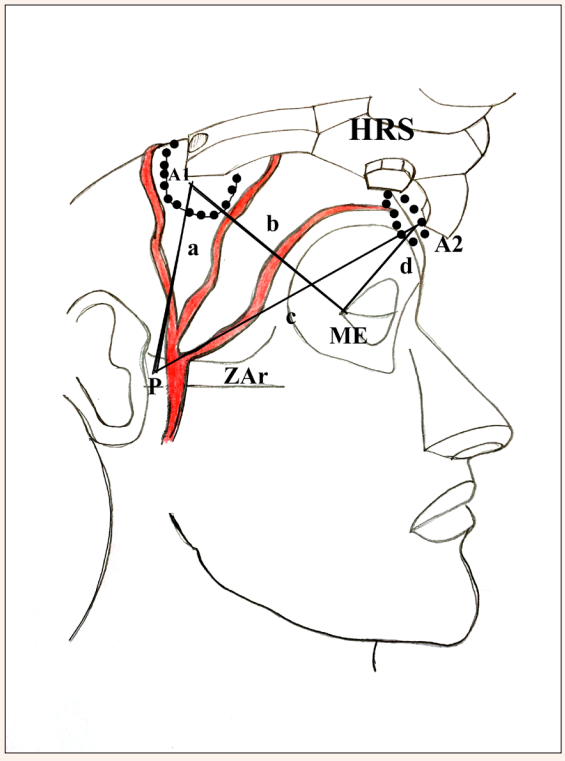
Journal of Experimental and Clinical Medicine

Vol: 33 • Issue: 3 • September: 2016



p-ISSN 1309-4483 • e-ISSN 1309-5129 JECM-DKTD http://jecm.omu.edu.tr

Journal of Experimental and Clinical Medicine

p-ISSN 1309-4483
e-ISSN 1309-5129

Vol: 33 Issue: 3 September 2016

p-ISSN 1309-4483
e-ISSN 1309-5129

Owner
On Behalf of Ondokuz Mayis University
Sait BİLGİÇ

Director in Charge
Ayhan DAĞDEMİR

Secretarial Staff
Işınsu ALKAN
Gamze ALTUN
Erkan ERENER
Sümeyye GÜMÜŞ UZUN
Arife Ahsen KAPLAN
Elfide Gizem KIVRAK
Neslihan KOÇ
Adem KOCAMAN
Kıymet Kübra YURT

Graphic Designer
Hamdi TANRIKULU

Publisher Administration Office
Ondokuz Mayıs University
Medical Faculty
Atakum / Samsun, Turkey

Publish Type
Periodical

Press
HT MATBAA
Hamdi TANRIKULU
Hançerli Mah. Atatürk Bulvarı No:112/A İlkadım / SAMSUN
www.htmatbaa.com

Press Date
XXXX

Acid-free paper is used in this journal.

Indexed: CEPIEC, Crossref, DOAJ, EMBASE, EBSCOhost, Google Scholar, Index Copernicus, J-Gate, NLM Catalog (PubMed), ResearchGate, Scopus, Turkiye Citation Index, TÜBİTAK ULAKBİM TR Dizin, World Cat.

Cover Art
Gokyar et al., Page 154; Figure 5

INTERNATIONAL ADVISORY BOARD

NATIONAL ADVISORY BOARD

CONTENTS ***Page***

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2016; 33(3): 123-128
doi: 10.5835/jecm.omu.33.03.001

Galectin-3 as a novel biomarker for the diagnosis of essential hypertension with left ventricular hypertrophy

Gokay Nar[a*], Gokhan Aksan[b], Ozkan Gorgulu[c], Sinan Inci[d], Korhan Soylu[e], Metin Ozdemir[f], Rukiye Nar[g], Serkan Yuksel[e], Mahmut Sahin[e]

[a] *Department of Cardiology, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey*
[b] *Department of Cardiology, Gazi State Hospital, Samsun, Turkey*
[c] *Department of Biostatistics and Medikal Informatics, Ahi Evran University, Kirsehir, Turkey*
[d] *Department of Cardiology, Aksaray State Hospital, Aksaray, Turkey*
[e] *Department of Cardiology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey*
[f] *Department of Microbiology, Gazi State Hospital, Samsun, Turkey*
[g] *Department of Biochemistry, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey*

ARTICLE INFO

Article History
Received 01 / 05 / 2016
Accepted 10 / 05 / 2016

*** Correspondence to:**
Gokay Nar
Department of Cardiology,
Faculty of Medicine,
Ahi Evran University
Kırsehir, Turkey
e-mail: gokay_nar@yahoo.com

Keywords:
Galectin-3
Hypertension
Left ventricular hypertrophy
Left ventricular muscle mass

ABSTRACT

Galectin-3 (Gal-3) is a carbohydrate-binding protein that has important regulatory roles in inflammation, immunity and cancer. The aim of this study was to investigate the relationship of Gal-3 level with left ventricular hypertrophy (LVH) related to hypertension (HT). Thirty seven patients (Group I) with newly diagnosed hypertension (HT) and left ventricular hypertrophy (LVH)were included in the study. Thirty eight patients with newly diagnosed hypertension without LVH (Group II) and 38 normotensive healthy volunteers (Group III) were included in the study as control group. Transthoracic echocardiography was performed and Gal-3 was measured in all patients.Although demographic characteristics of the groups were similar, systolic and diastolic blood pressure levels of Group I and Group II were significantly higher than Group III ($p<0.001$). Interventricular septum (IVS) thickness, posterior wall (PW) thickness and left ventricular mass index (LVMI) were also significantly increased in Group I compared to Group II and Group III. Serum Gal-3 levels in all three groups were seen to be different from each other ($p<0.001$). Increase of Gal-3 levels has a significant correlation with LVMI, IVS and PW thickness ($p<0.001$).We determined that Gal-3 levels, even at the newly diagnosed stage of HT,were increased. Moreover, we found strong correlation between Gal-3 levels and left ventricular muscle mass. These results may indicate that increased Gal-3 level is an important marker for target organ damage and high cardiovascular risk in patients with HT.

Introduction

Precise risk assessment should be done in order to manage the treatment of hypertension (HT) with proper density. For this determination, the risk factors such as diabetes, dyslipidemia and smoking as well as findings of subclinical organ damage should be examined. Left ventricular hypertrophy (LVH) is a HT-induced subclinical organ damage. It was shown that LVH was related with increased cardiovascular events and death in many previous studies (Lewington et al., 2002;

Kannel et al., 2000). Similarly, cerebrovascular events due to HT such as stroke are increased in patients with LVH (Devereux et al., 2004; Levy et al., 1990). Besides, antihypertensive treatment decreases left ventricular hypertrophy, and cardiovascular morbidity (Verdecchia et al., 1998; Bahlf and Pennert, 1992).

Galectin-3 (Gal-3) is a carbohydrate-binding protein that has significant regulatory roles in inflammation, immunity and cancer (Rabinovich et al., 2002). The relationship of Gal-3 with the severity and mortality of the disease in patients with heart failure both with preserved ejection fraction (EF) and with low EF was investigated in studies in recent years (Lok et al., 2010; Lopez-Andrès et al., 2012). Increased Gal-3 expression induces cardiac fibroblasts to proliferate and deposit type I collagen, contributing to myocardial fibrosis and adverse remodeling (De Boer et al., 2010). Therefore, increased collagen synthesis and myocardial fibrosis occurring during LVH may be related to Gal-3. Moreover, this condition may also be detected in patients in whom there is no detectable hypertrophy. We tried to clarify this issue by measuring galectin-3 levels in hypertensive patients with LVH in our study.

