroscopy was performed in 1977 by Veli Lök, the first publication of a cadaver study on ankle arthroscopy was published by Pinar et al. (1989), and the first clinical trial was conducted by Aydin (1991)

Ankle arthroscopy is an endoscopic method which allows the visualization of the joint without the necessity to perform arthrotomy or malleolar osteotomy, and performing surgical intervention in the joint and the surrounding soft tissues (Aydin and Gokkus, 2013). It is a minimally invasive technique which is applied by placing the necessary devices through two incisions of approximately. 0.5 cm. The reasons why ankle arthroscopy is preferred include the visualization of all intraarticular structures and the possibility of intervention, low post-operative morbidity, and easy and rapid rehabilitation (Aydin, 1996).

Patients with anterior ankle pathologies whom were admitted to our clinic between 2011-2013 were divided into three groups (anterolateral impingement, osteochondral lesions, arthrosis) and had been evaluated regarding the need for arthroscopic treatment. Beneficence of arthroscopic management for the patients have been evaluated with comparison of the preoperative and postoperative AOFAS and NPS scores. 40 patients who had could have been properly followed up, out of 52 patients have been included to the study. We do think that this study will make significant contribution to the literature regarding the beneficence of arthroscopic surgery for the anterior ankle pathologies.

Methods

Patients who admitted to our clinic with ankle problem between July 2011 and July 2013 were assessed using AOFAS scores after a detailed history taking and physical examination in the outpatient clinic. Patients with the possible need for arthroscopic treatment were referred to thesis resident. All patients were examined by same physician and the problems were determined. Arthro-MRI was taken and interpreted by an experienced radiologist in many patients who were deemed necessary to undergo MR to examine the problems in the region more clearly. Patients with reflex sympathic dystrophy syndrome (sudeck atrophy), excessive edema around the joint, peripheral vascular pathology, open wound in operation site, tinea pedis, or cellulitis underwent a therapy program based on their underlying condition and were referred to the related clinics. Patients with anterior ankle pain for at least 3 months who did not benefit or even worsened using conservative methods and physical therapy were informed about arthroscopic treatment and recommended to undergo arthroscopy if there is a possibility to find a solution using arthroscopic treatment.

Three groups were formed from patients with anterior ankle pathologies including impingement syndrome (including the ones of osseous and soft tissue origin), osteochondral talus defect, and ankle arthrosis due to various reasons, and included into the study. The radiological appearance of the lesions of the patients with osteochondral defect were grouped before the operation based on the classification specified by Berndt and Harty.

All patients were assessed before the operation using AOFAS (American Orthopedic Foot & Ankle Society) ankle-hindfoot scale and NPS (Numerical Pain Scale). Following the last vital sign control in the ward, the patients received antibiotic (IV, 1 gr cefazolin). Important anatomic structures were drawn using skin pen in the operating room. Using fourth toe traction and foot inversion, effort was made to observe superficial peroneal nerve. The patients received anesthesia of their choice after being informed. The patients were laid down on their back in a position as described by Parisen. A pillow was placed under the hip of the operation side (toes pointing towards the ceiling). Additionally, another pillow was placed on the cruris midline of the operation side to facilitate ankle manipulation and use the instruments effectively. The foot was in a position extending out of the table. The operation table was adjusted to a level so that the surgeon can manipulate the foot freely with his belly. Tourniquet was not used in any of the patients.

Four mm 30 degree scope which is used for knee arthroscopy was used. Manual traction device made of sterilized gauze was wrapped around the ankle and foot and prepared for the procedure. 10-15 cc of isotonic injection was injected into the joint through anteromedial soft point. The degree of easiness of the fluid flow was observed, and the ankle was observed to go into dorsiflexion with the ongoing fluid injection. The syringe was removed by leaving the needle inside, and fluid was observed to flow out.

Fluid entry to the joint was applied under control by Y-pump using 3000 mL of isotonic with 0,5 mg of adrenaline inside. During the case, shaver and RF device were kept ready to use. As the operation period did not reach 2 hours in any of the patients, no additional antibiotic was required during the

operation. Postoperative splint-plaster cast application was not necessary in any of the patients.

They were told they can walk for necessary activities for the first 3 days and gradually increase their walking without overdoing for the first 3 weeks. Osteochondral defect pathology patients with stage II and stage III lesion underwent debridement together with arthroscopic microfracture operation, and patients with stage IV lesion underwent curettage and arthroscopic microfracture operation after fragment removal. All patients who underwent microfracture operation were requested to start ankle exercise on the postoperative second day and recommended to avoid putting load on that ankle for three weeks. After three weeks, exercises for increasing joint range of motion and muscle strength were recommended for three months by putting partial load on the ankle. Sports activities were forbidden for 6 months.

Unless another problem arises, the patients were requested to return for 3-week, 3-month, 6-month and 1-year follow-up visits. In the follow-up visits, all patients were assessed using AOFAS ankle-hindfoot score and NPS score. Patients with a postoperative follow-up period less than a year were excluded from the study. After the completion of the study, the patients were grouped, and their AOFAS and NPS score progression was evaluated. As patients who underwent arthroscopic debridement and grafting due to mass in talus, and septic arthritis irrigation-debridement during the study were not suitable for any of the 3 groups or other diseases, and as their number was not as high as to make an evaluation, they were excluded from the evaluation.

Results

In descriptive statistics, numerical data were expressed as mean and standard deviation, and categorical data as number and percentage. For the comparison of disease groups and pathologies, Chi-Square and Fisher's Exact tests were used. For the comparison of the group scores, Kruskal-Wallis test was used. For the comparison of preoperative, and postoperative 3-month and 1-year data of the patients (for repeated samples), ANOVA test and Wilcoxon test were used. A p value of <0.05 was considered to be significant.

The study included 40 patients. The mean age of the cases was 44.1 ± 12.3 years. Twenty-six of the patients (65%) were males, and 14 (35%) were females. The pathological side which underwent operation was right ankle in 18 (45%) patients and left ankle in 22 (55%). The weights of the patients were grouped as thin, normal, overweight and obese. No significant relationship was found in the comparison of sides, sex and weights with disease groups.

The patients were classified by 3 disease groups and 8 different pathologies. The following table shows the percentage of the patients by group and pathology (Table 1).

Table 1. Patient distribution by pathology

	Number	Percentage
Anterior Impingement	5	12,5
Anterior Osseous Impingement	3	7,5
Anterolateral Impingement	7	17,5
Anteromedial Impingement	1	2,5
Medial OCD	8	20,0
Anterolateral OCD	3	7,5
Traumatic Arthritis	10	25,0
Degenerative Arthritis	3	7,5
Total	40	100,0

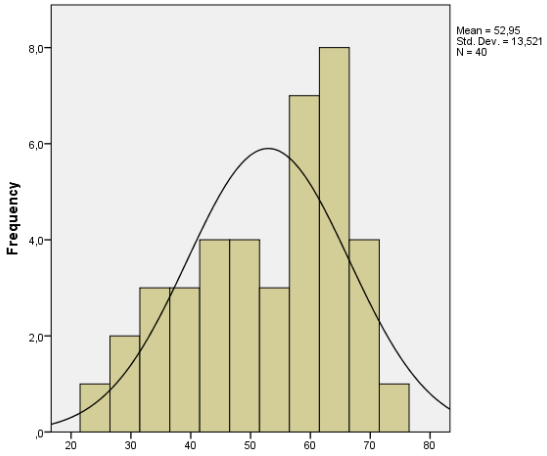
The patient's preoperative, postoperative 3-month, postoperative 1-years and last follow-up visit AOFAS values, and preoperative NPS and postoperative last follow-up visit NPS values were checked. Significant changes were observed in postoperative AOFAS values (Table 2).

Table 2. The mean AOFAS and NPS scores of the patients in pre- and postoperative period

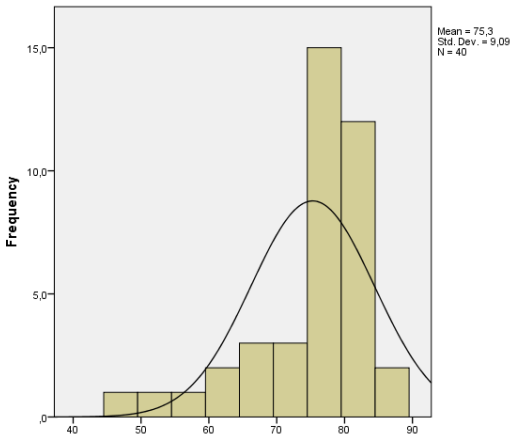
	N	Mean	SD	Min	Max
Preoperative AOFAS Score	40	52,95	13,521	24	72
Postoperative 3-Month AOFAS Score	40	75,30	9,090	47	87
Postoperative 1-Year AOFAS Score	40	74,50	11,239	41	89
Last Follow-Up Visit AOFAS Score	40	73,37	11,758	41	89
Preoperative NPS Score	40	5,25	1,171	3	8
Last Follow-Up Visit NPS Score	40	2,35	1,099	1	5

Min: Minimum; Max: Maximum; SD: Std. Deviation

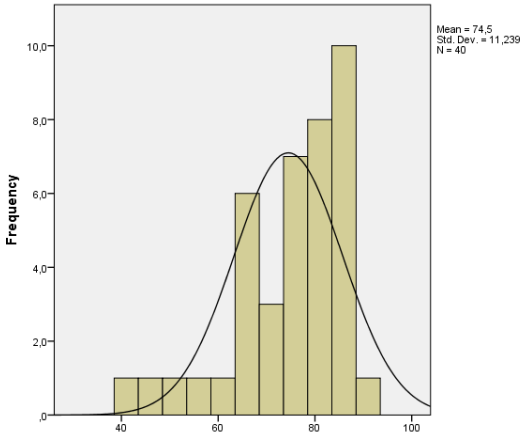
One-way ANOVA was planned to test the distribution of AOFAS and NPS scores in patient groups. Histogram graphs were studied to evaluate the data distribution. Some data was observed to be non-normally distributed. See below for histogram graphs (Graphs 1-2-3-4-5-6).



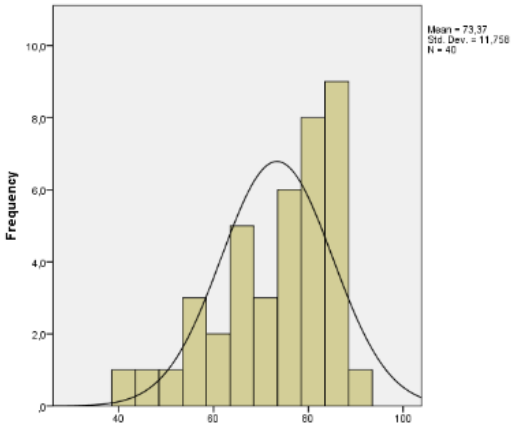
Graphs 1. Preop AOFAS score



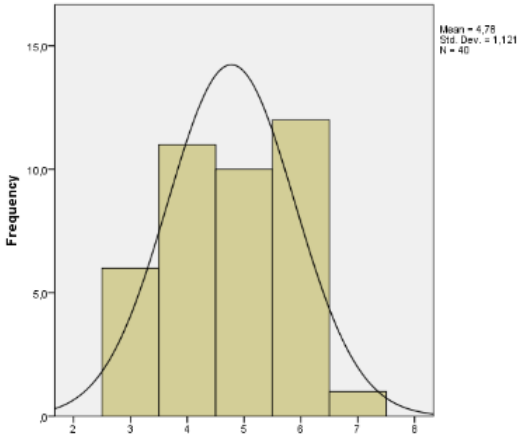
Graphs 2. Postop 3th month AOFAS score



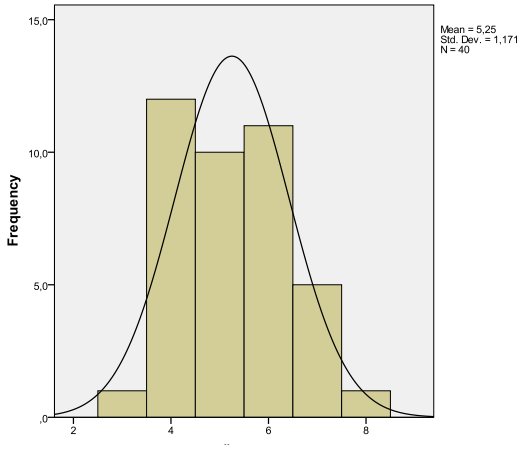
Graphs 3. Postop 1st year AOFAS score



Graphs 4. Last control AOFAS score



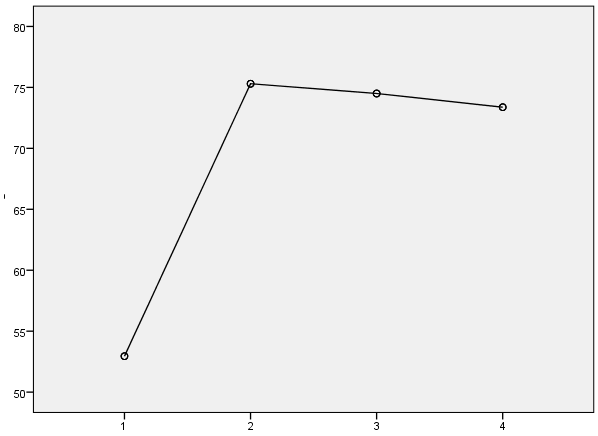
Graphs 5. NPS score before operation



Graphs 6. NPS score on the last check

Therewith, being a non-parametric alternative of one-way ANOVA, Kruskal-Wallis test was used. Preoperative AOFAS, postoperative 3-month AOFAS, postoperative 1-year AOFAS, and last follow-up visit AOFAS scores of the patients were observed to be significantly different. For the NPS scores, no significant difference was found between preoperative and last follow-up visit values of the patient groups.

The patients' AOFAS scores were evaluated in preoperative period, and at postoperative month 3 and year 1. These values were evaluated using ANOVA test for repeated samples. As p was <0.05 in the Mauchly's ANOVA, sphericity hypothesis could not be established, and Greenhouse-Geisser value in Tests of Within- Subjects Effects table was shown. Significant difference between the repeated measures of the scores. P< 0.001 (Graph 7)



Graph 7. Estimated Marginal Means of AOFAS (1: Preop, 2: Postop 3th month, 3: Postop 1st year, 4: Last control AOFAS)

As the preoperative and last follow-up visit NPS scores of the patients were non-normally distributed, Wilcoxon test which is the non-parametric alternative of the t-test for dependent samples. Significant difference was observed between the pre- and postoperative scores. P< 0.001

The total follow-up period of the patients was 17.60 months (min. 12, max. 30 months). The mean preoperative AOFAS score was 52.95 (±13.521, min. 24, max. 72). The mean postoperative 3-month AOFAS score was 75.30 (±9.09, min. 47, max. 87); the mean postoperative 1-year AOFAS score was 74.50 (±11.239, min. 41, max. 89); the mean last follow-up visit AOFAS score was 73.38 (±11.758, min. 41, max. 89). The mean preoperative NPS score was 5.25 (±1.171, min. 3, max. 8) and the mean last follow-up visit NPS score was 2.35 (±1.099, min. 1, max. 5).

When the patients were evaluated by disease subgroups, out of patients with impingement syndrome, 7 were diagnosed with anterolateral impingement in preoperative period, 1 with anteromedial impingement, and 5 with anterior impingement due to synovial hypertrophy in both regions. The diagnoses were confirmed postoperatively. Three patients had osseous anterior impingement. Thereby, the mean age of 16 impingement patients included into the study was calculated to be 45.63 (min. 32, max. 72) years. The total follow-up period of the patients was 18 months (min. 12, max. 30 months). The mean preoperative AOFAS score was 58.94 (±9.248, min. 36, max. 69). The mean postoperative 3-month AOFAS score was 79.31 (±5.82, min. 66, max. 87); the mean postoperative 1-year AOFAS score was 78.25 (±8.56, min. 52, max. 89); the mean last follow-up visit AOFAS score was 77.75 (±8.67, min. 58, max. 89). The mean preoperative NPS score was 4.88 (±1.20, min. 4, max. 8) and the mean last follow-up visit NPS score was 2.06 (±0.998, min. 1, max. 5).

In the second group consisting of 11 patients with osteochondral defect, 8 had medial and 3 had anterolateral pathology. The mean age of the patients was 43.55 (min.23, max.70). Male/female ratio was 8/3. The total follow-up period of the patients was 17.55 months (min. 12, max. 27 months). The mean preoperative AOFAS score was 57 (±11.045, min. 40, max. 69). The mean postoperative 3-month AOFAS score was 79.55 (±2,382, min. 76, max. 84); the mean postoperative 1-year AOFAS score was 81 (±5.34, min. 68, max. 86); the mean last follow-up visit AOFAS score was 79.82 (±5.67, min. 66, max. 84). The mean preoperative NPS score was 5.09

(±0.83, min. 4, max. 6) and the mean last follow-up visit NPS score was 1.91 (±0.944, min. 1, max. 4).

All 5 patients at stage II according to the radiological classification of Berndt and Harty had good clinical outcomes according to the clinical outcome classification of Berndt and Harty. Two of 4 patients at stage III had good, and the other 2 had moderate outcomes. Two patients with stage IV osteochondral lesion had moderate outcome. In subjective assessments, all patients were found to be satisfied with the ankle function in their daily activities. When evaluating the clinical outcomes, postoperative MRI was requested only in 4 patients. A complete correlation was not observed between clinical and MRI findings of these patients. The patients returned their daily life activities 6 weeks to 3 months after the surgery. Completely returning to sports activities was allowed 6 months after the surgery.

In the last group consisting of 13 patients with arthrosis, only 3 had arthrosis due to degenerative reasons and 10 due to traumatic reasons. The mean age of the patients was 42.85 (min. 25, max. 68 months) years. The total follow-up period of the patients was 17.15 months (min. 12, max. 30 months). These patients had a mean preoperative AOFAS score of 42.15 (±14.017, min. 24, max. 72), and the mean postoperative 3-month AOFAS score was 66.77 (±10.22, min. 47, max. 78); the mean postoperative 1-year AOFAS score was 64.38 (±11.25, min. 41, max. 82); the mean last follow-up visit AOFAS score was 62.54 (±11.73, min. 41, max. 82). The mean preoperative NPS score was 5.85 (±1.21, min. 3, max. 7) and the mean last follow-up visit NPS score was 3.08 (±1.38, min. 2, max. 5). In this group, one patient with arthrosis had a low AOFAS score at postoperative month 3 due to severe pain at 3-month follow-up visit because of a loose body falling in the joint due to trauma approximately 3 months after the operation. After the removal of the loose body, same patient was evaluated to have an AOFAS score of 82 in the next follow-up visit.

Three patients developed postoperative complications. One of them had transient superficial peroneal nerve paralysis, 1 had complaint worsening, and the other one described locking ankle while walking. The male OCD patient with superficial peroneal nerve paralysis was followed up using Vitamin B supplement. His complaints regressed starting from the first month and at month 3, he had no complaint at all. As the female impingement syndrome patient with complaint worsening stated that her pain is more than before when standing up in the first follow-up visits, additional imaging was requested. However, no pathology was detected that may cause pain. Conservative treatment methods were recommended. In the 3-month follow-up visit, the patient stated that her pain level is at the same level before the operation. The condition was unchanged in 1-year follow-up visit. The remaining patient with complication who had no complaint in the early follow-up visits was a female patient with impingement syndrome. Stating that she has to stop for a few seconds due to locking ankles while walking, the complaints started approximately 6 months after the operation. There was no pathology found in the additional imaging which can explain the symptoms. As the study only included patients with 1-year follow-up, the patient who developed reflex sympathic dystrophy in postoperative period and did not return for follow-up visits after month 3 was excluded from the study.

The complication rate seen in 40 patients was 7.5% (3 patients); and the complication rates by group were 12.5% (2 patients) in 16 patients with impingement syndrome, 9.09% (1 patient) in 11 patients with osteochondral defect, and 0% (0 patients) in 13 patients with arthrosis. It should be remembered that transient superficial peroneal nerve paralysis improved without any sequela in approximately 3 months, and 2 patients with worsening pain and locking ankles while walking were considered to have complication without any detectable medical pathology. The only major complication seen in 52 patients including 12 patients who were excluded from the study due to various reasons (lost-to-follow-up, follow-up period less than 1 year) was reflex sympathic dystrophy seen in 1 patient.

Among the patients included into the study; one patient who underwent operation for degenerative arthritis had FMF and possibly missed old Tillaux fracture. While this patient has significantly improved AOFAS value and life quality in the early postoperative period, the complaints in the region were worsened again after another trauma. Another patient who underwent operation for traumatic arthritis had bilateral ankle arthrosis due to old trauma and stated that he cannot carry out the postoperative resting recommendations as we requested.

Increase of AOFAS scores from the mean of 52.95 to 74.50 at the first year follow up and decrease of NPS scores from the mean of 5,25 to 2,35 at the last follow up visit can be interpreted as

significant beneficence. Also minimally invasive technique of the procedure, low rate of complications, shorter period of hospitalization and returning to work are major advantages of the technique. Groups had also been evaluated separately. AOFAS score has increased from 57 to 81 and NPS score has decreased from 5 to 1,91 in the group with osteochondral lesions. AOFAS score has increased from 59 to 78,2 and NPS score has decreased from 4,88 to 2,06 in the group with anterior impingement. AOFAS score has increased from 42 to 64 and NPS score has decreased from 5,85 to 3,08 in the group with arthrosis. Evaluating the difference between the first year follow up and preoperative AOFAS scores the group had the most beneficence was the arthrosis group (%54).

Discussion

Being gradually more used in the last 25 years for ankle pathologies, ankle arthroscopy became an important alternative for open surgeries as it is a minimally invasive procedure. The reasons why ankle arthroscopy is preferred include the visualization of all intraarticular structures and the possibility of intervention, low post-operative morbidity, easy and rapid rehabilitation, and the patients being able to return their work, social and sports activities as soon as possible (Aydin,1996; Tosun and Yilmaz, 2009). It is a sensitive matter that the portals to be opened for arthroscopy procedure allow the most effective way to perform the procedure and also avoid damaging important anatomic structures. Thereby, it becomes more favorable method compared to open surgical interventions, and the complications of open surgical procedure can be reduced.

Arthroscopy procedure is usually performed under tourniquet and in aqueous setting (Aydin and Gokkus, 2013). The basis of seeing tourniquet use as a rule is undoubtedly image quality. Conditions such as acute hemorrhagic synovitis and pigmented villonodular synovitis also support this opinion (Lawrence and Albert, 2011).

There are also surgeons who do not wish their patient go through tourniquet pain and try to avoid DVT risk caused by tourniquet by performing arthroscopy applications without tourniquet (Tecimer at al., 1995). Hence, a recent publication stating that tourniquet use does not provide any extra benefit was published by Zaidi et al. (Zaidi at el., 2014). However, there are also publications that cannot state tourniquet use is unnecessary but exhibit impartial attitude due to its risks (Smith and Hing, 2010). We did not use tourniquet in our cases, and we believe that image quality for procedure was obtained by adding adrenalin inside the fluid to provide vasoconstriction. Because we did not encounter a problem caused by not using tourniquet in any of our ankle arthroscopy cases. The necessary intraarticular fluid pressure can be adjusted by hanging the irrigation solution up, and using pump Y-catheter or arthropump pressure-adjusted electronic system (Aydın and Gokkus, 2013).

For ankle arthroscopy, standard imaging system used for the knee and knee arthroscopy instruments would be enough. Optically, standard 4 mm 30-degree optic is adequate. However, for convenience, short-barrel (14 mm) optic and sleeves can be used. Microfracture apparatus at different angles designed for ankle, ring curettes, guides for drilling using special Kirschner wire (Ferkel's retrograde drilling guide), Hembfling's intraarticular distractor, fine shaver tips, Boehler and radiofrequency (RF) probes are also used (Aydin, 1996; Aydin, 2009; Aydın and Gökkuş, 2013). We perform our ankle arthroscopy procedures without using any special instrument and by completely using knee arthroscopy instruments in our clinic.

In a study in 2009, Glazebrook et al. investigated the outcomes of different treatments for ankle pathologies using Pubmed database, and graded the generally accepted treatment methods by acceptability. In the study named 'Evidence-based indications for ankle arthroscopy', fair evidence-based literature (Grade B) is present to support using ankle arthroscopy for the treatment of 'impingement syndrome' and osteochondral lesions, and ankle arthrodesis. Ankle arthroscopy for ankle instability, septic arthritis, arthrofibrosis and removing free bodies is only supported by low-quality evidence (Grade C). Ankle arthroscopy treatment for isolated bone impingement is not effective, and thereby, this indication is not recommended (Grade C opposing). Lastly, they concluded that evidence-based literature to support or reject the hypothesis that arthroscopy is beneficial in the management of synovitis and fracture is not adequate (Class I) (Glazebrook, 2009). While ankle arthroscopy is definitely contraindicated in periarticular soft tissue infection (e.g., cellulitis, chronic or acute open wound, periarticular dermatitis) and degenerative arthritis in several joints, it is relatively contraindicated in moderate arthritis (narrowing which restricts joint movement), edema, peripheral vascular diseases, peripheral neuropathy, reflex sympathic

dystrophy/complex regional pain syndrome, and toe infections.

Complications predominantly consist of nerve injuries (approx. 50-60%). And majority of nerve injuries (approx. 50-60%) is at superficial peroneal nerve (Ferkel et al., 1996). Among the complication rate stated to be 9% by Ferkel in their series consisting of 612 cases, the rate of neurological complications was high, 49%. Out of these 27 cases, 56% had injury to superficial peroneal nerve, 22% to sural nerve, and 18% to long saphenous nerve. Deep peroneal nerve injury has only been reported in 1 case (4%). These injuries have been stated to be observed especially in cases where anterocentral or posteromedial portals, and invasive distraction devices are used (Ferkel et al., 1996). There are many studies stating that complications rates dramatically drop when non-invasive distractors are used (Sprague et al., 1989; Ferkel et al., 1996; Deng et al., 2011; Zengerink and van Dijk, 2012).

As most of the complications are related with portals, the anatomy of the region should be known very well and routine portals should be used (Aydin, 1996). It is recommended to visualize and mark superficial peroneal nerve which is at high risk for injury during anterolateral portal opening by traction of the 4th toe, plantar flexion and inversion (in 1/3 of the patients) before starting the operation if possible; and in addition to this or if the nerve cannot be visualized by this method, it is known that the subcutaneous structures can be protected by entering through anteromedial portal and giving out light from inside (Ferkel et al., 1996; Lawrence and Albert, 2011).

As a nuance, when we put the foot in plantar flexion and inversion to mark the superficial peroneal nerve and then leave it back to neutral position, the nerve shifts to lateral side by 3.6 mm on average. And this leads to injury to the nerve which we marked to avoid injury during portal opening. It has been recommended to stay on the medial side of the mark to avoid this risk (Zengerink and van Dijk, 2012). In the light of this information, we tried to open anterolateral portal rigorously in every case. We believe the fact that superficial peroneal nerve injury, which has been reported at various rates in several publications and is almost the most common complication, was only seen in 1 of our cases is a result of our sensitivity.

Anatomic studies have demonstrated that anterior tibial artery passes close to anterolateral portal in 6.7% of the cases, and the complications such as pseudoaneurysm or vascular injury are caused by this closeness (Son et al., 2011).

There are inconclusive matters in postoperative period, and some authors state that they are important for complication control. While Ferkel recommended 1 week immobilization and prophylactic antibiotic use in postoperative period (Ferkel et al., 2001), van Dijk did not recommend postoperative antibiotic use and starts in case of superficial infection. He recommends the patients should start postoperative movement on the same day, put the foot in active dorsiflexion every few hours, put load on the foot as much as they can tolerate, and use the crutches for 4-5 days maximum (Zengerink and van Dijk, 2012). We recommended every patient we discharged to change the dressing every 3 days, suture removal on the 15th day, and to use prophylactic antibiotic during this period. Including the patients who refused or forgot using antibiotics, none of our cases had superficial or deep infection. We also believe that by trying to mobilize our patients as soon as possible, the regional perfusion was improved and the resistance to infections was increased. Excluding the patients who underwent microfracture operation due to chondral defect, we tried to apply a policy of putting load on the day after the operation and allowing active walking only 3 weeks after as much as possible.

Conclusion

We believe that the outcomes of ankle arthroscopy performed properly by experienced hands after determining the patient population who were accurately diagnosed by careful clinical examination and necessary imaging methods are greatly satisfactory, and the acceptability is high thanks to complications being less and usually transient.

Ethics Committee Approval: Ethics committee approval was received for this study from Faculty of Medicine Clinical Research Ethics Committee of Ataturk University. B.30.2.ATA.0.01.00/93 30.05.2014
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RESEARCH ARTICLE

Effects of Autologous Platelet-Rich Plasma on Endometrium Thickness and Pregnancy Rates During Intrauterine Insemination

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Abstract

Objective: Evaluation of the effect of platelet-rich plasma (PRP) on the endometrium and pregnancy outcomes in patients undergoing insemination due to unexplained infertility.

Methods: 24 patients who were admitted to the clinic due to unexplained infertility, analyzed retrospectively between March 2018 and October 2018. Gonadotropin induction was initiated on day 3 of the cycle for follicular growth. Human chorionic gonadotropin (hCG) was applied for ovulation induction at the point that at least 1 follicle that is over 16 mm was detected by transvaginal ultrasound. 17.5 ml of blood from the patient's venous system was drawn for the preparation of the PRP which includes 4-5 times more platelets than regular blood. PRP was administered to 12 patients (Group 1) on the hCG day, while hCG was solely administered to the other group (Group 2) and both groups were inseminated 36 hours later.

Results: The demographic properties of all patients were determined as follows: mean age; 29.13 years old (±3.4), mean infertility period; 1.96 years (±1.08), mean ovulation induction period; 7.92 days (±1.76), mean antral follicle count; 14.54 (±6.56), mean dominant follicle count; 2.04 (±0.75). Although there was no significant difference between the groups in terms of clinical pregnancy (3/12 vs 2/12, p: 0.623), the change in endometrial thickness was significantly higher in the PRP administered group (1.95 mm vs 0.44 mm, p< 0.001).

Conclusion: PRP application before the insemination seems promising for the preparation of the endometrium in patients having an inadequate endometrial thickness or in patients experiencing recurrent implantation failure.

Key words: Intrauterine insemination, endometrial thickness, autologous platelet-rich plasma, infertility, pregnancy rate

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Introduction

Unexplained infertility; is the failure to achieve pregnancy despite having unprotected regular sexual intercourse for the period of 12 months (which is the period of 6 months for the women that are above the age of 35) without a definable cause according to American Society for Reproductive Medicine (ASRM, 2008). 15% of the all infertility reasons are included in this group (Collins and Crosignani, 1992). Factors such as folliculogenesis, luteal phase defect, cervical factor and implantation failure are considered as possible causes. The rate of spontaneous pregnancy in these patients without a

treatment is 1% to 3% per month (Evers, 2002; van Eekelen et al., 2018). It is expected that the treatment option will increase the pregnancy rate above this level. Therefore, to eliminate infertility reasons including cervical factor, intrauterine insemination is a commonly used treatment modality for the unexplained infertility cases.

Recurrent implantation failure is also a frequently discussed topic at in vitro fertilization. Embryo quality, endometrial receptivity, and immunological factors have been subject to research in etiology and, although there have been different treatment options such as blastocyst transfer, pre-implantation genetic screening (PGS), assisted hatching, different culture mediums, endometrial scratching and immunotherapy, no consensus has been achieved yet (Margalioth et al., 2006; Green et al., 2015; Choi et al., 2016). Recently, intrauterine administration of PRP has been described as to accelerate endometrial growth and improve implantation achievement (Chang et al., 2015). PRP is an autologous serum, which contains various growth factors and cytokines such as fibroblast growth factor, platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor, insulin-like growth factor I-II, connective tissue growth factor and IL-8 (Chang et al., 2015; Nazari et al., 2016) . Although PRP has found widespread use for tissue healing, especially in areas such as ophthalmology, orthopedics and plastic surgery, there are very limited number of existing studies in the field of infertility. (Dhillon et al., 2012; Chang et al., 2015; Ronci et al., 2015; Garcia-Velasco et al., 2016; Sfakianoudis et al., 2018).

The aim of our study was to investigate the effect of PRP on endometrium and pregnancy outcomes in patients undergoing intrauterine insemination IUI due to unexplained infertility

Methods

24 patients, who were admitted to Giresun University Gynecology and Obstetrics Clinic due to unexplained infertility, were analyzed retrospectively since March 2018 to October 2018. PRP was administered to 12 patients (Group 1) on the hCG day, while hCG was solely administered to the other group (Group 2) and both groups were inseminated 36 hours later. Being exposed to clomiphene citrate treatment at least twice, existence of tubal patency, and being under the age of 35 were defined as inclusion criteria, while the exclusion criteria was determines as existence of a structural or anatomical condition that could prevent reproduction, any sort of systemic disease, patients with BMI over 30, having total progressive sperm concentration (TPSC) under 10 million and having an endometrium under 7 mm at hCG day.

Gonadotropin induction was initiated on day 3 of the cycle for follicular growth. Recombinant hCG (Ovitrelle 250 µg, Merck) was applied for ovulation induction at the point that at least 1 follicle that is over 16 mm was detected by trans-vaginal ultrasound. Endometrium thicknesses on hCG and insemination day were also noted.

17.5 ml of blood from the patient's venous system was drawn for the preparation of the PRP. Then, centrifugation at 1200 rpm for 12 minutes was applied to eliminate red blood cells and remaining plasma was centrifugated at 3000 rpm for 7 minutes to achieve PRP having 4-5 times more platelets when compared to regular blood (Nazari et al., 2016). hCG was administered after 0.5 mL PRP was given to endometrial cavity with a soft catheter (Medbar, Turkey) without touching to the fundus of uterus and (IUI) was applied 36 hours later. Microfluidic sperm sorting chip was used for sperm preparation in all patients (Fertil Plus Koek Ltd., Turkey).

Ethics committee approval was received from Giresun University Ethical Committee (KAEK-2017/32) and all patients have signed their volunteer consent forms as being informed.

Results

The demographic properties of all patients that underwent IUI due to unexplained infertility were determined as following: mean age; 29.13 years old (Standart Deviation: [SD]: ±3.4), mean infertility period; 1.96 year (SD: ±1.08), mean follicle stimulating hormone (FSH) level; 6.38 mIU/mL (SD: ±1.55), mean estradiol (E2) level; 47.75 pg/mL (SD: ±31.26), mean ovulation induction period; 7.92 days (SD: ±1.76), mean gonadotrophin dose; 775,52 IU (SD: ±198.85), mean antral follicle count; 14.54 (SD: ±6.56), mean dominant follicle count; 2.04 (SD: ±0.75). The distribution of demographic properties and clinical findings of groups are summarized below in Table 1.

Although there was no significant difference between the two groups in terms of clinical pregnancy (p: 0.623), the change in endometrial thickness was significantly higher in the PRP implemented group (1.95 mm vs 0.44 mm, p <0.001).

Table 1. Demographic properties and findings of groups

	Group 1 (±SD)	Group 2 (±SD)
Age (year)	29.58 (3.6)	28.67 (3.2)
Infertility period (year)	2.33 (1.2)	1.58 (0.7)
FSH 3. day (mIU/mL)	6.50 (1.5)	6.25 (1.6)
TPSC	94.10^6	124.10^6
Induction period (day)	8.25 (2.1)	7.58 (1.2)
AFC (N)	13.83 (3.5)	15.25 (8.7)
End thickness at hCG day (mm)	9.15 (1.6)	10.49 (2.2)
End thickness at IUI day (mm)	11.10 (1.5)	10.93 (2.3)
Change of end thickness (mm) *	1.95 (0.4)	0.44 (0.1)
Clinical pregnancy (%) – [N] Ł	25 (0.4) – [3/12]	17 (0.3) – [2/12]

* p<0.001; Ł p:0.623; FSH, follicle stimulating hormone; TPSC, total progresive sperm count; AFC, antral follicle count; End, endometrial; hCG, human chorionic gonadotrophine; IUI, intrauterine insemination.

Discussion

IUI is a frequently used treatment modality for couples who are admitted for unexplained infertility. Although it has a low success rate comparing to in vitro fertilization, IUI is an economical and less invasive method. Since the cause of infertility cannot be fully established, it is still arguable as when the treatment should be initiated and how to proceed. There was no significant difference for expectant management between the group that started the treatment with IUI as delayed for 6 months and the group that initiated treatment with IUI immediately in a study that compared 253 patients to find out the effect of pre-treatment expectant period. (RR: 0.99, 95% CI: 0.85–1.1) (Custers et al., 2012).

Similarly, 14 studies and 1867 women were examined in a meta-analysis comparing IUI, timely intercourse and expectant management in terms of live birth rates and multiple pregnancies in natural and stimulated cycles. The only significant increase in live birth rates were detected when stimulated IUI cycles (25%) were compared to IUI (9-21%) in the natural cycles (OR: 0.48, 95% CI:0.29-0.82; 4 RCT, n:396, I2:0) (Veltman-Verhulst et al., 2016).

This study has designed IUI treatment for 24 patients, who had been failed for pregnancy with clomiphene citrate at least 2 times without tubal factor with unexplained infertility. Assuming that it could increase endometrial growth and receptivity, the study also applied PRP to the group 1. Although there was no significant difference between the two groups in terms of pregnancy, the increase in endometrial thickness was higher in the PRP implemented group (0.44 mm vs 1.95 mm; p<0.001).