2. Material and method

Study Population

This study was designed as cross-sectional trial. Patients diagnosed with new hypertension and healthy individuals who were admitted to the Kırşehir outpatient clinics of cardiology of Ahi Evran University Training and Research Hospital between January 2013 and March 2014 were enrolled in this study. Their ages were between 18 and 70 years. They were investigated in three different groups. Group I was consisted of new hypertension patients with LVH. Group II was consisted of hypertension patients without LVH. Group III was consisted of healthy individuals matched for age and gender.

Permanent cardiovascular disease, diabetes mellitus, renal failure (serum creatinine>2.0 mg/dL), liver disease, autoimmune diseases, hematologic disorders, obesity (BMI>30 kg/m^2), cancer, presence of systemic inflammatory disease, and history of drug use were considered as a criteria for exclusion from the study. The study was conducted in accordance with the ethical principles described by the Declaration of Helsinki (Williams, 2008).

Description of hypertension

Diagnosis of hypertension in an office location was made in accordance with ESC/ESH 2007 arterial hypertension guidelines (Mancia et al., 2007). Hypertension was defined as an average systolic blood pressure (SBP)≥140 mmHg or an average diastolic blood pressure (DBP)≥90 mmHg.

Echocardiography

All patients experiencedtransthoracic echocardiographic examination in the left lateral decubitus position by using GE Vingmed Vivid 7 (GE Vingmed Ultrasound, Horten, Norway) echocardiography. All measurements were achieved by two cardiologists experienced in echocardiography who were uninformed of the clinical status of patients. The calculation was done rendering to the criteria of the American Society of Echocardiography (Quinones et al., 2002).

Description of left ventricular hypertrophy:

Left ventricular mass index (LVMI) was measured by using the calculation previously defined Devereux et al. (1991). Therefore, left ventricular mass index were associated to body surface area. LVM's higher than 125 gr/m^2 in men, 110 gr/m^2 in women were observed as the occurrence of LVH.

Biochemical measurements

The biochemical parameters were measured using commercial kits in ARCHITECT C8000 Abbott (Abbott Laboratories, IL, USA) autoanalyzer.

Serum Galectin-3 measurements

Blood samples were taken in the morning from 7:00 to 9:00, after 8-12 hours of fasting with the purpose of escape to be affected by diurnal rhythm. Blood samples serums were separated by centrifuging samples for 10 minutes at 4000'g. Biochemical and hematological parameters were calculated on the same day. The serums were protected at -80^{0}C until Galectin ELISA study was done. Serum Galectin-3 levels were resolute by studying consistent with producer's instructions by using Multiwash (Tricontinent Scientific, USA) etc. Synergy 4 Microplate Reader (Biotek, USA) procedures and Platinum Human Galectin-3 ELISA kit (eBioscience, Inc. San Diego, USA) with enzyme-linked immunosorbent assay (ELISA) method.

Statistical analysis

Statistical analyses were based on SPSS 15.0 (Statistical Pack age for Social Sciences) program. Kolmogorov-Smirnov test was used to check normal distribution of all parameters. Categorical variables were expressed in percentage, whereas numerical variables were presented as mean ± standard deviation (SD). Categorical variables of the patients were compare dusing the Chi-Square test. Comparison of groups was based on One-way ANOVA or Kruskal-Wallis Test and multiple comparisons were made using either the Student's t-test or the Mann-Whitney U-test. $P<0.05$ was accepted as statistically significant. Bonferroni's correction was performed when statistical comparisons of the three groups were made as <0.016. The correlation between data was tested with Pearson or Spearmen correlation analysis.

Table 1. Baseline clinical and laboratory characteristics of study population and comparison between groups

	Group I (n=37)	Group II (n=38)	Group III (n=38)	p
Ages, years	55.32 ± 7.34	56.73 ± 8.62	53.05 ± 8.48	0.166
Male sex % (n)	45 (17)	47(18)	50 (19)	0.622
Body mass index (kg/m²)	26.71 ± 2.81	25.73 ± 2.53	26.39 ± 3.01	0.356
Smoking % (n)	55 (20)	53 (20)	53(20)	0.556
Heart rate (beats/minute)	74.25 ± 10.91	72.36 ± 11.61	73.78 ± 9.76	0.684
Mean systolic blood pressure (mmHg)	157.16 ± 12.55*	155.78 ± 14.63*	116.57 ± 14.75	<0.001
Mean diastolic blood pressure (mmHg)	99.45 ± 8.23*	98.81 ± 9.68*	70.78 ± 11.99	<0.001
Serum glucose (mg/dL)	89.54 ± 11.09	90.36 ± 20.40	89.18 ± 8.85	0.791
Creatinine (mg/dL)	0.86 ± 0.26	0.89 ± 0.28	0.95 ± 0.16	0.371
Triglyceride (mg/dL)	148.00 ± 57.47	127.39 ± 61.47	140.26 ± 68.92	0.182
Low-density lipoprotein cholesterol (mg/dL)	93.37 ± 25.74	103.10 ± 32.39	94.36 ± 27.42	0.359
High-density lipoprotein cholesterol (mg/dL)	34.01 ± 10.07	36.21 ± 8.17	38.13 ± 9.97	0.073
Total cholesterol (mg/dL)	178.29 ± 35.19	186.78 ± 36.30	184.01 ± 24.27	0.667
Hemoglobin (g/L)	13.72 ± 1.96	14.21 ± 1.48	13.84 ± 1.43	0.308
Sodium (mmol/L)	136.94 ± 3.64	137.39 ± 3.11	138.15 ± 3.56	0.277
Potassium (mmol/L)	4.28 ± 0.35	4.15 ± 0.47	4.24 ± 0.50	0.407
AST (U/L)	31.59 ± 14.88	35.92 ± 13.79	33.21 ± 17.04	0.330
ALT (U/L)	25.83 ± 10.43	32.13 ± 17.51	30.15 ± 14.52	0.214

* p<0.001between Group III
ALT: Alanine aminotransferase; **AST:** Aspartate aminotransferase

3. Results

Totally 113 subjects, consisting of 75 patients newly diagnosed HT and 38 healthy individuals, were enrolled in this study. There were 37 newly diagnosed hypertensive patients with LVH (Group I), 38 newly diagnosed hypertensive patients without LVH (Group II), and 38 healthy normotensive controls (Group III). The demographic, clinical and laboratory characteristics of the patients are as long as in Table 1. There is no significant difference between the groups at the values of heart rate, lipid parameters, creatinine, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), hemoglobin, age, gender, body mass index (BMI), smoking (p>0.05). But systolic and diastolic blood pressure levels were significantly higher in Group I and II (p<0.001).

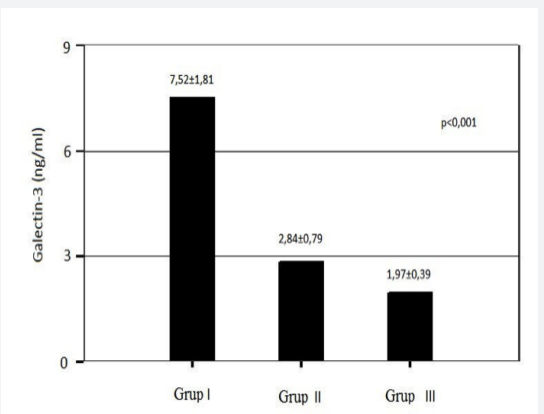


Fig. 1. Galectin-3 levels of the groups.

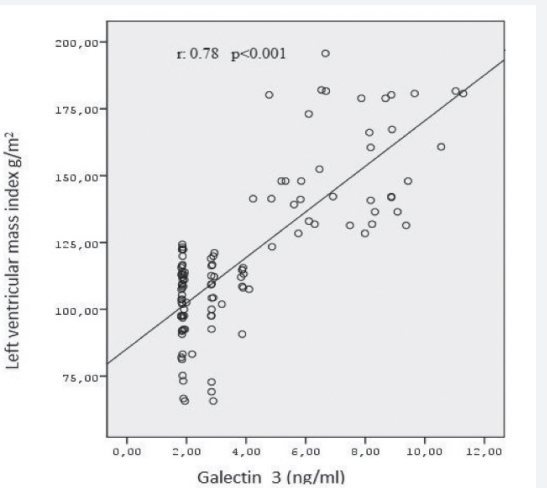


Fig. 2. Correlation graphs of left ventricular mass index with Galectin-3 levels

When the groups were evaluated in terms of echocardiographic measurements, left ventricular diameters and systolic functions were parallel. But, IVS thickness, PW thickness, left ventricular mass index, left atrium dimension, deceleration time, isovolumetric relaxation time, early diastolic flow, atrial contraction signal were significantly higher in Group I and II (P<0.016). Also, IVS thickness, PW thickness, left ventricular mass index values were also different between Group I and Group II (P<0.016) (Table 2).

A significant difference was observed between three groups in terms of serum Gal-3 levels (p<0.001). While the serum Gal-3 level was 7.52±1.81 ng/ml in Group I, it was significantly higher than both Group

Table 2. Comparison of echocardiographic parameters and Galectin-3 levels between groups

	Group I (n=37)	Group II (n=38)	Group III (n=38)	p
IVS thickness (mm)	13.72 ± 1.23*ɣ	10.81 ± 0.86 ˜	10.13 ± 1.01	<0.001
PW thickness (mm)	12.97 ± 1.06* ɣ	10.60 ± 0.91°	9.65 ± 1.34	<0.001
LVEDD (mm)	48.21 ± 4.58	46.39 ± 3.69	46.94 ± 4.29	0.162
LVESD (mm)	33.75 ± 4.85	32.10 ± 3.61	32.28 ± 4.56	0.324
LA dimension (mm)	37.43 ± 4.74	33.84 ± 3.75⁺	34.18 ± 3.43⁺	0.002
LVEF (%)	58.72 ± 5.66	60.32 ± 4.92	61.57 ± 3.92	0.107
DT (msn)	236.40 ± 27.29 ɣ	233.07 ± 38.55 ɣ	176.18 ± 28.74	<0.001
IVRT (msn)	124.56 ± 25.20 ɣ	117.75 ± 20.35 ɣ	82.44 ± 15.43	<0.001
E (m/sn)	66.13 ± 10.83 ɣ	66.71 ± 12.59 ɣ	78.21 ± 11.54	<0.001
A (m/sn)	81.08 ± 12.42 ɣ	81.65 ± 16.73 ɣ	67.15 ± 8.73	<0.001
E/A	0.82 ± 0.13 ɣ	0.82 ± 0.12 ɣ	1.18 ± 0.23	<0.001
Galectin-3	7.52 ± 1.81* ɣ	2.84 ± 0.79 ɣ	1.97 ± 0.39	<0.001
LVMI (g/m²)	154.38 ± 20.19* ɣ	104.66 ± 13.36ɣ	101.84 ± 16.24	<0.001

* p<0.0001 for the two-way comparison with Group II
ɣ p<0.0001 for the two-way comparison with Group III
° p=0.0011 for the two-way comparison with Group III
˜ p=0.0071 for the two-way comparison with Group III
⁺ P=0.0021 for the two-way comparison with Group I
A: Atrial contraction signal; **DT:** Deceleration time; **E:** Early diastolic flow; **IVRT:** Isovolumetric relaxation time; **IVS:** Ventricular septal thickness; **LA:** Left atrium; **LVEDD:** Left ventricular end-diastolic dimension; **LVEF:** Left ventricular ejection fraction; **LVMI:** Left ventricular mass index; **LVESD:** Left ventricular end-systolic dimensions; **PW:** Posterior wall thickness

II and III (P<0.001). Furthermore Galectin-3 level of Group II was higher than Group III (2.84±0.79 ng/ml versus 1.97±0.39 ng/ml, P<0.001) (Table 2, Fig 1).

In the hypertension group, A strong correlation was observed between Gal-3 levels and LVMI (r=0.78, p<0.001), IVS thickness (r=0.77, p<0.001), PW thickness (r=0.72, p<0.001) and a moderate level of correlation between mean systolic blood pressure (r=0.47, p<0.001), mean diastolic blood pressure (r=0.45, p<0.001), IVRT (r=0.45, p<0.001), DT (r=0.44, p<0.001) in the correlation analysis (Table 3, Fig 2).

Table 3. The univariate correlations of the Galectin-3 level in the hypertension group

Variables	R value	P value
LVMI (g/m²)	0.78	<0.001
IVS thickness (mm)	0.77	<0.001
PW thickness (mm)	0.72	<0.001
Mean systolic blood pressure (mmHg)	0.47	<0.001
Mean diastolic blood pressure (mmHg)	0.45	<0.001
IVRT (msn)	0.45	<0.001
DT (msn)	0.44	<0.001

DT: Deceleration time; **IVRT:** Isovolumetric relaxation time; **IVS**: Ventricular septal thickness; **PW:** Posterior wall thickness

In the hypertension group, a strong correlation was observed between Gal-3 levels and LVMI (r=0.78, p<0.001), IVS thickness (r=0.77, p<0.001), PW thickness (r=0.72, p<0.001) and a moderate level of correlation between mean systolic blood pressure (r=0.47, p<0.001), mean diastolic blood pressure (r=0.45, p<0.001), IVRT (r=0.45, p<0.001), DT (r=0.44, p<0.001) in the correlation analysis (Table 3, Fig 2).

4. Discussion

We have achieved two important results in this study. First, Gal-3 levels were found higher in HT patients with LVH. Second, there was a strong correlation between Gal-3 and LV mass, septal and posterior wall thickness. Galectin-3 is a soluble β-galactoside-binding lectin obtainable by activated macrophages in the heart. It functions as a probable mediator in the inflammation. It is expressed by activated macrophages and encourages cardiac fibroblasts to proliferate and deposit type I collagen in the myocardium (Sharma et al., 2004). Thus, the researches on the cardiovascular effects of Gal-3 have focused on heart failure patients. The correlation between the increased serum Gal-3 and the increased mortality and prolonged hospitalization in heart failure patients with preserved EF, other than heart failure patients with low EF, has been shown in the studies (van der Velde et al., 2013;de Boer et al., 2011). Even, stronger correlation was observed between Gal-3 and the heart failure patients with preserved EF than heart failure patients with low EF (de Boer et al., 2011; Shah et al., 2010). Playing of fibrosis and matrix markers such as Gal-3 a more significant role in HF patients with preserved ejection fraction was supposed as a reason for this. Higher Gal-3 levels in patients with LVH may also be related to increased fibrosis and remodeling in our study.

The relationship of Gal-3 with LVH in patients with newly diagnosed hypertension has not been investigated in previous studies. But De Boer et al., (2012) have showed a relation between Gal-3 and age, blood pressure, serum lipids, body mass index, renal function and cardiovascular risk factors such as N-terminal pro-

B-type natriuretic peptide in their large observational study. The Gal-3 levels of 3353 people, composed of the children of the original volunteers of Framingham heart study, were evaluated in study Ho et al. (2012). Gal-3 levels were seen to be associated with heart failure and increased mortality in the analysis. In addition, increased Gal-3 levels have been found associated with increased cardiac fibrosis in asymptomatic patients. Another interesting point in the study was to find a relationship between increased levels of Gal-3 and increased LV mass. The relationship of Gal-3 with LVH was investigated in rats with cardiac hypertrophy with endocardial biopsy in an animal study and Gal-3 levels were found increased (Sharma et al., 2004). Similarly, Gal-3 levels were higher in HT patients with LVH in our study. Additionally, a strong relation between Gal-3 and LVMI, septum and posterior thickness was determined.