During the period, the endometrium is getting ready for implantation of the embryo between the 19th and the 23rd days of the menstrual cycle, which is known as implantation window at the mid-secretory phase (Nazari et al., 2016). Cytokines, growth factors, prostaglandins and adhesion molecules increase at this stage in the endometrium for embryo implantation and development. Among the possible causes of unexplained infertility, PRP was tried at in vitro fertilization in order to treat implantation failure, immunological factors and thin endometrium (Chang et al., 2015; Nazari et al., 2016; Zadehmodarres et al., 2017). Numerous cytokines and growth factors in the PRP may increase the proliferation and receptivity of the endometrium. In a study, PRP was applied to 5 patients that had not received embryo transfer because the endometrial thickness remained below 7 mm despite estrogen treatment was administered in the previous IVF trial. The expected increase in endometrium thickness was achieved after 48-72 hours of PRP administration. Then, 4 patients received blastocysts and 1 patient received 3rd-day embryo and all had clinical pregnancies (Chang et al., 2015).

In a similar study, 10 patients, whose frozen embryo transfer was canceled due to insufficient endometrial growth in the previous cycle, were examined. The endometrial thickness was enhanced above 7 mm and embryo transfer was performed in all patients after PRP was given twice as the second dose was applied 48 hours later than the first one. As a result, pregnancy was detected in 5 patients but one resulted in premature abortion (Zadehmodarres et al., 2017). Consequently, PRP can be used for maturation of the thin endometrium according to these studies.

In a different study, 20 patients, who underwent IVF because of recurrent implantation failure, were given PRP 48 hours prior to transfer in frozen-thawed cycles. Although the total number of pregnancies were 18, clinical pregnancy number was 16 because of an early abortion and a molar pregnancy (Nazari et al., 2016). In another study conducted in patients with recurrent implantation failure, it was found that these patients had fewer growth factors in endometrium comparing to fertile patients (Sak et al., 2013). Thus, increased cytokines and growth factors with PRP may have a critical role in the implantation of the embryo.

Since there is a limited number of studies on the use of PRP in the field of infertility, having a small number of patients included in the study was a con, while the first application of PRP for IUI and the use of the microfluidic sperm sorting chips were the significant pros of this study

Conclusion

PRP application before the insemination seems promising for the preparation of the endometrium in patients with inadequate endometrial thickness or in patients with recurrent implantation failure. It is believed that more studies involving more patients are needed to make the use of autologous PRP more reliable in clinical practice.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Giresun University. KAEK-2017-32.
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RESEARCH ARTICLE

Antimicrobial Activity of Silver Nanoparticles Synthesized with Extract of Tomato plant Against Bacterial and Fungal Pathogens

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Abstract

Objective: Silver nanoparticles (AgNPs) have a wide range of applications. Environmental-friendly synthesis methods for these nanoparticles are more preferable due to their various advantages. This study aimed to synthesize AgNPs using the extract of the tomato plant in an easy and economical way. and testing this AgNPs against some human pathogens.

Methods: Silver nanoparticles were synthesized using aqueous silver nitrate and reducing tomato plant extract. The characterization of AgNPs was determined by ultraviolet-visible spectrophotometry (UV-Vis), X-ray crystallography (XRD) Scanning electron microscopy (SEM), Fourier transform infrared Spectroscopy (FT-IR), energy dispersive X-ray spectrum (EDAX), thermogravimetric - differential thermal analysis (TGA-DTA) data. The effects of the particles on pathogenic microorganisms were determined by minimum inhibition concentration (MIC).

Results: These data, with a maximum absorbance of 450.51 nm, in the spherical view, with the peaks and values of 111°, 200°, 220° and 311° (38.08, 44.28, 64.42 and 77.34), AgNPs showed a cubic crystal structure and, using the Debye-Scherrer equation, it was determined that they had a crystal size of 21.11 nm AgNPs had an antimicrobial activity on hospital pathogens gram negative, gram positive and Candida albicans yeast.

Conclusion: We found that these particles showed antimicrobial activity on various microorganisms even at very high concentrations. As a solution to the antimicrobial search, it can be developed in medical industry.

Key words: Antimicrobial activity, XRD, SEM, TGA-DTA, Silver nanoparticle

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Introduction

Nanoparticles (NPs) are structures smaller than 100 nm. Nanotechnology is a branch of technology that examines the properties of these structures (Beyene et al., 2017). The increasing use of NPs makes these structures more important every day (Tovar-Corona et al., 2018). NPs such as gold, silver, platinum, copper, palladium etc. are widely used in a wide range of areas including personal care, clothing, cosmetics, food industry, catalysis and medical, optic and electronic industries (Lloyd et al., 1998; Song et al., 2010; Gopinath et al., 2016;

Vetchinkina et al., 2016; Prakash et al., 2018). Silver nanoparticles (AgNPs) have large surface areas and a high conductivity capacity and they can be synthesized using various biological sources (Nanda et al., 2018). In addition, AgNPs can be synthesized both physically and chemically, however this may have various disadvantages compared to biological methods.Chemical methods lead to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in medical applications (Jain et al., 2009; Baran, 2019). Phytochemicals in plant reduce Ag+ to AgO structure and provide the stabilityof AgNPs. (Ali et al., 2015; Saha et al., 2017). The use of colloidal silver for the treatment of diseases has been the subject of research for more than 50 years. Recent developments in the chemical, biological and material characterization techniques have allowed this subject to be explored better and silver has begun to be used more widely in the medical fields (Brandt et al., 2012). These particles have a strong antimicrobial activity thus, approximately 320 tons of AgNPs are produced every year (Gliga et al., 2014).

In the present study, an AgNP was synthesized in an easy way using the extract of the tomato plant during the autumn period and an inexpensive, simple and environmentally friendly method and the effects of this particle on various microorganisms were examined.

Methods

Preparation of the Tomato Plant Extract and Silver Nitrate Solution

The tomato plants used in this study were collected in the agricultural region of Yenişehir in the district of Diyarbakır, Turkey when they were still unripe during the autumn period They were washed several times with tap water and then distilled water and were left to dry at room temperature. 50 gr of the plant was mixed with 500 ml distilled water and boiled. After being filtered for several times, it was kept at + 4ºC for synthesis. 1 mm aqueous solution was prepared with silver nitrate (AgNO3) of 99.8% purity was purchased from Alfa Aesar."

Synthesis and Characterization

The extract and the AgNO3 solution were mixed with a ratio of 1:4. The color change of the solution was monitored based on time. The formation and presence of AgNPs were observed using Perkin Elmer UV- Vis spectrophotometer. The FTIR analysis and functional groups responsible for the reduction in the synthesis were examined with Perkin Elmer Spectrum One. After the synthesis, AgNPs were centrifuged at 10.000 rpm for 15 min with OHAUS FC 5706 model device. The bottom precipitate was washed with distilled water and dried at 75ºC. The evaluation of content of the particles was carried out with Bruker-125 eV (EDX).). The formation of AgNPs was examined with scanning electron microscope (SEM) EVO 40 LEQ data. The crystal structures were examined by RadB-DMAX II computer-controlled X-ray diffractometer (XRD) analysis and the crystal particle size was determined using the Debye-Scherrer equation and TGA-DTA decay temperature values were checked with Shimadzu TGA-50 device data.

Determination of the Antimicrobial Effects of AgNPs

The effects of AgNPs on pathogenic microorganisms were examined by using the micro dilution method to determine the minimum inhibition concentration (MIC). The effects of the particles were investigated on gram negative Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and gram positive Staphylococcus aureus ATCC 29213, Streptococcus pyogenes ATTC 19615 strains and Candida albicans ATTC 10231 strains For the bacteria in the microplate wells, Mueller Hinton broth and for the fungus RPMI broth were added. The solutions prepared from AgNPs were placed on plates at the appropriate concentrations and a mixture of microorganisms was added according to MC Farland standard 0.5 concentration (Nishanthi et al., 2019) and waited for one night at 37 oC. The MIC values of Vancomycin for gram positives, colistin and flucanozol antibiotics for gram negatives and fungi, and the aqueous solution of 1 mM AgNO3 were also investigated.

Results

After taking 500 ml from the 1 mM $AgNO_3$ solution and 100 ml from the plant extract and mixing 1000 ml in conical flask, a dark brown discoloration was observed after 75 min. The absorbance data of UV-Visible measurement results are given in Figure 1. The dark brown discoloration is a characteristic data showing the formation of AgNPs due to vibrations on the plasma surface and

the maximum peak value at 450.51 nm and the absorption data also support this fact (Figure 1).

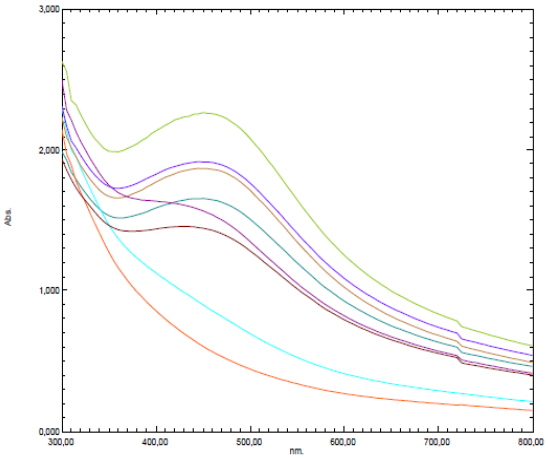

Figure 1A. Time Dependent of AgNPs in UV Visible Spectroscopy

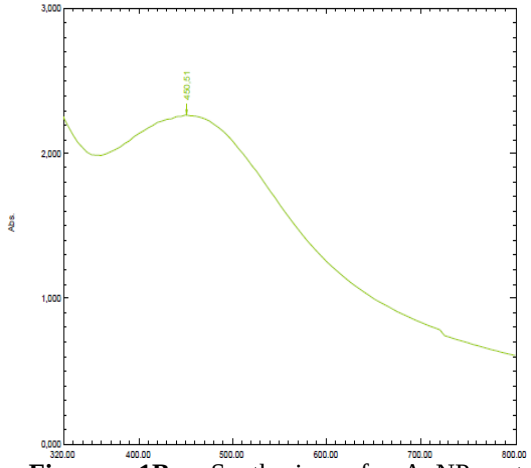

Figure 1B. Synthesis of AgNP at UV Spectrophotometry Maximum Absorbance Value

In this study, when the functional groups participating in the reduction were examined, it was determined that the shifts at 3511, 3206, 2148 and 1618 cm-1 corresponded to -OH, -NH, -CN and C=O respectively (Figure 2.)

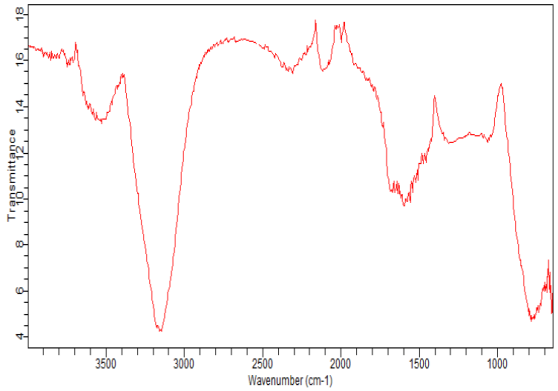

Figure 2A. FT-IR spectrum of extract spectrum.

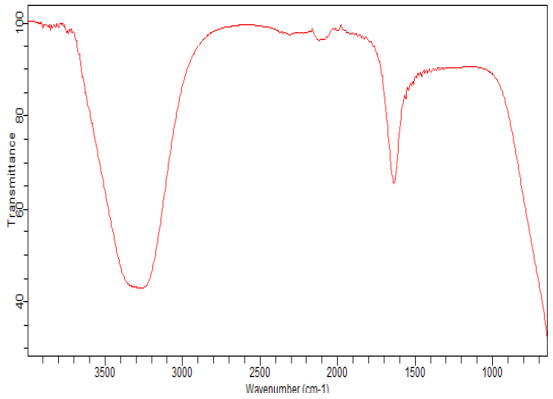

Figure 2B. FT-IR spectrum of synthesised AgNPs.

In the XRD analysis data, the peaks of 111º, 200º, 220º and 311º are the peaks that correspond to the peaks at 2θ which represent the cubic crystal structure of silver (Figure 3). The crystal size of AgNPs was calculated by the Debye-Scherrer equation given below and was found to be 22.11 nm. $D = K\lambda / (\beta \cos\theta)$

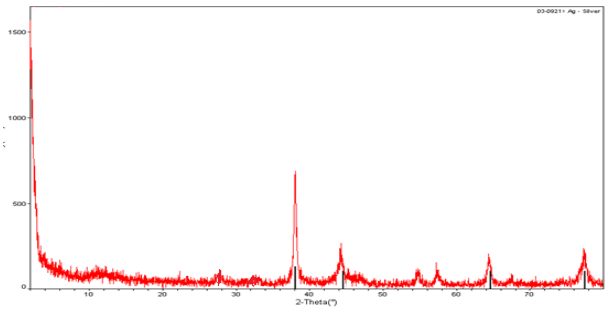

Figure 3. Investigation of crystal structure and silver phases of AgNPs by XRD analysis

The SEM results for examining the morphological views of AgNPs give us that NPs are in spherical view and have an average 52.74 nm of dimensions (Figure 4).

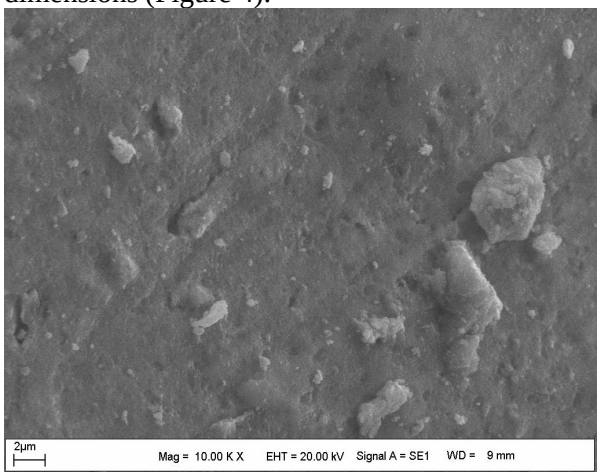

Figure 4A. Evaluation of morphology of AgNPs in SEM results A) 10,000 times magnified view of AgNPs

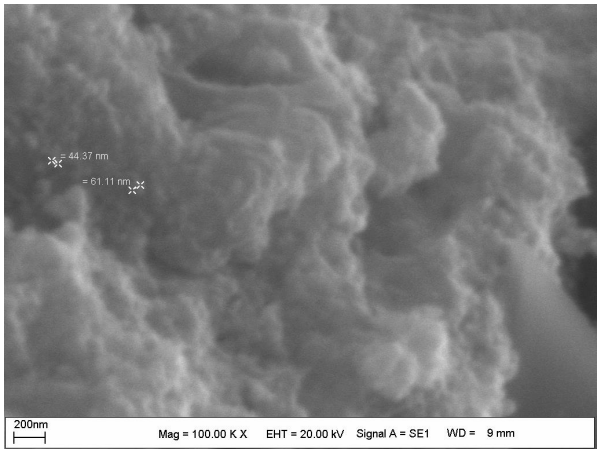

Figure 4B. Evaluation of morphology of AgNPs in SEM results 100,000 times magnified view of AgNPs

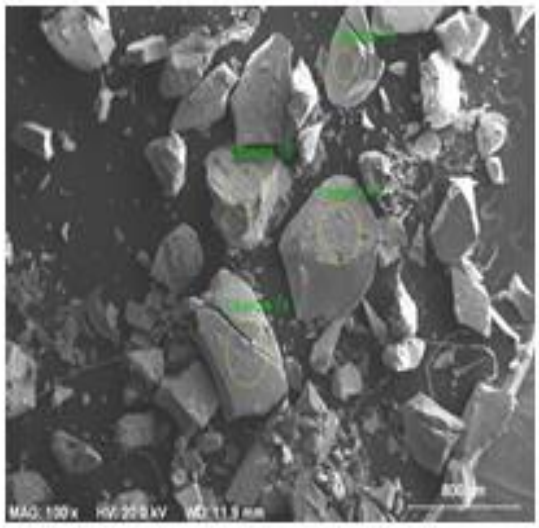

Figure 5 A. Analysis of the elemental composition by the EDX analysis of AgNPs. EDX measurement with three different points,

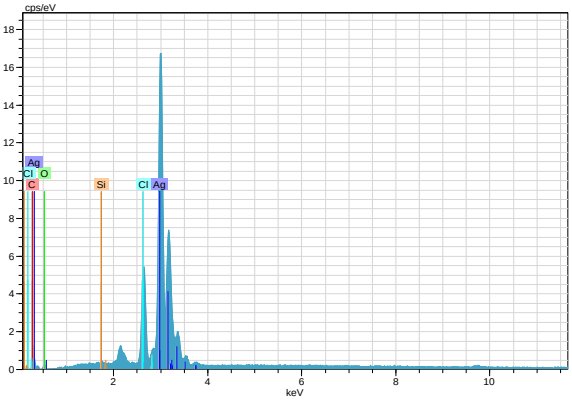

Figure 5B. Analysis of the elemental composition by the EDX analysis of AgNPs. EDX pattern showing the presence of silver

When the element content was examined according to the results of EDX (figure 5), it was found that it was mostly composed of silver the TGA-DTA curve was examined, it was thought that the mass loss of 2.24% at 12-227ºC was due to the hydration by moisture and water, the mass loss of 3.8% at 227-355ºC was due to phytochemicals present in the plant extract, and the mass loss of 23% at 355-846ºC was due to the structure of the substance deteriorating (Figure 6). AgNPs showed a suppressive effect against microorganisms. When the AgNPs obtained in this study were compared with the antibiotic and 5 mM AgNO3 solution, the effect of Ag NPs was examined in low concentrations (Table 1).

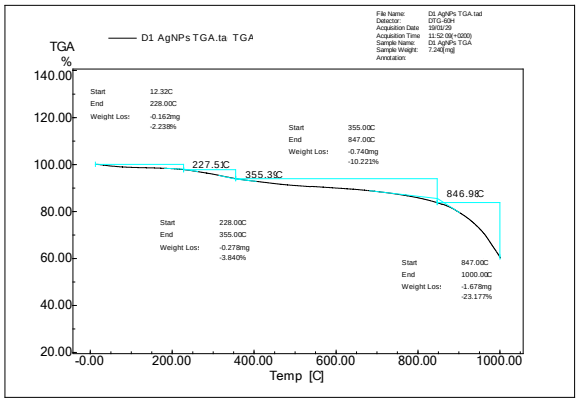

Figure 6A. TGA-DTA data of the synthesized nanoparticle. Nanoparticle TGA curve at different temperatures

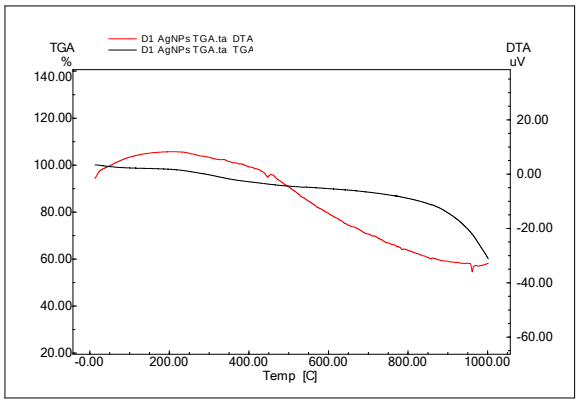

Figure 6B. TGA-DTA data of the synthesized nanoparticle. Nanoparticle DTA curve at different temperatures

Table 1. MIC values of Synthesized silver nanoparticles (AgNP) (mg mL^{-1}) on Silver nitrate solution and vancomycin, fluconazole, colistin antibiotics, *S. Aureus*, *S.pyogenes*, *S. albicans* and *E. Coli*, *P.aeruginosa* microorganisms.

	ORGANISM	AgNPs	Silver Nitrat	Antibiotic
Gram Pozitive	*S. aureus ATCC 29213*	0.035	2.65	1
	S.pyogenes ATTC 19615	0.035	1.32	1
Gram Negative	*E. coli* ATCC25922	0.017	0.66	2
	P.aeruginosa ATCC 27853	0.009	0.66	2
Fungi	*C. albicans*	0.018	0.66	2

Discussion

The presence of AgNPs was determined by detecting dark brown color changes by UV-Vis. The maximum absorbance values at the 345 nm wavelength in the results are the data showing the character of AgNPs. The dark brown color changes are a characteristic data showing the formation of AgNPs due to vibrations on the plasma surface (Begum et al., 2009; Prakashet al., 2013; Sinsinwar et al., 2018). Similar values found as a result of other studies conducted regarding the synthesis of AgNPs using plants are also in correlation with the results of the present study (Ferreyra et al., 2018; Shao et al., 2018). The XRD results show us the peaks: 111º, 200º, 220º, 311º and 38.08, 44.28, 64.42 and 77.34. The values of these peaks indicate the characteristic of AgNPs. The results of different studies in literature interpreted these peaks as AgNPs (Sengottaiyan et al. 2016; Khan et al., 2018). In accordance with this data, the crystal size of AgNPs was calculated 22.11 nm. In similar studies, NPs were evaluated using Debye-Scherrer equation (Sagar and Ashok, 2012; Pugazhendhi et al., 2018). In this study, when the functional groups participating in the reduction were examined, it was determined that the shifts at 3511, 3206, 2148 and 1618 cm^{-1} corresponded to -OH, -NH, -CN and C = O respectively. It was observed that the reduction of the tension in 3511 cm^{-1} did not occur at the end of the reaction, and other functional groups were also involved in the reduction when the FTIR images in Figure 2 were examined. These groups were evaluated in studies for the synthesis of other AgNPs (Baran, 2019). The SEM results showed that AgNPs were global in appearance. Some studies mention that AgNPs are in spherical view and have an average 52.74 nm of dimensions (Hemmati et al., 2019; Singh et al., 2017). Other studies also state that AgNPs are in global view (Kumar et al., 2016; Kobashigawa et al., 2018). When the TGA-DTA curve was examined, it was thought that the mass loss of 2.24% at 12-227ºC was due to the hydration by moisture and water, the mass loss of 3.8% at 227-355ºC was due to phytochemicals present in the plant extract, and the mass loss of 23% at 355-846ºC was due to the structure of the substance deteriorating In one study, the results are almost identical (Baran, 2019). Various studies in literature showed that AgNPs had a repressive effect on the reproduction of various microorganisms (Singh et al., 2017; Alsammarraie et al., 2018; Baran, 2019). AgNPs increase the formation of reactive oxygen species (ROS) by disrupting the membrane structure of the microorganisms, and negatively affect the functions of structures such as DNA and proteins, which provide other vital activities (Ahmed et al., 2018 ; Sarkar et al., 2018).

Conclusions

AgNPs are used in a wide range of areas. Due to their antibiotic resistance properties, these nanomaterials can play a key role in the search for antimicrobial agents. Due to the advantages of environmentally friendly methods, the interest in AgNPs increases every day. In this study, AgNPs were synthesized from the green parts of the tomato plant using simple, inexpensive and environmentally friendly methods. AgNPs were characterized and it was determined that, these particles hada maximum absorbance of 450.51 nm, a spherical size of 52.74 nm, a cubic crystal structure and a crystal size of 21.11 nm

It was determined that AgNPs had an antimicrobial activity on various microorganisms even at very high concentrations. In conclusion, AgNPs can be developed in the medical industry for the antimicrobial search.

Peer-review: Externally peer-reviewed.
Author Contributions: Concept - MFB, HA Design - MFB, HA Supervision- MFB, HA ; Materials MFB, HA ; Data Collection and/or Processing - MFB, HA ; Analysis and/or Interpretation - MFB, HA Literature Review - MFB, HA Writing - MFB, HA ; Critical Review - MFB, HA
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study hasn't received no financial support.

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RESEARCH ARTICLE

Retrospective Evaluation of the Treatment of Wharton's Duct Stones with Transoral Approach

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Abstract

Objective: Sialolith is one of the most common causes of salivary gland obstruction and often leads to sialadenitis. It usually seen in the submandibular gland around 80-90 percent. In this retrospective study, we aimed to retrospectively evaluate the treatment of sialoliths in different parts of the Wharton duct with transoral approach using minimally invasive techniques.

Methods: After the clinical and radiological examination of eight patients, six male and two female patients, transoral removal of sialoliths detected in Wharton duct was decided. All surgical interventions were performed with a transoral approach using minimally invasive surgical techniques. Six patients were treated under general anesthesia and two patients were treated under local anesthesia.

Results: 8 patients aged between 29-81 years who were transoral surgically removed Wharton duct stones. During the 20-month follow-up period, no intraoperative or post-operative complications such as bleeding and lingual nerve injury were observed. According to the results of the survey, 75% of the patients were very satisfied, 12.5% were satisfied and 12.5% were dissatisfied with the result**.**

Conclusion: The transoral approach may be considered as a more effective option for the treatment of Wharton duct sialoliths because of the high success rate and the wider use indication compared to non-invasive procedures such as ESWL and sialendoscopy.

Key words: Wharton duct, sialolith, transoral

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Introduction

Sialolith is a calcified structure of salivary glands, often causes acute and chronic infection and obstruction of salivary gland duct (Im et al., 2017). The sialoliths is usually seen in the submandibular gland around 80-90 percent, followed by the parotid gland 5-20 percent (Lustmann et al., 1990; Matsunobu et al., 2014). The reason of the high rate in the submandibular gland is the secretory content is rich in calcium, two perpendicular curves during the course of the duct and the long channel length (Fonseca, 2000; Liao et al., 2007).

Patients usually refers to dentist complaint of swelling and pain during eating, however sialolith can be noticed without any symptoms while routine clinical and radiological examination (Kraaij et al., 2014; Goodstein et al., 2017). Standard x-ray films, computed tomography, sialography, ultrasonography which is a noninvasive method of

diagnosis, and magnetic resonance sialography, is a new diagnostic procedure, can be used for diagnosis of sialoliths (Marchal and Dulguerov, 2003). 40 percent of the submandibular sialolith are seen in the anterior third of the Wharton duct and can be easily removed by intraoral approach. Removable of the submandibular sialolith in the proksimal part via intraoral approach, especially posterior third, is more difficult. At the same time, this treatment modality, is the minimally invasive traditional surgical technique, increases the submandibular gland and lingual nerve damage (McGurk et al., 2005).

Traditionally, the sialoliths are treated with medical drugs or intraoral-extraoral surgical procedures such as the excision of the salivary gland (Matsunobu et al., 2014; Goodstein et al., 2017). Lingual or facial nerve damage and morbidity are usually seen after major surgeries that are worrying for patients and requiring hospitalization (Hald and Andreassen, 1994; Combes et al., 2009; Matsunobu et al., 2014). Recently, minimal invasive techniques which are Extracorporeal Shock Wave Lithotripsy (ESWL) and sialendoscopy, are use for the treatment modalities of the salivary gland stones (McGurk et al., 2005; Goodstein et al., 2017). ESWL and sialendoscopy are successful procedure in a limited number of patient (ESWL, <8mm stones; Sialendoscopy, <4mm stones). However, combined therapy is usually required for this patient.

In this retrospective study, we aimed to evaluate retrospectively the sialoliths which were located in different regions of the Wharton's duct has treated using minimal invazive techniques with transoral approach.

Methods

Study design

The data were analyzed retrospectively at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Ordu University. The medical records for all patients treated with transoral approach due to sialolithiasis in the wharton's duct between 2012 and 2017 were evaluated. The patients without systemic disease who were decided to remove the sialoliths after the clinical and radiological examination, were included in the study and who had psychiatric illness and systemic disease, failed to fill out the forms for any reason, had incomplete data, wanted to withdraw from the study and could not be cooperation, were excluded. Also, the patients with insufficient follow-up data were excluded from the follow-up study cohort. The study conducted with the approval of ethics committee of Ordu University (2019-27).

Surgical procedure

All surgical interventions were performed with a transoral approach using minimally invasive surgical techniques. The sialoliths in the distal part of the canal (3/8) under local anesthesia; the sialoliths in the proximal part of the canal (5/8) were taken under general anesthesia (Figure 1).

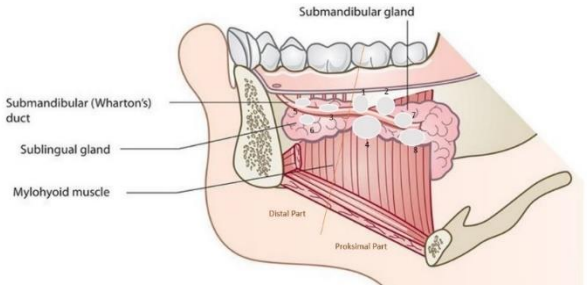

Figure 1. Schematic view of the localization of all excised sialoliths.

Briefly; local anesthetic infiltration was performed in the sublingual region near the salivary calculus with injection of 4% articaine, associated with 1:100,000 epinephrine to effect local vasoconstriction. Then, the duct was sutured by posterior of the sialolith to prevent escaping into the proximal part of the canal. Intraoral access was obtained by making a linear incision along the path of Wharton's duct in the floor of the mouth posterior to the sublingual caruncle. The Wharton ducts membrane was passed, after the soft tissue was dissected carrefully, the sialolith was reached and removed (Figure 2,3). The drainage catheter was inserted into the Wharton duct for normal saliva flow from submandibular gland, after the sialolith was removed (Figure 4). An antibiotic (amoxicillin + clavulanate, 2000 mg/day) and an analgesic (parasetamol + propifenazon 400 mg/day) were prescribed postoperatively for 5 days. The drainage catheter was removed after 48 hours. Patient controls were performed at the end of the first week after surgery.

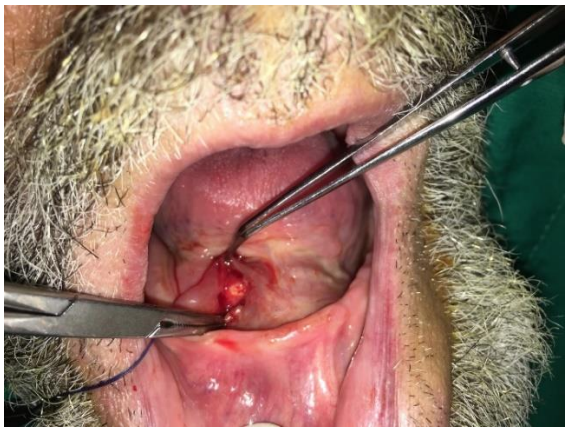

Figure 2. The duct was sutured by posterior of the sialolith. After blunt disection, the sialolith was identified.

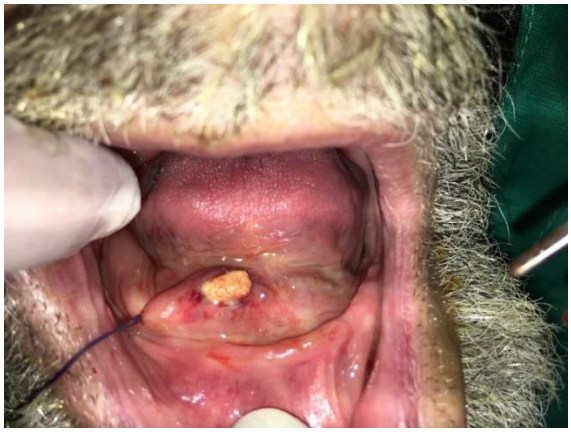

Figure 3. It is showed the removal of the sialolith.

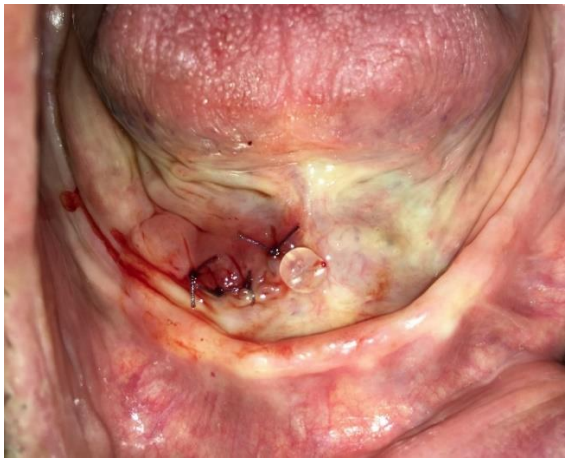

Figure 4. The catheter was inserted for submandibular gland drainage

Statistical analysis

Age, location of the sialoliths, preoperative and postoperative symptoms which were swelling, pain, edema and pus as well as complications and recurrences were noticed. Furthermore, the patient's satisfaction is recorded according to the patient satisfaction index, is shown in Table 1.

Table 1. Patient satisfaction index

Dissatisfied	Resolving of 50% or less of the pre-operative symptoms
Normal	Resolving of 50% - 75% of the pre-operative symptoms
Satisfied	Resolving of 75% or more of the pre-operative symptoms
Very satisfied	No remaining of the pre-operative symptoms

Results

This study group consists of 8 patients (6 males and 2 females) aged between 29-81 years who were transoral surgically removed submandibular canal stones from 2012 to 2017. After clinical and radiological examination, 38% of all sialoliths were determinated in the distal part of the Wharton's duct (anterior third) and 62% in the proximal part (middle and posterior third). The mean size of the sialoliths at the distal part were 1.41 cm and the proksimal part were 2.51 cm. Six of the patients had pain and swelling, especially during eating, and one of them had pus formation, one patient has no symptoms and the other patient has only pain.

In addition, submandibular gland stones which were located at the proximal part (62%), were treated under general anesthesia, and the stones of the distal part (38%) were treated under local anesthesia. These characteristics of patients are evidenced in Table 2.

There was no intra-operative or post-operative complication, such as lingual nerve damage and bleeding, in our cases during the follow-up period, average 20 months. As a result of the survey, 75% of the patients were very satisfied, 12.5% satisfied and 12.5% dissatisfied with the result. Postoperative pain and swelling occurred in one patient. The patient's clinical symptoms and ultrasound images revealed that improved the recurrent sialolith formation in the same submandibular duct. The result evaluation is shown on Table 3.

Table 2. Patients characteristics

Patients	Sex	Age	Pre-operative Symptoms	Enfection	Localization of Sialolith	Anesteshia
1	M	74	Swelling, Pain	-	Middle Third	G
2	M	36	Swelling, Pain	-	Middle Third	G
3	M	81	Swelling, Pain	Edema, Pus	Anterior Third	L
4	M	45	Swelling, Pain	Edema	Middle Third	G
5	F	29	Swelling, Pain	Edema	Anterior Third	L
6	F	75	-	-	Anterior Third	L
7	M	45	Swelling, Pain	Edema	Posterior Third	G
8	M	53	Pain	-	Posterior Third	G

Table 3. Evaluation of clinical outcomes

Complication	Satisfaction	Recurrence	Follow-up (Months)
-	Very Satisfied	-	24
-	Very Satisfied	-	36
-	Satisfied	-	5
-	Very Satisfied	-	18
-	Dissatisfied	+	6
-	Very Satisfied	-	28
-	Very Satisfied	-	24
-	Very Satisfied	-	24

Discussion

We aimed to evaluate the results of the transoral approach in the treatment of Wharton's duct stones and to evaluate this application against other minimally invasive techniques. This procedure is a simple and successful treatment option that eliminates the possible disadvantages of variable procedures which are extraoral approach, ESWL, sialendoscopy or laser fragmentation, are used in the treatment modalities of salivary stones. In particular, the major techniques, submandibular gland excision with extraoral approach, used in the treatment of stones in the proximal part of the Wharton's duct or intraglandular stones have disadvantages such as facial nerve damage and scarring (Eun et al., 2010). However, it has been reported that inflammation and mucosel or retention cyst formation occur due to the fact that the gland is not completely removed by the extraoral approach (Blatt, 1966; Berini-Aytes and Gay-Escoda, 1992). Hong and Kim (2000). reported no facial nerve damage, minimal residual gland formation (3%) and abscess formation (3%) in the postoperative period of the patients underwent submandibular gland excision with intraoral approach. In the same study, decreasing of lingual nerve sensitivity and tongue movements were stated in the early post-operative period. In our study, for the purpose of removing the stones in the proximal part of Wharton's duct (62%), we performed the treatment using the intraoral approach without the excision of the submandibular gland and all patient were very satisfied and there were no complications such as nerve damage, abscess formation and taste changes.

Successful results were obtained with sialendoscopy, especially in the treatment of minor sialoliths (<4mm) (Matsunobu et al., 2014; Gerni et al., 2017). Goodstain et al (2017). reported that they obtained successful results in stones up to 6 mm in their study. Furthermore, the sialendoscopy procedure in Wharton's duct was more difficult than Stensen's duct (Chossegros et al., 2006) and narrowing of the duct during the post-operative period and traumatic ranula development have been reported (Nahlieli et al., 2006). It was stated that only sialendoscopy treatment is not sufficient in the majority of cases and may be used in combination with other techniques such as ESWL or laser fragmentation (Marchal and Dulguerov, 2003; Matsunobu et al., 2014; Schwartz et al., 2015). In ESWL method, it is aimed to dispose the dissected sialolites into small pieces from salivary gland duct with normal saliva by using shock waves without the need for surgical treatment. In the literature, it was reported that using in the treatment of sialoliths which are smaller than 8mm has been shown and damaged the vital structures in cases with incorrect focus (Lafont et al., 2018; Foletti et al., 2018). Also, ESWL is unsuccessful in some cases and limited application in submandibular glands has been described in previous publications (Zenk et al., 2001; Escudier et al., 2003). Ottaviani et al (Ottaviani et al., 1996). stated that in order to treat larger sized sialolites more effectively, surgical treatment should be preferred instead of expensive and time-consuming ESWL procedure.

We extracted Wharton's duct sialoliths with an average size of 2.01 cm, directly from the duct without auxiliary equipment as used other minimal invasive procedures. In the previously published studies, the success rate of treatment with a transoral approach ranged from 85% to 100% has shown by Gerni at al. (2017). Thus, according to the data of our study, the success rate (87.5%) is consistent with the literature.

Conclusion

The transoral approach may be considered as a more effective option for treatment of Wharton's duct sialoliths because of high success rate and the wider use indication compared to non-invasive procedures such as ESWL and sialendoscopy.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University.
Informed Consent: Oral and written informed consent was obtained from the participants.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – A.F., K.K.; Design- A.F., K.K.; Supervision- A.F., K.K.; Funding- A.F., K.K.; Materials- A.F., K.K.; Data Collection/Data Process- A.F.; Analyze or Comment- A.F., K.K., Literature Scanning- A.F., K.K.; Writer of Paper- A.F.; Critical Review- A.F.
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RESEARCH ARTICLE

Comparison of Identification of *Toxoplasma gondii* by Commercial Realtime PCR and Inhouse Realtime PCR Methods

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Abstract

Objective: This study aimed to compare the diagnosis of toxoplasmosis with a commercial kit and inhouse realtime PCR methods to determine molecular methods with high diagnostic accuracy for use in addition to serologic tests for routine diagnosis.

Methods: The study included a total of 116 samples of blood, CSF or amniotic fluid with 19 identified positive and 97 negatives for *T. gondii* sent to our laboratory. Due to the low number of positive samples, DNA samples from an external quality control program that our laboratory participates in were included in the study. First to all samples, realtime PCR method were applied with commercial kit used primers for *T. gondii* rep529 gene, and then inhouse realtime PCR were applied with TG-F and TG-R primers and Taqman probe, targeting the insertion sequence region of *T. gondii* B1 gene.

Results: The results for the total of 116 samples studied with both methods was that 17 were identified as positive with commercial realtime PCR and 19 were determined as positive with inhouse realtime PCR. Accordingly, two cases with the commercial realtime PCR method were determined as false negative. The limit of detection for both methods used in our study was determined as 10-3 dilution (0.028 copy/reaction). There was a high level of compatibility determined between the inhouse and realtime PCR methods (kappa value: 0.934).

Conclusion: In conclusion, though there was perfect compatibility observed between the results with the two methods, disadvantages of the commercial realtime PCR method included isolates where the target gene was not found, deletion or mutation of all or part of this gene or different numbers of repeats causing false negative results and high cost. Considering this, our laboratory decided to use the inhouse realtime PCR using primers for the B1 gene to research *T. gondii* with molecular methods. A significant limitation of the study is the low number of positive samples. For DNA samples belonging to the External Quality Control Program, the commercial kit was 66.66% successful, while the inhouse realtime PCR method was 100% successful.

Key words: *Inhouse realtime PCR, realtime PCR, T. gondii*

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Introduction

Toxoplasma gondii (T. gondii) is an obligate intracellular apicomplexan parasite responsible for opportunistic infections in patients with immune suppression and infects all warm-blooded vertebrates including mammals and birds (Sails, 2004; Su et al., 2010; Robert-Gangneux and Belaza, 2016; Rajendran et al., 2018). Cats are definite hosts (Rajendran et al., 2018). Infection may occur by eating raw or poorly cooked meat containing tissue

cysts, drinking water contaminated by oocysts from feces of infected cats or eating fruit-vegetables washed in this water. Just as infection may be observed through blood or organ transplantation, the tachyzoite form may be transferred in congenital infection from mother to child (Hill and Dubey, 2002; Sails, 2004; Su et al., 2010; Rajendran et al., 2018; Rostami et al., 2018). Infection is generally asymptomatic and self-limiting, especially in hosts with stable immune system (Hill and Dubey, 2002; Su et al., 2010; Rajendran et al., 2018). However, lymphadenopathy, fever, fatigues, muscle pain, throat pain and headache or ocular toxoplasmosis may be observed in some patients (Hill and Dubey, 2002; Su et al., 2010). Due to the presence of tissue cysts, it may remain as lifelong latent infection (Switaj et al., 2005). When congenital infection is obtained in the first trimester, it causes more severe clinical findings compared to transmission in the second and third trimester (Hill and Dubey, 2002). It may cause miscarriage especially in early pregnancy, stillbirth and congenital anomalies (Hill and Dubey, 2002; Su et al., 2010; Rajendran et al., 2018). For patients with suppressed immune systems such as those infected during pregnancy, fetuses and neonates with intrauterine infection, HIV patients, patients with organ transplantation and retinochoroiditis patients, the diagnosis is critical (Rostami et al., 2018). Diagnosis may be made with microscopic investigation of smears from tissue obtained by biopsy or autopsy stained with Giemsa (Hill and Dubey, 2002). Parasite may isolated with inoculation of laboratory animals and tissue cultures with these clinical samples, and additionally by secretions and body fluids obtained from patients (Hill and Dubey, 2002; Su et al., 2010). However, these methods are not practical, require intense effort and a few days to obtain results (Calderaro et al., 2006; Fallahi et al., 2015). Routine diagnosis of disease is based on serologic methods especially (Su et al., 2010; Rostami et al., 2018). Diagnostic methods like Sabin Feldman Dye testi (SFDT), IFAT, latex agglutination test (LAT), IHA, ELISA, modified agglutination test (MAT), western blot (WB), and IgG avidity tests are used (Hill and Dubey, 2002; Rostami et al., 2018). In recent years, immunological methods like CLIA, ELFA, immunochromatography tests and ISAGA have been developed (Rostami et al., 2018). Antibody titrations remaining high after clinical amelioration of infected people make it difficult to interpret the results of these serologic tests (Hill and Dubey, 2002). The most important limitation of these methods is the inability to fully estimate the infection duration. Serologic diagnosis is difficult for patients with congenital infection or immune failure (Wastling et al., 1993; Switaj et al., 2005). In these patients with insufficient or disrupted immune response, effective, rapid, and accurate diagnosis is important to be able to begin treatment. Molecular techniques are very beneficial for these patients as evaluation of results is not linked to the immune status of the host (Wastling et al., 1993; Switaj et al., 2005; Calderaro et al., 2006; Fallahi et al., 2015). Especially, realtime PCR methods are commonly used due to high sensitivity and specificity (Fallahi et al., 2015; Rostami et al., 2018).

Just as around the world, toxoplasmosis is a significant health problem in Turkey and the use of serology and molecular methods together is recommended for cases to strengthen diagnosis, especially for cases experiencing diagnosis problems. This study aimed to compare diagnosis of toxoplasmosis with a commercial and inhouse realtime PCR methods to determine molecular methods with high diagnostic accuracy for use in addition to serologic tests for routine diagnosis.

Methods

The study, included a total of 116 samples of blood, CSF or amniotic fluid with 19 identified positive and 97 negative for *T. gondii* sent to our laboratory. Due to the low number of positive samples, DNA samples from an External Quality Control Program that our laboratory participates in were included in the study. Samples were applied DNA exraction with a commercial DNA extraction kit (QIAmp DNA mini kit, Qiagen, Germany) used in accordance with the manufacturer's. DNA isolation was performed with commercial kit from *T. gondii* strain which was carried out with mouse passage in our laboratory. Limit of detection study was performed with serial dilutions of this DNA sample prepared with 1/10 dilutions. The obtained DNA samples used two different realtime PCR methods for verification in our laboratory for identification of *T. gondii*. Realtime PCR method was applied to all samples first by using commercial *T. gondii* rep529 gene primers (Genesig, Primer Design, UK), according to manufacturer's recommendations. Then as recommended by Lin et al. TG-F (5'-CTTCGTCCAAGCCTCCGA-3') and TG-R (5'-GACGCTTTCCTCGTGGTGAT-3') primers and Taqman probe (6-FAM-TCTGTGCAACTTTGGTGTATTCGCAG-BHQ-1) were used targeting the insertion sequence region of the *T. gondii* B1 gene for inhouse realtime PCR.

Optimization studies were performed to determine the synthesized primers and best study concentrations for the probe. According to the results of this study for 20 µl reaction mix, reaction volume was set with LightCycler probe mastermix (Roche, Mannheim, Germany) 10 µl, 1 µl of each primer (10 pmol), probe (4 pmol) 0.4 µl, molecular grade water 5.6 µl and DNA 2 µl. The amplification cycles of PCR was 95 °C 5 min initial denaturation, 40 cycles of two stage 95 °C 15 s denaturation, 60 °C 30 s annealing- extension studied in a LightCycler realtime device (Roche, Mannheim, Germany) (Lin et al., 2000).

Results

DNA extraction was performed with a commercial kit using 200µl (2,800 tachyzoite) *T. gondii* strain (14,000 tachyzoite/ml) obtained by mouse passage in our laboratory. At the end of extraction, DNA was obtained within 200 µl elution buffer (2,800 genomic DNA). In PCR reaction used 2 µl DNA. Limit of detection studies were performed with serial dilutions prepared with 1/10 dilution of the DNA sample. The limit of detection for both methods used in our study was determined as 10^{-3} dilution (0,028 copy/reaction). The comparison of CT values belonging to limit of detection of samples with both methods is given in Table 1. The inhouse realtime PCR limit of detection studies determined 3.51-3.44 difference between CTs of dilutions, while the commercial realtime PCR limit of detection study found 4.72-9.79 difference between CTs of dilutions. The results for the total of 116 samples studied with both methods was that 17 were identified as positive with commercial realtime PCR and 19 were determined as positive with inhouse realtime PCR. Accordingly, two cases with the commercial realtime PCR method were determined as false negatives (Table 2). There was a high level of compatibility determined between the inhouse and commercial realtime PCR methods (kappa value: 0.934). The results of studying DNA samples belonging to the external quality control program identified five positives and one negative correctly as determined by the program organizer with the inhouse realtime PCR, while the commercial realtime PCR method determined two positive samples as being negative (Table 3).

Table 1: Comparison of CT values of Inhouse realtime PCR and commercial kit

	10^{-1} dilution (2,8 copy)	10^{-2} dilution (28 copy)	10^{-3} dilution (0,028 copy)*
Inhouse Realtime PCR CT values	25.05	28.56	32
Commercial Realtime PCR CT values	18.73	23.45	33.24

*Limit of detection

Table 2: Comparison of Inhouse realtime PCR method with commercial kit.

		Inhouse Realtime PCR		
Commercial Realtime PCR		**Positive**	**Negative**	**Total**
	Positive	17	0	17
	Negative	2	97	99
	Total	19	97	116

Table 3: Results of External Quality Control Program samples with commercial and inhouse realtime PCR method.

	Intended Results	**Inhouse Realtime PCR Results**	**Commercial Realtime PCR Results**
Sample 1	Positive	Positive	Positive
Sample 2	Positive	Positive	Positive
Sample 3	Positive	Positive	**Negative***
Sample 4	Negative	Negative	Negative
Sample 5	Positive	Positive	**Negative***
Sample 6	Positive	Positive	Positive

* False negative samples

Discussion

Among molecular methods for diagnosis of toxoplasmosis, the most commonly used are conventional PCR, nested PCR and realtime PCR methods (Su et al., 2010). Conventional PCR method is beneficial, but has low sensitivity and specificity and is a very laborious and time-consuming method (Sails, 2004). Nested PCR increases specificity of DNA amplification and is used to identify very small amounts of the pathogen. However, this method involves higher contamination risk and as a result has low specificity (Robert-Gangneux and Belaza, 2016; Rostami et al., 2018). Realtime PCR is a method making it possible to diagnose the pathogen and determine parasite load in clinical samples and has high diagnostic accuracy. It is the most sensitive molecular method to identify target DNA especially at low concentrations (Sails, 2004, Calderaro et al., 2006; Su et al., 2010; Ivović V et al., 2012; Rostami et al., 2018). Quantitative realtime PCR determines decreasing parasite loads in clinical samples like

blood, BAL and CSF offering the opportunity to monitor treatment efficacy (Sails, 2004; Robert-Gangneux and Belaza, 2016; Rostami et al., 2018). Due to the closed system, there is low risk of amplicons contaminating the environment and low possibility of false positive results with probes able to measure amplification products in each cycle leading to the superiority of realtime PCR (Sails, 2004; Rostami et al., 2018). This method also can identify the DNA of more than one pathogen in the same tube. Due to this, the number of laboratories using realtime PCR methods is rapidly increasing.

In this study, we aimed to compare two PCR methods to select a realtime PCR with high diagnostic accuracy for use in our laboratory. When both methods are compared in our study, four samples had incompatible results. The reasons for these incompatible results may be inhibitors not removed during DNA extraction from the matrix of the examined sample, target regions used and master mix composition used in the method or inability to optimize the amplification thermal cycle well.

When sample matrix is investigated, two of the samples with incompatible results were blood, one was amniotic fluid and one was CSF. Due to the low number of samples, it is notable that two samples with incompatible results were whole blood samples. Heme is a significant inhibitor for PCR (Cardona et al., 2011). Inhibitors from the samples in the DNA extraction stage may prevent good shaping of the reaction. There may not be sufficient reagents in the mastermix used to prevent effects from inhibitors. Incompatibility may be observed among results obtained in this situation. Procedures used when studying blood samples (isolation of leukocyte cells from 5 or 10 mL whole blood or direct DNA extraction from full blood) may change the PCR results. Parasite load identified in the buffy coat obtained from 1200 µl blood is reported to be higher than that obtained from 200 µl blood. As *T. gondii* proliferates in leukocyte cells and whole blood contains PCR inhibitors, it should be remembered that sensitivity may reduce (Robert-Gangneux and Belaza, 2016).

Another important factor affecting sensitivity and specificity is the selection of DNA target and primers (Calderaro et al., 2006; Mousavi et al., 2016). Identification of the single copy gene on the surface of P30 protein was the first target gene used for identification of *T. gondii* DNA with PCR (Robert-Gangneux and Belaza, 2016). However, the sensitivity of single copy genes like P30 is low and as a result it is chosen less often today (Su et al, 2010; Ivović et al., 2012). Later, 30 to 300 repeated sequence targets are recommended in the parasite genome (Robert-Gangneux and Belaza, 2016). One of the target genes still most commonly used for molecular identification of *T. gondii* is the B1 gene with 35 repeats in the genome. This gene is conserved at high rates in all *T. gondii* strains (Switaj et al., 2005; Calderaro et al., 2006; Su et al., 2010; Ivović et al., 2012; Rostami et al., 2018). Following this, a few multiple copy target genes including 18S rRNA and ITS-1 genes, rep529 repeated fragment or AF146527 element (300 copy target gene) are used (Su et al., 2010; Ivović et al., 2012; Rostami et al. 2018). Studies have reported PCR of ITS-1 and 18S rDNA sequences have similar sensitivity for the B1 gene (Su et al., 2010; Switaj et al., 2005; Liu et al., 2015). PCR targeting the multiple copy gene of rep529 gene is reported to be 10-100 times more sensitive than the B1 gene (Switaj et al., 2005; Su et al., 2010; Ivović et al., 2012; Liu et al., 2015; Rostami et al., 2018). Due to the high sensitivity and specificity of realtime quantitative PCR, currently these two target genes are more commonly used and comparisons of these are found in many studies. When these studies are assessed, in addition to studies reporting the rep529 gene is more successful (Hierl et al., 2004; Belfort et al., 2008; Sterkers et al., 2010; Robert-Gangneux et al., 2017), there are studies reporting the B1 gene is more successful (Wahab et al., 2010; Cardona et al., 2011; Mousavi et al., 2016; Kalantari et al., 2017). Diagnostic performance for identification of *T. gondii* with PCR may be affected by many factors like repeat number of the target and target series polymorphism or absence (Teixeira et al., 2013). The true repeat numbers of the target genes are still a topic of debate and may be lower than expected or may display differences between parasite strains (Robert-Gangneux and Belaza, 2016). Some recent studies have reported the copy number for the rep529 gene in the Toxoplasma genome is lower than the 5 to 12 times found in previous studies (Ivović et al., 2012). Again, some studies comparing the B1 gene and AF146527 gene reported false negative results for some samples with the AF146527 gene. The reason for this is considered to be the rep529 gene not being found in all isolates analyzed, deletion or mutation of all or fragments of it or repeat numbers being different in parasite strains (Wahab et al., 2010; Ivović et al., 2012). Before a diagnostic laboratory brings a protocol using AF146527 repeated fragments, it is necessary to reveal the specificity very well (Wahab et al., 2010). In our study, four samples with false

negatives determined are considered to be due to absence or polymorphism of the rep529 gene.

One of the factors affecting sensitivity and specificity is the amplification conditions (Calderaro et al., 2006). In amplification conditions, the annealing temperature of primers is important. Optimization studies were performed to determine the best annealing temperature for the inhouse PCR method. Annealing temperatures may display differences from device to device. Although commercial kits are based on recommendations in the manufacturer's instruction, sometimes may not have optimum efficiency. The incompatibility of results obtained in our study may due to this reason.

When calculating the amplification efficiency of realtime PCR, the CT differences between dilutions are important. A 100% efficient reaction will provide 10-fold increase (log10= 3.3219) in PCR amplicon in every 3.32 cycles in the amplification exponential phase. The mean CT difference between DNA samples diluted 10-fold should be 3.32. CT difference larger than 3.32 (i.e. 3.9) shows efficiency lower than 100%, while CT difference lower than 3.32 (i.e. 2.5) shows problems with sample quality or pipetting (ThermoFisher manual). For our inhouse realtime PCR, limit of detection studies determined 3.51-3.44 CT difference between dilutions, while for the commercial realtime PCR limit of detection studies found 4.72-9.79 CT difference between dilutions. According to this data, the CT difference being above 3.9 may be assessed as the commercial kit having low efficiency. The result of studying DNA samples with known content from the External Quality Control Program identified five positives and one negative with inhouse realtime PCR, while two positive samples were determined as negative with the commercial realtime PCR method. The efficiency of the commercial kit was not found to be high when assessing positive samples.

Conclusion

In conclusion, though there was perfect compatibility observed between the results with the two methods in terms of kappa value, disadvantages of the commercial realtime PCR method included isolates where the target gene was not found, deletion or mutation of all or part of this gene or different numbers of repeats causing false negative results and high cost. Considering this, our laboratory decided to use the inhouse realtime PCR using primers for the B1 gene to research *T. gondii* with molecular methods. A significant limitation of the study is the low number of positive samples. For DNA samples belonging to the External Quality Control Program, the commercial kit was 66.66% successful, while the inhouse realtime PCR method was 100% successful. As a result, it is considered that more comprehensive studies increasing the the number of positive samples will increase the reliability of the results.

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RESEARCH ARTICLE

Clinical and Pathologic Evaluation of Adnexal Torsion Patients in Adolescence, Reproductive and Postmenopausal Periods

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Abstract

Objective: The objective of our study was to compare the adnexal torsion patients in different age groups clinically and pathologically.

Methods: Sixty-eight patients who had applied with pelvic pain and adnexal torsion diagnosis had been confirmed intraoperatively were included in the study. Patients were divided into three groups based on their ages as the adolescent period (Group 1), reproductive period (Group 2) and postmenopausal period (Group 3). Ages, history of torsion, history of past operations, periods between the date of hospitalization and operation, periods of hospital stay, adnexa as the localization of torsion and number of tours of torsion were examined in groups. Mass lesions found during the operation and pathologic consequences of such masses were examined retrospectively and compared.

Results: No statistically significant differences in torsion history, past operations, WBC and platelet values, neutrophil/lymphocyte ratios, preoperative fever, preoperative nausea and vomiting and preoperative Doppler US findings between the groups included in the studies (p>0,05). Although not statistically significant, WBC values were higher in the postmenopausal patient group, and it was notable that symptoms including fever, nausea and vomiting were absent in this group. The adolescent age group was the group with the least confirmation of torsion diagnosis following the operation with 13 (52%) patients. Upon evaluation of the operations carried out on patients, it was seen that mostly fertility preserving approaches were preferred for adolescent and reproductive patients with 88%, while more radical methods such as USO (72.7%) and TAH+BSO (18.2%) were preferred in postmenopausal patients. Ovarian tissue with torsion without any pathologic findings is more frequent in the adolescent period as compared to other groups (48%). In the postmenopausal period however, some pathology was found in the entire portion of the adnexa with torsion. In the postoperative evaluation of the pathology results of patients, no significant differences were observed as regards the diameters of cysts (p:0,207). There are significant differences between the histopathological types.

Conclusion: Although statistically significant differences have not been observed as regards the clinic al presentation and laboratory findings based on age groups; it must be kept in mind that a mass lesion can accompany the torsion and the pathology of this mass can vary. Torsion must be remembered in pelvic pain in the adolescent age group because of the indistinct clinical findings and inadequacy of imaging methods.

Key words: Adnexal Torsion, Pelvic Pain, Gyneacologic Emergency

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Introduction

Adnexal torsion constituting about 2.5-7.4% of patients applying to emergency room is defined as the partial or complete rotation of the ovaries, Fallopian tubes or both on the ligamentous supports and vascular pedicles (Hibbard, 1985). The reason for adnexal torsion being one of the most important emergencies of gynecology is the ischemia and necrosis developing as a consequence of the reduced blood flow (Hibbard, 1985). While adnexal torsion can be seen in every age group from intrauterine life to postmenopausal period, it faces us most frequently in the reproductive period (Günay et al., 2018). The diagnosis and treatment is an issue that maintains its actuality based on the facts that it is seen in adolescents and women in reproductive ages (mean age 26 years) and its adverse effects on ovarian functions (Hibbard, 1985).

Conditions leading to adnexal torsion are not clearly known; however, the most common predisposing factor is adnexal mass lesions. Functional ovarian cysts and benign cystic teratoma are frequently seen among the causes of adnexal torsion while malignant cysts and endometrioma are seen less frequently (Huchon and Fauconnier, 2010). Furthermore, the first trimester of pregnancy and assisting reproductive techniques are the other predisposing factors of adnexal torsion (Houry and Abbott, 2001). Hydrosalpinx, hematosalpinx, paraovarian cysts are among the other risk factors of isolated tubal torsion (Ekmekci et al., 2010; Noviello et al., 2018).

Ischemia related to torsion and loss of ovarian functions in relation with the former is the most important complication of torsion, and such adverse effects are reduced significantly in patients who are diagnosed in the early period (Huchon and Fauconnier, 2010). This is particularly important for young patients with fertility expectations (Santra et al., 2018). At the same time, diagnosis is difficult because of the clinical presentation mostly involving nonspecific findings.

Abdominal pain, nausea, vomiting and fever are typical for the clinical presentation of adnexal torsion. Pain may not be present in every patient, and this causes delays in diagnosis (Bodur et al., 2016). Possibility of necrosis increases in patients who are taken to operation about 10 hours later than the onset of pain (Mazouni et al., 2005). Nausea and vomiting are most common, and can be seen in 70% of patients (Lomano et al., 1970). Fever, which is an indicator of necrosis, can also be seen as a symptom (Houry and Abbott, 2001). There is no specific laboratory findings; however, leukocytosis is seen frequently (Chiou et al., 2007). The most commonly used imaging technique is ultrasonography (USG). However, according to studies, diagnostic benefit of US is quite limited. This benefit ranges between 46% and 77% according to one study, and it has not been possible to show the diagnostic benefit of Doppler US in addition to conventional US (Albayram and Hamper, 2001; Mashiach et al., 2011; Wilkinson and Sanderson, 2012). MRI and CT studies are useful in cases where an accompanying mass lesion is present.

The value of this study lies in the fact that studies comparing the adnexal torsion cases seen in adolescents, adults and postmenopausal individuals are lacking in the literature. In this context, our study will be the first. We aimed at comparing the adnexal torsion cases in different age groups in clinical and pathologic aspects.

Methods

Files of patients operated due to adnexal torsion in Liv Hospital Ankara between January 2016 and August 2018 were evaluated retrospectively. Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Liv Hospital Ankara. Demographic data, past torsion histories, clinical presentations, findings in operations, post-operative periods and pathology results of patients were reviewed on the computer system and patient files.

Sixty-eight patients applied with pelvic pain and diagnosis of adnexal torsion confirmed intraoperatively were included in the study. All operations conducted on patients included in the study were performed by gynecologists and obstetricians. Patients were divided into three groups according to their ages, namely, the adolescent period (Group 1), reproductive period (Group 2) and the postmenopausal period (Group 3). Ages, history of torsion, history of past

operations, periods between the date of hospitalization and operation, periods of hospital stay, adnexa as the localization of torsion and number of tours of torsion were examined in groups. Mass lesions found during the operation and pathologic consequences of such masses were examined and compared retrospectively.

Groups were compared in terms of the types and times of operations performed on patients, preoperative symptoms, laboratory markers including leukocyte (WBC) and platelet counts and neutrophil/leukocyte ratio and absence of Doppler flow suggesting torsion.

Mean value, standard deviation, the lowest and the highest median values, frequencies and ratios were used in the descriptive statistics of the data. Distribution of values were measured with Kolmogorov-Simirnov test. ANOVA (Tukey test) and Kruskal-Wallis test were used in the analysis of quantitative data. Chi-square test was used for the analysis of qualitative data; and if the conditions required for the chi-square test could not be met, Fischer test was used for the same. SPSS 22.0 program was used for the analyses. P value <0.05 was accepted as the limit of significance in all analyses.

Results

Demographic characteristics of patients included in the study are summarized in Table 1. No statistically significant differences were found between the past torsion histories, past operations, WBC and platelet counts, Neutrophil/Lymphocyte ratios, preoperative fever, preoperative nausea and vomiting, and absence of Doppler flow in the adolescent, reproductive and postmenopausal period groups. (p value <0,05 was accepted as the level of statistical significance.) (Table 1)

Upon evaluation of patients based on their age groups, higher WBC values in the postmenopausal group is notable, although not statistically significant. In the evaluation of the preoperative fever, this was more marked only in the adolescent group, again not statistically significant (12%). Furthermore, absence of symptoms including fever, nausea and vomiting in the postmenopausal patient groups is notable. US findings suggesting torsion were seen in the preoperative Doppler US study in the adolescent age group only with a ratio of 32%. Preoperative diagnoses are given in Table 1 based on age groups. The adolescent age group is the one that torsion diagnosis was the least confirmed intraoperatively with 13 patients (52%). In addition, total of 51 patients were operated with the diagnosis of preliminary torsion in all the age groups within the same period, and torsion diagnosis had become definite in 44 patients (86%).

No significant differences were observed in the operation time, period between admission and operation, side of the torsion, number of tours of torsion and hospital stay periods in the intraoperative evaluation of patients. (Table 2) Upon evaluation of the procedures performed on patients however, it was found that fertility-preserving approaches had been more commonly preferred in the adolescent and reproductive patients (88%), while more radical methods had been preferred such as USO (72.7%) OR TAH+BSO were preferred in postmenopausal patients. It was observed that the period between admission and performance of the operation was longer in the postmenopausal patients, although not statistically significant (17,6+20,9; 14,3+14,6; 25,3+22,0 respectively).

Evaluation of postoperative pathology results of patients showed no significant differences in the diameters of cysts (p = 0,207); however, there are significant differences in histopathological types (p = 0.015). (Table 3) In the histopathological evaluation, mature cystic teratoma (20%) and simple serous cysts (12%) were found in the adolescent age group, mature cystic teratoma (21.9%), mucinous cystadenoma (18.8%) and simple serous cyst (18.8%) in the reproductive age group, and widespread hemorrhagic infarct (63%) in the postmenopausal age group come to the front. One (9.1%) malignant ovarian tumor was found in the postmenopausal age group. Ovarian tissue with torsion and without any other pathologic finding is more frequent in the adolescent period as compared to other groups (48%). In the postmenopausal period however, some pathology was found in the entire portion of the adnexa with torsion.

Table 1. Demographic Characteristics and Preoperative Findings of Patients

		Adolescence Period (n:25)	Reproductive Period (n:32)	Postmenopausal Periods (n:11)	P
Age		15,0+1,7	31,4+4,8	52,2+5,9	
Past torsion history		2 (%8)	4 (%12,5)	0 (%0)	0.566
Past operations					0,078
	No	22 (%88)	22 (%68,8)	6 (%54,5)	
	USO	0 (%0)	2 (%6,3)	1 (%9,1)	
	Appendectomy	1 (%4)	2 (%6,3)	1 (%9,1)	
	Cesarean	2 (%8)	4 (%12,5)	3 (%27,3)	
	Cystectomy	0 (%0)	2 (%6,3)	0 (%0)	
WBC (*10^3)		15,6+27,6	13,7+14,5	22,6+41,7	0,985
Platelet Count (*10^3 /mm^3)		272,0+53,9	267,2+61,3	279,1+79,5	0,836
Neutrophil/Lymphocyte ratios		6,8+5,2	5,9+3,7	7,5+4,8	0,519
Preoperative Diagnosis					0.082
	Torsion	13 (%52)	25 (%78,1)	6 (%54,5)	
	Adnexal Mass	6 (%12)	4 (%12,5)	4 (%36,4)	
	Rupture	2 (%8)	3 (%9,4)	1 (%9,1)	
	Appendicitis	4 (%16)	0 (%0)	0 (%0)	
Preoperative Fever		3 (%12)	0 (%0)	0 (%0)	0,612
Preoperative Nausea-Vomiting		3 (%12)	7 (%21,9)	0 (%0)	0,292
Absence of Doppler Flow		8 (%32)	13 (%40,6)	6 (%54,5)	0,093

Table 2. Intraoperative Evaluation of Patients

	Adolescence Period (n:25)	Reproductive Period (n:32)	Postmenopausal Periods (n:11)	p
Operation				0,136
Detorsion	11 (%44)	8 (%25)	0 (%0)	
Detorsion + Cyst excision	11 (%44)	13 (%40,6)	0 (%0)	
Salpingectomy	1 (%4)	1 (%3,1)	1 (%9,1)	
Unilateral Salpingo-oophorectomy	2 (%8)	10 (%31,3)	8 (%72,7)	
Total Abdominal Hysterectomy + Bilateral Salpingo-oophorectomy	0 (%0)	0 (%0)	2 (%18,2)	
Duration of Operation (min)	54,6+29,6	56,3+29,2	70,0+31,1	0,263
Duration of Admission to Operation (hours)	17,6+20,9	14,3+14,6	25,3+22,0	0,499
Torsion Side				0,506
Right	13 (%52)	19 (%59,4)	8 (%72,7)	
Left	12 (%48)	13 (%40,6)	3 (%27,3)	
Torsion Type				0,318
Over	12 (%48)	12 (%37,5)	5 (%45,5)	
Tubal	0 (%0)	1 (%3,1)	0 (%0)	
Adnexa	13 (%52)	19 (%59,4)	6 (54,5)	
Number Of Torsion Cycles	2,5+1,5	2,3+1,3	2,3+1,3	0,918
Hospitalization Period (hours)	40,8+26,6	38,0+19,8	38,1+17,7	0,993

Table 3. Postoperative Pathology Results of Patient

	Adolescence Period (n:25)	Reproductive Period (n:32)	Postmenopausal Periods (n:11)	p
Cyst Diameter (Avg. ± SD; Med.)	75.6±24.2; 67	89.9±31.8; 86	87.4±32.9; 75	0.207
Cyst Pathology				0.015
Paratubal cyst + hydrosalpinx	1(%4)	0 (%0)	1 (%9.1)	
Mature Cystic Teratoma	5 (%20)	7 (%21.9)	1 (%9.1)	
Mucinous Cystadenoma	2 (%8)	6 (%18.8)	1 (%9.1)	
Common Hemorrhagic İnfarct	0 (%0)	4 (12.5)	7 (%63.6)	
Simple Serous Cyst	5 (%20)	6 (%18.8)	0 (%0)	
Cancer	0 (%0)	0 (%0)	1 (%9.1)	
No Pathology	12 (%48)	9 (%28.1)	0 (%0)	

Discussion

Diagnosis and treatment in the early period are important in torsion patients for preservation of organ functions. While clinical symptoms and findings are nonspecific for diagnosis, torsion presents with some signs including nausea, vomiting and pelvic pain and some findings including muscular defense, rebound and tenderness. Clinically, patients commonly apply with abdominal pain with sudden onset. Pain is localized in the lower right quadrant as sharp and stinging pain with sudden onset generally following a physical activity. Fever is added to the clinical picture in some cases. (White and Stella, 2005) In our study, while abdominal or pelvic pain was present in all the patients, nausea and vomiting found 10 patients (14.7%) and fever was found in 3 (4.4%). Nausea and vomiting were found in none of the postmenopausal patients. Since the clinical findings other than pain rarely accompanies and creates confusion in many other clinical pictures, torsion must be remembered in women with acute lower abdominal pain.

US, Doppler US, magnetic resonance imaging and computerized tomography can be used for the diagnosis of adnexal torsion as assisting imaging methods (Chang et al., 2011). B-mode US and Doppler US are imaging methods that should be selected in the first place in patients with suspected torsion based on their advantages of being inexpensive, easily accessible and ability to evaluate the blood flow (Bronstein et al., 2015). The most frequently-seen findings in imaging include the expansion in ovaries and absence of Doppler flow. However, normal blood flow can be seen in partial torsion with preserved arterial blood flow and interrupted lymphatic drainage (Shadinger et al., 2008), which can lead to delayed diagnosis or evaluation based on an erroneous preliminary diagnosis. PPV is reported as 19-35% for adnexal torsion with transabdominal ultrasound and 94% with transvaginal ultrasound (Naiditch and Barsness, 2013) Use of TV US was limited for the adolescent age group in our study, 4 patients (16%) were operated with the preliminary diagnosis of appendicitis, possibly because of the similarity between the findings and the limitation of the use of imaging methods in this age group. Furthermore, it was found that the positive Doppler finding involving loss of flow consistently with torsion was the lowest in the adolescent period with 32%. (Table 1). CT and MRI can be used as assisting diagnostic tools in the adolescent patient group; however, routine use of these methods in the diagnosis of adnexal torsion is not recommended because of their lower specificities and sensitivities and higher costs.(Moribata et al., 2015) Moreover, in spite of these methods, the rate of confirmation of the diagnosis in operations carried out with the preliminary diagnosis of adnexal torsion is reported between 23% and 62% in the literature.(Huchon and Fauconnier, 2010)

Diagnosis was confirmed in 44 patients (44/51 – 86%) in total who were operated with the preliminary diagnosis of torsion in all the age groups for the period covered by the study. We think that this high rate of confirmation of the preliminary diagnosis is that our hospital is a tertiary healthcare center and its experience in this area. Furthermore, while hemorrhagic infarct was seen in pathology results of none of the patients, hemorrhagic infarct was seen in 7 patients (63%) in postmenopausal period. The reason for this is that surgical procedures are planned for later times in postmenopausal patients because of additional symptoms including nausea, vomiting and fever accompanying the imaging findings are absent in the postmenopausal period and there is no expectation of fertility. In parallel with this, the period between admission and operation in our study was the longest for the postmenopausal period.

The right ovary undergoes torsion more frequently than the left ovary. The reason for this is the greater length of the right utero-ovarian ligament and localization of the sigmoid colon at the left side (Huchon and Fauconnier, 2010). Consistently with the literature, torsion was observed in the right adnexa in 40 patients (58.8%).

An adnexal mass lesion is present in the great majority of torsion cases in adults. It has been reported that no adnexal pathology is found only in 8 to 18% of cases. Majority of these masses are benign ovarian masses and tubal or paraovarian cysts (Anders and Powell, 2005; White and Stella, 2005). Cystic teratomas (60%) and cystadenomas (30%) constitute majority of benign ovarian masses (Oltmann et al., 2010). Torsion in ovaries in physiologic sizes is seen rarely. It is considered that adnexal torsion can be secondary to congenitally long ovarian ligament or extraordinarily loose pelvic ligaments in cases where there are no cysts or tumors (Bayer and Wiskind, 1994). In our study, no pathologies were found in 12 patients (48%) in the adolescent period (48%) and in 9 patients (28.1%) in the reproductive period. The most frequent pathology in the adolescent (5/25, 20%) and reproductive (7/32, 21.9%) periods was mature cystic teratoma.

Malignancy potential and ovarian masses is an important issue in the management of patients diagnosed with torsion. No malignancy was found in several case series and studies in the pathology results after operations carried out because of torsion (Rody et al., 2002; Oelsner et al., 2003). Malignancy is extremely rare particularly in torsion cases in adolescent cases (0-3%) (Savic et al., 2008). A possible explanation is that malignant lesions and endometriomas cause more fibrosis and leads to adhesions to the surrounding tissues, and torsion is seen less in such cases (Hibbard, 1985). The increased malignancy potential is obvious only in postmenopausal women, and malignancy rates reaching 22% has been reported in case series evaluating the postmenopausal patients diagnosed with adnexal torsion (Eitan, et al. 2007). In our study, 1 (9.1%) malignant tumor is reported for the postmenopausal period, and no endometrioma was reported.