As is known, the main purpose of the HT treatment is to prevent the target organ damage. Thus, the risk rating is the first step of treatment. In particular, hypertensive patients with target organ damage such as left ventricular hypertrophy, irrespective of their blood pressure levels, bring high cardiovascular risk. Therefore, accurate and early detection of patients at high cardiovascular risk is very important. The association between Gal-3 and LVMI in our study has shown that Gal-3 may be helpful as an early marker of target organ damage in recognition of patients with high cardiovascular risk. Another remarkable point in our study is the high level of Gal-3 even in hypertensive patients without organ damage related to HT. The reason of this condition may be diastolic weakening in hypertensive patients and the detection of the relationship between Gal-3levels and diastolic parameters IVRT, DT supports this. The increased Gal-3 level has been associated with the deterioration of diastolic parameters particularly in heart failure patients with preserved EF in previous studies. Sharma et al., (2004) have showed that Gal-3 was related to the diastolic functions in patients with acutely decompensated heart failure in their study. Moreover, the myocardial changes in patients with no detectable hypertrophy may be also responsible for this increase.

Increased angiotensin-aldosterone system takes an important place in the pathophysiology of HT. Clinical and preclinical studies particularly have revealed that increased aldosterone causes cardiac hypertrophy and fibrosis, plays an vital role in cardiovascular diseases and cardiovascular remodeling by increasing arterial stiffness (Struthers and Mac Donald, 2004; Young, 2008). Recent studies have revealed the importance of Gal-3 on fibrotic effects of aldosterone. Laurent al., (2013) in their study, have determined that Gal-3 was necessary in the inflammatory and fibrotic response of vascular smooth muscle cells to aldosterone and Gal-3 had a key role in vascular fibrosis. Similarly, Gal-3 has been shown to play a critical role in renin angiotensin aldosterone (RAAS) system by increasing salt and water retention in another study (Sherwi et al., 2012). This interaction of Gal-3 with RAAS may help us understand the mechanism of increased Gal-3 level in our study.

Another reason of the increased Gal-3 level in HT patients may be the inflammation. As is known, inflammation takes an important place in many diseases of cardiovascular system. The relationship between HT and many inflammatory cytokines were determined in studies conducted in recent years. Gal-3 is also an inflammatory marker and Gal-3, released from blood vessels other than the heart, plays a systemic role in proliferation and inflammation (Yang et al., 2008). Gal-3 improves neutrophil-endothelial interaction by temporary as a proinflammatory agent (Sato et al., 2002) and activates multiple cell types involved in immune response and inflammation that causes fibrosis (Suzuki et al., 2008). Increasing evidence shows that inflammatory changes in the vascular system take an important place in the pathophysiology of HT. Being of Gal-3 a direct mediator of profibrotic pathways and the effects of Gal-3 on inflammation may help us to understand the increased Gal-3 level in HT patients in our study.

Study limitations

Our study population is comparatively small and cross-sectional study. Larger study population with long-term follow-up of patients may be suggested for more influential statistical data. In addition, lack of assessing the relationship between Gal-3 and the parameters (microalbuminuria, carotid intima-media thickness of the carotid-femoral pulse wave velocity, etc.) that show non-cardiac subclinical organ damage is another limiting factor.

5. Conclusion

We have found that Gal-3 has increased in patients with newly diagnosed hypertension in our study. Moreover, we have determined a strong correlation between Gal-3 level and LVH. These results indicate that increased Gal-3 levels may be a marker in noticing subclinical cardiac damage in patients with newly diagnosed HT. Mainly, results to be achieved by prospective follow-up studies would more explain this topic.

REFERENCES

Bahlf, B., Pennert, K., 1992. Reversal of left ventricularhy pertensive patients. A meta analysis of 109 treatment studies. Am. J. Hypertens. 5, 95-102.

De Boer, R.A., Yu, L., van Vel dhuisen, D.J., 2010. Galectin-3 in cardiac remodeling and heart failure. Curr. Heart. Fail Rep. 7, 1-8.

De Boer, R.A., Lok, D.J., Jaarsma, T., van der Meer, P., Voors, A.A., Hillege HL, van Veldhuisen, D.J. 2011. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserve dejection fraction. Ann. Med. 43, 60-68.

De Boer, R.A., van Veldhuisen, D.J., Gansevoort, R.T., Muller, Kobold, A.C., van Gilst, W.H., Hillege, H.L., 2012. The fibrosis marker galectin-3 and outcome in the general population. J. Intern. Med. 272, 55-64.

Devereux, R.B., de Simone, G., Koren, M.J., Roman, M.J., Laragh, J.H., 1991. Left ventricular mass as a predictor of development of hypertension. Am. J. Hypertens. 4, 603-607.

Devereux, R.B., Wachtell, K., Gerdts, E., Boman, K., Nieminen, M.S., Papademetriou, V., 2004. Prognostic significance of left ventricular mass change during treatment of hypertension. JAMA. 292, 2350-2356.

Ho, J.E., Liu, C., Lyass, A., Courchesne, P., Pencina, M.J., Vasan, R.S,, 2012. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. J. Am. Coll. Cardio. l60, 1249-1256.

Kannel, W.B., 2000. Risk stratification in hypertension: New insights from the Framingham Study. Am. J. Hypertens. 13, 3-10.

Laurent, C., Maria, M., Pascal, R., Victoria, C., 2013. Galectin-3 Mediates Aldosterone-Induced Vascular Fibrosis. Arterioscler Thromb. Vasc. Biol. 33, 67-75.

Levy, D., Garrison, R.J., Savage, D.D., Kannel, W.B., Castelli, W.P., 1990. Prognostic implication of echocardiographic determined left ventricular mass in the Framingham Heart Study. N. Eng. J. Med. 323, 1706-1707.

Lewington, S.,Clarke, R., Collen, S.R., Peto, R., Collins, R., 2002. Age-specific relevance of usual blood pressure to vascular mortality: A meta analysis of individual data forone million adults in 61 prospective studies. Prospective studies collaboration. Lancet. 360, 1903-1913.

Lok, D.J., Van, Der, Meer, P., de la Porte, P.W., Lipsic, E., Van Wijngaarden, J., Hillege, H.L., 2010. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: Data from the DEAL-HF study. Clin. Res. Cardiol. 99, 323-328.

Lopez-Andrès, N., Rossignol, P., Iraqi, W., Fay, R., Nuée, J., Ghio, S., 2012. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: Insights from the CARE-HF (Cardiac Resynchronization in Heart Failure) trial. Eur. J. Heart Fail. 14, 74-81.

Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., 2007. Guidelines for the management of arterial hypertension: The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European Heart J. 28, 1462-1536.

Quinones, M.A., Otto, C.M., Stoddard, M., Waggoner, A., Zoghbi, W.A., 2002. Recommendations for quantification of Doppler echocardiography: A report from the doppler quantification task force of the nomenclature and standards committee of the american society of echocardiography. J. Am. Soc. Echocardiogr. 15, 167-184.

Rabinovich, G.A., Rubinstein, N., Toscano, M.A., 2002. Role of galectins in inflammatory and immune modulatory processes. Biochim. Biophys. Acta. 1572, 274-284.

Sato, S., Ouellet, N., Pelletier, I., Simard, M., Rancourt, A., Bergeron, M.G., 2002. Role of galectin-3 as a adhesion molecule for neutrophil extra vacation during strep to coccalpneumonia. J. Immunol. 168, 1813-1822.

Shah, R.V., Chen-Tournoux, A.A., Picard, M.H., van Kimmenade, R.R., Januzzi, J.L., 2010. Galectin-3, cardiac structure and function, andlong-termmortality in patients with acute lyde compensated heart failure. Eur. J. Heart Fail. 12, 826-832.

Sharma, U.C., Pokharel, S., van Brakel, T.J., van Berlo, J.H., Cleutjens, J.P.M., Schroen, B., 2004. Galectin-3 mark sactivated macro phages in failure-pronehypertrophied hearts and contribut estocardiacdys function. Circulation. 110, 3121-3128.

Sherwi, N., Merali, S., Wong, K., 2012. Personalizing biomarker strategies in heart failure with galectin-3. Future Cardiol. 8, 885-894.

Struthers, A.D.,Mac Donald, T.M., 2004. Review of aldosterone- andangiotensin II-inducedtarget organ damageandprevention. Cardiovasc Res. 61, 663-670.

Suzuki, Y.,Inoue, T., Yoshimaru, T., Ra, C., 2008. Galectin-3 but not galectin-1 induces mast cell death by oxidative stress and mitochondrial permeability transition. Biochim. Biophys Acta. 1783, 924-934

Van der Velde, A.R., Gullestad, L., Ueland, T., Guo, Y., Adourian, A., Muntendam, P., van Veldhuisen, D.J., de Boer, R.A., et al. 2013. Prognostic value of changes in galectin-3 levels over time in patients with heart failure data from CORONA and COACH. Circ. Heart Fail. 6, 219-226.

Verdecchia, P., Schilacci, G., Borgioni, C., Ciucci, A., Gattobigio, R., Zampi, I. 1998. Prognosticsignificance of serialchanges in leftventricularmass in essentialhypertension. Circulation. 97, 48-54.

Williams, J.R., 2008. The Declaration of Helsinki and public health. Bulletion of the Wprld Health Organization. 86, 650-651.

Yang, R.Y., Rabinovich, G.A., Liu, F.T., 2008. Galectins: Structure, function and therapeutic potential. Expert Rev Mol Med. 13, 17-39.

Young, M.J., 2008. Mechanisms of mineralo corticoid receptor-mediated cardiac fibrosis and vascular inflammation. Curr. Opin. Nephrol. Hypertens1. 7, 174-180.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2016; 33(3): 129-137
doi: 10.5835/jecm.omu.33.03.002

Effects of aspirin on oxidative and nitrosative stress in vascular endothelial cell cultures

Gunnur Demircan[a*], Emine Diraman[b], Yeliz Yilmaz Miroglu[c], Sabri Demircan[d], Zafer Yazici[e], Ahmet Yilmaz Coban[f], Tuba Yildirim[g], Ayhan Bilir[h]

[a] *Department of Medical Biology and Genetics, Faculty of Medicine, Istanbul Bilim University, Istanbul, Turkey*
[b] *Department of Biology, Faculty of Science, Ondokuz Mayis University, Samsun, Turkey*
[c] *Ministry of National Education, Ordu, Turkey*
[d] *Department of Cardiology, Faculty of Medicine, Istanbul Bilim University, Istanbul, Turkey*
[e] *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
[f] *Department of Microbiology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey*
[g] *Department of Biology, Faculty of Science, Amasya University, Amasya, Turkey*
[h] *Department of Histology and Embryology, Faculty of Medicine, Zirve University, Gaziantep, Turkey*

ARTICLE INFO

Article History
Received 26 / 04 / 2016
Accepted 11 / 05 / 2016

*** Correspondence to:**
Gunnur Demircan
Department of Medical Biology and Genetics,
Faculty of Medicine, Istanbul Bilim University, Istanbul, Turkey
e-mail: gunnurbio@yahoo.com

Keywords:
Aspirin
Cell culture
Endothelium
Nitrosative Stress
Oxidative Stress

ABSTRACT

In this study, it was aimed to investigate that whether any change in activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), lipid peroksidase and nitric oxide (NO) over time, and whether any difference between the different doses by giving different doses of aspirin to endothelial cells. Endothelial cells (HUVEC) in 24 wells microplates used in this study and 25, 50, 100, 250, 500, 750, 1000 and 1500 µM aspirin to 2 of 4 wells at each row were given, the other 2 wells were included as controls. Accordingly, while CAT, SOD, GSH-Px levels and lipid peroxidation were being measured, NO release from cell media was observed. The significant differences were not found between the baseline (0 hour) CAT, SOD, GSH-Px and lipid peroxidase levels measured from lisates that obtained from the cells that different drug doses given and controls ($p>0.05$). Also, CAT, SOD, GSH-Px and lipid peroxidase levels at 24 ($p>0.05$), 48 and 72 hours did not show any difference among different drug doses and control ($p>0.05$). In the control group significant differences were found between CAT, SOD and GSH-Px levels measured at 0, 24, 48, 72 hours ($p<0.05$, $p<0.05$ and $p<0.05$ respectively) but lipid peroxidase activity and NO levels showed no difference. Increase in antioxidant enzyme activity in the cells that aspirin was not given, caused by raised free radical formation due to increase in number of cells by time was observed. Aspirin prevented the increase in reactive enzyme activity which increases by time. These results suggest that nontoxic doses of aspirin might protect the cells.

Introduction

Free radicals are molecule or molecular portions that carry one or more unpaired electrons in their atomic or molecular orbital (Valko et al., 2007). The most important free radicals in biological systems are derived from oxygen. High doses or redundant reactive oxygen

species cause oxidative stress that result in damage of biological macromolecules and metabolic dysfunction (Singal et al., 1983; Singal et al., 1998). There are protective mechanisms against the harmful effects of free radicals in the organism. Most studies used anti-oxidants to modulate side effects which results from free radicals production and inflammation (Yapislar et al., 2016). Antioxidant mechanisms are assessed by measuring the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) like enzymes in practice. (Mates et al., 1999, Mates et al., 2000; Valko et al., 2007). Lipid peroxidase activity is used as a marker of membrane damage caused by free radicals.

Endothelium protects vascular homeostasis by providing local maintains that regulate the vascular tonus, platelet adhesion, inflammation, fibrinolysis, and vascular proliferation. In case of oxidative stress, endothelial cells lose their protective phenotype and synthesize pro-inflammatory molecules (Kunsch and Medford, 1999). Endothelial dysfunction forms the cardiovascular risk increases because of its negative effects on all of these events (Irani, 2000; Nedeljkovic et al., 2003).

Aspirin treatment is important in heart disease caused by vascular problems such as atherosclerosis. In addition to the known anti-clotting effects, it was shown that aspirin provides protection of the vascular endothelium, and therefore, the prevention of occlusive cardiovascular and cerebrovascular diseases, and also it is very effective on damages developed at the beginning of the atherosclerosis (Watala and Gwozdzinski, 1993). Based on the clinical data showing that aspirin provided improvement of endothelial functions, we designed our study to determine mechanisms of this improvement at cellular level.

In this study, by giving different doses of aspirin to endothelial cell culture, whether there are any changes over time in CAT, SOD, GSH-Px, lipid peroxidase and nitric oxide (NO) activities and whether there are differences between the different doses are investigated.

2. Methods

The cell culture phase

Human Umbilical Vascular Endothelial Cell (HUVEC) provided from the Istanbul University Istanbul Faculty of Medicine, Biophysics Department were used in the experiments. Cells were cultured in DMEM-F12 raw medium (Dulbecco's Modified Eagle Medium, nutrient mixture F12 Ham medium) containing 10% inactivated fetal calf serum (FCS), 0.2 mM glutamine, 100 mg/ml streptomycin, 100 IU/ml penicillin at 37^{0}C, under 5% CO_2 and 1 atm pressure. The cells were routinely passaged 2 times per week. The cells were used in experiments when their densities occupy half of the flask surface.

1 ml DMEM-F12 raw medium was added to each well of the 24-well culture plates and there were two wells for each dose. One ml of medium without active substance was added to control wells and again there were two wells for each dose. HUVEC from the 100% living single cell suspension with calculated cell size as 1 ml containing 100.000 cells were seeded to each. As mentioned above, two wells were used for each concentration of active substance, and 0-1, 24, 48, 72-hour experimental groups were formed for each concentration in the experiments.