In our times, aggressive treatment approaches are less preferred in the treatment of adnexal

torsion, and organ-preserving conservative procedures like detorsion are preferred in the first place. Long-term results of conservative treatment and more radical treatment methods are conflicted in the literature (Bellati et al., 2014; Parelkar, et al. 2014). Classical theory mentions increase in the thromboembolic event risk after detorsion, and as a possible reflection of this, preference by many gynecologists and obstetricians of oophorectomy in the treatment of torsion is between 30% and 86% (Rossi et al., 2012). Pulmonary embolism incidence in torsion cases is reported in studies as 0.2%, while no differences have been reported reaching this rate after detorsion (McGovern et al., 1999). In our study also, no thromboembolic events were observed after conservative procedures.

Conclusion

Adnexal torsion is a clinical condition that will always be actual for gynecologists and obstetricians because of its prevalence and diagnostic difficulties. Currently, rapid management and laparoscopic and organ-preserving approaches are important particularly for patients with fertility expectations. Although significant differences are not observed in clinical presentation and laboratory findings between age groups, mass lesions accompanying torsion and differences in pathologies of such masses must be kept in mind. While the clinical findings are nonspecific in the adolescent age group, suspected patients must be evaluated in detail for the sake of early diagnosis, and surgical intervention must be considered in the first place if the diagnosis of torsion cannot be eliminated and an early diagnosis is not possible with the purpose of preserving the ovarian reserve. Presence of a mass lesion accompanying the adnexal torsion must be searched for by all means in postmenopausal women, and pathology results of this mass must always be followed up because of the malignancy potential.

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RESEARCH ARTICLE

Surgically Treated Posterior Acetabular Fractures Via Iselin's Modified Approach with A Short-Term Follow-Up

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Abstract

Objective: The main purpose of the surgical treatment of posterior acetabular fracture is to achieve anatomical reduction to attain a functional and stable hip joint without pain. Although Kocher-Langenbeck (K-L) approach is the most commonly used surgical exposure, various modified approaches have been described in the literature. The aim of this study to determine the early surgical results of the acetabulum posterior fractures surgery via Iselin's modified K-L approach.

Methods: We reviewed the hospital records of patients who were operated for acetabulum posterior wall fractures via Iselin's modified approach between 2016 and 2018. All patients had detailed radiological, clinical evaluation and fractures were classified by AO/ASIF classification. All patients had radiological and clinical evaluation at the end of the postoperative 1st year.

Results: There were 16 men and 4 women with an average age of 42.8±18.0 (range 18-77) years. The average follow-up was 14.8±6.1 (range 6-28) months. The right hip was involved in 12 (60%) patients and the left in 8 (40%) patients. The average operative time was 78.6±16.7 (range, 54–115) minutes. Average blood loss during the operation was 179.22±51.9 (range, 100–260) ml. The postoperative reduction was graded as anatomic (0–1 mm of displacement) for 17 hips and imperfect (2–3 mm of displacement) for 3 patients. No patient had a deep infection, implant loosening, recurrent dislocation, deep venous thrombosis (DVT), pulmonary embolism (PE) or revision fixation**.**

Conclusion: The modified approach of Iselin is a successful approach with its ease in the surgery of displaced fractures that extending proximally and early radiological and functional results.

Key words: Acetabulum Fracture, Posterior Wall, Modified Method of Iselin

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Introduction

Because of their low incidence, accompanying injuries and their complex and deep anatomy, acetabular fractures are still challenging cases for the orthopaedic surgeons. The most common type of acetabular fractures is posterior wall fracture, that accounts for 25% of all (Letournel, 1992). Thirty percent of posterior wall fractures occur in single fragment; the rest is multi fragment or contains

impaction areas. In the emergency setting, orthopaedic treatment is adapted to the procedures to maintain hemodynamic stability and to improve the overall clinical status. Long-term morbidity of acetabular fractures is high when the treatment is insufficient and ineffective (Porter, 2008).

The main purpose of surgical treatment is to provide anatomical reduction and functional, stable hip joint without pain. Fracture type and dislocation, femoral head injury, intra-articular fragments, injury duration, quality of reduction, associated injuries and surgical approach are the most common factors affecting the long term results of operative treatment (Matta,1996). Despite good fracture reduction and fixation, osteoarthritis, avascular necrosis (AVN) and heterotopic ossification (HO) may cause poorer outcome (Kaempffe, 1991).

The preferred surgical approach is one of the most important step of treatment. Anatomical reduction and rigid fixation are very important criteria for a good clinical outcome in the displaced posterior acetabular fractures. Although Kocher - Langenbeck (K-L) approach is the most commonly preferred surgical technique, various modified approaches have been described in the literature (Magu, 2011). The aim of this study to evaluate the early clinical and radiological results of the surgically treated posterior acetabular fractures via Iselin's modified K-L approach.

Methods

This study was carried out with the approval of the local ethics committee of clinical research of Duzce University with the decision numbered 113/2018 as a retrospective clinical study. We reviewed the data of patients who underwent open reduction and internal fixation (ORIF) for posterior acetabular wall fractures via Iselin's modified approach between 2016-2018 retrospectively. The informed consent form was taken for all individuals. Exclusion criteria included patients with severe osteoporosis, low-energy trauma, pathological fractures, and previous history of hip injuries, as well as dementia and other disease processes.

The treatment protocol for fractures and hip dislocation initially involved closed reduction under sedation/anaesthesia, followed by upper tibial skeletal traction with weights ranging from 7.5 to 10 kg.

All patients had plain pelvic radiographs (anterior-posterior, and two 45° oblique Judet views) and 2 and 3-dimensional computer tomography (CT) preoperative and fractures were classified by AO/ASIF classification.

All patients were operated by the same surgical team using the Iselin's modified K–L surgical approach. Patients were in the prone position on the orthopaedic table with the knee flexed at 90°. The curved incision began about 4 cm from the posterior superior iliac spine and follows the demarcation line between the gluteus maximus and the gluteus medius, once the greater trochanter was reached, the incision was extended downwards as far as the gluteal plica (Figure-1). The gluteal aponeurosis was detached from the linea aspera. The musculocutaneous flap was then completely detached and two retractors were used to lift it, so as to entirely expose the external rotator muscles and the sciatic nerve. At this point, proceeding from top to bottom, it is best to section the short rotators. Thus the bone plane was revealed by placing the two retractors in the greater and in the lesser sciatic notches, straddling the spine. Observe in the surgical field with a wide view of the skeletal plane. In proximity to the angle of the greater sciatic notch, reduction procedures carried out with care, in order to avoid accidental injury to the superior gluteal artery (Fıgure2).

Closed-suction surgical drains were used for 24–72h. Thorough prophylactic antibiotics, tranexamic acid and low-molecular-weight heparin (LMWH) were used during the perioperative period and LMWH continued 4 more weeks. No prophylaxis against HO (indomethacin or radiation) was used.

Patients were informed and encouraged to perform intermittent, pain-free quadriceps, hip and knee flexion exercises with traction starting on the second postoperative day. Partial weight bearing was permitted 6 weeks after surgery, gradually progressing to full weight bearing at 12 weeks. All patients were evaluated in the outpatient clinic once a month in the first 6 months and then once every 3 months.

For the evaluation of fracture reduction on the postoperative radiographs we used Matta's criteria. In any of the three radiographs of the hip, displacement of fracture was evaluated and recorded in millimeters for quality of reduction. ≤ 1 mm displacement defined as anatomic reduction, displacement in the range of 2 to 3 mm categorized as imperfect reduction, and >3 mm displacement categorized as poor reduction (Matta, 1996).

Brooker et al documented the degree of HO classification scheme. Finally, Helfet and Ficat/Arlet stages 3 or 4 defined posttraumatic arthritis and femoral head AVN, respectively (Brooker,1973; Helfet,1996; Ficat 1985).

Statistical analysis was mainly conducted using Statistical Package for Social Sciences (SPSS) v.22 software package (SPSS/PC Inc., Chicago, IL.). Continuous data were summarized as mean and standard deviation while categorical data were summarized as frequency and percentage. Fisher's Exact test was used to analyze categorical data. P values below 0.05 were considered as statistically significant.

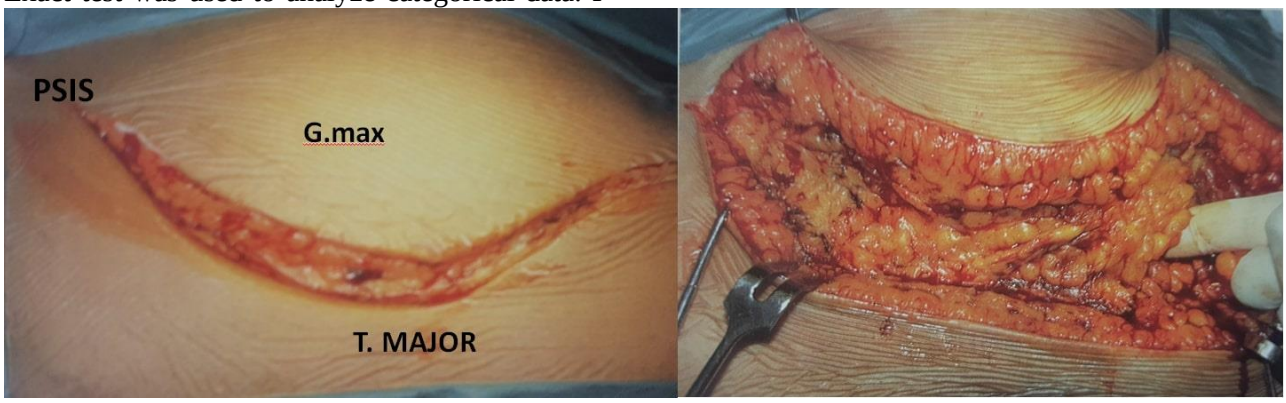

Figure -1: Incision of the modified Iselin's method: Skin incision and subcutaneous dissection. PSIS: Posteior Superior Iliac Spine G.max: Gluteus Maximus T. Major: Trochanter Major

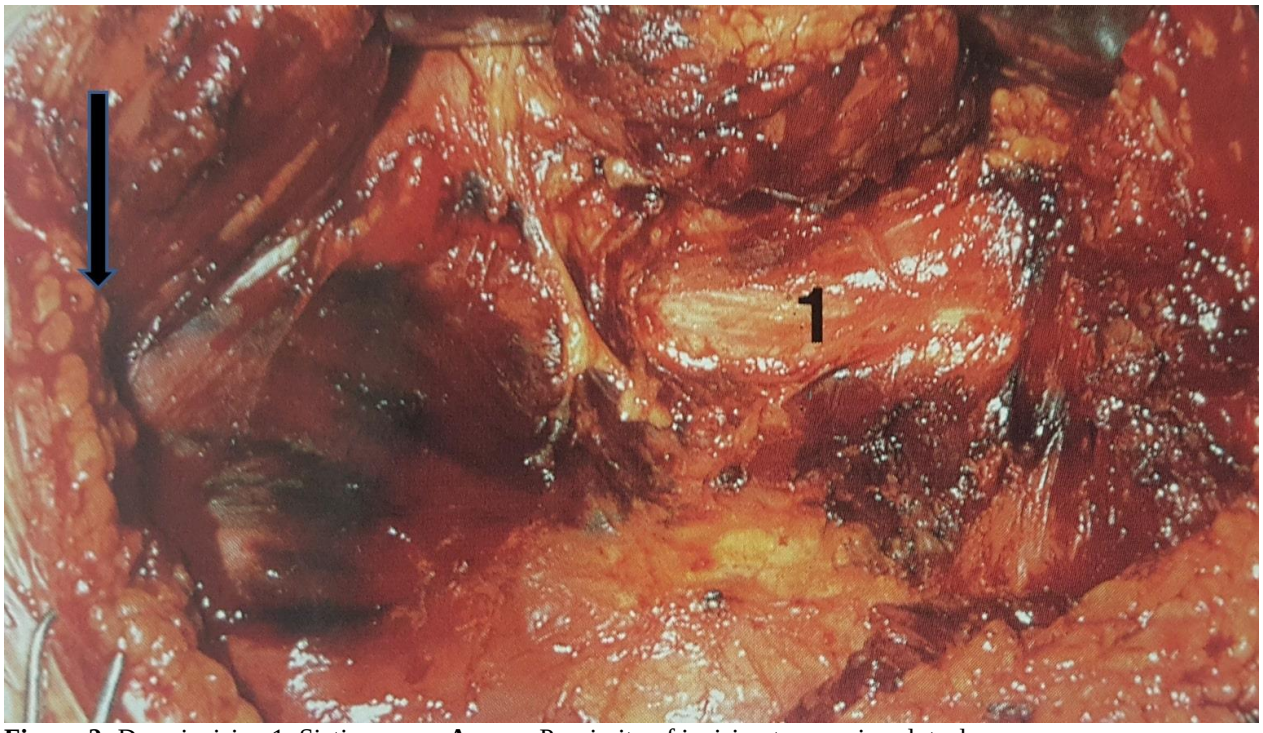

Figure-2: Deep incision 1: Siatic nerve - **Arrow:** Proximity of incision to superior gluteal a,v

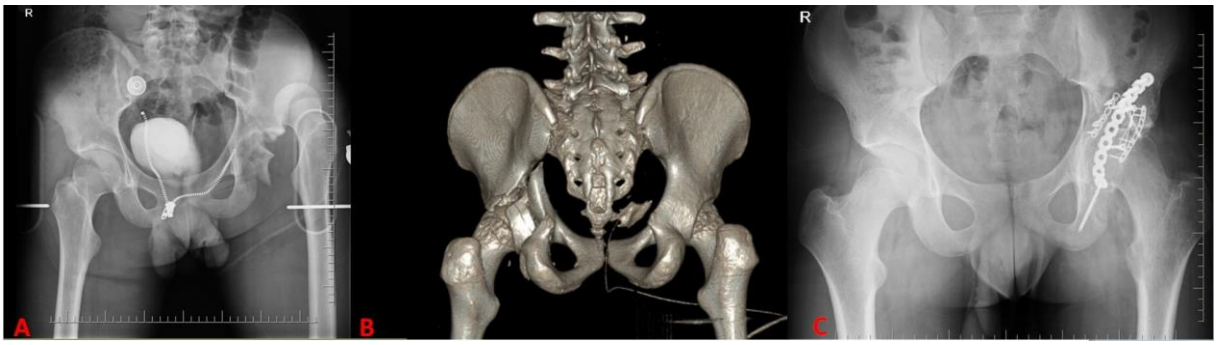

Figure-3: A: Posterior wall fracture and dislocation **B**: Preoperative 3-D CT screening **C:** Postoperative X-Ray

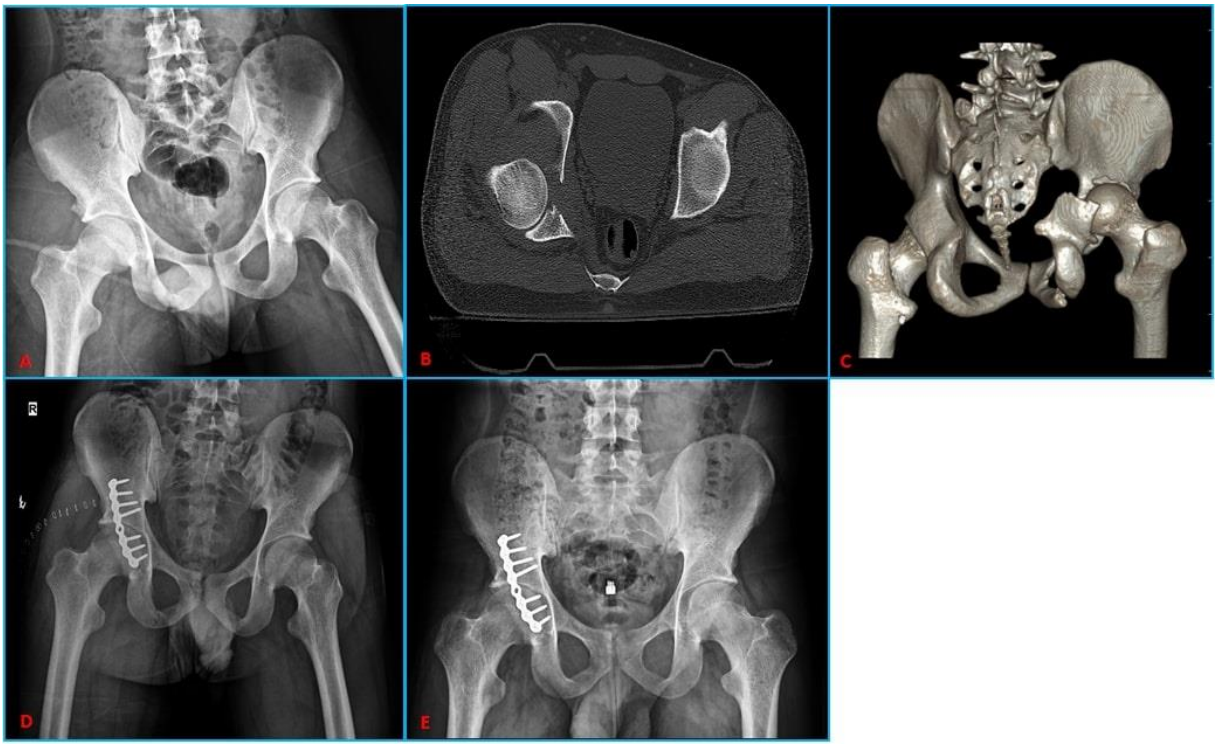

Figure-4: A: Preoperative x-ray B: Preoperative CT C: Preoperative 3-D CT screening D: Postoperative 2nd day x-ray E: Postoperative 11th month x-ray

Results

Twenty patients (16 men, 4 women) were enrolled in the study. Mean age of the patients was 42.8±18.0 years old (range=18-77 years old). The mean follow-up was 14.8±6.1 (range 6-28 months) months. The operation sites were right hip in 12 patients (60%), and left hip in 8 patients (40%). The etiology of hip injuries were traffic accidents in 13 (65%), motorcycle accident in 4 (30.8%), and falls in 7 (53.8%) patients. The mean time between the injury and surgical procedure was 3.2±0.8 (range 2-5) days. (Table-1)

Preoperative evaluations revealed posterior dislocation of the injured hip in 5 (25%) patients which were reduced within 12 h of injury. None of the patients had a fracture of the femur head. The associated injuries were present in 8 (40%) patients, which included lower-extremity injuries in 6 (75%) patients. The preoperative neurologic deficit of the sciatic nerve was observed in 2 patient.

Fractures were stabilized with lag screws in 5 patients and lag screws and reconstruction plate in 15 patients (Figure 3,4). The mean operation time was 78.6±16.7 (range, 54–115) minutes. Mean blood loss during the operation was 179.22±51.9 (range, 100–260) mL.

The postoperative reduction was graded as anatomic (0–1 mm of displacement) for 17 hips and imperfect (2–3 mm of displacement) for 3 patients. None of the patients had a deep infection, implant loosening, recurrent dislocation, deep venous thrombosis (DVT), pulmonary embolism (PE) or revision fixation.

At the last follow-up visit, Matta's radiographic outcomes (Matta,1996) revealed excellent results in 11 hips, good in 5, fair in one and poor in three. Final Harris hip scores were excellent in 14 hips, good in 2, fair in 2 and poor in 2 (Table-2).

In patients with anatomical reduction, AVN was seen only 1 (5.9%) patient and in 2 (66.7%) patients in the imperfect group. The anatomical reduction was found to be associated with better short term functional outcome compared with non-anatomical reduction (p=0.004). Moreover, the presence of associated injuries in the lower limbs adversely affected the final functional outcome in these patients when compared with the patients suffering from isolated posterior wall fracture (p=0.046). Quality of reduction directly affected the functional outcomes of our patients

Discussion

Although there are multiple factors affecting the surgical outcome of acetabular fractures, the proper surgical approach is very important for anatomic reduction and stable fixation.

The most commonly used approach for posterior acetabular fractures is the K-L incision but there are many modified approaches defined in the literature (Brooker,1973; Helfet,1996; Ficat,1985; Moed,2002; Magu,2011). The aim of all these new methods is to have less surgical complications with convenient access to the fracture site, stable fixation and effective use of fixation materials.

The defined modified approaches have various advantages and disadvantages. In literature, complications of various posterior approaches were reported at a frequency of 18-32%, including HO, iatrogenic sciatic nerve palsy, AVN, posttraumatic osteoarthritis (Baumgaertner, 1999).

Sciatic nerve damage is one of the most important complications of posterior acetabular approaches. Iatrogenic sciatic nerve palsies were reported with an incidence of 3% to 18% in literature (Kaempffe,1991; Giannoudis,2005).

Table 1 Clinico-radiological workup of patients with acetabular fractures

Sr.	Sex/Age	Postoperative reduction	Associated lower-limb injury/Dislocation	BMI	Complications	Follow-up (month)	Final radiological outcome	Harris Hip Score
1	38/M	Anatomical	Tibia plateu	36,3	-	24	Excellent	Excellent
2	18/F	Anatomical	Dislocation	19,9	-	6	Excellent	Excellent
3	42/M	Anatomical	-	26,4	-	14	Excellent	Excellent
4	31/M	Imperfect	IL/ Fem neck	24,2	AVN	12	Poor	Poor
5	66/M	Anatomical	Tibia shaft	27	-	28	Good	Good
6	50/M	Anatomical	-	28,2	-	24	Excellent	Excellent
7	49/F	Anatomical	-	29,7	-	16	Excellent	Excellent
8	45/M	Anatomical	-	27,8	-	17	Excellent	Excellent
9	21/M	Anatomical	Dislocation	25,9	Sciatic neuropraxia	18	Excellent	Excellent
10	20/M	Imperfect	IL/ Femur shaft	22	AVN	12	Poor	Good
11	22/M	Anatomical	-	23,4	-	9	Fair	Excellent
12	59/M	Anatomical	-	24,7	-	8	Excellent	Excellent
13	46/M	Imperfect	Dislocation	24,2	Cocxarthrosis	14	Poor	Poor
14	66/M	Anatomical	-	31,2	-	12	Excellent	Excellent
15	77/F	Anatomical	IL/ Fem neck	30,9	-	16	Good	Good
16	24/M	Anatomical	Dislocation	27,1	AVN	12	Good	Fair
17	40/M	Anatomical	Dislocation	24,7	Sciatic	13	Good	Excellent
18	54/M	Anatomical	-	30,1	neuropraxia	14	Excellent	Excellent
19	43/F	Anatomical	IL/ Femur shaft	22	-	12	Excellent	Excellent
20	35/F	Anatomical	-	23	- -	11	Good	Excellent

Table 2 Radiological and functional outcome of patients at final follow-up

Fracture Reduction	Radiological Outcome				Functional Outcome				AVN	HO
	Excellent	Good	Fair	Poor	Excellent	Good	Fair	Poor		
Anatomical	11	5	1	-	14	2	1	-	1	-
Imperfect	-	-	-	3	-	1	-	2	2	-

AVN: Avascular Necrosis **HO:** Heterotopic Ossification

Stretching of the retractor placed in the sciatic notch during the surgical procedure or the reduction of unstable posterior colon fracture can cause injury of the sciatic nerve. In the Iselin's approach, gluteus maximus muscle is detached at the beginning of the surgery and nerve is dissected by a finger that allows the safe insertion of the retractors to the sciatic notches. In our cases, sciatic nerve neuropraxia was detected in 2 patients with posterior fracture and dislocation and recovered at 6 months after the accident. None of the patients had iatrogenic nerve injury.

Surgery time and amount of blood loss are important parameters for early postoperative hemorrhagic shock and infection. Although the accident and fracture mechanisms are mentioned as the main reasons in the literature, iatrogenic vascular injuries are also an important factor (Rommens, 2004). It has been mentioned that there may be a superior gluteal artery injury in the proximal part of the incision in Iselin method (Zinghi,2004). By careful dissection, no iatrogenic vascular injury or postoperative hemorrhagic shock were observed in our cases. If surgical approach provides safety and easy reach to the fracture site, it shortens the surgery time and thus decreases the associated complications such as hemorrhagic shock, wound infection and PE (Carr, 2006).

In our study, the mean interval between injury and the surgical procedure was found to be 3.2 days with a mean surgical time of 79.8 minutes, and blood loss of 179.15cc. The time of surgery and amount of bleeding are lower than the average of the standard and modified approaches specified in the literature, in particular (Negrin, 2017). Complications such as DVT and PE, which were reported as 8% in the literature, were not detected in any of our cases (Negrin, 2017). In 2 patients, superficial wound infections were observed and the mean duration of surgery and total blood loss were up to the average (Magu,2011). Regarding our results, we hypothesized that the mechanism of injury, the interval between injury and the surgical procedure are important parameters in the risk of complications.

Body mass index (BMI) and advanced age are the personal characteristics that can affect the surgical outcome. In elderly patients with anatomical reduction, the presence of associated injuries in lower limbs and a BMI >25 adversely affected the final functional outcome (Magu,2014). This group of patients should be advised about weight loss.

The goal of operative treatment is to achieve precise anatomical reduction to attain a painless, mobile and stable hip joint. Arthosis, AVN and HO are complications that can develop even in case of a good reduction, stable fixation, and can worsen the clinical outcome (Harris,1969). In patients with anatomical reduction, we recorded 93% excellent or good results in Harris hip score, whereas there were worse clinical results in all of the patients with poor reduction.

In the early postoperative period, the femoral head AVN is an important complication that adversely affects the clinical outcome. This complication has a frequency of 2-10%. Possible associations leading to this complication are reported as injury mechanism, and iatrogenic injury of the medial circumflex femoral artery (Im,2004). Giannoudis,2005 and Negrin,2017 reported approximately 20% posttraumatic osteoarthritis and 5,5% AVN in their series. In our study, 85% anatomic and 15% imperfect reductions were determined in the postoperative radiological evaluation according to the Matta classification. The mean Harris hip score was found to be 89.25 at 12 months postoperatively. This ratio is consistent with the literature data. Four patients underwent total hip arthroplasty, in whom 3 had AVN of the femoral head and one had coxarthrosis. Among these patients, all had a history of motorcycle accident as the cause of fracture and dislocation.

Heterotopic ossification was reported as 4% to 31% after the surgery of posterior acetabular fractures and racial features, wide incisions and dissection of gluteus minimus muscle during the surgery were also reported to be a factor of this complication (Ghalambor, 1994). All patients in our study were white and gluteus minimus dissection was performed according to the location of the fracture line and no prophylactic medication was introduced. There were no HO in our patients. However, short-term follow up may be the limitation of our study restricting the results. As previously stated in the literature, HO cases may evolve 22 years after surgery (Liebergall,1999; Chiu, 2000).

Limitations of our study can be summarized as the study design which was conducted retrospectively, not comparing the results with patients operated on through the conventional Kocher– Langenbeck approach and the relatively small population size. However, these are limitations in most series on acetabular posterior wall fractures (Iselin,2013; Magu,2014;).

Conclusion

An appropriate surgical approach is the major predictor of stable fixation and successful clinical outcome in posterior acetabular wall fractures. The modified approach of Iselin is a successful approach with its ease in the surgery of displaced fractures that extend proximally. Furthermore it has good clinical and radiological outcomes

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Düzce University. Ethics no: 113/2018

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RESEARCH ARTICLE

miR-1267 Induces Tumorigenicity and Contributes to Risk of Clear Cell Renal Cell Carcinoma

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Abstract

Objective: Dysregulated microRNA signatures in different cancer types are being uncovered continually implying their significance in cancer pathogenesis. miR-1267 was not previously associated with RCC. In this study, it is aimed to obtain the expression profile of miR-1267 in patients with ccRCC and its correlation with patient parameters.

Methods: Kidney Cancer cDNA Array consisting of cDNA samples obtained from healthy kidney tissues of 4 healthy individuals and tumoral kidney tissues of 5 Stage I, 5 Stage II, 3 Stage III and 2 Stage IV ccRCC patients was used. Hsa-miR-1267 and SNORD48 (as housekeeping gene) expressions were analyzed. miR-1267 expression was statistically correlated with the clinical parameters of patients. miRGator 3.0 database was used to compare miR-1267 expression patterns of different urological cancer types.

Results: The expression of miR-1267 was significantly higher in male than female ($p=0.027$). Also, there were statistically significant increase in miR-1267 expression in stage IV when compared to stage I ($p<0.001$). Moreover, increased platelet/lymphocyte ratio and calcium level, which were parameters giving information about the occurrence of ccRCC, are significantly associated with increased miR-1267 expression ($p<0.001$ and $p=0.003$, respectively). The expression of miR-1267 in kidney tumor tissues was higher approximately three times than normal kidney tissues ($p>0.05$).

Conclusion: miR-1267 could have oncogenic function, have predictive value for RCC development and be predictive about aggressiveness in ccRCC.

Key words: miR-1267; ccRCC; platelet/lymphocyte ratio; high calcium level

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Introduction

Renal cell carcinoma (RCC) is different from kidney cancer with the involvement of renal pelvis or renal medullary and it is also the only type of cancer that occurs in cells (renal tubules) that extend into the kidney bed. RCC includes a range of heterogeneous cancers arising from renal tubular cells. RCC is the third most common cause of death after prostate and bladder cancer among the urological cancers and accounts for about 2% of all adult cancer patients. Moreover, its clinical course is the most fatal one among urological cancers. RCC is caused by the accumulation of many genetic and

epigenetic alterations as similar to other cancer types (Shingarev and Jaimes, 2017).

Clear cell renal cell carcinomas (ccRCCs) are ordinarily globular masses that may originate anywhere in the renal cortex and frequently extrude beyond the normal form of the kidney. ccRCC ofttimes attacks the renal venous system, sometimes filling the renal vein and growing longer into the vena cava or even the right atrium. ccRCC is the most frequent type of kidney cancer in adults by far (Znaor et al., 2015).

In the last twenty years, genetic and clinical researches have presented that ccRCC is both heterogeneous in its histology and clinical course, and heterogeneous in its genetic changes. The identification of various histological subtypes of ccRCC ensures a better comprehension of the molecular mechanism of these distinct subtypes of cancer and one or more crucial mutations were defined for each subtype. Sporadic cancers originate from multiple (epi)genetic alterations. Therefore, promoter hypermethylation of genes is considered to be involved in sporadic or hereditary forms of ccRCC. The epigenetic alterations that regulate the formation and progression of ccRCC are in the initial stages of reconnaissance yet. More detailed specification of epigenetically changed genes and pathways in ccRCC may canalize to the development of new and minimally invasive diagnostic and prognostic tools for ccRCC. For the future, epigenetic therapies may provide an additional treatment preference for advanced ccRCC that does not respond to standard therapy (Shingarev and Jaimes, 2017). Factors are potentially related with the pathophysiology of ccRCC and probably usable as biomarkers in the development of the disease need to be researched.

MicroRNAs (miRNAs) are functional RNA molecules of 18-28 nucleotides in length which are transcribed from exonic or intronic regions of protein coding genes and non-coding regions of the genome. miRNAs perform their functions by virtue of their ability to recognize complement genes to their nucleotide sequences. RISC complex formed by the addition of miRNA to the structure binds to mRNA and causes the inhibition of the protein translation of interested gene and / or the destruction of the mRNA (Garzon et al., 2009). Some studies have shown that miR-21, which has proven oncogenic properties in many cancers, triggers the emergence of tumorigenic properties by targeting tumor suppressor genes such as RCC-specific PTEN, SATB1, PDCD4, TCF21 and KISS1 (Yu et al., 2014; Kowalczyk et al., 2016; Asangani et al., 2008; Zhang et al., 2012). miR-21 is a miRNA that has been extensively studied. However, the exact location in the pathological pathways of RCC molecular mechanism cannot be determined, and it is necessary to identify new miRNA intermediate molecules in which miR-21 and its targets interact. Upon that, we identified miR-1267, another miRNA that targets all tumor suppressor genes such as PTEN, SATB1, PDCD4, TCF21 and KISS1 in RCC like miR-21 by using biostatistical approaches. Relying on biostatistical data and literature review, we know that miR-1267 was not previously associated with RCC, and we estimate that it may have oncogenic function for RCC like miR-21. This suggests that this miRNA can be a reliable agent for the diagnosis and treatment of disease, indicating that it is an informative molecule for RCC. In this study, the expression profiles of miR-1267 in patients with RCC, and its correlation with patient parameters, were investigated. In this way, it is aimed to determine the new candidate molecules that may play a role in the pathology and progression of RCC.

Methods

In this study, Kidney Cancer cDNA Array (Origene Technologies Inc., Rockville, MD, USA) consisting of cDNA samples obtained from healthy kidney tissues of 4 healthy individuals and tumoral kidney tissues of 5 Stage I, 5 Stage II, 3 Stage III and 2 Stage IV ccRCC patients (15 ccRCC patients and 4 healthy controls in total) was used. Upon this cDNA panel, Real-Time PCR (qRT-PCR) method was used for hsa-miR-1267 and SNORD48 expression analyzes and Rotor-Gene Q (Qiagen GmbH, Manheim, Germany) was used for this purpose. As the procedure, hsa-miR-1267 and SNORD48 expression primers (Origene Technologies Inc., Rockville, MD, USA) were added separately to the panels containing all Real-Time PCR reaction ingredients except the primers and the device was switched on under the conditions specified in kit procedure. All Real-Time PCR experiments were performed in three replicates. Since the study was carried out using the commercially available ccRCC cDNA panel, the approval of the ethics committee was not required. The number of patients to be included in the study was detected with 80% test power and 95% confidence interval. The statistically significant number of patients was calculated as at least 19.

In the method based on comparative expression, measured values of hsa-miR-1267 were normalized with SNORD48. In qRT-PCR method, Ct (Cp,

Crossing points) values were obtained. The comparison between healthy and ccRCC tumor samples with different stages in cDNA panel was performed. The concerned miRNA expression levels were statistically compared using Ct values obtained from the groups defined via 2-ΔΔCt formula.

Formula 1. 2-ΔΔCt calculation

2-ΔΔCt=2-[Tumor ΔCt (miRNA - Reference) – Control ΔCt (miRNA – Reference)]

The statistically significance analysis of differences in miRNA expressions between tumor and normal samples was performed and statistically correlated with the clinical parameters of patients. All demographic and histopathological data of the patients were obtained with the purchased panel.

SPSS 21 program (IBM software, Pointe Claire, Quebec, Canada) was used in the statistical analysis of measured hsa-miR-1267expression levels. Normal distribution of data was evaluated statistically by Kolmogorov-Simirnov test. It was decided to use non-parametric tests because the data were not suitable for normal distribution ($p<0.05$) and the number of samples was below 30. Wilcoxon Signed Rank Test was used in binary comparisons and Kruskal Wallis Test was applied in multi-comparisons. $p<0.05$ was accepted as a statistical significant value and the evaluation was made at 0.95 confidence interval.

Finally, miRGator 3.0 database, which collected 73 deep sequencing datasets on human samples from Gene Expression Omnibus (GEO), Sequence Read Archive (SRA) and The Cancer Genome Atlas (TCGA) archives, was used to compare miR-1267 expression patterns of different urological cancer types, including ccRCC, papillary renal cell carcinoma (PRCC), bladder urethelial carcinoma and prostate adenocarcinoma (Cho et al., 2012).

Results

Expression levels of miR-1267 in ccRCC tumor tissues and the healthy kidney tissues were compared and the possible association between the clinical parameters of the patients and miRNA expression levels were investigated.

Demographic (gender, age) and clinicopathological [Tumor node metastasis (TNM) staging, Fuhrman nuclear grade, platelet/lymphocyte ratio, calcium level] characteristics of patients enrolled in this study were presented in Table 1. The expression of miR-1267 was significantly higher in male than female (20.12-fold) ($p=0.027$). Also, there were statistically significant associations between stage I-IV with respect to miR-1267 expression (32.45 fold higher in stage IV than stage I) ($p<0.001$). Moreover, increased platelet/lymphocyte ratio and calcium level, which were parameters giving information about the occurrence of ccRCC, are significantly associated with increased miR-1267 expression ($p<0.001$ and $p=0.003$, respectively).

Table 1. Demographic and clinicopathological characteristics of patients and the statistical significance of the associations of these data with hsa-miR-1267 expressions of the patients

		Patients (n=19)	p value
Gender	Male	10 (52.6%)	0.027
	Female	9 (47.4%)	
Age	35-44	6 (31.6%)	0.642
	45-64	8 (42.1%)	
	>65	5 (26.3%)	
TNM staging	Healthy	4 (21.1%)	< 0.001 (between stage I and IV)
	Stage I	5 (26.3%)	
	Stage II	5 (26.3%)	
	Stage III	3 (15.7%)	
	Stage IV	2 (10.6%)	
Fuhrman nuclear grade	Healthy	4 (21.1%)	0.082
	Grade 2	8 (42.1%)	
	Grade 3	5 (26.2%)	
	Grade 4	2 (10.6%)	

(Abbreviations.TNM: Tumor Node Metastasis)

Tumor samples of patients with ccRCC were compared to healthy kidney tissues in cDNA panel with respect to the expression levels of miR-1267 (Figure 1-2). The expression of miR-1267 in kidney tumor tissues was increased approximately three times compared to normal kidney tissues ($p>0.05$).

According to miRGator 3.0 database, miR-1267 expression patterns of different urological cancer types, including ccRCC, papillary renal cell carcinoma (PRCC), bladder urethelial carcinoma and prostate adenocarcinoma, were compared. Among these urological cancer types, ccRCC showed the highest miR-1267 expression profile (Figure 3).

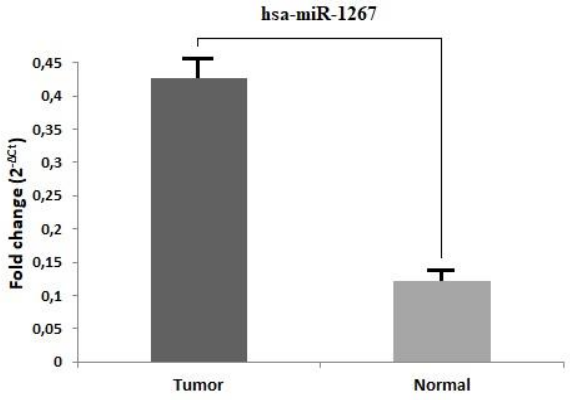

Figure 1. Quantitative expression levels of hsa-miR-1267 in tumor tissues as compared to adjacent healthy kidney tissues of patients with ccRCC (Approximately 3-fold increase in hsa-miR-1267 expression) (p=0.084).

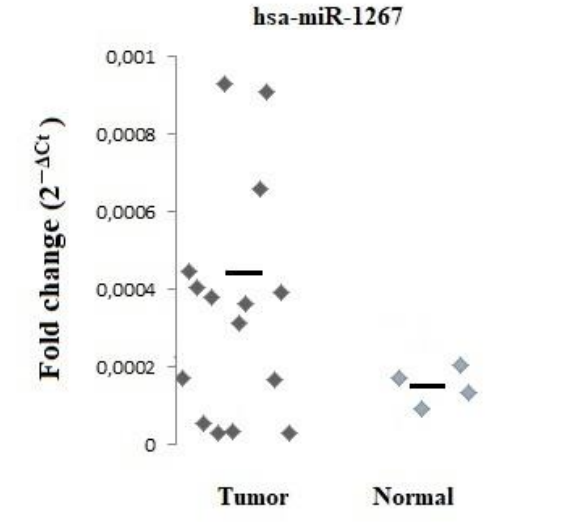

Figure 2. Distribution of patients' quantitative expression levels of hsa-miR-1267 in tumor tissues as compared to adjacent healthy kidney tissues**.**

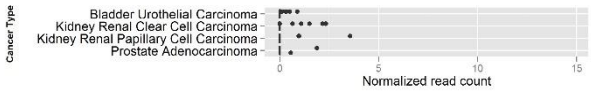

Figure 3. Comparative expression levels of hsa-miR-1267 in tumor tissues as compared to adjacent healthy tissues of patients with different types of urological cancer according to deep sequencing datasets provided by miRGator v3.0 database

Discussion

Renal cell carcinoma is one of fifteen most frequent cancer types arising globally. The most aggressive subtype, ccRCC comprises about 70% of all kidney tumors. ccRCC is potentially medicable by resection, however approximately 30% of patients show recurrence after first nephrectomy. Unhappily, ccRCC is often non-symptomatic in the early stages, and is repeatedly stated in advanced phase frequently with metastases. In case of metastasis, ccRCC is radiation- and chemo-resistant and remains incurable in most cases, resulting in a 95% mortality ratio. Up to now, no effective ccRCC therapy has been created and none of the probable biomarkers have been approved for clinical administration (Moch, 2013).

In our study, tumor tissues of the patients with ccRCC were compared with healthy kidney tissues in terms of expression levels of miR-1267 gene and the possible association between the clinical parameters of the patients and miR-1267 expression levels were analyzed.

According to the association analysis between demographic (gender, age) and clinicopathological (TNM staging, Fuhrman nuclear grade, platelet/lymphocyte ratio, calcium level) parameters, and the expression level of miR-1267, interesting results were obtained. The expression of miR-1267 was significantly higher in male than female (p=0.027). This significant expression change of miR-1267 between genders might be caused by different hormonal status between male and female. According to the study performed by Znaor et al., RCC incidence in men varied from approximately 1/100 000 in African countries to >15/100 000 in several Northern and Eastern European countries and among US blacks. Similar patterns were observed for women, although incidence rates were commonly half of those for men. Moreover, kidney cancer is currently the ninth most common cancer in men and the 14th most common in women worldwide (Znaor et al., 2015). This data shows consistency with high miR-1267 expression in men when compared to women in our study because of the fact that we suggest an oncogenic function to miR-1267 for ccRCC.

Moreover, platelet/lymphocyte ratio and calcium level, which were parameters giving information about the occurrence of ccRCC, are significantly associated with miR-1267 expression (p<0.001 and p=0.003, respectively). All studies correlating platelet/lymphocyte ratio and calcium level with RCC in the literature are consistent to our results. According to a study realized by Wang et al (2018) on a total of 1528 patients with RCC, a high preoperative platelet/lymphocyte ratio is correlated with poor prognosis in RCC patients. Also, the pooled analysis showed that an elevated platelet/lymphocyte ratio is an effective prognostic marker of both overall survival (OS) and

progression free survival (PFS). When we look at another study, high platelet/lymphocyte ratio was associated with shorter survival of metastatic RCC (mRCC) patients receiving first-line TKI (Park et al., 2016) So, miR-1267 may be an effective independent prognostic factor in this setting, like platelet/lymphocyte ratio, based on their significant association in our cohort. If we pass to the association of high calcium level and ccRCC in our study, there are some investigations correlating high calcium levels with ccRCC progression. For example, Motzer et al. (1999) reported in their study conducted on 670 RCC patients that high serum calcium level (>10 mg/dL) was one of the pretreatment features associated with a shorter survival in the multivariate analysis. So, all these findings support that miR-1267 could have predictive value for RCC development in accordance with its correlation to platelet/lymphocyte ratio and calcium level in RCC.

Tumor samples of patients with ccRCC were compared to healthy kidney tissues in cDNA panel with respect to the expression levels of miR-1267 (Figure 1-2). The expression of miR-1267 in kidney tumor tissues was increased approximately three times compared to normal kidney tissues ($p>0.05$). Also, there were statistically significant associations between stage I-IV with respect to miR-1267 expression. The expression of miR-1267 was significantly higher than in stage IV than that of stage I ($p<0.001$). According to our results, miR-1267 not only has an oncogenic feature but also provide significantly discrimination between ccRCC stages consistently. All these results are consistent with the literature. For instance, the mitochondrial-processed miRNAs are likely to contribute to some post-transcriptional regulation of gene expression related to the mitochondrial functions. It was obtained a list of 33 human pre-miRNAs and 25 miRNAs. The most significant alignments with human miRNAs were obtained with four pre-miRNAs (pre-mir-302a, pre-let-7b, pre-mir-1267 and pre-mir-1296) (Barrey et al., 2011). Also, low mitochondrial respiratory chain content correlates with tumor aggressiveness in ccRCC (Simonnet et al., 2002). So, this mitochondrial dysfunction could be related with miR-1267, one of the most mitochondrial function related miRNAs, consistently with our study findings. Moreover, some studies have shown that miR-21, which has proven oncogenic properties in many cancers, triggers the emergence of tumorigenic properties by targeting tumor suppressor genes such as RCC-specific PTEN, SATB1, PDCD4, TCF21 and KISS1 genes (Yu et al., 2014; Kowalczyk et al., 2016; Asangani et al., 2008; Zhang et al., 2012). Like miR-21, miR-1267 also targets all of these tumor suppressor genes. Relying on biostatistical data and literature review, miR-1267 was not previously associated with RCC, and we estimate that it may have oncogenic function for RCC like miR-21.

Conclusion

ccRCC is the most common and apparently most aggressive RCC subtype with the highest rates of local invasion, metastasis and mortality (Protzel et al., 2012). In our study, we found that ccRCC showed the highest miR-1267 expression profile in ccRCC among different urological cancer types, including ccRCC, papillary renal cell carcinoma (PRCC), bladder urethelial carcinoma and prostate adenocarcinoma (Figure 3). Upon this consistency with the literature finding, we can suggest that miR-1267 could also be predictive about aggressiveness of RCC.

Consequently, the changes in the expression levels of miR-1267 analyzed and RCC-specific parameters are needed to be confirmed in the larger study groups. If verified, the expression changes of miR-1267 may be possible to be used as biomarkers for the prognosis of ccRCC. The results of this study may propose that molecular applications can be designed to change the level of the expression of miR-1267 to affect the development of ccRCC in future projects.

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RESEARCH ARTICLE

In Silico Analysis of Biomarker Potentials of miRNA-Mediated ceRNAs in Gastric Neoplasms

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Abstract

Objectives: The objective of this study is to define novel biomarkers for gastric neoplasm (GN) via *in silico* analysis that takes GN-specific miRNAs, finds their combinatorial target genes (potential ceRNAs), selects ones containing T-UCR among them and potentiates their relevance with GN. Based on this study we can plan new in vitro and in vivo studies.

Methods: Four miRNAs of which clinical relevances with GN were proved experimentally were exported via mirTarbase. Using the ComiR database, 1008 genes targeted by these 4 miRNAs simultaneously were identified. Genes containing T-UCR and showing potential ceRNA activity were extracted. Among GN-associated ceRNAs including T-UCR, we identified genes with significant expression differences between GN and normal stomach tissue using the GEPIA database. The statistical evaluation of the association of *NFAT5* and *CLK3* genes with GN was performed by Spearman correlation test in GEPIA database.

Results: GN-associated ceRNAs cross-matching with genes including T-UCR in their exonic regions were *NFAT5* and *CLK3*. We identified genes with significant expression differences between GN and normal stomach tissues among GN-associated ceRNAs including T-UCR. According to this analysis, only *NFAT5* gene was significantly higher expressed in GN than in normal stomach tissue while the other didn't show any significant differential expression pattern. *NFAT5* and *CLK3* genes were found to be significantly correlated with GN ($p<0.001$; $R=0.22$)

Conclusion: All in all, this is the study associating *NFAT5* gene with GN for the first time and giving it ongogenic potential for GN. Still, larger and more comprehensive studies are needed on this issue.

Key words: Gastric neoplasms; miRNA; ceRNA; T-UCR; *In silico* analysis

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Introduction

Gastric neoplasms may manifest in various different forms, depending on the cell of origin. The most common form is adenocarcinoma, while lymphoma, gastrointestinal stromal tumors (GISTs), carcinoids and other neoplasms are less frequently seen. Gastric adenocarcinoma is a particularly common cancer across the world, but particularly in the Far East. A lower incidence has been reported in the United Kingdom, but poor prognosis when the disease is in the late stage results in a significant impact on population health. Advanced disease is observed in the majority of patients at time of diagnosis (Schiller, 2017).

MicroRNAs (miRNAs) are small RNAs that are not encode a protein, but nevertheless potent coordinating capacities. They perform vital regulatory functions in a range of malign cancers, involving gastric cancer. Abnormal stated miRNAs are also involved in gastric carcinogenesis through modification of growth of cells, cell cycles, apoptosis, and migration of cells. Epigenetic and genetic alteration has been identified as one of the mechanisms responsible for miRNA dysregulation. MiRNA performs essential functions in the progression of gastric cancer by targeting oncogene or tumor suppressor gene expression. The first step in determining the roles of miRNAs in gastric cancer is to investigate differences in miRNA expression profiles between normal and tumor gastric tissues (Pan et al., 2013). Tumor suppressor miRNAs inhibit tumor formation by suppressing oncogenes. Relationship of microRNAs within cancer changes protein-encoding oncogene or tumor suppressor genes are known to cause cancer. Genetic cause of cancer with the recent demonstration of miRNAs in tumor formation.

Competing endogenous RNAs (ceRNAs) are transcripts capable of mutual regulation at the post-transcription level through competition for shared miRNAs. CeRNA networks link protein-coding mRNA functions with those of non-coding RNAs (ncRNAs), including microRNA, long ncRNA, pseudogenic RNA and circular RNA. Since any transcripts containing an miRNA response component are in theory capable of acting as ceRNAs, these may represent a widespread form of post-transcriptional gene expression regulation in physiological and pathological terms. A number of factors are known to be capable of affecting ceRNA activity, including the abundance and subcellular localization of ceRNA components, the binding affinity of miRNAs to their sponges, RNA editing, RNA secondary structures and RNA-binding proteins. Disturbance in these may lead to deregulation of ceRNA networks and thus to human diseases, including cancer (Qi et al., 2015).

In recent years, ncRNAs have generated considerable interest in terms of cell transformation. Ultraconserved regions (UCRs) were first discovered in 2004 following bioinformatic investigation of mouse, rat, and human genomes. UCRs consist of a minimum of 481 genomic sequences at least 200 bp in length (range 200-779 bp), and which are fully conserved (100% identity without any insertions or deletions) among the above three vertebrate species. A significant proportion of UCRs are transcribed (T-UCRs) in normal human tissues, and their expression levels have been observed to exhibit a ubiquitous and tissue-specific pattern. While the functions of T-UCRs are largely unclear, the high level of trans-species conservation they exhibit appears to suggest that they are of significant importance to ontogenesis/phylogenesis in mammals. Recent research into genome-wide expression has revealed that T-UCRs exhibit distinct profiles in different human cancers, representing further evidence of their role in carcinogenesis in humans (Fassan et al., 2014).

In recent years understanding their role in cancer, miRNAs have been hopeful in understanding the molecular pathology of cancer and developing molecular targeted therapies. Based on this feature of miRNAs, we aim to identify genes with potential oncogenic activity not previously identified *in silico* in gastric cancer. In line with our data, we aim to conduct further in vitro and in vivo studies on these miRNAs

Methods

Selection of miRNAs involved in the pathogenesis of gastric neoplasms

Four miRNAs clinically associated with gastric neoplasm and authenticated experimentally were exported over the MiRTarBase database. The miRTarBase database submits estimated and verified data concerning miRNA-target interaction. This enables researchers to confirm novel miRNA targets. The ‘Comfirmed Target module’ showed in this study by Chou et al. (2018).

Analysis of gastric neoplasm-specific miRNA-mediated ceRNAs

One thousand eight genes projected by these four miRNAs simultaneously were described using the ComiR database. ComiR is an online system employed for the purpose of combinatorial miRNA target estimation. It computes the potency of targeting by a group of miRNAs. When calculating the relay impact of one mRNA from a group of several miRNAs, the application employs utilizer-defined miRNA expression levels in a combinatorial manner based on appropriate machine learning techniques and thermodynamic modeling to elicit more accurate estimates. ComiR admits the opportunity of constituting a operational target for a group of miRNAs, based on relevant miRNA expression levels, for every gene (Coronnello and Benos, 2013).

We hope that the RNA transcripts of these genes will exhibit potential ceRNA activity for these miRNAs and that their arrangement is organized on the basis of miRNA-sponging mechanisms.

Matching of GN-associated ceRNA with genes including T-UCR

Bejerano et al. identified the UCRs in the human genome. Genes including these regions are graded as upstream, exonic or downstream in accordance, depending on the site of fixation within the gene (Bejerano et al., 2004). Genes with T-UCR in their exonic areas were also identified, and those exhibiting latent ceRNA activity were excerpted in our previous research.

Analysis of gastric neoplasm-related ceRNAs *including T-UCR in the sense of differential gene expression between gastric neoplasm and normal gastric tissues*

Genes exhibiting significant expression differences between gastric neoplasm and normal stomach tissue from GN-associated ceRNAs, including T-UCR were identified with the assistance of the GEPIA database. GEPIA (Gene Expression Profiling Interactive Analysis), a web-based tool to deliver fast and customizable functionalities based on The Cancer Genome Atlas (TCGA) and The Genotype-Tissue Expression (GTEx) data. All plotting features in GEPIA are developed using R (version 3.3.2) and Perl (version 5.22.1) programs. (Tang et al., 2017).

Correl tests of NFAT5 and CLK3 genes in gastric neoplasm

Methods for analyzing gene expression are numerous and diverse. Expression-based clustering, for example, can be divided into supervised and unsupervised methods. Gene expression differential analysis is a classical supervised method, leading to the finding tumor-specific genes by comparing tumor to normal groups. Statistical analysis of the association between *NFAT5* and *CLK3* genes and gastric neoplasm was showed using the Spearman correlation test in the GEPIA database.

Results

A list of four miRNAs experimentally linked to gastric neoplasm using the miRTarbase database is shown in Table 1.

Table 1. List of miRNAs taking role in gastric neoplasms pathogenesis

1. hsa-miR-148a
2. hsa-miR-23a
3. hsa-miR-370
4. hsa-miR-429

A list of 1008 genes simultaneously targeted by these four miRNAs is shown supplementary 1. Wedeclared genes with T-UCR in their exonic regions from those listed by Bejerano et al., and these are shown in supplementary 2. Then, we extracted ones showing potential ceRNA activity in our previous analysis among them (Table 2). Genes exhibiting significant expression variation between gastric neoplasm and normal gastric tissues among gastric neoplasm-related ceRNAs with T-UCR were identified. In agreement with that analysis, expression of *NFAT5* was significantly higher in gastric neoplasm compared to normal stomach tissue, while no significant differential expression patterns were detected in the other genes (Table 3).

Table 2. List of gastric neoplasms-associated ceRNAs cross-matching with genes including T-UCR in their exonic regions

NFAT5
CLK3

Table 3. Expression values of GN-associated ceRNAs including T-UCR between gastric neoplasms and normal stomach tissues.

Gene ID	GN	Normal stomach
*NFAT5**	**8,94**	**4,03**
CLK3	30,1	36,53

*shows significantly differential expression pattern between GN and normal stomach tissues

Statistical analysis of the link between *NFAT5* and *CLK3* genes and gastric neoplasm was conducted through the GEPIA database. Spearman correlation analysis revealed that the *NFAT5* and CLK3 gene pair exhibited significant association with gastric neoplasm (Figure 1) (p=0.000; R=0.22).

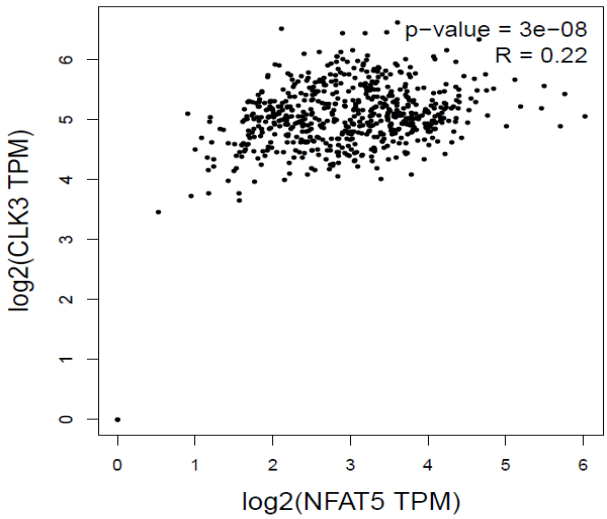

Figure 1: Spearman correlation analysis of *NFAT5* and *CLK3* genes with GN.

Supplementary 1. List of genes targeted bu these 4GN-associated miRNAs simultaneously

AVPR1A	0.9048	AK4	0.9053	ATF7	0.9127
AAK1	0.9202	AKAP5	0.9172	ATG9A	0.9127
ABCC3	0.9049	AKAP6	0.9054	ATP10A	0.9051
ABI2	0.9239	AKT2	0.9114	ATP11A	0.9198
ACADSB	0.9122	AMER2	0.9131	ATP11B	0.9117
ACER3	0.905	ANK2	0.9172	ATP2A2	0.9051
ACP6	0.9129	ANKFY1	0.9125	ATP8A1	0.9198
ACSS3	0.9052	ANKRD11	0.9201	ATP8A2	0.9054
ACVR1C	0.9173	ANKRD9	0.9049	ATRX	0.923
ACVR2B	0.9216	ANO5	0.9111	ATXN1	0.9216
ADAM10	0.9201	ANTXR2	0.9131	ATXN1L	0.9054
ADAM12	0.9212	AP1M1	0.9178	ATXN3	0.9202
ADAM22	0.9054	AP5M1	0.9201	ATXN7L3B	0.9175
ADAMTS4	0.9054	APC	0.9052	B3GALT5	0.9201
ADAMTS5	0.9197	APOL3	0.9104	B4GALT4	0.905
ADAMTS6	0.9213	APOL6	0.9055	B4GALT5	0.9163
ADARB1	0.9051	ARHGAP19	0.9051	BACH2	0.913
ADCY1	0.9202	ARHGAP20	0.9174	BAG2	0.9125
ADCYAP1R1	0.9128	ARHGAP31	0.9049	BCAR1	0.9128
ADRBK2	0.9055	ARHGAP32	0.9052	BCAS4	0.9054
AFF1	0.9197	ARIH1	0.9227	BCL2L11	0.9209
AFF2	0.9133	ARL10	0.9134	BICD1	0.9197
AFF3	0.9051	ARL5A	0.9167	BICD2	0.905
AGAP1	0.923	ARL8B	0.9146	BMF	0.9049
AGFG1	0.9172	ARNT2	0.9213	BMP1	0.912
AGO1	0.9217	ARPIN	0.9174	BMP2K	0.9166
AGO2	0.9116	ARSD	0.9117	BMPR1A	0.923
AGO3	0.9231	ASAP1	0.9165	BNC2	0.9216
AJAP1	0.9201	ASRGL1	0.909	BNIP2	0.917

BRCA1	0.9171
BRWD1	0.9173
C12orf49	0.9176
C14orf37	0.9125
C16orf52	0.9168
C17orf51	0.92
C17orf70	0.9048
C18orf25	0.9116
C18orf32	0.9195
C1orf95	0.9056
C2orf71	0.9162
C4orf32	0.9132
C5orf56	0.9116
C9orf114	0.9208
CA12	0.9049
CA5B	0.9051
CAB39L	0.9113
CACNA1E	0.9176
CACNG8	0.9177
CACUL1	0.923
CADM1	0.9132
CADM2	0.9056
CAMK4	0.9201
CAMSAP2	0.911
CAPRIN2	0.913
CARD8	0.905
CASK	0.913
CASP10	0.9166
CBFA2T2	0.9052
CBX5	0.9177
CCDC127	0.9201
CCDC144A	0.9115
CCDC50	0.9175
CCDC85C	0.9217
CCDC93	0.9128
CCNT2	0.9127
CCSAP	0.9125
CD226	0.9055
CD84	0.9053
CDCP1	0.9117
CDH23	0.9173
CDH7	0.9214
CDH8	0.9232
CDHR1	0.9211
CDKL5	0.9177
CDS2	0.9133
CDYL2	0.9174
CECR2	0.9173
CELF1	0.9225
CELF2	0.9132
CENPO	0.9117
CENPP	0.9176
CEP192	0.9132
CEP250	0.9133
CEP78	0.913
CEP85L	0.9207
CERS6	0.9125
CFL2	0.9232
CFLAR	0.9057
CHML	0.9123
CHRM3	0.905
CHRNA7	0.9167
CHST11	0.9127
CHST9	0.9056
CIITA	0.9133
CLCN3	0.9104
CLCN6	0.912
CLEC16A	0.905
CLEC2D	0.9049
CLK3	**0.9177**
CLMN	0.9134
CLOCK	0.9201
CLVS2	0.9237
CNKSR3	0.9238
CNNM2	0.9227
CNOT4	0.9103
CNOT6L	0.9228
CNTNAP2	0.9049
CNTNAP3B	0.9119
CNTROB	0.9121
COL20A1	0.9195
CPD	0.9125
CPEB3	0.9053
CPM	0.9127
CPSF6	0.9127
CRTAP	0.9198
CSRNP3	0.9134
CTNNA3	0.9211
CUL3	0.9049
CUX1	0.9216
CXCL12	0.9162
CXorf23	0.9196
CYB561D1	0.905
CYB5R4	0.9132
CYTH3	0.9119
DAPK2	0.9175
DBNL	0.9226
DBT	0.9133
DCAF7	0.9052
DCN	0.917
DCP2	0.9233
DCUN1D3	0.905
DCX	0.9053
DDI2	0.9177
DDX53	0.9165
DGKE	0.9131
DGKH	0.9227
DGKI	0.9176
DIRAS2	0.9113
DIS3	0.9215
DISC1	0.9052
DLG5	0.9174
DLGAP2	0.9055
DNAJC10	0.9231
DNAJC15	0.9131
DNAJC18	0.9167
DNAJC5	0.9196
DNASE1	0.9177
DNM3	0.9127
DNMT3A	0.9176
DOK6	0.9176
DR1	0.9216
DRP2	0.9122
DSC2	0.9215
DTNA	0.917
DTWD1	0.9227
DYRK2	0.9174
EEF2K	0.9051
EGFR	0.9176
EIF2AK2	0.9133
EIF4E3	0.9216
EIF4G1	0.905

EIF5	0.9048	FGF7	0.9121	GOLGB1	0.9133
ELFN2	0.9129	FGFR1OP	0.9217	GOLT1B	0.9106
ELK4	0.9174	FIGN	0.9215	GPATCH2L	0.9238
ELP2	0.9052	FILIP1	0.9123	GPR107	0.913
EMC10	0.9056	FKBP15	0.905	GPR161	0.9049
ENAH	0.9176	FKTN	0.913	GPR180	0.9197
ENTPD1	0.9056	FLNA	0.9056	GPRC5B	0.9125
EPHA5	0.9123	FLRT2	0.9241	GPRIN3	0.9134
EPHA8	0.9106	FMN1	0.9177	GRAMD1B	0.9049
EPHB6	0.9122	FNTA	0.9049	GREM1	0.9227
EPN1	0.9217	FOSL2	0.9128	GRIK3	0.9229
EPT1	0.9053	FOXK1	0.9177	GRIN2A	0.9132
ERBB2	0.9049	FOXP2	0.913	GRIN2B	0.9241
ERBB2IP	0.916	FREM2	0.9052	GTDC1	0.9176
ERBB4	0.9175	FRK	0.9231	GTF2H5	0.9132
ERI1	0.911	FRY	0.9192	GTF3C4	0.9123
ESRRG	0.9162	FSD1L	0.9209	GTPBP10	0.9213
ETNK1	0.913	FTO	0.9056	GUCY1A2	0.9134
ETV5	0.9109	FUT4	0.9224	GXYLT1	0.9197
EXOC5	0.9052	FUT9	0.9233	HDAC2	0.9053
EXOSC9	0.9111	FXR1	0.9175	HDAC9	0.9198
EXT1	0.9171	FZD3	0.9134	HECW2	0.9174
FAM126A	0.9133	GAB1	0.9051	HEG1	0.9194
FAM126B	0.9225	GABRA4	0.9054	HELB	0.9176
FAM168B	0.917	GABRG3	0.9176	HELZ	0.9174
FAM179A	0.9176	GALR1	0.9133	HEMK1	0.9231
FAM193B	0.9049	GAN	0.9217	HFE	0.9116
FAM204A	0.9202	GAS2L3	0.9189	HHIP	0.9051
FAM217B	0.9103	GCC2	0.9054	HIF1AN	0.9227
FAM26E	0.9201	GDAP2	0.9199	HIPK2	0.9234
FAM63B	0.92	GDF11	0.9056	HIPK3	0.9123
FAM83F	0.9178	GFOD1	0.9176	HLA-A	0.908
FAM9C	0.9209	GFPT1	0.9053	HLA-A	0.9194
FARP1	0.9175	GJA3	0.9122	HMGA2	0.9053
FARP2	0.9126	GLRA3	0.9173	HMHA1	0.9049
FAT3	0.9198	GMFB	0.9214	HNRNPA3	0.9049
FBXL4	0.9172	GMPPB	0.9051	HOOK3	0.9056
FBXO22	0.923	GMPS	0.905	HS2ST1	0.9171
FBXO25	0.9177	GNAI3	0.9238	HS6ST3	0.9131
FBXO30	0.9174	GNAO1	0.9168	HSBP1	0.9132
FBXO32	0.9129	GNB1L	0.9054	HSD17B2	0.9055
FEM1A	0.9055	GNB5	0.9132	HSPA12A	0.9122
FER	0.9216	GNPDA2	0.9118	HTT	0.9194
FGF14	0.9236	GOLGA6L2	0.9228	ICA1L	0.9173

ICE2	0.9175
ICOSLG	0.913
IDS	0.9127
IFITM10	0.9164
IGF2BP1	0.9129
IGSF10	0.9119
IKZF1	0.9125
IL17RD	0.9054
IL6R	0.9124
IL6ST	0.913
ILDR2	0.9201
IMPG1	0.9125
INO80D	0.9201
INPP4A	0.9176
INTS6	0.9217
INTU	0.9133
IPCEF1	0.9172
IPMK	0.9125
IPO9	0.9216
IRAK3	0.9051
IRGQ	0.9053
ITGA11	0.9172
ITGA9	0.9212
ITM2B	0.9132
ITSN1	0.9178
IYD	0.9213
JAKMIP2	0.9168
JMY	0.905
KALRN	0.9053
KAT7	0.9132
KATNAL1	0.913
KCNA1	0.9052
KCNB1	0.9132
KCNC1	0.9052
KCNC4	0.9238
KCND3	0.9113
KCNH5	0.92
KCNJ15	0.9131
KCNJ6	0.924
KCNK5	0.9103
KCNMA1	0.9055
KCNN3	0.9134
KCNQ3	0.9215
KCNQ4	0.9099
KCNQ5	0.9119
KCTD15	0.9121
KCTD16	0.9226
KDM3B	0.9199
KDM5A	0.9051
KDM7A	0.9213
KIAA0930	0.9198
KIAA1045	0.9124
KIAA1244	0.9216
KIAA1456	0.9053
KIAA1462	0.9171
KIAA1549	0.9054
KIAA1614	0.9175
KIAA1958	0.9175
KIAA2018	0.9129
KIDINS220	0.9199
KIF1B	0.9128
KIF26B	0.9054
KIF6	0.9215
KLC1	0.9234
KLF12	0.9216
KLHL21	0.9051
KLHL28	0.9116
KLHL42	0.905
KLHL6	0.9126
KMT2C	0.9236
KPNA4	0.913
KRR1	0.9226
KRT222	0.9105
KSR1	0.9212
KSR2	0.9134
KYNU	0.9236
LANCL3	0.9056
LCOR	0.9054
LCORL	0.9048
LDLRAD4	0.9053
LGALS8	0.9127
LPGAT1	0.9214
LPHN3	0.9131
LPP	0.9231
LRIG2	0.9199
LRRC58	0.9197
LRRC8B	0.9051
LRRK1	0.9133
LRRK2	0.9173
LSAMP	0.9176
LYNX1	0.9123
LYRM2	0.9126
MACC1	0.9171
MAP3K2	0.9201
MAP3K9	0.9133
MAPK1	0.9201
MAPK13	0.9052
MAS1	0.9056
MBNL3	0.9176
MBOAT2	0.9054
MBP	0.9177
MCC	0.905
MCFD2	0.9205
MCTP2	0.9169
MDGA1	0.9055
MDM2	0.9131
MDM4	0.9133
MECP2	0.9056
MED12L	0.9123
MED13L	0.9201
MEGF9	0.919
MEIS1	0.9123
MESDC2	0.9122
METTL8	0.9175
MEX3C	0.9121
MGAT4A	0.9131
MGAT4C	0.9238
MGAT5	0.9173
MGLL	0.9124
MIEF1	0.9165
MITF	0.9114
MKLN1	0.923
MLEC	0.9128
MLXIP	0.9126
MLYCD	0.9134
MMP16	0.9057
MOB1B	0.913
MON2	0.9131
MOSPD2	0.9103
MPP6	0.9128
MPRIP	0.9176
MR1	0.9131

MRE11A	0.9167	NIPA1	0.9197	PCNXL4	0.9236
MROH5	0.9124	NKD1	0.9174	PCYT1B	0.9122
MRPL35	0.9114	NKTR	0.9131	PDE4B	0.9098
MRPL42	0.9217	NLGN4X	0.9116	PDE4DIP	0.9049
MRPS25	0.9126	NOL4L	0.9049	PDE5A	0.919
MTF1	0.9198	NOVA1	0.9214	PDE7A	0.915
MTMR10	0.9211	NOVA2	0.9198	PDIK1L	0.9101
MTMR9	0.9197	NOX5	0.9174	PDK1	0.9231
MTR	0.9054	NQO2	0.9216	PDPR	0.9174
MTUS1	0.9118	NR6A1	0.905	PDXK	0.9131
MXD1	0.9125	NRDE2	0.9227	PDZD8	0.9166
MYLK	0.9199	NRXN3	0.9175	PEAK1	0.9231
MYO18A	0.92	NT5DC1	0.905	PELP1	0.9159
MYO18B	0.9225	NT5DC3	0.9053	PEX11A	0.9152
MYO5C	0.9172	NTNG2	0.9157	PEX26	0.9234
MYO9A	0.916	NTPCR	0.905	PGBD5	0.9056
N4BP2	0.9176	NTRK3	0.9238	PHACTR1	0.9167
N4BP2L2	0.92	NUCKS1	0.9127	PHACTR2	0.92
NA	0.9049	NUDCD2	0.9051	PHC3	0.9176
NA	0.9053	NUDT3	0.92	PHEX	0.9121
NA	0.9055	NUDT4	0.9132	PHF3	0.9053
NA	0.9125	NUFIP2	0.9198	PHKG2	0.9126
NA	0.913	NUPL1	0.9171	PIGP	0.9131
NA	0.9216	ODF2L	0.9196	PIK3C3	0.9173
NA	0.9239	OGFRL1	0.9176	PIK3CA	0.9223
NABP1	0.9201	ONECUT2	0.9134	PITPNM3	0.9048
NACC2	0.9051	ORAI2	0.9134	PLCXD3	0.9048
NAP1L1	0.9215	ORC4	0.9049	PLEKHA1	0.9057
NCKAP1	0.924	OSBPL8	0.9122	PLEKHA3	0.9057
NCOA2	0.9122	OTUD4	0.9115	PLEKHA8	0.9174
NDST1	0.9171	OTUD7A	0.9216	PLEKHG4B	0.9233
NDUFA5	0.9127	OTULIN	0.9175	PLEKHM1	0.9128
NDUFA9	0.9055	PAG1	0.9216	PLLP	0.9052
NDUFS1	0.9133	PAK3	0.9132	PLXNA4	0.9199
NEDD4	0.9198	PANK3	0.9133	PNRC2	0.9146
NEGR1	0.9226	PAPD5	0.9051	POLE	0.9226
NF1	0.9176	PARD3B	0.9168	POLR1A	0.9175
NFASC	0.9215	PAX5	0.9226	POLR3D	0.9048
NFAT5	0.9236	PAXIP1	0.9122	POU2F1	0.9178
NFIA	0.9233	PBX1	0.9198	PPARA	0.9175
NFIB	0.9175	PCDH10	0.9194	PPIP5K2	0.9202
NFIC	0.9053	PCDH19	0.912	PPM1A	0.9175
NHLRC2	0.9055	PCDH9	0.9239	PPM1F	0.9169
NIN	0.9127	PCDHA4	0.9052	PPP1CB	0.9166

PPP1R12B	0.9177
PPP1R13B	0.9128
PPP2R1B	0.9188
PPP2R5E	0.921
PRDM11	0.9132
PRDM15	0.9226
PRDM16	0.9052
PRKAA2	0.9054
PRKCA	0.9132
PRKCB	0.9052
PRLR	0.9176
PRPF38A	0.9122
PRRC2B	0.9048
PRRG3	0.9049
PRTG	0.9233
PSD3	0.92
PSMG4	0.9049
PTAR1	0.9215
PTBP2	0.9133
PTBP3	0.905
PTCH1	0.9057
PTCHD1	0.923
PTEN	0.9172
PTGER3	0.9198
PTK2	0.9171
PTPN11	0.9125
PTPN14	0.923
PTPN23	0.9048
PTPRT	0.9133
PURA	0.9201
PURB	0.9054
PVRL1	0.9048
PYGO1	0.9174
QKI	0.9226
RAB11FIP2	0.9165
RAB11FIP4	0.92
RAB15	0.9106
RAB21	0.9217
RAB3C	0.9201
RAB3IP	0.9132
RAB6B	0.9173
RAD51D	0.9131
RALY	0.9052
RAP1A	0.9206
RAP1B	0.9227
RAPGEF1	0.9162
RASAL2	0.9133
RASGEF1B	0.9049
RASSF5	0.9128
RASSF8	0.9166
RBBP4	0.9129
RBM25	0.9124
RBM28	0.9227
RBMS2	0.9129
RBMS3	0.905
RC3H2	0.9212
REL	0.9055
REPS1	0.9125
REPS2	0.913
RET	0.9116
REV1	0.9127
REV3L	0.9216
RFX7	0.92
RGMA	0.9057
RICTOR	0.9053
RIF1	0.9199
RILPL2	0.9051
RIMKLA	0.9198
RIMS2	0.9168
RNF115	0.9055
RNF150	0.9054
RNF152	0.9132
RNF165	0.9055
RNF217	0.92
RNF24	0.9175
RORA	0.9133
RORB	0.9175
RPAP2	0.9217
RPS6KA5	0.9241
RPS6KB1	0.9118
RRP15	0.9199
RTEL1-TNFRSF6B	0.9118
RTKN2	0.9049
RUNX1T1	0.9126
S100A7A	0.9049
SAMD12	0.9131
SAR1A	0.9052
SARM1	0.9201
SCAI	0.9176
SCN3B	0.9048
SCN8A	0.9209
SCO1	0.9056
SCOC	0.9119
SCUBE1	0.9132
SDHC	0.9057
SDK2	0.905
SDR42E1	0.9216
SEC22C	0.9049
SEMA3A	0.9051
SEMA5A	0.9133
SEMA6D	0.9169
SERINC3	0.9116
SERINC5	0.9052
SESN2	0.9096
SESN3	0.9225
SF3B3	0.9053
SGCD	0.9199
SH3BP2	0.9056
SH3PXD2A	0.9215
SH3TC2	0.9132
SHE	0.913
SHPRH	0.9216
SHROOM4	0.9198
SIK2	0.9175
SIK3	0.9209
SIM1	0.9129
SIX4	0.9122
SKP1	0.9216
SLC16A7	0.9133
SLC1A2	0.9201
SLC24A4	0.9214
SLC30A4	0.9052
SLC30A9	0.9126
SLC35B4	0.9194
SLC35C2	0.9129
SLC35E3	0.9217
SLC39A9	0.9122
SLC43A2	0.9214
SLC44A1	0.9132
SLC4A4	0.912
SLC4A7	0.9104
SLC4A8	0.9056