A stock solution of 1 M Aspirin was prepared. Final concentrations of 25 μM, 50 μM, 100 μM, 250 μM, 500 μM, 750 μM, 1000 μM and 1500 μM were prepared by diluting with culture medium.

Evaluation of the cytotoxic doses

For each concentration of the active ingredient, at the end of all periods, cells were photographed under an inverted microscope and cytotoxic dose specified areas are defined on images. Cytotoxic effect was detected by measurement of 3-(4 5-dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide (MTT). 96-well microplates were used for this measurement. After waiting for 24 hours for attachment of the cells to the wells, 100 μl cell suspensions were transferred to each well. At the end of the test period, upper medium was withdrawn from wells and 100 ml MTT solution was added and incubated for 4 h at 37°C and at 5% CO_2. MTT solution was prepared to be 5 mg/ml dissolved in PBS and transferred to a flask by sterile filtration. At the end of four hours incubation, MTT solution was withdrawn from the wells, 200 ml of dimethyl sulfoxide (DMSO) was added to solve formazon crystals formed with MTT on wells, immediately after, it was read by the 540 nm wavelength microplate reader. The level of cytotoxicity was determined by the absorbance value. Concentration having 50% cytotoxic effect compared to the control was accepted as cytotoxic dose.

Determination of the enzyme activities

Media of experimental groups were removed at the end of the incubation period. These media were used as the supernatant for NO measurement. The cells of which media were removed were taken from the attached region. Homogenization of removed cells was provided by adding 2 ml lysis buffer and vigorous pipetage. The homogenized cells were collected in this mixture and centrifuged at 10.000 g for 15 minutes at +4^{0}C. Supernatants were separated to measure enzyme activity. Supernatants stored at -80^{0}C until measurement.

NO production was evaluated by measuring the levels of nitrite (NO_2) which is a stable product of NO. For this purpose, 50 ml supernatant was taken and an equal amount of Griess reagent (1% sulfanilamide /

naftiletilen diamine dihydrochloride 0.1%/ 2.5% H_3PO_4) was added and measured spectrophotometrically at a wavelength of 550 nm. Lipid peroxidation levels defined by Varshey and Kale (1990) with thiobarbituric acid (TBA) method were measured spectrophotometrically. Method based on malondialdehyde (MDA) and TBA reactivity which is the aldehyde product of lipid peroxidation. Results were expressed as the amount of MDA per mg protein. Determination of GSH-Px, (Cayman, GSH-Px measurement kit, U.S.A) CAT (Cayman, Catalase measurement kit, U.S.A) and SOD activities (Cayman, Superoxide Dismutase measurement kit, U.S.A) were performed according to the manufacturers' recommendations. The results obtained were compared with control.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation. Differences in continuous variables between the study and control groups were investigated with the Kruskal-Wallis test. Differences in antioxidant enzyme activity values within the groups according to the time were measured using the Friedman test. p values <0.05 were considered statistically significant. The "Statistical Package for Social Sciences (SPSS) version 15.0 program" was used for statistical analysis.

3. Results

In this study; 2, 25, 50, 100, 250, 500, 750, 1000 and 1500 µM doses of aspirin were given to endothelial cells in 2 of 4 wells in each row of 24 well microplate, and the other two wells were used as controls. Accordingly, while the CAT, SOD, GSH-Px and lipid peroxidation measurements in cell lysates were being performed at basal conditions (0 hour), 24th hour, 48th and 72nd hours, the release of NO were measured from the cell medium. For the measurement of MTT; 25, 50, 100, 250, 500, 750, 1000 and 1500 µM aspirin doses given to cells in three wells of 96 wells microplate 3 wells served as control wells. Absorbance measurements were done at 0, 24th, 48th and 72th hours.

MTT measurements

The toxic dose level which is determined by decrease in absorbance by 50% compared to control group by MTT analysis was found as 1500 µM in analysis at 0, 24th and 72nd hours and it was found as 1000 µM in 48th hour analysis (Table 1, Fig. 1).

Morphologic evaluation of vascular endothelial cells to which 25, 50, 100, 250, 500, 750, 1000 and 1500 µM aspirin were given did not show any visible toxic effect between drug groups and controls. The observed finding is the better opening of the cells especially in higher doses such as 1000 µM and 1500 µM compared to lower doses.

Table 1. Absorbance values measured in different aspirin doses and controls according to time

Dose	0. Hour	24th Hour	48th Hour	72nd Hour
Control	0.394	0.685	1.776	2.157
25 µM	0.333	0.814	1.987	2.246
50 µM	0.332	0.669	1.897	2.266
100 µM	0.231	0.103	1.547	2.357
250 µM	0.222	0.644	1.549	1.437
500 µM	0.418	0.407	1.321	1.749
750 µM	0.257	0.558	1.327	1.484
1000 µM	0.289	0.508	0.857	1.42
1500 µM	0.117	0.187	0.841	0.779

Evaluation of the catalase activity

The catalase levels measured at basal (0 hour) conditions were not significantly different between the control wells and the wells that different dosages of drugs were given (p>0.05). Additionally, the catalase levels measured at 24th hour (p>0.05), 48th hour (p>0.05) and 72nd hour (p>0.05) were not different between the control and different dosages of drugs.

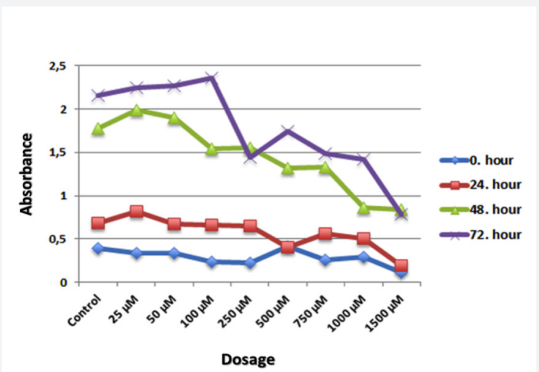


Fig. 1. Variation of absorbance values according to different aspirin doses

When the catalase levels measured at 0, 24th, 48th and 72nd hours from each well to which 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs given were compared, they did not show difference according to time (p>0.05). The significant difference was determined in catalase levels measured at 0, 24th, 48th and 72nd hours in control groups to which aspirin was not given (p<0.05). The data related to catalase activities measured at different time and dosages were presented in Table 2 and Figure 2.

Evaluation of the superoxide dismutase activity

SOD levels measured at basal (0 hour) conditions were not significantly different between the control wells and the wells that 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs were given (p=0.494). Additionally, the SOD levels measured at 24th hour (p>0.05), 48th hour (p>0.05) and 72nd hour (p>0.05) were not different between the control and different dosages of drugs.

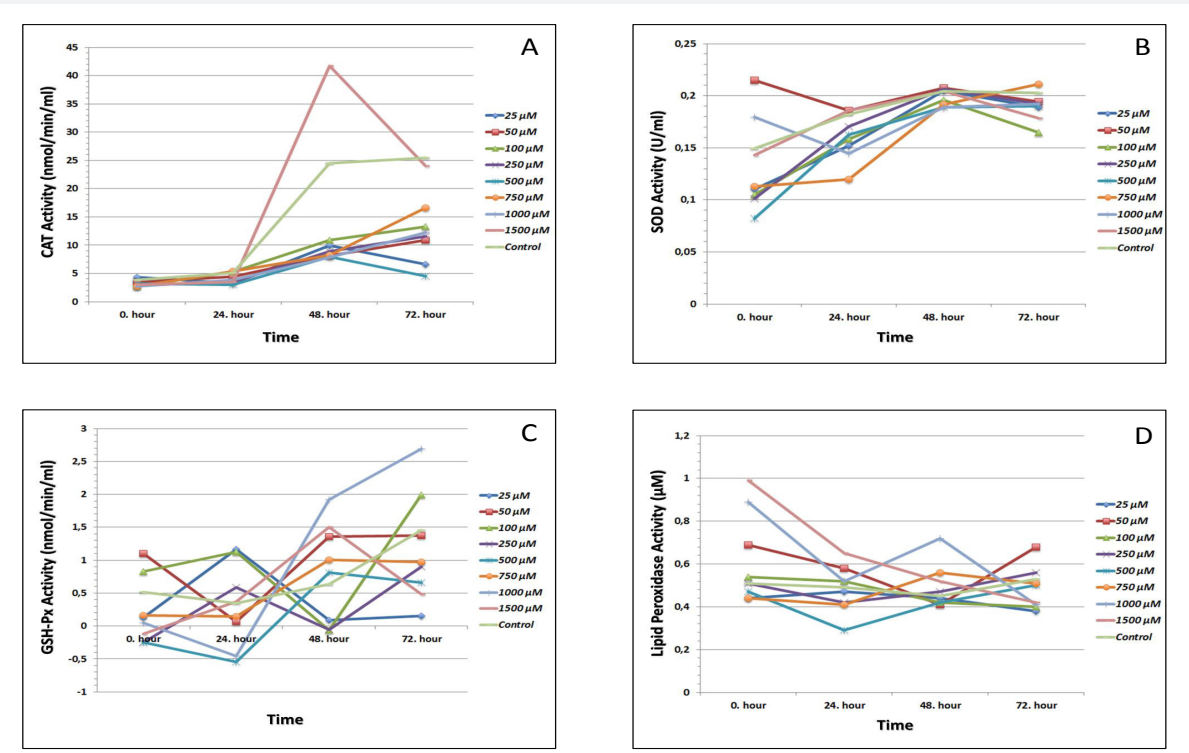


Fig. 2. Change of antioxidant enzyme activitiy. **2A:** Change of catalase (CAT) activity measured at different drug doses and controls by time; **2B:** Change of superoxide dismutase (SOD) activity measured at different drug doses and controls by time; **2C:** Change of glutathione peroxidase (GSH-Px) activity measured at different drug doses and controls by time; **2D:** Change of lipid peroxidase activity measured at different drug doses and controls by time

When the SOD levels measured at 0, 24th, 48th and 72nd hours from each well to which 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs given were compared, they did not show difference according to time ($p>0.05$). The significant difference was determined in SOD levels measured at 0, 24th, 48th and 72nd hours in control groups to which aspirin was not given ($p<0.05$). The data related to SOD activities measured at different time and dosages were presented in Table 3 and Figure 2B.

Evaluation of the glutathione peroxidase activity

The glutathione peroxidase (GSH-Px) levels measured at basal (0 hour) conditions were not significantly different between the control wells and the wells that 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs were given ($p>0.05$). Additionally, the GSH-Px levels measured at 24th hour ($p>0.05$), 48th hour ($p>0.05$) and 72nd hour ($p>0.05$) were not different among to the control and different dose groups.

When the GSH-Px levels measured at 0, 24th, 48th and 72nd hours from each well to which 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs given were compared, they did not show difference according to time ($p>0.05$). The significant difference was determined in GSH-Px levels measured at 0, 24th, 48th and 72nd hours in control groups to which Aspirin was not given ($p<0.05$) (Fig. 2C). The data related to GSH-Px activities measured at different time and dosages were presented in Table 4.

Table 2. The catalase activity measured at 0, 24th, 48th, and 72nd hours in different aspirin doses and controls (nmol/min/ml)

Dosages	0. hour	24th hour	48th hour	72nd hour	p value
25 µM (n=2)	4.373 ± 2.295	3.291 ± 0.765	9.953 ± 5.595	6.594 ± 1.899	> 0.05
50 µM (n=2)	3.515 ± 0.073	4.478 ± 0.289	8.105 ± 1.780	10.893 ± 2.176	> 0.05
100 µM (n=2)	2.750 ± 0.000	5.307 ± 3.616	10.926 ± 4.120	13.240 ± 3.379	> 0.05
250 µM (n=2)	2.909 ± 0.615	3.468 ± 0.006	8.865 ± 0.709	11.523 ± 4.172	> 0.05
500 µM (n=2)	3.160 ± 0.000	3.030 ± 0.395	7.977 ± 3.327	4.504 ± 1.583	> 0.05
750 µM (n=2)	2.726 ± 0.350	5.380 ± 0.007	8.193 ± 0.767	16.599 ± 0.193	> 0.05
1000 µM (n=2)	2.750 ± 0.000	3.851 ± 1.557	7.826 ± 1.108	12.230 ± 1.739	> 0.05
1500 µM (n=2)	3.030 ± 0.395	3.477 ± 1.028	41.685 ± 11.504	24.025 ± 12.197	> 0.05
Control (n=16)	3.892 ± 2.409	5.145 ± 2.812	24.496 ± 10.023	25.489 ± 22.013	< 0.05
***p* value**	0.571	0.696	0.249	0.118	

Table 3. The SOD activity measured at 0, 24th, 48th, and 72nd hours in different aspirin doses and controls (U/ml)

Dosages	0. hour	24th hour	48th hour	72nd hour	p value
25 µM (n=2)	0.11026 ± 0.06867	0.15190 ± 0.05561	0.20460 ± 0.15555	0.18910 ± 0.01711	> 0.05
50 µM (n=2)	0.21464 ± 0.10142	0.18601 ± 0.01054	0.20755 ± 0.01441	0.19408 ± 0.01081	> 0.05
100 µM (n=2)	0.10485 ± 0.08803	0.15838 ± 0.03368	0.19597 ± 0.14750	0.16485 ± 0.07177	> 0.05
250 µM (n=2)	0.10149 ± 0.03956	0.17064 ± 0.06477	0.20619 ± 0.00642	0.19199 ± 0.00772	> 0.05
500 µM (n=2)	0.08213 ± 0.01707	0.16235 ± 0.07035	0.18895 ± 0.03768	0.18990 ± 0.36345	> 0.05
750 µM (n=2)	0.11296 ± 0.05007	0.11981 ± 0.00704	0.19185 ± 0.00777	0.21092 ± 0.00533	> 0.05
1000 µM (n=2)	0.17940 ± 0.00339	0.14435 ± 0.04886	0.18885 ± 0.00063	0.19230 ± 0.03295	> 0.05
1500 µM (n=2)	0.14318 ± 0.09604	0.18600 ± 0.03945	0.20408 ± 0.01482	0.17845 ± 0.00063	> 0.05
Control (n=16)	0.14913 ± 0.06912	0.14913 ± 0.06912	0.18193 ± 0.02845	0.20430 ± 0.01358	< 0.05
***p* value**	0.494	0.565	0.566	0.659	

SOD: Superoxide dismutase

Table 4. The GSH-Px activity measured at 0, 24th, 48th, and 72nd hours in different aspirin doses and controls (nmol/min/ml)