SLC5A3	0.9177
SLC7A11	0.9133
SLC7A14	0.9172
SLC7A2	0.9048
SLC7A6	0.905
SLC8A1	0.9236
SLCO5A1	0.905
SLITRK5	0.9238
SMAD2	0.9241
SMAD5	0.9225
SMC1A	0.9198
SMG9	0.9121
SMURF2	0.9131
SNAP91	0.9125
SNTB2	0.9199
SNX1	0.9133
SNX27	0.9173
SNX30	0.9054
SNX33	0.913
SNX8	0.9191
SOD2	0.9134
SOGA3 KIAA0408	0.9055
SORT1	0.9128
SOS1	0.9194
SOX5	0.9122
SP3	0.9115
SPATA2	0.911
SPEF2	0.9097
SPRY3	0.9131
SREK1IP1	0.9131
SRGAP1	0.9237
SRRM4	0.9172
SSBP2	0.9132
SSH2	0.9124
SSTR2	0.9052
ST6GALNAC3	0.9124
ST8SIA1	0.9132
ST8SIA3	0.9176
ST8SIA5	0.9057
STAM2	0.9116
STARD8	0.9197
STK24	0.9216
STK35	0.9212
STOX2	0.913
STRN	0.9049
STX7	0.9217
STXBP4	0.9134
STXBP6	0.9155
SUGT1	0.9202
SULT1B1	0.905
SV2B	0.9228
SV2C	0.9176
SYK	0.9121
SYNE3	0.9216
SYNJ1	0.9119
SYT14	0.9134
SYT16	0.9134
TAB3	0.9052
TACR3	0.9166
TBC1D15	0.9119
TBC1D16	0.9201
TBC1D32	0.9173
TBC1D5	0.9121
TBX18	0.9169
TCF4	0.913
TET2	0.9198
TET3	0.913
TEX14	0.905
TFDP2	0.9104
TFEC	0.9195
TG	0.9195
TGFBR3	0.913
THBS1	0.9124
THRB	0.9051
THSD7A	0.9168
THUMPD3	0.9101
TMED3	0.924
TMED5	0.9171
TMED7	0.9181
TMEM120B	0.9053
TMEM127	0.913
TMEM132B	0.9174
TMEM154	0.9235
TMEM164	0.9196
TMEM168	0.9049
TMEM170A	0.921
TMEM170B	0.9133
TMEM178B	0.9177
TMEM184A	0.9052
TMEM192	0.9176
TMEM200C	0.9052
TMOD1	0.9101
TMOD2	0.9132
TMOD3	0.92
TNFAIP8	0.92
TNKS1BP1	0.921
TNPO1	0.9053
TNRC6A	0.9226
TNRC6B	0.9134
TOM1L2	0.9194
TPCN1	0.9116
TPPP	0.9052
TREM1	0.909
TRHDE	0.9234
TRIL	0.9102
TRIM33	0.9168
TRIM44	0.9227
TRIOBP	0.9237
TRMT5	0.9122
TROVE2	0.9199
TRPM3	0.9129
TRPS1	0.9127
TSC1	0.9053
TSC2	0.9051
TSC22D2	0.9177
TSPAN14	0.9231
TSPAN3	0.9129
TTBK2	0.9173
TTC39B	0.9176
TTC7B	0.924
TTL	0.9057
TTPAL	0.9128
TXLNG	0.9108
TXNDC15	0.9115
TXNL1	0.9128
UBA6	0.9051
UBN2	0.9238
UBXN10	0.9048
UBXN7	0.9132
UFM1	0.9151
UHMK1	0.9131
UNC119B	0.9124

UNC13A	0.905
USP15	0.9215
USP31	0.9174
USP35	0.9163
USP38	0.9052
USP42	0.9049
USP45	0.905
USP46	0.913
USP49	0.9054
USP6	0.9053
USP6NL	0.9227
USP8	0.9231
UVSSA	0.9126
VAMP4	0.9049
VANGL1	0.9176
VAPA	0.9199
VASH2	0.9181
VCPIP1	0.9173
VGLL3	0.9175
VKORC1L1	0.9126
VLDLR	0.9129
VPS35	0.9123
VTA1	0.9131
VTI1A	0.9164
VWC2	0.9132
WASF3	0.9161
WDFY2	0.9133
WDR11	0.9161
WDR62	0.9197
WDR7	0.9216
WDR82	0.916
WHSC1L1	0.9126
WNK3	0.9196
WNT2B	0.9233
WSCD1	0.9118
WTIP	0.9177
XIAP	0.9174
XKR4	0.9234
XPO1	0.917
XPO4	0.9225
XYLT1	0.9131
YIPF4	0.9217
YIPF6	0.9051
YOD1	0.9198
YY1	0.913
ZADH2	0.9131
ZBED3	0.9126
ZBTB25	0.9215
ZBTB34	0.9195
ZBTB37	0.9239
ZBTB44	0.9053
ZBTB8A	0.9049
ZBTB8B	0.9202
ZC3H12C	0.9172
ZC3H14	0.9239
ZC3H6	0.9215
ZC3H8	0.917
ZDHHC17	0.9183
ZDHHC18	0.9121
ZDHHC2	0.9127
ZDHHC21	0.9215
ZEB1	0.9232
ZFHX4	0.9114
ZFP90	0.9127
ZFYVE20	0.9167
ZFYVE26	0.9054
ZHX3	0.9175
ZNF107	0.9164
ZNF117	0.9238
ZNF138	0.9226
ZNF142	0.913
ZNF189	0.9114
ZNF207	0.923
ZNF223	0.9106
ZNF226	0.9231
ZNF230	0.9125
ZNF233	0.9182
ZNF257	0.9232
ZNF26	0.9234
ZNF268	0.9057
ZNF273	0.9227
ZNF286A	0.9128
ZNF286B	0.9127
ZNF292	0.9111
ZNF37A	0.9131
ZNF431	0.9177
ZNF445	0.9176

Supplementary 2: List of genes containing T-UCR in their exonic regions according to the study of Bejerano et al.

uc.143	218	AB014560
uc.203	203	AB067798
uc.135	201	AK096400
uc.339	252	ATP5G2
uc.413	272	BC060758
uc.49	207	BC060860
uc.61	326	BCL11A
uc.324	225	C11orf8
uc.285	232	CARP-1
uc.233	266	CENTG3
uc.393	**275**	**CLK3**
uc.185	411	CLK4
uc.184	230	CPEB4
uc.471	239	DDX3X
uc.331	218	DLG2
uc.13	237	EIF2C1
uc.194	201	EPHA7
uc.183	236	FBXW1B
uc.333	270	FLJ25530
uc.478	252	GRIA3

uc.479	302	GRIA3
uc.282	207	GRIN1
uc.97	442	HAT1
uc.144	205	HNRPDL
uc.186	305	HNRPH1
uc.263	207	HNRPK
uc.264	267	HNRPK
uc.443	239	HNRPM
uc.45	203	HNRPU
uc.46	217	HNRPU
uc.409	244	L32833
uc.174	260	MATR3
uc.129	212	MBNL1
uc.356	251	MBNL2
uc.375	300	MIPOL1
uc.292	217	MLR2
uc.406	**211**	**NFAT5**
uc.473	222	NLGN3
uc.378	251	NRXN3
uc.475	397	OGT
uc.280	220	PBX3
uc.338	223	PCBP2
uc.376	290	PRPF39
uc.377	217	PRPF39
uc.33	312	PTBP2
uc.102	338	PTD004
uc.48	298	PUM2
uc.477	209	RAB9B
uc.395	249	RBBP6
uc.330	207	RBM14
uc.455	245	RNPC2
uc.419	289	SFRS1
uc.138	419	SFRS10
uc.28	355	SFRS11
uc.189	573	SFRS3
uc.456	320	SFRS6
uc.50	222	SFRS7
uc.454	208	SLC23A1
uc.193	319	SYNCRIP
uc.436	210	TCF4
uc.414	246	THRA
uc.313	231	TIAL1
uc.208	218	TRA2A
uc.209	250	TRA2A
uc.77	296	ZFHX1B
uc.151	214	ZFR
uc.474	210	ZNF261

Discussion

Gastric neoplasm is the leading cause of cancer-related deaths. According to research conducted in 2008, gastric neoplasm is the fourth most common cancer in the world and ranks second among cancers that cause death. The death rate from this cancer is higher than that from malignant tumors such as colon, breast and prostate cancers. The development of this cancer is complex, involving a number of genetic and epigenetic alterations of oncogenes, tumor suppressor genes, deoxyribonucleic acid (DNA) repair genes, cell cycle regulators, and signaling molecules. Oncogenes are activated at different stages of the course of gastric neoplasm, and some tumor suppressor genes are inactivated. Numerous studies have shown that miRNAs can be effective in carcinogenesis. Changes in expression levels of miRNAs in different types of cancer have been investigated, and miRNAs have been observed to differ between normal and pathological tissues (Sevignani et al., 2006; Zhou et al., 2010). Various miRNAs have been shown to play a specific role in tumor progression and metastasis in the differentiation of cancer cells (Kim et al., 2011; Calin et al., 2002; Michael et al., 2003; Metzler et al., 2004; Chan et al., 2005; He et al., 2005; Sevli et al., 2010; Lamy et al., 2006; Iorio et al., 2005) The purpose of this study was to describe novel biomarkers for GN through *in silico* analysis involving gastric neoplasm-specific miRNAs, by determining their combinatorial target genes (potential ceRNAs), selecting those with T-UCR and potentiating their association with gastric neoplasm using statistical correlation techniques.

Four miRNAs experimentally related to gastric neoplasm were identified through the miRTarbase database (Table I). Genes with equal ComiR abundance were listed through 1008 genes targeted concurrently by these four miRNAs. Genes with T-UCR in their exonic regions were described from the genes containing T-UCR listed by Bejerano et al. (Bejerano et al., 2004). We then considered those exhibiting probable ceRNA activity in our earlier analysis (Table II). Next, we choosed genes with significant differences in expression between gastric neoplasm and normal gastric tissues from GN-related ceRNAs involving T-UCR. This test revealed significantly higher NFAT5 expression in gastric neoplasm than in normal stomach tissue, while the other exhibited no significantly different

expression pattern. In addition, the NFAT5 and CLK3 gene pair were substantively associated with gastric neoplasm based on the Spearman correlation analysis findings.

These NFAT5 genes have not previously been experimentally linked to gastric neoplasm. Ours is the first study to associate these two genes with gastric neoplasm. NFAT family contains five different proteins one of them is NFAT5 protein. But, NFAT1 to 4 proteins are regulated by calcineurin, NFAT5 is controlled by osmotic pressure at the nuclear localization, transcriptional and expression levels. When stimulated, NFAT5 triggers target gene transcription by binding to tonicity enhancer elements) in various coordinator domains which are all responsible for supplying cells in order to facilitate their survival under hypertonic conditions (Cheung and Ko, 2017). NFAT5 gene shows its oncogenic role via different pathways in such diseases as renal cell carcinoma, breast cancer, lung adenocarcinoma and colon cancer. NFAT5-related expression of S100A4 projects the migration and proliferation of renal carcinoma cells (Küper et al., 2014). Additionally, NFAT5/STAT3 interaction soften synergism of high salt with IL-17 towards induction of VEGF-A expression in breast cancer cells (Amara S et al., 2016). NFAT5 also stimulates the migration and proliferation of pulmonary adenocarcinoma cells, in part by modulating AQP5 expression (Guo and Jin, 2015). The Src kinase pathway is also involved in NFAT5-mediated S100A4 induction through hyperosmotic stress in colon cancer cells (Chen et al., 2011). NFAT5 is also a tumor suppressor that functions by suppressing invasion and triggering apoptosis in hepatocellular carcinoma.

Conclusion

The NFAT5 gene was correlated with gastric neoplasm in our study, and *in silico* analysis results predict that they may potentially play an oncogenic role in gastric neoplasm. The inconsistent results concerning their roles in varying forms of cancer suggests that our study findings will be preliminary for subsequent in vitro and in vivo studies performed to determine the roles of the NFAT5 gene in gastric neoplasm progression. Fatal one among urological cancers. RCC is causedby the accumulation of many genetic and

Ethics Committee Approval:
Since it is a in silico study, there is no need for an ethics committee approval
Peer-review: Externally peer-reviewed.
Author Contributions: Externally peer-reviewed. Author Contributions: Concept- D.U.A.; Design D.U.A., S.E.; Supervision-D.U.A., S.E.; Materials D.U.A., S.E.; Data Collection and/or Processing D.U.A., S.E.; Analysis and/or Interpretation-D.U.A; Literature Review-D.U.A.; Writing-D.U.A.; Critical Review- S.E
Conflict of Interest: No conflict of interest was declared by the author.
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RESEARCH ARTICLE

Investigation of Resistance and Clonal Relatedness Among Nosocomial Acinetobacter Isolates

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Abstract

Objective: This study focused on *Acinetobacter* spp. that were isolated from inpatients to determine the resistance rates to antibiotics and to monitor the resistance increase over the years.

Method: To determine whether there was a clonal relationship between the strains using pulsed field gel electrophoresis (PFGE), to evaluate the epidemiological and clinical data of patients, and to determine the factors that may be effective in the formation and spread of infection, antibiotic susceptibilities of *Acinetobacter* strains were evaluated by the Kirby Bauer disk diffusion method. Molecular typing was studied with PFGE.

Results: Netilmicin had the least resistance (7%) among the strains, while the highest resistance was found against piperacillin (92%), ceftriaxone (81%), and doxycycline (69%). When resistance profiles of strains were compared according to year (2005-2007), no significant changes were found for resistance rates to piperacillin, ceftriaxone, gentamicin, doxycycline, and trimethoprim / sulfamethoxazole. Of all the 131 tested *Acinetobacter* spp., 82 (62.6%) of the strains were clustered with the PFGE method and 72.3% of the strains were clonally related. The increase in MDR, XDR and PDR rates among the isolated *Acinetobacter* spp. is remarkable. It was determined that the degree of transmission of the strains was quite high among patients. The clone in the hospital was able to remain in the environment for long periods and the general patient mortality rates were higher than other studies.

Conclusions: According to results and with the support of molecular typing studies, the need for more effective prevention and control measures in our hospital was demonstrated.

Key words: Acinetobacter, PFGE, cluster, resistant

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Introduction

Acinetobacter species are important opportunistic pathogens that are responsible for severe hospital infections in various units, especially in intensive care units. It may cause various infections such as endotracheal tube- or tracheostomy-associated pneumonia, endocarditis, meningitis, skin and wound infections, peritonitis and urinary tract infections. Sporadic cases such as conjunctivitis, osteomyelitis and synovitis have also

been reported (Buxton et al., 1978; Lyons, 1985; Bergogne-Berezin and Towner, 1996; Villegas and Harstein, 2003). *Acinetobacter* species play an important role in colonization and infection of hospitalized patients. Trauma, mechanical ventilation, and surgical procedures are important risk factors for nosocomial infections of this microorganism (Bergogne-Berezin and Towner, 1996; Parvez and Jarvis, 1999).

A. baumannii is the most common bacteria responsible for hospital-acquired infections (Beck-Sague et al., 1990; Lortholary et al., 1995; Villegas and Harstein, 2003). Other species such as *A. johnsonii*, *A. lwoffii* and *A. radioresistens* occur naturally on human skin and can also be found commonly in the oropharynx and vagina. Compared to other *Acinetobacter* species, *A. lwoffii* is more commonly associated with meningitis (Siegman-Igra et al., 1993).

The number of multidrug-resistant *A. baumannii* infections as opportunistic pathogens has increased globally in recent years. It has become one of the most difficult species to control and treat among nosocomial gram-negative pathogens (Jain and Danziger, 2004; Li et al., 2005). Patients infected with *A. baumannii* are often immunocompromised or have severe disease status and it is associated with mortality (Lyons, 1985; Bergogne-Berezin and Towner, 1996). Many of the outbreaks of *A. baumannii* have environmental origin, such as patient beds, air conditioners, and mechanical ventilation equipment (Villegas and Harstein, 2003). *Acinetobacter* spp. can survive for between 3 days to 5 months on dry inanimate surfaces (Beck-Sague et al., 1990; Siegman-Igra et al., 1993; Lortholary et al., 1995). This contributes to the emergence of outbreaks. In hospitalized patients and in various outbreaks, cases with high colonization in the skin, throat, respiratory system and digestive system were recorded. In patients receiving mechanical ventilation in intensive care units, colonization of the airways is very high due to contamination of these devices. Skin contamination is also common in these patients and is transmitted and spread to the hands of health personnel. Also, oropharyngeal and digestive tract colonization of *Acinetobacter* spp. is an important reservoir for outbreaks in patients hospitalized in intensive care units (Bergogne-Berezin and Towner, 1996; Corbella et al., 1996; Webster et al., 1998).

Acinetobacter species tend to resist many antibiotics. Almost all group members are resistant to penicillin, ampicillin and cephalothin and most of the strains are resistant to chloramphenicol (Seifert et al. 1993). Sensitivities to second and third generation cephalosporins and trimethoprim / sulfamethoxazole have been reported to be variable. In recent years, resistance to aminoglycosides has tended to increase among *Acinetobacter* species. Again, in nosocomial outbreaks, an increase is seen in strains showing multidrug resistance, including carbapenem-resistant *Acinetobacter* species (Bou et al., 2000; Hsueh et al., 2002). No international consensus seems to have been achieved yet for terms used to describe resistance in gram negative bacteria. The general trend in definition of antibiotic resistance is multi drug resistant (MDR) when resistant to ≥3 drug groups (Giske et al., 2008). The term "extreme resistance, extensive resistance" (XDR) was adopted for resistance to all antibiotics except tigecycline and colistin. For resistance to all existing antibiotics, including tigecycline and colistin, "pan drug resistance" (PDR) is used (Falagas et al., 2005; Giske et al., 2008).

The increase of *A. baumannii* infections is due to incorrect infection control applications (Villegas and Harstein, 2003). In addition to the classical epidemiological information in the prevention of outbreaks, strain typing methods that provide determinative information about the source and transmission pathways of the causative microorganism are also important. Strain typing reveals the relationship between different sources in which microorganisms are isolated. Typing methods are important for defining the source and spread of epidemic strains. Pathogens associated with an epidemic have been identified by using molecular typing methods that support clinical-epidemiological data. A hypothesis is then generated based on the cause-and-effect relationship for the epidemiology of infection, including the shape of the contamination (reservoir and vector) and specific control measures. Continuous surveillance is essential to assess the effectiveness of the infection control measures and the treatment regimen (Jarvis, 1994; Aparajita et al., 2006).

The determination of the epidemiological relationship between nosocomial pathogens isolated from different sources in the past was based on the comparison of phenotypic characteristics such as biotype, serotype, bacteriophage or bacteriocin types and antimicrobial susceptibility profiles. This approach has begun to change over the last 20 years with new DNA-based technologies or developments in molecular analysis applications. For DNA-based molecular typing, Pulsed Field Gel Electrophoresis

(PFGE) and other restriction-based methods consist of plasmid analysis and polymerase chain reaction (PCR) based typing methods (Aber and Mackel, 1981; Goering, 1993; Arbeit, 1995).

In this study, *Acinetobacter* spp. were isolated from inpatients in various wards in a research hospital. The aim of this study was to determine the resistance rate of the strains to antibiotics, to observe the increase in resistance during the years, to determine whether there is clonal relationship between the strains by PFGE method, to evaluate the epidemiological and clinical data of the patients, and to determine the factors that may be effective in the formation and spread of infection.

Methods

***Acinetobacter* spp. and Patient selection criteria.** A total of 135 *Acinetobacter* strains belonging to 130 patients were recruited from January 2005 to December 2007 in İnönü University Turgut Özal Medical Center. All patients were evaluated by the Infection Control Committee in our hospital and it was decided whether they had a hospital infection according to the criteria of the Centers for Disease Control and Prevention (CDC) in the United States (CLSI, 2008).

Evaluation of Epidemiological Relationship and Risk Factors. For each patient with accepted hospital infection, an epidemiological study form was prepared with some questions such as gender, age, hospital ward, clinical diagnosis, sample type, and culture results.

Identification of strains. Clinical specimens taken from hospitalized patients were inoculated in blood agar and kept for 24 hours at 35 ºC. Gram staining, colony morphologies, oxidase and sugar fermentation activities, and catalase reactions of *Acinetobacter* spp. were investigated. Identification of strains was performed by using a Phoenix 100 system (Becton Dickinson Microbiology Systems, USA).

Antibiotic Susceptibility Profiles of Strains. The antibiotic susceptibilities of *Acinetobacter* strains isolated from clinical samples were investigated according to Clinical & Laboratory Standards Institute: CLSI Guidelines using the Kirby Bauer disk diffusion method (CLSI, 2008).

Imipenem resistant strains were confirmed with the imipenem E-test method. *Acinetobacter* spp. strains with the probability of pandrug resistance were evaluated by the disc diffusion method for sensitivity to colistin and tigecycline. The colistin sensitivity was evaluated according to the zone diameter in nonfermentary bacteria and for tigecycline sensitivity the study by Jones and colleagues (2007) was used as a reference.

Molecular Typing. *Acinetobacter* strains which were grown from the samples of patients who were hospitalized in the wards were examined by PFGE. The protocol of Durmaz et al. (2007) was used for the PFGE application.

Monitoring and Analysis of Results. After electrophoresis, the gel was taken up in 400 ml of ultrapure water containing 5 mg/ml ethidium bromide and stained for 20 minutes. Gel images were observed under UV light. Images of DNA band strips were taken using Gel Logic 2200 Imaging System (1708x1280 pixel, Kodak Company, NY, USA). Images were saved in TIFF format. Band profiles were analyzed using the GelCompar II software system (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). First of all, normalization between the images was done with the help of three standards (1, 7, and 15th wells) in each image. The dendrogram of the PFGE profiles were generated using "Unweighted pair group method with mathematical averaging (UPGMA)" and clustering analysis was completed. The relationship between the strains was determined according to the Dice similarity coefficient. In the calculation of the similarity coefficient, band and profile tolerance was taken as 1-1.5%. Using criteria developed by Tenover et al. (1997), isolates were considered to be identical, closely related, possibly related, and unrelated.

Statistical analyses. Pearson's chi-square analysis (exact test) was used to determine the relationships between categorical variables. The analysis of the data was performed with the statistical software TURCOSA (Turcosa Analytical Solutions Ltd., www.turcosa.com.tr). "$p<0.05$" was accepted as statistically significant.

Results

Epidemiological Results. Of *Acinetobacter* strains evaluated as infectious agents, 131 (97%) were identified as *A. baumannii* and 4 (3%) as *A. lwoffi*. Of all the strains, 1/3 of *A. baumannii* isolates were isolated from tracheal aspiration culture and about 1/4 were isolated from blood culture. Two *Acinetobacter baumannii* were isolated from

cerebrospinal fluid (CSF) culture of patients hospitalized in the neurosurgery and neurosurgery intensive care unit. Two patients with *A. baumannii* isolated underwent invasive procedures. One patient had immunodeficiency and the other patient had Arnold-Chiari syndrome. Unlike other studies, *A. lwoffii* was not isolated as a meningitis agent.

Antibiotic Susceptibility Results. In this study, the most effective antibiotics against the strains that tested were netilmicin (7%) and the least effective antibiotics were piperacillin (92%), ceftriaxone (81%), doxycycline (69%), and gentamicin (67%).

According to year, comparison of the change in antibiotic resistance profiles for *Acinetobacter* spp. found no significant change in resistance rates to piperacillin, ceftriaxone, gentamicin, doxycycline, and trimethoprim / sulfamethoxazole. When we compared 2005 and 2007, the resistance rate rose from 47% to 76% for piperacillin / tazobactam, rose from 42% to 77% for cefepime, rose from 47% to 77% for ceftazidime, rose from zero to 39% for imipenem, rose from zero to 14% for netilmicin, rose from 18% to 53% for amikacin, rose from 18% to 47% for tobramycin, rose from 24% to 55% for levofloxacin, and rose from 16% to 46% for ampicillin/sulbactam. The antibiotic resistance profiles of *A. baumannii* isolates changed over time.

Molecular Typing Results. As a result of PFGE typing, about three quarters of the strains were found to be clonally related. Cluster-forming strains were predominantly isolated from respiratory (42.7%) and blood culture (29.3%) samples. The number of strains increased to 27 in some clusters. This situation demonstrates the magnitude of the cross-contamination severity. Twenty-seven strains in this cluster were present in our hospital for approximately 9 months. The duration of other clusters in hospital extended to 27 months. These data emphasize that an important nosocomial pathogen, such as *A. baumannii*, can be easily disseminated in the hospital and can survive in the hospital environment for many years if proper prevention and control are not ensured.

Clonally associated strains displayed variety in susceptibility profiles to drugs over time. Our twelfth cluster was also found to be the most antibiotic-resistant cluster. All isolates found in this cluster were found to be MDR. Only two of the isolates in the cluster were susceptible to imipenem (Table 1). Significant changes in the susceptibility profiles of the strains found in the cluster were recorded (Table 1).

Table 1. Statistical analyses of antibiotics relation with time

Table 2. Antibiotic susceptibility profiles of isolates in the same cluster

Antibiotics	2005 N=38	2006 N=23	2007 N=74	*p*	2005–2007 N=135
PIP	34(89.5)	19(82.6)	70(94.6)	0.168	92
TZP	20(52.6)	12(52.2)	56(75.7)	**0.019**	64
FEP	16(57.1)	14(60.9)	57(77.0)	0.087	64
CAZ	18(47.4)	14(60.9)	57(77.0)	**0.006**	66
CRO	30(78.9)	17(73.9)	62(83.8)	0.551	81
İPM	38(100.0)	5(21.7)	29(39.2)	**<0.001**	25
NET	38(100.0)	23(100.0)	13(17.6)	**<0.001**	7
GM	25(65.8)	11(47.8)	55(74.3)	0.050	67
AK	7(18.4)	5(30.4)	39(52.7)	0.001	39
TOB	7(18.4)	5(21.7)	35(47.3)	0.004	35
DOX	26(68.4)	19(82.6)	49(66.2)	0.340	69
LVX	9(23.7)	11(47.8)	41(48.8)	**0.028**	45
SXT	25(65.8)	14(60.9)	51(60.7)	0.888	67
SAM	7(18.4)	1net1(47.8)	34(45.9)	**0.011**	38

Stock no	PFGE type	PIP	TZP	FEP	CAZ	CRO	IMP	NET	GM	AK	TOB	DOX	LVX	SXT	SAM
28	V	R	R	R	R	R	S	S	R	I	R	R	R	R	S
35	V	R	R	I	R	R	S	S	R	I	R	R	R	R	S
22	V-a	R	R	I	R	R	S	S	R	I	R	R	R	R	S
17	V-b	R	R	R	R	R	R	S	R	R	R	S	R	R	R
24	VII	R	R	R	R	R	R	S	R	R	S	R	R	R	R
27	VII	R	R	R	R	R	S	S	R	S	S	R	R	R	R
85	VII-a	R	R	R	R	R	S	S	R	S	S	I	R	I	R
86	VII-a	R	R	R	R	R	S	S	R	S	S	S	S	R	R
34	VII-b	R	R	R	R	R	S	I	R	S	I	R	R	R	R
93	VII-b	R	R	R	R	R	S	S	R	S	S	S	S	S	R
21	VIII	R	I	I	I	R	S	S	R	S	S	R	S	R	I
52	VIII	R	R	R	R	R	S	S	S	S	S	R	S	S	S
16	VIII-a	R	S	S	I	R	S	S	R	S	S	R	S	S	S
2	IX	R	R	R	R	R	S	S	R	S	S	I	R	S	R
11	IX	R	R	R	R	R	S	S	R	S	S	R	S	S	R
10	IX	R	R	R	R	R	S	S	S	S	S	R	R	S	S
19	IX	R	I	R	R	R	S	S	S	S	S	R	R	I	S
20	IX	R	R	R	R	R	S	S	S	S	S	R	R	S	S
65	IX	R	R	R	R	R	S	S	R	S	S	S	S	S	R
66	IX	R	R	R	R	R	S	S	R	S	S	S	S	S	R
67	IX	R	R	R	R	R	S	S	R	S	S	S	I	S	R
80	IX	R	S	R	R	R	S	S	R	S	I	S	S	S	R
103	IX	R	R	R	R	R	S	R	S	S	S	I	R	S	I
138	IX	R	R	R	R	R	S	S	R	R	S	R	S	R	R
30	X	R	S	S	S	I	S	S	S	S	S	R	S	R	S
60	X	R	S	S	S	I	S	S	S	S	S	S	S	S	S
5	XII	R	R	R	R	R	S	S	R	S	S	R	S	R	I
7	XII	R	I	I	I	R	S	S	S	S	S	R	S	R	I
12	XII	R	I	I	I	R	S	S	R	R	S	R	S	R	I
13	XII	R	R	R	R	R	S	S	R	R	S	R	S	R	I
14	XII	R	R	R	R	R	S	S	R	R	S	R	S	R	I
15	XII	R	R	R	R	R	S	S	R	S	S	S	S	R	S
25	XII	R	R	I	I	R	S	S	R	S	S	R	S	R	I
40	XII	R	I	I	I	R	S	S	R	S	S	R	S	R	I
53	XII	R	R	R	R	R	S	S	R	R	S	S	S	R	S
63	XII	R	I	R	I	R	S	S	R	S	S	R	S	R	I
125	XII	R	I	I	I	R	S	S	I	S	S	S	R	S	S
127	XII	R	R	R	R	R	R	S	R	R	R	R	R	R	I
1	XII-a	R	R	R	R	R	S	S	S	S	S	R	S	I	S
33	XII-a	R	R	R	R	R	S	S	S	S	S	R	S	S	S
58	XII-a	R	R	I	I	R	S	S	R	S	R	R	S	R	I
114	XII-a1	R	R	I	I	R	S	S	R	I	S	S	S	R	I
50	XIII	R	I	R	R	R	S	S	R	S	S	S	S	S	S
38	XIII-b	R	S	I	I	R	S	S	S	S	S	R	R	R	S
137	XX	R	R	R	R	R	S	R	R	R	R	R	R	R	S
140	XX	R	R	R	R	R	S	R	S	I	R	R	R	R	I
68	XXIX	R	R	R	R	R	R	I	R	R	R	R	R	R	I
70	XXIX	R	R	R	R	R	R	S	R	R	R	R	R	R	R
72	XXIX	R	R	R	R	R	R	I	R	S	R	R	R	R	S
73	XXIX	R	R	R	R	R	S	S	R	R	R	R	R	R	I
75	XXIX	R	R	R	R	R	R	S	R	R	R	R	R	R	I
76	XXIX	R	R	R	R	R	R	S	R	R	R	R	R	R	R

79	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
83	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
91	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
92	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
97	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**
98	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**I**
102	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
106	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
109	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
111	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
113	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
115	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
118	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
119	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
120	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
123	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
132	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**I**
142	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
144	**XXIX**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**S**
145	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
116	**XXIX**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
3	**XXIX-b**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
8	**XXIX-b**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
69	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**I**	**S**	**R**	**S**	**R**	**I**
74	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**I**	**S**	**R**	**S**	**R**	**R**
77	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**I**	**S**	**R**	**S**	**R**	**R**
94	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**I**	**R**	**R**	**S**	**R**	**S**	**R**	**I**
105	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	**S**	**R**	**S**	**R**	**S**
124	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**S**
135	**XXV**	**R**	**I**	**R**	**R**	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**
141	**XXV**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	**S**	**R**	**S**	**R**	**S**
104	**XXV-a**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**S**
41	**XXXXIV**	**R**	**R**	**I**	**I**	**R**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**R**	**I**
42	**XXXXIV**	**R**	**I**	**I**	**I**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**I**
48	**XXXXIV**	**R**	**I**	**I**	**I**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**I**
59	**XXXXIV**	**R**	**R**	**I**	**I**	**R**	**S**	**S**	**R**	**R**	**S**	**R**	**S**	**R**	**R**
62	**XXXXIV**	**R**	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	**S**	**R**	**R**	**R**	**I**
49	**XXXXIV-a**	**R**	**I**	**I**	**I**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**S**
44	**XXXXV**	**R**	**I**	**R**	**R**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**R**
46	**XXXXV-b**	**R**	**I**	**R**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**I**	**S**

Four of 135 strains could not be classified with PFGE. The cultures of these four resultant bacteria were checked for purity and these strains were confirmed as *A. baumannii* again. The same PFGE protocol was applied 3 times with markers but failed to obtain a result. The dendrogram of the 131 *Acinetobacter* spp. isolates is shown in Figure 1 and the antibiotic susceptibility profiles of the bacteria in the same cluster are given in Table 1. Also, PFGE results of *Acinetobacter* species are given in Figure 2.

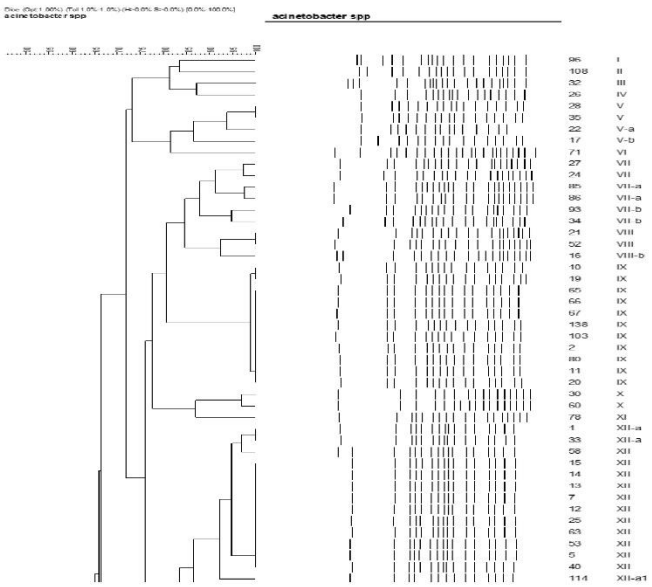

Fig. 1a. The dendogram of *Acinetobacter* strains

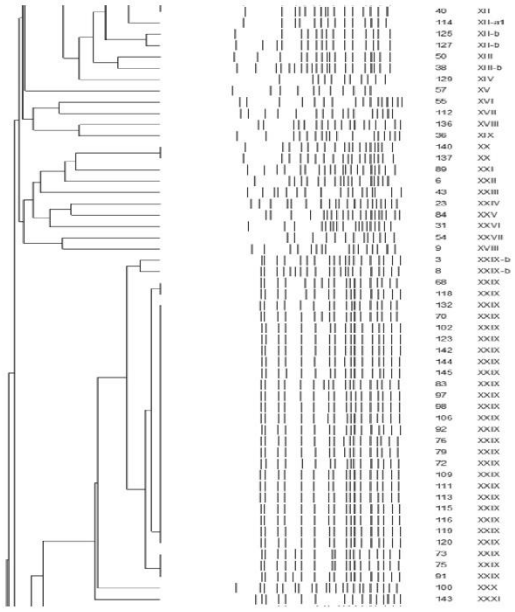

Fig. 1b. Continued. The dendogram of *Acinetobacter* strains

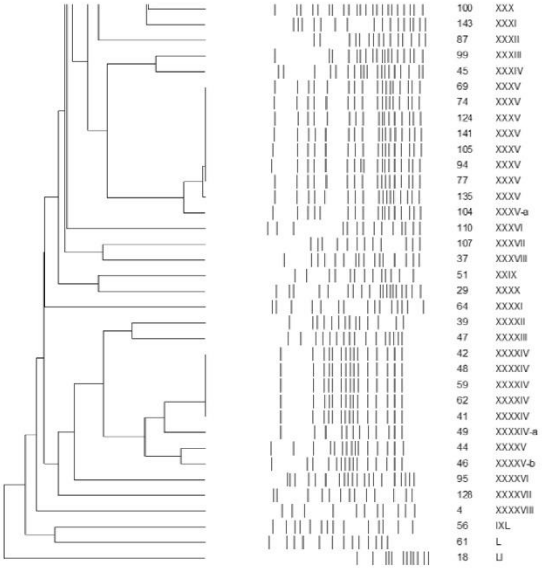

Figure1c. Continued. The dendogram of *Acinetobacter* strains

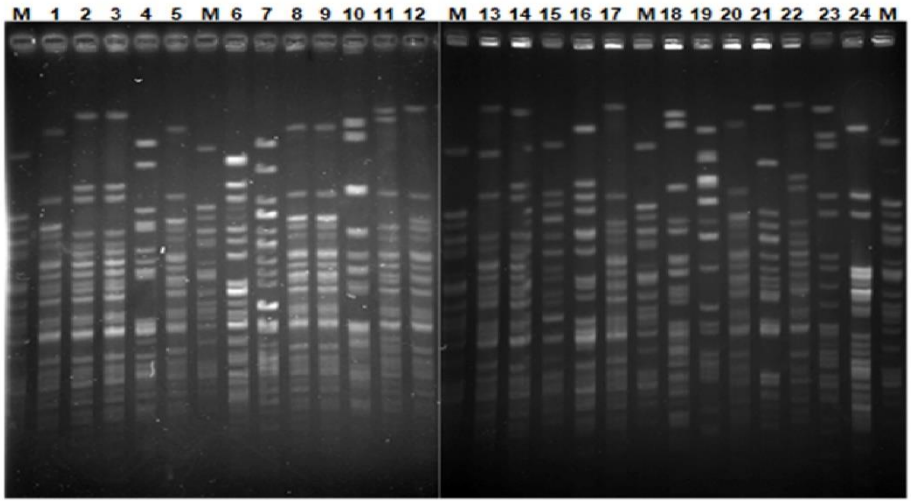

Fig. 2. PFGE results of Acinetobacter species

Of the 131 *Acinetobacter* spp. strains, 82 (62.6%) were in clusters. These strains are located in 15 clusters. The number of strains varies between 2 – 27 in the cluster. Two of the fifteen clusters (VII-a and XII-a) were also close to another two clusters and three (VII-b, XII-b, XXIX-b) were the possibly related with the other three clusters. Five of the strains were closely related and seven were related. According to these data, 72.3% of the strains were clonally related. The isolates of 37 strains (28%) showed a specific PFGE profile. In total, 64 (48.9%) PFGE patterns were determined for 131 strains.

The maximum number of strains in a cluster (27 strains) were in the twelfth cluster. The longest surviving clone was in the sixth cluster and survived twenty-seven months. The shortest period was in the eleventh cluster with sixteen days.

Strains 111 and 123, typed by PFGE, belonged to the same patient. Both strains are in the twelfth cluster. The first strain from the patient was isolated on 30.7.2007 in the paracentesis fluid culture of the patient who was hospitalized with diagnosis of liver failure and diabetes. The second strain was isolated on a blood culture sample after transplantation on October 15, 2007.

Strains 63 and 70, which were typed by PFGE, belonged to the same patient. The first strain was isolated on 12.1.2007 in the urine culture of the patient hospitalized in the neurosurgical intensive care unit. The strain is in the eighth cluster. The second strain was isolated in a blood culture sample three months later. The strain is located in the twelfth cluster with a different set.

Strains 28 and 29 were isolated from the same patient at an eight-month interval. Although the 28th isolate was in the first cluster, strain 29 was not included in any cluster.

Strains 47 and 48 were isolated in the blood and tracheal aspiration culture samples of a patient at a one-week interval. The first strain was not included in any cluster. The second strain was in the fifteenth cluster.

Strains 82 and 139, which were typed by PFGE, were isolated from the same patient at seven-month intervals. Both strains could not be identified by the PFGE method.

Four of the 130 patients included in the study had liver transplantation. The strains isolated from these four patients are located in the twelfth cluster and belong to patients who were hospitalized in the organ transplantation and anesthesia intensive care unit.

Twenty-five of the 27 strains were resistant to imipenem and amikacin in the twelfth cluster. Compared to other clusters, the most resistance to antibiotics was observed in this cluster.

Discussion

In this study, the number of strains resistant to three or more groups of antibiotics (MDR) number was 77 (57%). XDR strains and pandrug resistant strains numbered 23 (17%) and 2 (1.5%), respectively. Also, 34 *Acinetobacter* spp. had colistin and tigecycline sensitivities evaluated by using the disk diffusion method. Two strains were found to be resistant to colistin, while nine were resistant to tigecycline.

In the literature, the resistance profiles of 402 *A. baumannii* strains were compared. This profile has statistically significantly increased for all tested antibiotics compared to the previous year (Gazi et al., 2005).

Gülhan et al. (2007) monitored the resistance changes from 2004 to 2006. They found a statistically significant increase in carbapenem resistance from 7% to 25% and for ciprofloxacin from 54% to 82%.

In a study comprising 1532 clinical isolates over a 6-year period (1991 to 1996), resistance rates increased for ciprofloxacin from 54.4% to 90.4%, for amikacin from 21% to 83.7%, for trimethoprim / sulfamethoxazole from 41.1% to 88.9%, for imipenem from 1.3% to 80%, and for ampicillin / sulbactam from 65.7% to 84.1% in Spain (Ruiz et al., 1999).

Infections caused by MDR *Pseudomonas aeruginosa*, *A. baumannii* and *Klebsiella pneumoniae* strains have become a common problem in health institutions. These strains have consistently developed resistance to antibiotics, leading to the emergence of PDR isolates that are susceptible to only one antimicrobial agent and are resistant to all available drugs. The frequency of PDR clinical isolates, the treatment options of the infections associated with these isolates, and the mortality and morbidity rates are of great importance in terms of clinical and public health (Falagas and Bliziotis, 2007).

Gales et al. (2006) detected a polymyxin resistance rate of 2% and PDR rate of 0.3% in 2621 *Acinetobacte*r spp. isolates in a surveillance study between 2001 and 2004. Henwood and colleagues (2002) identified the polymyxin resistance rate as 44% among 443 *A. baumannii* isolates in a surveillance study involving 25 laboratories.

In a surveillance study conducted in hospitals in ten different geographic regions, it was shown that imipenem resistance increased to 18.2% in 2004 from 4.5% in 2003 (Catchpole et al., 1997). In a study conducted in a 1600-bed tertiary education hospital in Beijing, imipenem resistance was 5% in 1993 to 2003 and increased rapidly to 50% in intensive care units and to 20% in non-intensive care units in 2004.

While imipenem resistance was not found in our hospital in 2005, it increased to 22% in 2006 and to 39% in 2007. These isolates were also MDR Acinetobacter isolates. In our hospital, carbapenem treatment is begun prophylactically and empirically

for patients who are thought to have infection due to Gram negative bacteria. This contributes to the increase in carbapenem resistance among *Acinetobacter* species.

In a study, a clone survived for about 6 years in a hospital (Wang et al., 2007). Prashanth et al. (2005) identified 71 *Acinetobacter* spp. isolated in intensive care units using PFGE, and 59 (83%) were determined to have different patterns.

The reasons for different antibiotic patterns of isolates in the same clones can be explained in several ways. The first is that the genetic event that leads to resistance development in the strains is different for the resistance profile and is different than the rate of genetic change that causes the PFGE profile although they are clonally related. The second reason is that the source of resistance in the strains exhibiting a common resistance profile, may be in the form of plasmid transfer, although they are different clonally. It is generally accepted that mutual resistance and PFGE profile is parallel to the clonal spread in strains showing common resistance (Maslow et al., 1993; Falagas and Kopterides, 2006).

In a similar study, 36 PFGE patterns were present in 66 *A. baumannii* isolates studied and their genotype analysis found twelve clusters. They found the epidemiological relationship rate to be 80.3% (Çetin et al., 2009).

In our hospital, all intensive care wards are on the same floor and hospital staff and ventilator devices are used together. Patient transfers are made frequently between intensive care units. This increases the spread of *A. baumannii* clones in our hospital. In addition, 35 isolates were produced from the cultures of respiratory tract samples and patients were connected to the ventilator device. This is compatible with the literature which described that "the biggest risk factor for *A. baumannii* infections was mechanical ventilation" (Villari et al., 1999).

In our study, the antibiotic resistance profiles of the strains belonging to the same cluster were substantially similar and it was shown that antibiotic profiles in the same cluster could be different. Additionally, strains isolated from the same patient may belong to the same cluster or belong to a different cluster.

Hui Wang et al. (2007) identified 221 imipenem resistant isolates and 15 patterns that contain two or more subtypes in 11 hospitals between 1999 and 2005 with PFGE. During this period, they found clonal extension in 10 hospitals in four cities. Approximate mortality rates were 22.1% and 40.2%, respectively. Twenty-eight cases were classified as colonized. The development time of the infection was 27.8 days. All patients had an underlying disease and 70% used broad-spectrum antibiotics. It was also found that a clone maintained its existence in the hospital for 6 years. However, no patient transfers were found between hospitals with clonal extension.

In our study, the PFGE analysis of 34 (26%) isolates that had imipenem resistance consisted of fifteen patterns and two clusters. The first cluster with imipenem resistance consists of twenty-five isolates. The second cluster consists of two isolates. The first cluster is likely related to the second cluster. The clone survived in our hospital for about nine months. There was clonal invasion in the ten services. The propagation and transmission are probably associated with patient transfer between wards, shared hospital staff, and common use of ventilator devices. Twenty-two of the isolates were produced in the intensive care units and twelve from other wards. Fifteen isolates were found in lower respiratory tract culture samples, nine in blood culture samples, four in urine culture samples, and three isolates in paracentesis and wound culture samples. The mortality rate (47%) for this clone was found to be significantly higher than the average found in the hospital (36%).

During the three years of this study, *A. baumannii*-related hospital infections were encountered, especially in intensive care units. Acinetobacter strains produced as a hospital agent have high antibiotic resistance and resistance increases significantly over the years. The increase in MDR, XDR and PDR rates among the strains is noteworthy. It was determined that the degree of transmission of the strains was quite high among the patients, the clones in the hospital were able to stay in the environment for long periods and the general patient mortality rates were higher than the other studies. The necessity of ensuring more effective protection and control measures in our hospital was shown with the support of molecular typing studies.

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RESEARCH ARTICLE

Antimicrobial Resistance of Enterococcus Species Isolated from Urine Cultures

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Abstract

Objective: Enterococcus species are among the common causes of nosocomial urinary tract infections mainly, along with other infections and have been observed to have increasing rates of resistance against antimicrobial agents in recent years. The aim of this study is to determine the Enterococcus spp. isolated from urine cultures in our hospital and to determine antibiotic resistance rates.

Methods: Enterococcus isolates identification and antibiogram results obtained from urine samples sent to Ordu University Education and Research Hospital Microbiology laboratory from inpatients and outpatients were retrospectively evaluated. The identification and antimicrobial susceptibility tests of the isolates were completed on a VITEK 2 Compact (Biomerieux, Marcy l'Etoile, France) system. Resistance rates against ampicillin, ciprofloxacin, nitrofurantoin, tigecycline, linezolid, teicoplanin and vancomycin were analyzed.

Results: Our study identified 346 Enterococcus strains. These strains were defined as 195 *Enterococcus faecalis* (56%), 127 *Enterococcus faecium* (37%) and 24 other enterococci (7%). The antimicrobials with highest resistance were ciprofloxacin (51%), ampicillin (42%), nitrofurantoin (14%) and tigecycline (1%), in order, with no resistance encountered for linezolid, teicoplanin and vancomycin.

Conclusion: Glycopeptide resistance were not encountered among enterococci isolated from urine cultures in our hospital, with quinolone resistance at the fore. The results of antimicrobial susceptibility tests are important to select appropriate treatments.

Key words: antimicrobial resistance, Enterococcus species, urine culture

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Introduction

With the reduction in the efficacy of antibiotics, hospitals around the world have seen increasing numbers of infections due to drug-resistant bacteria. Effective treatment of these infections is more difficult, which causes morbidity and mortality in the patient and increasing health care costs. The most common antimicrobial resistant hospital pathogens are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acetinobacter baumanni, Pseudomonas aeruginosa* and Enterobacter species and are called ESKAPE (Rice, 2008). In humans the enterococcus species causing most infections are *Enterococcus faecalis* (80-90%) and one of the two gram positive

ESKAPE pathogens of *Enterococcus faecium* (5-10%). These bacteria are among the common causes of nosocomial urinary tract infections mainly, along with other infections (Aykut Arca et al., 2009). Enterococcus species carry a range of intrinsic and acquired resistance genes and may transfer these genes to other bacteria (van Harten et al., 2017). Enterococci may be resistant to commonly used antibiotics including ampicillin and vancomycin and currently resistance has begun to be determined against last-chance antibiotics like daptomycin and linezolid (Gonzales et al., 2001; Long et al., 2005; van Harten et al. 2017).

Due to increasing resistance against commonly used antibiotics, note should be taken of culture results for antimicrobial treatment of Enterococcus infections and the regional resistance phenotypes should be considered when deciding on empirical antibiotic treatment until culture results are obtained. This study aimed to identify the resistance status against a variety of antimicrobials of Enterococcus isolates from urine samples in Ordu University Education and Research Hospital microbiology laboratory.

Methods

From January 2014 to June 2018, enterococci isolates identifications and antibiogram results obtained from urine samples sent to Ordu University Education and Research Hospital microbiology laboratory from inpatients and outpatients were retrospectively evaluated.

Midflow urine samples taken under appropriate conditions from patients with preliminary diagnosis of urinary tract infections were inoculated on the surface of 5% sheep's blood agar (Salubris, Istanbul, Turkey) using standard loops taking 1 µl urine. The media plates were incubated in an aerobic environment for 18-24 hours at 37 °C and cultures with single type proliferation and colony account 10^5 CFU/ml were taken for investigation. The identification and antimicrobial susceptibility tests of the obtained isolates were completed in line with Clinical and Laboratory Standards Institute (CLSI) until January 2017 and then in line with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations on a VITEK 2 Compact (Biomerieux, Marcy l'Etoile, France) system. Resistance rates against ampicillin, ciprofloxacin, nitrofurantoin, tigecycline, linezolid, teicoplanin and vancomycin were analyzed. Strains with intermediate resistance were accepted as resistant. Only one strain from each patient was included in the study. For quality control, the *E. faecalis* ATCC 29212 standard strain was used.

Results

Our study identified 346 enterococci strains. These strains were identified as 195 *E. faecalis* (56%), 127 *E. faecium* (37%) and 24 other enterococci (7%) (9 *E. gallinarum*, 7 *E. casseliflavus*, 3 *E. avium*, 3 *E. raffinosus*, 2 *E. durans*).

The *E. faecalis* strains were most common among outpatients, while *E. faecium* strains were isolated mainly from the intensive care units, with the clinical distribution of these strains given in Table 1.

Table 1. Distribution of E.faecalis and E.faecium strains according to clinics

Clinics	*E.faecalis*	*E.faecium*
Polyclinics	149	42
Intensive Care Units	20	51
Internal medicine Services	17	22
Surgical Services	6	7
Pediatric Services	3	5
Total	195	127

The antimicrobial resistance rates for the isolated *E. faecalis* and *E. faecium* strains are shown in Table 2.

Table 2: Resistance rates of *E.faecalis* and *E.faecium* strains

	E.faecalis			*E.faecium*			Total*		
	Number of strains	Resistant		Number of strains	Resistant		Number of strains	Resistant	
Antibiotic	n	n	%	n	n	%	n	n	%
Ampicillin	170	-	0	124	124	100	294	124	42
Ciprofloxacin	184	56	30	114	95	83	298	151	51
Tigecycline	182	-	0	103	3	3	285	3	1
Nitrofurantoin	36	3	8	15	4	27	51	7	14
Linezolid	192	-	0	122	-	0	314	-	0
Teicoplanin	184	-	0	124	-	0	308	-	0
Vancomycin	191	-	0	121	-	0	312	-	0

n :Number, %:Percent, * Sum of *E.faecalis* and *E.faecium*

Discussion

Enterococci are bacteria forming the normal flora in the gastrointestinal system, vagina and urethra of humans but may also cause a variety of infections. They may survive for long periods on inorganic material like stethoscopes, door handles, and beds in the hospital environment. As a result, enterococci may cause epidemics as a hospital infection vector carried on both inorganic materials and from patient to patient by health personnel (Butler, 2006). In recent years, the observation of an increase in vancomycin resistant strains in isolation and the variation in antibiotic susceptibility according to species have led to the requirement for species level identification. Studies about the topic have shown that in urine samples generally *E. faecalis* isolation rates are higher compared to *E. faecium* (Yuksel Ergin et al., 2013; Etiz et al., 2014; Yenisehirli et al., 2016). In our study the results were similar with 56% *E. faecalis* and 37% *E. faecium* identification rates.

Compared to other Enterococcus species, *E. faecalis* is found at higher rates in feces. Epidemiologic studies in recent years have shown that the presence of these bacteria in normal intestinal flora is a basic risk factor for the spread of enterococci from patient to patient and even between hospitals (Butler, 2006). Some studies have found higher rates of *E. faecium* strains among enterococci from hospital isolates compared to *E. faecalis* (Aykut Arca et al., 2009). In our study, *E. faecalis* was isolated more from outpatients, while *E. faecium* was isolated more from inpatients. This situation may be linked to the ability of *E. faecium*, found at high rates in intestinal microbiome, to spread between patients.

Treatment of Enterococcus infections has become complicated since the emergence of strains with high levels of resistance to nearly all antibiotics used in clinical practice, especially aminoglycosides, β-lactams and glycopeptides. Enterococci are the most common vector for infections of the urinary tract. Enterococci are known to be more resistant to antimicrobials affecting inhibition of cell wall synthesis compared to other streptococci and the use of penicillin or ampicillin is recommended for susceptibility tests (Murray, 1997). Studies in Turkey have noted that enterococci are increasingly resistant to beta-lactam antibiotics and a variety of studies have reported ampicillin resistance from 16% to 84% (Baykan, 2001; Agus et al., 2006; Aktepe et al., 2011; Kalayci et al., 2011; Yuksel Ergin et al., 2013; Etiz et al., 2014). In our study, rates of 42% were between these two values.

Quinolones are found to have limited efficacy against enterococci. As a result, though they are effective in vitro, their use for treatment of infections caused by these bacteria is limited (Gordon et al., 1992). Ciprofloxacin is approved for use for both uncomplicated and complicated urinary tract infections, including cystitis, pyelonephritis, and chronic bacterial prostatitis (Andriole, 2005). Among enterococci infections, they are among alternative treatment choices only for urinary tract infections (Gordon et al., 1992). A variety of studies have identified ciprofloxacin resistance from 48% to 87% (Baykan, 2001; Yavuz et al., 2006; Aktepe et al., 2011; Kalayci et al., 2011; Yuksel Ergin et al., 2013; Etiz et al., 2014). In our study, ciprofloxacin resistance was close to the lower limit at 51%.

Tigecycline is an antibiotic derived from the first member of the glycylcyclines of minocycline and a promising new antibiotic of last resort, active against many bacteria including *Enterococcus* spp. (Tunger, 2012). Studies by Karaoglan et al. and

Aktepe et al. did not report resistance for enterococci against tigecycline, while Etiz et al. reported 0.3% resistance (Karaoglan et al., 2008; Aktepe et al., 2011; Etiz et al., 2014). In our study, Enterococcus spp. resistance to tigecycline was 1%, and all resistant isolates were observed to be *E. faecium*. Nitrofurantoin may be used for uncomplicated urinary tract infections (Tunger, 2012). The use of nitrofurantoin with appropriate indications will reduce the use of new antimicrobials and as a result their risk of resistance developing. In our study, 14% rates of resistance against nitrofurantoin were encountered and this resistance was observed more for the *E. faecium* isolates.

Glycopeptides are still known as the most effective antibiotics against enterococci, with increased rates of vancomycin and teicoplanin resistant strains reported (Agus et al., 2006; Yuksel Ergin et al., 2013; Etiz et al., 2014). In this study, vancomycin and teicoplanin resistance was not encountered among *E. faecalis* and *E. faecium* isolates.

Linezolid is an oxazolidinone group antibiotic effective against many gram positive bacteria including vancomycin-resistant enterococci (VRE) (Dilek et al., 2007). It is recommended for treatment of infections caused by VRE (Contreras et al., 2019). In our study, similar to many studies performed to date, linezolid resistance was not encountered among enterococci (Dilek et al., 2007; Aktepe et al., 2011; Yuksel Ergin et al., 2013). Contrary to this, Etiz et al. in a 2014 study found 5% resistance to linezolid and reported the necessity to use this agent with appropriate indications and sufficient doses by performing antibiotic susceptibility tests, as for other antimicrobials to prevent development of resistance (Etiz et al., 2014).

Conclusion

Enterococci have become microorganisms threatening health and causing problems with treatment today. They are resistant to many drugs and the increase in this resistance will continue to increase problems like causing difficult clinical infections. As a result, as for all infection agents, rational antibiotic use is necessary for treatment of infections linked to Enterococcus species.

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RESEARCH ARTICLE

The Relationship Between Body Mass Index and Lower Urinary Tract Symptoms in Men

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Abstract

Objective: The studies evaluating, association between obesity and lower urinary tract symptoms (LUTS) are limited. Our study's objective was to determine the correlation between obesity and LUTS in men.

Methods: Information of 639 patients who were aged between 37 and 92, had not been treated for BPH before, had moderate or severe LUTS, had International Prostate Symptom Score (IPSS) ≥8 and had prostate-specific antigen (PSA) value less than 4 ng/ml was evaluated retrospectively. Measured Body Mass Index (BMI) was classified in accordance with World Health Organization (WHO).

Results: A statistically significant difference was present between BMI groups with respect to post-void residual urine volume (PVR) ($p=0.017$). PVR level of the obesity group's PVR level was found to be significantly lower when compared to the normal group ($p<0.05$). In patients with diabetes mellitus (DM), only PVR parameter among LUTS was found to differ significantly from BMI groups ($p=0.037$). In patients with DM, the mean of PVR of obese patients was detected to be significantly lower when compared to the mean of normal patients ($p<0.05$). In patients with cardiovascular disease (CD), only Qmax and Qave parameters were found to differ significantly from BMI groups. ($p=0.001$ and $p<0.001$, respectively). In patients with CD, the mean Qmax of obese patients was significantly higher than the average of normal-weight patients ($p<0.05$).

Conclusion: Although there is no association between obesity and LUTS except PVR, we think that the risk of obesity associated with DM and CD would significantly increase the risk of LUTS.

Key words: Lower urinary tract symptoms, Body mass index, Obesity, Urology

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Introduction

LUTS are one of the most important health problems frequently seen in adult men and affecting quality of life negatively. It is known that older men are suffering from at least one of LUTS. The level of discomfort may vary from "too mild to negligible" to "severe discomfort". The change and development of information about LUTS over time has led to some problems in the definition and terminology of LUTS, and a number of studies were published by Standardization Subcommittee of "International Continence Association (ICS)" to eliminate the terminology problems.

"According to ICS 2002 standardization report", LUTS classification is as follows (Abrams et al., 2002);

I. Symptoms of filling phase (urinary incontinence, nocturia, increase in the frequency of daytime urination, urinary compression)

II. Symptoms of voiding phase (terminal drip, delayed urine initiation, forked-scattered urine flow, discontinuous urine flow, poor urine flow, forced urine discharge)

III. Post-voiding symptoms (post-void drip, feeling of incomplete urinary excretion).

The prevalence of these symptoms detected in both males and females has been reported to be approximately 19.2% of males and 13.7% of females, although it has changed from country to country in the population screenings (Abrams et al., 2002). However, the high prevalence of disease and drug use accompanied by aging contributes to the increase in the prevalence of LUTS during the old age (Takeda et al., 2003).

Obesity has been defined as "excessive or abnormal increase of body fat content to disrupt health" by WHO. Obesity is a condition which the body fat ratio is 25% for men and 35% for women (Yuksel, 2016). The study of "Turkey Obesity Profile" performed on 13878 individuals over the age of 20 in 6 provinces (Gaziantep, Konya, Denizli, Kastamonu, Kirklareli and Istanbul) by Turkish Association for the Study of Obesity (TASO) between 2000-2005 years. It was found that 30.9% of the individuals had BMI> 25 kg/m^2. Obesity causes damage to the urethral mucosa, decrease in the amount of collagen and loss of urethra elasticity. However, obesity is an important predisposing factor for urinary incontinence and increases the severity of the condition. Chronic strains caused by pelvic muscles and nerves affected by excessive weight trigger stress, stretching and weakening. Body mass index showed significantly higher values in stress urinary incontinence (Bilge and Beji, 2016).

Obesity and LUTS are frequent in elder men and might significantly influence their quality of life. In the cohort studies, it was reported that there was a positive association between LUTS and anthropometric obesity measurements (Giovannucci et al., 1994; Gann et al., 1995). In addition, Hammarsten et al. suggested in a clinical-based study that an expanded prostate may be the consequence of prostate development, impaired insulin management and other sides of the metabolic syndrome according to the results of 158 patients more frequently identified in men with constituents of a metabolic syndrome like hypertension needing therapy, insulin-dependent diabetes mellitus (IDDM), low HDL-cholesterol levels, obesity and high fasting insulin levels (Hammarsten et al., 1998).

There are limited number of studies investigating the association between obesity-associated diseases and LUTS in the literature. Moreover, the number of studies emphasizing which LUTS are affected is much less. It is observed in the literature that there are different results between obesity and accompanying diseases, and LUTS development. The aim of our study is to determine the association between obesity and LUTS in men.

Methods

This study was a cross-sectional study conducted from January 2015 to June 2018. Data of 639 patients who admitted to Urology Clinic of Medical Faculty Hospital in Ordu University, were aged between 37 and 92, had not been treated for BPH before, had moderate or severe LUTS, had IPSS ≥8 and had PSA value less than 4 ng/ml was evaluated retrospectively. This planned research complies to the Declaration of Helsinki rules including patient's rights and ethical guidelines and were confirmed by Local Ethics Committee of Ordu University (Date: Dec 2018, Number: 2018/265).

639 male patients between the ages of 37 and 92 were evaluated. BMI, which is calculated by dividing the weight in kilograms by the square of the height in meter, is classified according to WHO: underweight (<18.5 kg/m2), normal weight (18.5-24.9 kg/m2), overweight (25 to 29.9 kg/m2) and obese (> 30 kg/m2), however there wasn't any underweight patient in this study.

The patients in the study were split into two groups as with or without hypertension (HT), diabetes mellitus (DM), cardiovascular disease (CD) and drug use (DU). Ages, IPSS values, prostate volumes, urinary flow rates (Qmax, Qaverage), PVR and PSA data of patients in each group were evaluated.

In patients with HT, DM, CD and positive DU, LUTS variables were examined in terms of BMI groups.

Statistical Analysis

For the continuous variables, Kolmogorov-Smirnov test for normal distribution control of the data and Levene test for the homogeneity of the group variances were performed. Independent samples t-test was utilized to compare two groups.

One-way ANOVA and following Tukey post-hoc test were used to compare the averages of more than two independent groups. Pearson correlation coefficients were calculated to evaluate the relationships among the continuous variables. Pearson's chi-square test (χ^2) was used to determine the relationship between the categorical variables. The statistical significance level was accepted as 5% for calculations and interpretations. All data analyses were conducted using the SPSS (Demo version 25.0, IBM Corp., Armonk, NY, USA) statistical software.

Results

According to one-way ANOVA, no statistically significant difference was observed between BMI groups with respect to the mean age (p=0.091). When the prevalences were evaluated, the prevalence of normal weight, overweight and obese groups were 19.4% (n = 124), 49.5% (n = 316) and 31.1% (n = 199), respectively (Table 1).

Table 1. Comparison of the prevalences and ages of the patients among BMI groups

	n (%)	Mean±SD	Min.-Max.	p
Normal weight	124 (19.4)	63.00±10.64	44.0-92.0	0.091^{NS}
Overweight	316 (49.5)	62.00±9.21	39.0-85.0	
Obese	199 (31.1)	61.00±9.12	37.0-84.0	
Total	639 (100.0)	62.00±9.52	37.0-92.0	

^{NS}; p>0.05

One-way ANOVA test was performed to detect statistically significant difference among BMI subgroups in terms of LUTS. No statistically significant difference was detected among BMI groups for all variables, except PVR (p>0.05). A statistically significant difference was observed among BMI groups with respect to PVR (p=0.017). According to Tukey test, no significant difference was found between the normal group and overweight group (p>0.05); however, PVR level of the obesity group was detected to be significantly lower than normal group (p<0.05) (Table 2).

IPSS was divided into mild (0-7), moderate (8-19) and severe (20-35) symptoms. Chi-square test was performed to analyze the frequency distribution of IPSS groups in BMI groups. It was observed that the frequency distribution of IPSS groups did not change in terms of BMI groups (p=0.730) (Table 3).

Table 2. Descriptive statistics and comparison results for LUTS among BMI groups

	Normal weight (n=124)	Overweight (n=316)	Obese (n=199)	p
	Mean±SD	Mean±SD	Mean±SD	
IPSS-obstructive total	6.28±5.94	5.63±4.84	5.44±4.96	0.333^{NS}
IPSS-irritative total	6.09±3.16	6.27±3.64	6.26±3.31	0.879 ^{NS}
IPSS total	11.94±6.74	11.74±6.85	11.60±7.18	0.913 ^{NS}
Prostate volume	34.09±16.15	37.02±18.73	38.10±25.57	0.822 ^{NS}
Qmax	14.38±6.54	15.88±7.53	16.66±9.89	0.229 ^{NS}
Qave	6.27±3.68	7.09±4.39	6.87±3.07	0.051 ^{NS}
PVR	43.47±97.55 ^{a}	33.94±51.41 ^{ab}	23.54±38.95 ^{b}	**0.017***
PSA	2.01±2.05	2.29±4.23	2.37±4.37	0.333 ^{NS}

^{NS}; p>0.05, *; p<0.05, According to Tukey test, means that do not share a common letter are significantly different (p<0.05)

Table 3. Frequency distribution of IPSS total score for the patients in BMI groups

	Normal weight	Overweight	Obese	p
Mild	36 (5.6%)	108 (16.9%)	69 (10.8%)	0.730^{NS}
Normal	70 (11.0%)	160 (25.0%)	97 (15.2%)	
Severe	18 (2.8%)	48 (7.5%)	33 (5.2%)	

^{NS}; p>0.05

According to One-way ANOVA results, there was no statistically significant difference among BMI groups in terms of LUTS parameters in HT and DU positive patients (p>0.05). In patients with positive DM, only PVR parameter showed a significant change among BMI groups (p=0.037), yet there was no significant difference in the remaining parameters (p>0.05). In patients with DM, the mean PVR of obese patients was significantly decreased when compared to the average of normal patients (p<0.05). In the patients with positive CD, only Qmax and Qave parameters showed a significant change among BMI groups (p=0.001 and p<0.001, respectively). In patients having CD, the mean Qmax of obese patients was significantly increased when compared to that of normal-weight patients (p<0.05). The mean Qmax of overweight patients was not significantly different from normal and obese patients (p>0.05). In patients with CD, there was no significant difference between overweight and obese patients (p>0.05) while they had significantly higher Qave average than normal weight patients (p<0.05) (Table 4).

Table 4. Descriptive statistics and comparison results of the patients with HT, DM, CD and DU in BMI groups

		HT +			DM +			CD +			DU +		
		n	Mean±SD	p	n	Mean±SD	p	n	Mean±SD	p	n	Mean±SD	p
IPSS-obs.	N	33	6.73±5.87		18	5.72±4.20		15	11.27±11.16^{A}		48	7.29±7.38	
	OW	113	5.48±4.71	0.406^{NS}	62	5.10±4.14	0.768^{NS}	60	5.43±4.61^{B}	0.004**	115	6.87±4.78	0.771^{NS}
	O	92	6.11±5.22		63	5.67±5.42		34	5.35±5.75^{B}		75	6.28±4.60	
IPSS-irrit.	N	33	6.18±3.14		18	6.83±3.30		15	6.47±4.02		238	6.77±5.34	
	OW	113	6.51±3.31	0.873^{NS}	62	6.84±3.35	0.981^{NS}	60	6.80±3.31	0.867^{NS}	48	6.23±3.23	0.909^{NS}
	O	92	6.48±3.26		63	6.73±3.13		34	7.03±3.40		115	6.46±3.32	
IPSS Total	N	33	12.85±7.83		18	12.44±6.16		15	15.27±9.45		75	6.32±3.35	
	OW	113	11.97±6.66	0.751^{NS}	62	11.94±6.34	0.921^{NS}	60	12.25±6.61	0.337^{NS}	48	12.75±7.05	0.732^{NS}
	O	92	12.59±7.47		63	12.40±7.57		34	12.18±7.71		115	13.33±6.71	
Prostate volume	N	33	35.76±15.72		18	38.78±17.64		15	34.27±14.86		75	12.57±6.85	
	OW	113	35.24±17.10	0.211^{NS}	62	32.24±13.23	0.164^{NS}	60	35.85±15.33	0.758^{NS}	48	37.77±17.90	0.684^{NS}
	O	92	40.85±31.04		63	39.06±27.08		34	38.62±30.76		115	41.10±21.28	
Q_{max}	N	33	14.01±4.58		18	14.53±5.97		15	9.93±4.78^{B}		75	40.52±26.42	
	OW	113	15.18±6.62	0.301^{NS}	62	16.00±7.08	0.439^{NS}	60	14.03±6.17^{AB}	0.001**	48	13.59±5.17	0.687^{NS}
	O	92	16.09±7.64		63	14.58±6.22		34	17.12±7.06^{A}		115	13.61±6.77	
Q_{ave}	N	33	5.95±2.42		18	5.88±2.89		15	3.87±2.03^{B}		75	14.70±12.15	
	OW	113	6.57±2.97	0.535^{NS}	62	7.98±7.05	0.093^{NS}	60	6.12±2.30^{A}	p<0.001***	48	5.41±2.55	0.477^{NS}
	O	92	6.56±2.96		63	6.16±2.79		34	7.28±2.97^{A}		115	6.16±5.51	
PVR	N	33	26.67±33.43		18	44.17±41.92^{A}		15	38.47±26.82		75	5.57±2.30	
	OW	113	30.24±43.06	0.170^{NS}	62	31.85±41.25^{AB}	0.037*	60	29.38±43.44	0.081^{NS}	47	38.28±53.86	0.271^{NS}
	O	92	20.26±31.55		63	20.24±31.55^{B}		34	15.79±20.12		115	42.44±54.32	
PSA	N	33	1.99±1.80		18	2.41±2.49		15	1.76±1.32		75	29.87±48.18	
	OW	111	1.88±2.28	0.136^{NS}	61	1.51±1.22	0.177^{NS}	60	1.92±1.66	0.383^{NS}	48	2.37±2.27	0.917^{NS}
	O	90	3.02±6.07		63	1.75±2.00		32	2.70±4.49		114	2.57±2.86	

N; Normal weight, OW; Overweight; O; Obese, Hypertension; HT, DM; Diabetes mellitus, CD; Cardiovascular disease, DU; Drug use (Prostate), NS; p>0;05, *; p<0.05, **; p<0.01, ***; p<0.001, According to Tukey test, means that do not share a common letter are significantly different (p<0.05)

Pearson correlation coefficients were calculated to investigate the correlations between LUTS and BMI. The correlation coefficients given in Table 5 represent that LUTS variables are very weakly correlated with BMI and most of them have no statistically significant association (p>0.05). The correlation coefficients of some variables that are statistically significantly correlated with BMI are quite small (PVR, r=-0.105; p=0.008). These associations have emerged from the high sample size and are too weak to be considered in practice (Table 5).

Table 5. Correlation coefficients between BMI and LUTS (n=639)

	r	p
Age	-0.086	**0.030***
IPSS-obstructive total	-0.075	0.058
IPSS-irritative total	0.075	0.058
IPSS total	0.002	0.956
Prostate volume	0.057	0.149
Qmax	0.093	**0.019***
Qave	0.036	0.358
PVR	-0.105	**0.008****
PSA	-0.100	**0.012***

r; Pearson correlation coefficient, *; p<0.05, **; p<0.01

Discussion

Our study was realized to determine the association between BMI and LUTS in men. According to the results of our study, there was a statistically significant difference among BMI groups in terms of PVR (p=0.017). PVR level of the obesity group was found to be significantly decreased when compared to the normal group (p<0.05). Although many factors have been charged until now, the real causes of LUTS are not known precisely and LUTS is considered as a multifactorial event. The two risk factors taking role in the etiology of BPH are aging and the presence of functional testes (androgens). In recent years, the importance of metabolic syndrome, DM, obesity, smoking and lifestyle, heredity and genetic factors are stated as other etiological factors (Konwar et al., 2008; Parsons, 2010; Cetinkaya and Oztekin, 2011).

In many studies, a significant relationship was detected between obesity and LUTS (Altunkaynak and Ozbek, 2006). In a study conducted by Bart et al. in France, the prevalence of LUTS was found to be 44% (Bart et al., 2008). In addition, severe weight loss in morbidly obese patients with LUTS significantly was observed to reduce intravesical pressure. This is a step that emphasizes the importance of obesity-induced intra-abdominal pressure in the development of stress LUTS (Yalcın, 2009). In a case-control study performed on African-American men, Sarma et al. expressed that BMI was directly related to prostate volume (Sarma et al., 2002). No relation was found between BMI and LUTS in the studies realized in China and Greece (Signorello et al., 1999; Dahle et al., 2002). While obesity reduces free and total testosterone and serum globulin binding protein levels, it increases estrogen levels as well as free and total estradiol concentrations (Pasquali et al., 1991). Higher estrogen levels can affect prostate cell growth in the environment of low testosterone levels due to age-related and obesity. In particular, it increases the rate of estrogen/androgen in abdominal obesity and may increase the sympathetic nerve activity, which is known to affect both the development of BPH and the severity of LUTS (Giovannucci et al., 1994; Barqawi et al., 2005). In our study, no significant relationship was observed between prostate volume and obesity. However, a statistically significant difference was detected between obesity and PVR, which is one of LUTS (p=0.017).

Serum PSA levels can be affected by many factors such as age, prostate volume, and obesity. Nowadays, it has been reported in many studies that there has been a negative correlation between PSA levels and BMI (Barqawi et al., 2005; Kristal et al., 2006). However, Ochiai et al. expressed that anthropometric parameters were not directly correlated with PSA levels and BMI (Ochiai et al., 2005). Although obesity is an important anthropometric factor in the metabolic syndrome, there are complex associations among individual anthropometric parameters, partly due to their association with obesity. Crystal et al. declared that PSA levels were 0.2-0.4 ng/ml lower in obese patients compared to normal weight (Kristal et al., 2006). However, they informed that the magnitude of the association between serum PSA levels and the presence of each metabolic component could not be precisely determined. In our study, no significant relationship was detected between serum PSA level and BMI.

Obesity, which is a crucial risk factor of metabolic syndrome, causes hypertension, insulin resistance, hypertriglyceridemia and low HDL cholesterol. Metabolic syndrome of which prevalence has increased progressively in the world is seen in 28% of men over the age of 30 in Turkey (Onat et al., 2002). While LUTS in elderly men have made think direct benign prostate hyperplasia in previous years, later studies have proved that chronic illnesses such as diabetes, heart disease, and metabolic syndrome components, lifestyle factors such as alcohol, smoking and physical activity are effective in the development of LUTS (Chapple and Roehrborn, 2006; Fitzgerald et al., 2007).

In our study, it was found that only PVR parameter in LUTS was significantly different among BMI groups in patients with DM (p=0.037), however there was no significant difference in the remaining parameters (p>0.05). In patients with DM, the mean PVR of obese patients was significantly lower than the average of normal patients (p<0.05). In patients with CD, only Qmax and Qave parameters showed a significant difference among BMI groups (p=0.001 and p<0.001, respectively). In patients with CD, the mean Qmax of obese patients was significantly increased when compared to that of normal-weight patients (p<0.05).

Conclusion

All in all, although there is no relationship between obesity and LUTS except PVR, we believe that obesity associated diabetes mellitus and cardiovascular diseases will increase the risk of LUTS development significantly. We think that our study will provide a significant contribution to the

literature in terms of the high number of patients, the high number of parameters evaluated and the different results. In the future, more studies are needed to determine the etiology of LUTS development and contribute to the prevention of LUTS development.

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RESEARCH ARTICLE

The Clinical Predictive Value of the Neutrophil to Lymphocyte Ratio as a Biomarker in Lumbar Disc Herniation

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Abstract

Objective: Low back pain is a frequently seen problem in the society and causes loss of labor. Although etiology of lumbar disc herniation is multi-factorial it is known that intervertebral disc degeneration is an important determinant for herniation. In recent studies, inflammatory mediators and inflamation itself has an efficient role in the degeneration process. We aimed to investigate the association between the neutrophil to lymphocyte ratio level as an inflammatory biomarker in patients with lumbar disc herniation.

Methods: 394 patients between the age of 18-80 applying to our center because of low back pain complaint and having lumbar MR were included in the study. The patients were divided into two groups as having lumbar disc hernia and not having based on the lumbar MR result. Blood samples were taken from all patients during application and neutrophil lymphocyte rates were calculated.

Results. Average age of group with lumbar disc hernia was 46 and 55 of these cases were female and 45 of them were male. N/L rate was measured as 3.81+/-1.85 (p=0.001) in the group having lumbar disc hernia and significant difference compared to the control group was noted. It was found out that lifting weight (β=0.121 95% Cl (0.052-0.281), P<0.001),BMI (β=0.226, 95% Cl (0.080-0.640) P=0.005), DM (β=0.268 95% Cl (0.074-0.969), P=0.045), smoking (β=3.226 95% Cl (1,343-7.749), P<0.009), educational background (β=5.268 95% Cl (1.941-9.796), P=0.001) and NLR (β=1.302 95% Cl (1.013-1.673), P=0.039) were the independent predictors in the presence of lumbar disc herniation.

Conclusion: NLR may be used as a simple and reliable premise independent predictor of lumbar disc herniation in patients with low back pain.

Key words: Neutrophil to Lymphocyte Ratio, Back Pain, Lumbar Disc Herniation

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Introduction

Low back pain is a frequently seen problem in the society and causes loss of labor and significant burden on both the healthcare system and the economy. Its life-long prevalence is about 80% and rate of application to the hospital in adult population annually is 15% (Sarı et al.,2015).Lumbar disc herniation frequently occurs as a result of tear of annulus fibril not resisting to torsional forces in the end of degeneration and nucleus becomes herniated. (Boden SD et al., 1990).Although etiology of lumbar disc herniation is multi-factoral it is known that it is determinant for the etiology of

intervertebral disc degeneration and herniation (Andersson GB et al., 1999; Shen FH et al., 2006).In recent studies, it has been shown that in addition to mechanical effects on lumbar disc degeneration and herniation, extended inflammation and inflammatory cytokine (such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β) has had efficient role for lumbar disc degeneration and herniation (Karin W et al., 2013; Wei Li et al., 2014).

Previously, Neutrophil-to-lymphocyte ratio (NLR), known to be a systemic inflammatory marker, has been shown to play a role in the progression of many diseases and it was shown that it has a prognostic value in many acute and chronic diseases (Kaya A et al., 2014). NLR is measured by proportioning 2 inflammatory markers (neutrophil and lymphocyte), it has a stronger predictive value (Kaya A et al., 2014).

Although it was shown that predictive value of increased NLR in many diseases, the relationship between NLR and lumbar disc herniation has not been investigated comprehensively. Therefore, in this particular study, we aimed to investigate the association between the NLR level and lumbar disc herniation in patients with low back pain.

Method:

Study population

This was a case-control study whose subjects were sampled from neurosurgery department of our hospital. 394 patients (between the age of 18-80) applying to our center for low back pain complaint between the dates of September 2015 and January 2018 were included in the study.

Exclusion criteria were previous lumbar surgery (n: 42), epidural corticosteroid injection within the last 6 months (89), known inflammatory condition (n:51) (e.g. vasculitis, seronegative arthritis, rheumatoidarthritis, systemic lupuserythematosus, gout, osteomyelitis, discitis,buruselloz, pot disease), history of cancer (n:11) and pregnancy (n:2) respectively. Finally 199 patients were included in the study. Patients were divided into two groups as patients having discopathy and having normal lumbar MR results based on the MRI results. Lumbar MRI of selected patients examined by a neurosurgeon and a neuroradiolog.

Written informed consent was received from all patients, and the study protocol was approved by the hospital's local ethics committee Ordu University (70/04.05.2018) in accordance with the Helsinki Declaration and Good Clinical Practice Guidelines.

Definitions

Discopathy was defined;

1) Protrusion: <%25 of disc circumferences, base wider than herniation

2) Extrusion: Complete annular tear with passage of nuclear material beyond disc annulus, base narrower than herniation dome, disc material may extend above or below endplates or adjacent intervertebrae

3) Sequestered: Disc material that has no continuity with the parent disc and is displaced away from the site of extrusion. It corresponds to a subtype of disc extrusion (Spengler DM et al., 1990).

DM: When use of anti-diabetic medicine was in question or post-prandial blood sugar was above 200 mg/dl in any time or fasting plasma glycose was minimum 126 mg/dl, diagnosis of diabetes was established.

Laboratory Data

Blood samples were taken from patients that were admitted to our Neurosurgery Department with the complaint of low back pain for the whole blood count and the biochemistry parameter measurement. Blood samples were collected from the antecubital vein by and a traumatic puncture and were sent to the laboratory for analysis within 1 hour after collection. Hemoglobin, total WBC, neutrophils, lymphocytes, and monocytes were determined by an automated blood cell counter calledCoulter LH 780 Hematology Analyzer (BeckmanCoulterIrelandIncMervue, Galway, Ireland). Biochemical parameters were measured during the Abbott Architect C16000 autoanalyzer (Abbottlaboratories, Abbott park, IL, USA).

Statistical Analysis

The data analysis was conducted using SPSS (version 20.0, SPSS Inc., Chicago, IL, USA) and MedCal statistical software (trial version 12.7.8, Mariakerke, Belgium). Continuous variables data are expressed as the mean ± standard deviation. Categorical variables were compared using Chi-square or Fisher's exact tests and summarized as percentages. The Kolmogorov–Smirnov test was used to evaluate the distribution of the continuous variables. To predict lumbar disc herniation, gender, age, smoking, occupational motor vehicle driving, trauma history, heavy lifting, diabetes mellitus (DM), Neutrophil-lymphocyte ratio (N/L), and education status and body mass index (BMI) included in the univariate analysis. The parameters with $p < 0.05$ were included in the multiple logistic

analyses. Receiver operating characteristic (ROC) curves were used to predict the future incidence of CIN.

Results

Average age of the group with lumbar disc herniation was 46 and 55 of cases in this group were females and 45 of them were males. Rates of motor vehicle driving was found as 30%, trauma history was 31%, heavy lifting was 74%, smoking was 56% and DM rate was found as 27%. N/L rate was measured as 3.81+/-1.85 (p=0.001) in the group having lumbar disc herniation and a significant difference compared to the control group as noted. Educational level in this group was 76% primary-secondary school and BMI≥30 (obese) 33% and a significant difference was found compared to the control group (p<0.001). Clinical and laboratory characteristics of the groups are presented in Table 1

In multiple regression analysis, it was found out that heavy lifting (β=0.121 95% Cl (0.052-0.281), P<0.001),BMI (β=0.226, 95% Cl (0.080-0.640) P=0.005), DM (β=0.268 95% Cl (0.074-0.969), P=0.045),smoking (β=3.226 %95 Cl (1,343-7.749), P<0.009), educational background (β=5.268 95% Cl (1.941-9.796), P=0.001) and NLR (β=1.302 95% Cl (1.013-1.673), P=0.039) were the independent predictors in the presence of lumbar disc hernia (Table 2 and Figure 1).

Table 1. Comparison of Disc herniation Group And Control Group

		Lumbar mr is normal	Lumbar disc hernia	P value
Sex, male (n, %)		21(21.9)	45 (45)	0.001
Age (years)		42.99±13.77	46.89 ±9.91	0.025
Smoking		24 (24,24)	39(39)	0.019
Motor vehicle driving (n, %)		15 (15.6)	30 (30)	0.017
Trauma history (n, %)		14 (14.6)	31 (32.3)	0.004
Heavy lifting (n, %)		24 (24,24)	74 (74)	<0.001
Dm (n, %)		6 (6.3)	27 (27)	<0.001
N/L rate		2.05±1.02	3.81±1.85	0.001
Educational level	Primary-secondary school	40 (41.7)	76 (76)	<0.001
	High school and above	56 (58.3)	24 (24)	<0.001
BMI ≥30 (n, %)		9(9.4)	33 (33)	<0.001

Table 2. Evaluation of Independent Predictors of Disc herniation

	Univariate analysis		**Multivarite analysis**		
	QR	**P value**	**beta**	**95% Cl**	**P value**
Sex (% male)	0.342	0.001			
Age (years)	1.042	0.025			
Motor vehicle driving	0.432	0.017			
Trauma history	0.452	0.004			
Heavy lifting	0.117	<0.001	0.121	0.052-0.281	<0.001
Dm	5.548	<0.001	0.268	0.074-0.969	0.045
N/L rate	1.419	0.001	1.302	1.013-1.673	0.039
Education	0.226	<0.001	5.268	1.941-9.796	0.001
BMI	4.761	<0.001	0.226	0.080—0.640	0.005
Smoking	3.818	<0.001	3.226	1.343-7.749	0.009

Discussion:

In our study, we showed that NLR predict the lumbar disc herniation. It was found as an independent predictor of lumbar disc herniation.

Lumbar disc herniation is one of the pathologies occurring secondary to the degeneration frequently. This mechanism is in the form of tear of annulus fibril not resisting to torsional forces and deactivation of end plate-nucleus- annulus complex functioning as a closed-system accordingly. By the effect of incoming compressive force, nucleus become herniated from torn annulus region and end-plate microfractures occur the contact of nucleus with spongiform bone and blood members in vertebra corpus results in immune reaction and trigger inflammatory process. In the end of degenerative processes, nucleus migrates towards the canal and causes stenosis or root pressure. This pressure results in radicular pain and clinical symptom (Parker SL et al., 2004; En'Wezoh DC et al., 2016).

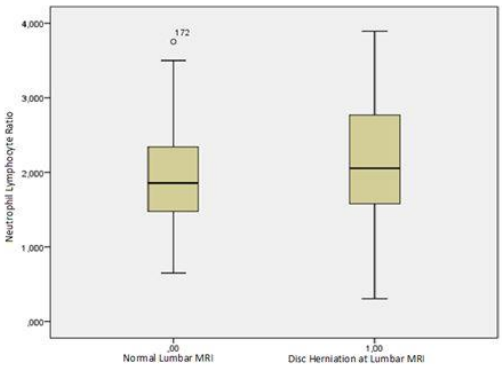

Figure 1: Comparison of mean NLR Between Control Group and in Disc Herniation Group

For the disc degeneration, genetic characteristics, environmental impacts and mechanical effects of heavy occupational conditions have been emphasized frequently. In the studies, it has been reported that dynamic and static loading might initiate the disc degeneration (Osterman H, et al., 2016; Shamji MF l et al., 2010). Similarly, end plato fracture is a significant factor for the onset of disc degeneration, high impact loading may deteriorate the natural matrix structure of disc tissue without end plate fracture have been shown on MRI scans (Stefan D et al., 2014).In our study, parallel with the studies in the literature, a significant relationship was found among motor vehicle driving, heavy lifting, trauma history and BMI≥30 with lumbar disc herniation presence as related to dynamic and static loading. Similarly, a significant relationship was found between DM, age and sex of male with the presence of lumbar disc hernia in our study. It is also known that cell – mediated mediators such as interleukin and TNF are efficient for the onset and progress of disc degeneration (Adams MA et al., 2016; Singh K et al., 2006).There are studies in the literature showing that inflammatory process has an important role in the process of degeneration (Ala-Kokko L et al., 2005; .Battie´ M et al., 2014).

Although the definite mechanism is not understood exactly, it is reported that there is a significant relationship between the degree of degeneration and levels of inflammatory mediators due to intervertebral disc pathology (Wei Li et al., 2014).It has been found out that inflammatory process increases cell aging and apoptosis and reduces disc anabolism and in this way, it inhibits expression of genes coding type II collagen and proteoglycan being the structural component of the intervertebral disc. Similarly, it has been shown that inflammatory process deteriorates the non-cellular matrix of intervertebral disc and facilitates catabolic processes and increases degeneration (LiW et al., 2014).

While effects of inflammation on disc generation are known, at which step it has a role and its role is not understood exactly. Whether the inflammation has an efficient role from the beginning of disc degeneration process or occurs as a result of changes due to mechanical effects or not should be evaluated with the new related studies in the future.

In chronic severe and continuous inflammation cases, number of neutrophil increases as secondary to the inflammation. Simultaneously, it contributes to the decrease of lymphocyte as a result of severe apoptosis and distribution of lymphocyte secondary to the stress inducing to lymphatic organs (Sen BB et al., 2014).

It is known that monocyte, lymphocytes and neutrophils being the while blood cell group have a critical role in inflammatory response. As it has been demonstrated in previous studies, following infiltration of stimulated macrophages, natural killer cells, lymphocyte and especially neutrophile to the tissue and excessive activation, release of many enzymes, cytokine, reactive oxygen products, protease, elastase (Harjai KJ et al., 2008; Russo D et al., 1995; Solomon R et al., 2010). All of these factors may contribute to disc degeneration by increasing damage of tissue.

In our study being compatible with the data of literature, a significant and independent relationship has been found between the rate of neutrophil-lymphocyte being an easy and fast detectable marker of systemic inflammation and presence of lumbar disc herniation. Presence of an independent correlation between these two parameters may be useful for taking protective actions for the individuals being at risk in terms of lumbar disc hernia. In the light of findings, it has been considered that NLR may be added to the diagnosis algorithm as a predecessor assistant marker in the risk group and initiation of anti-inflammatory based medical therapies and conservative approaches in early period in cases with high NLR may have a protective role in disc degeneration and lumbar disc hernia development. For this reason, our findings are of clinical importance.

Limitations

This is an observational, single-institution study, which had a relatively small sample size and was thus subject to various unaccounted confounders inherent in such an analysis. Additionally, we could not compare N/L with other inflammatory markers,

TNF alfa, interleukin, fibrinogen, or myeloperoxidase, because they were not routinely obtained in our study population.

Conclusion

In this study, it's determined that increased NLR is an independent predictor of lumbar disc herniation, which is as an easily applicable, simple and useful non-specific inflammatory marker. This finding is of clinical importance, since early initiation of anti-inflamatory-based preventive medical therapies and conservative therapies may provide time to prevent the progression of lumber disc herniation and improve its negative impact on outcome.

Ethics Committee Approval: Ethics committee approval was received for this study from Ordu Clinical Research Ethics Committee of ORDU University. Ethics no: 70/04.05.2018.
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RESEARCH ARTICLE

Protective Effects of Grape Molasses and Resveratrol Against DMBA Induced Oxidative Stress in Rat Ovarian Tissues

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Abstract

Objective: The polyphenolic compound, resveratrol (3, 4', 5-trihydroxystilbene), trans-resveratrol is a natural phytoalexin that exists in many different foods such as grape peel and seed, peanut, mulberry and blueberry. Molasses is a natural food obtained by boiling and concentrating fruit juices known to be rich in minerals such as iron, phosphorus, potassium, sodium, calcium and magnesium, and phosphoric acid, formic acid, organic acids and some vitamins. Polyphenolic compounds are natural antioxidants and are known to have protective effects against tissue damage caused by reactive oxygen species (ROS). In this study, we aimed to investigate the protective effects of grape molasses and resveratrol on ovarian damage induced by 7, 12-dimethylbenz [a] anthracene (DMBA).

Methods: A total of 42 old female Wistar Albino rats, aged 18 weeks were divided into six groups. 10 mg/kg DMBA was injected in the rats in DMBA group subcutaneously on day 0 and day 7 while grape molasses feed with 20% grape molasses was given along with DMBA application to the rats in DMBA + grape molasses group. Resveratrol was administered by subcutaneous injection at 10 mg/kg/day to the DMBA + Resveratrol group, while only feed with 20% grape molasses was given to the grape molasses group. Resveratrol was administered by subcutaneous injection at 10 mg/kg/day to the resveratrol group.

Results: GSH activity between the control group and molasses group, DMBA and DMBA + molasses groups and the control group and the DMBA + grape molasses groups was found statistically significant. Regarding the NO activity, the difference between the DMBA and resveratrol groups, DMBA and DMBA + resveratrol groups and the grape molasses and resveratrol groups was found statistically significant. MDA activity between DMBA + resveratrol and DMBA + grape molasses groups, and DMBA + grape molasses and grape molasses groups was found statistically significant.

Conclusion: Molasses as one of the most important nutrient sources of Eastern and Central Anatolia was observed to decrease ovarian tissue oxidative damage induced with DMBA compared to resveratrol.

Key words: DMBA (7, 12-dimethylbenz [a] antrasen), resveratrol, grape molasses, oxidative stress, ovarian

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Introduction

Rapid developments in technology, changing lifestyles, environmental pollution and chemicals cause negative impacts on human health and natural resources (Im et al., 2019). Therefore, people are exposed to many carcinogens and mutagenic substances of exogenous origin in their daily lives. This exposure may be air, water, food and soil borne and can transform into toxic compounds as a result of biotransformation reactions as well. Increased duration of exposure to such chemicals can cause irreversible and genetic disorders (David, 1999). Recent studies have demonstrated findings that consumption of green fresh vegetables and fruits protects the organism against toxic effects (Zhao, 1999).

Polycyclic aromatic hydrocarbons (PAH) are three or more aromatic ring compounds originating from different environmental and anthropogenic sources as a result of complete or incomplete combustion of carbon and hydrogen containing organic compounds and pyrolysis processes.

PAHs are carcinogens with tumor initiator, enhancer and promoter properties. PAHs inhibit cellular and humoral immunity (Armstrong et al., 2019). PAHs with low toxicity alone show tumor and mutagenic effect after undergoing metabolic activation. DMBA (dimethylbenz [a] anthracene), a PAH-member with environmental toxic effect, is known as procarcinogen and premutagenic. It is also effective as a teratogen, especially in the adrenal gland and fetal brain, which has been reported to exhibit high cytotoxicity and atherogenic properties in vivo - in vitro. DMBA causes genetic mutation as a result of the binding of intermediates from DMBA metabolism to DNA. It also destroys DNA structure and causes lipid peroxidation. It has been reported to exert a carcinogenic effect by increasing the formation of free radicals such as intracellular hydroxyl and superoxide anion radicals (Giovanni et al., 1980; Gao et al., 2007; Zeweil et al., 2019).

Resveratrol (3, 4', 5-trihydroxystilbene), a subpopulation of stilbenes, is a polyphenolic compound found in grapes, wine, peanuts and blueberries. Resveratrol is a non-flavonoid most intensively found in black grape clusters. Resveratrol is a compound of the phytoalexin group that the grape releases to protect against environmental stress and pathogenic attacks such as thirst, cold weather conditions, fungal infections, ultraviolet rays and ozone. There are many studies reporting that resveratrol presents a number of properties with different mechanisms of action. The inhibitory effect of resveratrol has been mainly associated with anti-xenobiotic ability (De-la-Lastra and Villegas, 2005; De-la-Lastra and Villegas, 2007).

Reactive oxygen species (ROS) are produced continuously in the body as a result of various metabolic and physiological processes. When the balance between ROS production and natural antioxidant activity deteriorates, a table of "oxidative stress" emerges that can lead to serious cellular damage, premature aging, and even cancer development. As a polyphenol, resveratrol is an antioxidant and free radical scavenger that inhibits reactive oxygen species (ROS) by activating AMP-activated protein kinase. It suppresses cyclooxygenase-2 (COX-2) and lipid peroxidation. Thus, resveratrol shows different pharmacological functions such as antiangiogenic, antioxidant, antitumor and cardio-protective and anticancer (Zadi et al., 2018; Al Fatease et al., 2019; Santos et al., 2019).

Molasses, which is a rich source of energy with organic acids, carbohydrates, minerals and various vitamins, is produced mostly from grapes in our country. Grape molasses contains Thiamin (B1) and Riboflavin (B2) and Niacin (B3) vitamins, Phosphorus (P), Iron (Fe), Copper (Cu), Zinc (Zn), Potassium (K), Sodium (Na), Magnesium Mg), and Calcium (Ca) minerals (Pharm et al.,2014). It is known that resveratrol in the content of black grape molasses inhibits the formation of reactive oxygen species (ROS) triggered by tumor necrosis factor (TNF), and lipid peroxidation in cells. There are also studies showing that resveratrol reduces tumor progression through the inhibition of cyclooxygenase-2 (Cox 2) as well as antimutagenic, cancer-inhibiting effects (Krishna et al., 2002; Yu et al., 2018; Zheng et al., 2018). There are several studies in the literature about protective effects of grape seeds and grape skin on various cancer types. However, we could not found any study about the effect of grape molasses on ovarian cancer. In the light of these information, the present study was designed to investigate the protective and preventive effect of resveratrol and molasses on DMBA toxicity. The aim of this study was to investigate the effect of resveratrol with grape molasses origin as antioxidant and anti-cancerogenic on the oxidative stress parameters in resveratrol and rat ovary tissue.

Methods

Chemicals, animals and diets

Wistar albino female rats used in this study were obtained from Inonu University Experimental Animal Production and Research Center. Guidelines of Inonu University Experimental Animal Ethics Committee was complied with during the study period. 18-weeks old female rats weighing 205 ± 13 g were kept in standard cages until the day of the experiment. Throughout the experiment, the drinking water was changed daily and the standard cage cleaning was done. The rats are housed in rooms with air conditioner in 24-27 °C room temperature with 12 hours of light and 12 hours of dark. A total of 42 rats were divided into 6 groups and fed with standard pellet food during the experiment except molasses groups. In the power analysis, the number of animals in each group considering % 90 power and 0.05 error margin.

Preparation of Resveratrol DMBA

In our study, a subcutaneously injected resveratrol mixture was prepared by dissolving 110 mg resveratrol in 110 ml DMSO, which is known as the solvent of many chemicals. 65 mg of DMBA was applied after being dissolved in 65 ml of sesame oil.

Preparation of Grape Molasses

Grape picked in the harvest period is cleaned out and the acidity is removed by adding grape marl after the crushing and squeezing process. After the resting and filtering process, the sun darkening process is applied and the molasses is packed. The black grape molasses used in the study was obtained from the Arapgir district of Malatya. Feed containing 20% molasses was prepared.

Experimental Design

Control Group (n=7): The rats in this group were injected subcutaneously 1 ml each day in a mixture of 20 ml of sesame oil and 30 ml of DMSO.

DMBA Group (n=7): 10 mg/kg DMBA was injected subcutaneously on day 0 and day 7.

DMBA + Molasses Group (n=7): 10 mg/kg DMBA was injected subcutaneously on day 0 and day 7. Molasses feed with 20% molasses was given every day.

DMBA + Resveratrol Group (n=7): Resveratrol was administered by subcutaneous injection at 10 mg/kg/day. 10 mg/kg DMBA was injected subcutaneously on day 0 and day 7.

Molasses Group (n=7): Molasses feed with 20% molasses was given every day.

Resveratrol Group (n=7): Resveratrol was administered by subcutaneous injection at 10 mg/kg/day.

Obtaining Ovarian Tissues and Preparing for Analyzes

On the 10^{th} day of the study, rats were sacrificed under general anesthesia. Ovarian tissues from rats were wrapped in aluminum foil and stored at -70 °C in deep freezing until the day when biochemical tests were to be carried out.

Tissue Homogenization and Tampons

Tissues weighed approximately 200 mg were homogenized at a rate of 16000 rpm by adding 2 ml of Tris - HCl tampon (pH: 7.0). After homogenization, the tubes were centrifuged at 4000 rpm for 10 minutes at +4 °C. Supernatants formed after centrifugation were taken into eppendorf tubes and kept in the freezer until the day of operation.

Estimation of Oxidative Stress Markers

Measurement of Reduced Glutathione

The absorbance of the yellow colored product resulting from the reaction of total sulphydryl content with Ellman's Reagent (DTNB) was determined by spectrophotometrically measuring at 412 nm (Tietze, 1969).

Measurement of Lipid Peroxidation

The measurement of MDA, a lipid peroxidation indicator, is based on the spectrophotometric evaluation of the resultant pink-red color absorbance at 532 nm as a result of the reaction of MDA in the sample with TBA at 95 °C (Uchiyama and Mihara, 1978).

Measurement of Nitric Oxide

NO formed by ambient NOS activity is measured at 545 nm in a color compound spectrophotometer, which is formed after the reaction with Griess reactivity by being reduced from nitrate to nitrite with cadmium garnets (Cortas and Wakid, 1990). For the standard measurement, the standard graphic was drawn with solutions prepared by a serial dilution of the 10 mmol/L $NaNO_3$ stock solution (5-200 μM).

Statistical Analysis

Because of the clinical variables are not normally distributed within groups, non-parametric statistics were used. Descriptive statistics were calculated by median, minimum and maximum. Group comparisons according to GSH, NO and MDA were evaluated by using Kruskal-Wallis Variance Analysis. For pairwise comparisons, Bonferroni adjustment was used. Type-I error rate was taken as α=0.05 for statistically significance. SPSS 21 software was used for statistical analyses (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.)

Results

Difference between study groups according to GSH, NO and MDA was statistically significant (p<0.001 for overall comparison). Significance values for pairwise comparisons of these groups were given in Table 1.

Table 1. Difference between study groups according to GSH, NO and MDA (p<0.001). Different letters (a, b, c) on the colons indicate statistical significance (p<0.05).

GROUPS		GSH (µmol/wet tissue)	NO (µmol/wet tissue)	MDA (µmol/wet tissue)
CONTROL	Median	1.89 [a,b]	3.07	16.83
DMBA	Median	1.79 [c]	4.79 [a]	22.44
DMBA + RESV.	Median	1.83	4.15 [c]	19.8 [b]
DMBA + G.MOLASSES	Median	1.84 [b,c]	4.09	19.14 [a,b]
GRAPE MOLASSES	Median	1.85 [a]	3.32 [b]	18.15 [a]
RESVERATROL	Median	1.85	3.51 [a,b,c]	17.66
Overall comparison		p<0.001	p<0.001	p<0.001
Pairwise comparison		[a] p=0.008	[a] p=0.018	[a] p<0.001
		[b] p<0.001	[b] p=0.022	[b] p=0.039
		[c] p=0.027	[c] p=0.004	

GSH activity between the control group and grape molasses group was found statistically significant (p=0.008). GSH activity between the DMBA and DMBA + grape molasses groups was found statistically significant (p=0.027). GSH activity between the control group and the DMBA + grape molasses groups was found statistically significant (p<0.001).

NO activity between the DMBA and resveratrol groups were found statistically significant (p=0.018) and was found statistically significant between DMBA and DMBA + resveratrol groups (p=0.004). NO activity was found to be significant between the grape molasses and resveratrol groups (p=0.022).

MDA activity between DMBA + resveratrol and DMBA + grape molasses groups was found statistically significant (p=0.039). MDA activity between DMBA + grape molasses and grape molasses groups was found statistically significant (p<0.001).

Discussion

Various animals are used in many in vivo studies in order to produce cancer models. The most commonly used animals for this purpose are rats. In this study we also used Wistar Albino rats in ovarian toxicity model induced with DMBA.

Recently protective effects of polyphenolic compounds obtained from various diet resources, against oxidative stress resulted from cancer and cancer drugs. Polyphenolic compounds are the most important part of natural plant products with known anti-inflammatory, antimicrobial, anti-alergenic and antioxidant effects (Francischi et al., 2017).

Resveratrol, which is one of the phenolic compounds, and richly found in grapes, wine, peanut and soy, has drawn attraction of scientists

and medical doctors for many years. Resveratrol is known to have anti-oxidative properties as scavenging reactive oxygen species (ROS) such as hydroxyl, superoxide and metal-induced radicals (Leonard et al., 2003; Truong et al., 2018). Grape molasses is a popular and traditional Turkish food produced in East and Middle Anatolia for long time (Ustun and Tosun, 1997). Grape molasse is mainly produced by concentrating the fruit juice with a soluble dry substance up to 70-80% (Batu et al., 2013). Although effects of various grape products grape seed, grape skin and grape pomac on oxidative stress have been studied in the literature, there is no any study directly investigating effects of grape molasses. In our study we investigated antioxidant effects of grape molasses in comparison of resveratrol, which is among the polyphenolic compounds found in grape molasses on ovarian cancer induced by DMBA in rats.

In the study by Kim et al., it was determined that grape seed extract was chemo-preventive against DMBA-induced breast cancer in adult rats and genistein exhibited similar activity in N-methyl-N-nitrosourea (MNU) breast cancer rat model, thus, it was concluded that both grape seed and genistein effects were based on diet (Kim et al., 2004). In another study conducted on rats, the anti-tumor effect of polyphenolic fractions isolated from grape seeds was investigated by establishing a two-step carcinogenesis protocol with DMBA and TPA (12-O-tetradecanoylphorbol). Extracted polyphenolic fractions have been found to have inhibitory effect on epidermal lipid peroxidation. In the present study we also used DMBA to induce ovarian cancer in rats.

The antioxidant activities of grape and grape seed phenolics have been studied in different models in in vitro systems, and low-density lipoprotein (LDL) has been determined to protect Cu^{+2} against SIN-1-mediated oxidation producing the oxygen-based radical generator 2, 2-azobis (2-amidinopropane) dihydrochloride (AAPH) or peroxynitrite. It has been found to protect spleen cells against DNA damage induced by hydrogen peroxide (H_2O_2) and reduce oxidative stress in PC12 cells that are stimulated by the addition of Fe^{2+} and t-butyl hydroperoxide (Shafiee et al., 2003; Chanvitayapongs et al., 1997). Anticancer effects of grape antioxidants in in vitro and in vivo models have been studied and have been shown to induce cell cycle blockage and apoptosis in cancer cells, and to inhibit carcinogenesis and cancer progression in rodent models (Aggarwal et al., 2004; Garvin et al., 2006; Ebeler et al., 2002). Male Wistar rats were fed with diets containing cellulose (control) and grape fiber for 4 weeks and GSH: GSSG ratio, GSSG / 2GSH pair redox status, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), lipid peroxidation (LPO) and apoptosis level were evaluated. In the results obtained, the cytosolic GSH: GSSG ratio increased in the grape fiber diet group, and the decline in apoptosis in relation to diet has been attributed to the modulation of the glutathione redox system and endogenous antioxidant enzymes (Lopez et al., 2010). In another study, a reduction was observed in inhibition of oxidative stress, immunosuppression, reduction of tumor growth, tumor diversity and malignant transformation of papillomas to carcinomas in UVB-induced skin cancer in rats administered with grape seed pro-anthocyanidin diet supplement (Katiyar, 2008). It was found in another study that grape pomac flour alleviated hepatic oxidative stress induced in carps, by improving antioxidant defence. It was reported that this effect occurred by prevention of overproduced ROS and NOx as well as by prevention of lipid damage (Souza et al., 2019).

A study investigating the effect of grape juice consumption on 7, 12-dimethylbenz [a] anthracene (DMBA)-induced female rat breast tumorigenesis has shown that tumor mass and tumor growth was inhibited. It has been concluded that specific components or phytochemicals in purple grape juice may interfere with the initial phase of DMBA-induced rat breast tumorigenesis (Jung et al., 2006).

In MCF-7 rat cells transfected with aromatase, grape seed extract has been found to inhibit enzyme activity and formation responsible for the transformation of androgens into estrogens and suppress estrogen biosynthesis of procyanidin B2 dimer obtained from grape seed and red wine (Eng et al., 2003; Kijima et al., 2006).

The medium-term oral carcinogenesis process initiated by 4-nitroquinoline 1-oxide (4NQO) was initiated to evaluate anti-tumor activity of grape juice concentrate. As a result of the study, there was a decrease in hyperplastic and dysplastic lesions, a decrease in COX-2 and TNF-alpha and eNOS gene expression, and an increase in SOD Cu / Zn and catalase activity in the grape juice supplement group (Pacheco et al., 2014).

In another study, grape seed pro-anthocyanidins (GSP) was reported to have increased pro-apoptotic Bax protein expression, decreased anti-apoptotic Bcl2, Bcl-xl protein expression, caused degradation in mitochondrial membrane potential, and induced apoptosis in NSCLC, A549, and H1299 cells in

vitro associated with activation of 9, 3 caspases, poly (ADP- ribose) polymerase (PARP) (Singh et al.,2011).

60 healthy volunteers aged 19-57 (16 females and 51 males) were given 480 ml of grape juice daily for an additional 8 weeks to the daily diet and blood samples were obtained from these patients. DNA damage was measured using a single cell gel (comet) test with alkaline electrophoresis. A significant reduction in lymphocyte DNA damage and reduction in the number of ROS/photons by 15% were determined compared to the beginning of the study (Park et al., 2003)

It has been found that grape seed proanthocyanidins have the ability to inhibit the invasion of human cutaneous HNSCC cells by reversing the epithelial-mesenchymal transition process targeting EGFR expression, can act as free radical scavengers and help reduce reactive oxygen species (ROS) (Sun et al., 2011).

In another study, the combination of resveratrol, quercetin and catechin (0.5, 5 or 20 μM) were found to significantly reduce cell proliferation, block in vitro cell cycle continuity and reduce primary tumor growth (Schlachterman et al., 2008). In a recent study it was demonstrated that resveratrol suppressed tumor growth and inhibited leiomyoma cells in vitro (Chen et al., 2019). Again in another study, resveratrol was shown to inhibit oxidative stress induced with aflatoxin B1 in bovine mammary epithelial cells (Zhou et al., 2019).

Our findings support the hypothesis that resveratrol, of the polyphenolic compounds found in molasses, restricts DMBA-induced oxidative stress possibly through a reduction in free radical levels. In the literature review, while there were studies on anticancer in the presence of resveratrol in fruits in grape and various fruit juice concentrates, there were no studies comparing the effects of grape molasses and resveratrol on the rat reproductive organ.

Understanding the preventing and improving mechanisms of ovarian-damage of resveratrol and grape molasses which is found at higher rates in some foods suggests that it will play an active role in preventing many diseases. It is known that in recent years there has been an increase in the interest of alternative herbal or local foods in order to prevent or reduce tissue damage caused by cancer or cancer treatments. For this reason, several studies have been conducted on the antioxidant properties of green tea containing catechin and its derivatives, red onion containing quercetin, apples, tomatoes containing lycopene, foods containing isoflavones such as soybean, chickpeas and lentils, broccoli and brussels sprouts containing indol-3-carbinol, pomegranate containing polyphenol and ellagic acid, and many food products containing selenium, vitamin E and D, anthocyanin, sulforaphane, citylbin and resveratrol. In addition to the above-mentioned food products, our study results suggest grape molasses containing resveratrol, also known as black miracle, as an important antioxidant source. By increasing the number of groups specified, the effects on the metabolism damage at different doses can be supported by further clinical trials.

Conclusion

It was supported by the findings that the molasses as one of the most important nutrient sources of Eastern and Central Anatolia played an effective role as an effective agent in both the protection and the healing process when its effect on the reproductive system was compared to its active ingredient, resveratrol.

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