Dosages	0. hour	24th hour	48th hour	72nd hour	p value
25 µM (n=2)	0.130285 ± 0.276797	1.164839 ± 4.044800	0.088341 ± 0.771087	0.158243 ± 0.434973	> 0.05
50 µM (n=2)	1.100569 ± 1.055960	0.063526 ± 1.020622	1.354878 ± 0.553523	1.376815 ± 2.687833	> 0.05
100 µM (n=2)	0.829307 ± 2.174858	1.122900 ± 0.810627	-0.065442 ± 0.395426	1.989691 ± 1.799198	> 0.05
250 µM (n=2)	-0.250178 ± 0.686040	0.589871 ± 0,229931	-0.052255 ± 0.744080	0.900379 ± 0.105861	> 0.05
500 µM (n=2)	-0.245208 ± 0.830400	-0.540780 ± 0.316345	0.815326 ± 1.324688	0.661543 ± 0.790856	> 0.05
750 µM (n=2)	0.163651 ± 0.128836	0.148060 ± 1.093653	1.003614 ± 0.892688	0.972225 ± 0.701923	> 0.05
1000 µM (n=2)	0.046401 ± 0.039542	-0.456900 ± 0.316345	1.919788 ± 2.609826	2.688716 ± 0.296571	> 0.05
1500 µM (n=2)	-0.121365 ± 0.158170	0.367952 ± 0.217485	1.500373 ± 1.937598	0.479794 ± 1.680572	> 0.05
Control (n=16)	0.519243 ± 0.821678	0.346849 ± 0.781278	0.633676 ± 1.265019	1.451028 ± 0.89589	< 0.02
***p* value**	0.624	0.513	0.590	0.375	

GSH-Px: Glutathione peroxidase

Evaluation of the nitric oxide activity

In the analysis performed at the wells that drugs given and control wells in which drugs were not given, the quantitative NO activity measurement in significant proportion of the samples could not be done because of the low level of activity. The analysis of present measurements revealed that; The nitric oxide levels measured at basal (0 hour) the conditions were not significantly different between the control wells and the wells that 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs were given (p=0.406). Additionally, the NO levels measured at 24th hour (p=0.392), 48th hour (p=0.392) and 72nd hour (p=0.416) were not different between the control and different dosages of drugs.

Table 5. The NO activity measured at 0, 24th, 48th, and 72nd hours in different aspirin dosages and controls (µM)

Dosages	0. hour	24th hour	48th hour	72nd hour
25 µM (n=2)	2.350	4.138	3.021	3.759
50 µM (n=2)	-	-	-	3,349
100 µM (n=2)	3.691	3.244	3.244	3.485
250 µM (n=2)	2.126	-	-	-
500 µM (n=2)	3.468	3.691	3.468	3.622
750 µM (n=2)	-	-	-	3.349
1000 µM (n=2)	3.244	3.691	3.759	3.554
1500 µM (n=2)	-	-	-	-
Control (n=16)	-	-	-	-
***p* value**	> 0.05	> 0.05	> 0.05	> 0.05

NO: Nitric oxide

The analysis of NO activity measurements obtained from samples to which different drug dosages given and controls to find out whether there was any difference between different time intervals could not be done because of the lack of data. The data related to NO activity measured at different time and dosages were presented in Table 5.

Evaluation of the lipid peroxidase activity

The lipid peroxidase levels measured at basal (0 hour) conditions were not significantly different between the control wells and the wells that 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs were given (p>0.05). Additionally, the lipid peroxidase levels measured at 24th hour (p>0.05), 48th hour (p>0.05) and 72nd hour (p>0.05) were not different between the control and different dosages of drugs.

When the lipid peroxidase levels measured at 0, 24th, 48th and 72nd hours from each well to which 25, 50, 100, 250, 500, 750, 1000 and 1500 µM drugs given were compared, they did not show difference according to time (p>0.05). The data related to lipid peroxidase activity measured at different time and dosages were presented in Table 6 and Figure 2D.

4. Discussion

Oxidative stress that is derived from reactive oxygen species causes cardiovascular tissue damage associated with cardiac and vascular myocytes. Oxidative stress plays an important role in various cardiovascular diseases such as atherosclerosis, ischemic heart disease,

Table 6. The lipid peroxidase activity measured at 0, 24th, 48th, and 72nd hours in different aspirin dosages and controls (μM)

Dosages	0. hour	24th hour	48th hour	72nd hour	p value
25 μM (n=2)	0.44 ± 0.01	0.47 ± 0.01	0.44 ± 0.10	0.38 ± 0.01	> 0.05
50 μM (n=2)	0.69 ± 0.32	0.58 ± 0.27	0.41 ± 0.12	0.68 ± 0.33	> 0.05
100 μM (n=2)	0.54 ± 0.05	0.52 ± 0.08	0.42 ± 0.07	0.40 ± 0.02	> 0.05
250 μM (n=2)	0.51 ± 0.18	0.42 ± 0.03	0.47 ± 0.15	0.56 ± 0.14	> 0.05
500 μM (n=2)	0.47 ± 0.01	0.29 ± 0.10	0.42 ± 0.07	0.50 ± 0.02	> 0.05
750 μM (n=2)	0.44 ± 0.07	0.41 ± 0.02	0.56 ± 0.03	0.51 ± 0.04	> 0.05
1000 μM (n=2)	0.89 ± 0.63	0.52 ± 0.18	0.72 ± 0.07	0.41 ± 0.17	> 0.05
1500 μM (n=2)	0.99 ± 0.30	0.65 ± 0.02	0.52 ± 0.27	0.42 ± 0.22	> 0.05
Control (n=16)	0.51 ± 0.10	0.49 ± 0.12	0.45 ± 0.11	0.53 ± 0.13	> 0.05
***p* value**	0.383	0.362	0.25	0.477	

hypertension, cardiomyopathy, cardiac hypertrophy, and congestive heart disease (Valko et al., 2007). Singal et al. (1983) and Hess et al. (1983) showed the importance of increased oxygen free radicals in pathogenesis of many heart disease in vitro and in vivo studies.

Complications of atherosclerosis are the basis of cardiovascular diseases (Singh and Jialal, 2006). Endothelial dysfunction is seen in the early stages of atherogenesis as well. Damaged endothelial function produces even more dangerous results when combined with several risk factors. Setting up a ground for the long-term atherosclerotic lesions by endothelial dysfunction is one of these results, and this condition is important in the diagnosis of coronary syndromes. (Vogiatzi et al., 2009).

Aspirin treatment is important in heart diseases caused by vascular problems especially atherosclerosis. Aspirin treatment is effective in reducing recurrent events in patients with one or more of the risk factors like hyperlipidemia, hypertension, diabetes and smoking, as well as patients who had coronary or other vascular events (stroke, peripheral vascular disease).

Most of the risk factors associated with atherosclerosis and cardiovascular morbidity and mortality are also associated with endothelial dysfunction. Many of the risk factors including hyperlipidemia, hypertension, diabetes, and cigarette smoking are associated with excessive production of reactive oxygen species and increased oxidative stress (Hink et al, 2001; Channon and Guzik, 2002).

In this study, aspirin was given to endothelial cells in different doses and the changes in CAT, SOD, GSH-Px lipid peroxidase and NO activities with respect to time, and the differences between the doses were investigated. The doses of aspirin given to cells were determined by MTT assay (McGahon et al., 1995). In the MTT analysis, the dose of aspirin leading to 50% decrease in absorbance compared to the control group was determined as cytotoxic dose. The cytotoxic doses were determined as 1500 μM at 0, 24th and 72nd hour analysis, 1000 and 1500 μM at 48th hour analysis, respectively. In this way, the number of demaged cells was evaluated by MTT assay.

When the CAT, SOD and GSH-Px enzyme activities were evaluated; there was a significant increase in activity when the enzyme activity results of control group at 0 and 24th hours compared to enzyme activity 48th and/or 72nd hours. The changes in the level these three enzyme activities over time measured at the cells to which aspirin 25, 50, 100, 250, 500, 1000 and 1500 μM given were not statistically significant. A remarkable, but not statistically significant increase in CAT activity at 48th and 72nd hours was observed only in the cells to which 1500 μM aspirin given. Additionally, there was no difference between the control and groups to which different drug doses given regarding the enzyme activities measured at 0, 24th, 48th and 72nd hours.

Endothelial cell proliferation in cell culture over time increases the amount of free radicals in the environment and at the same time increases the activities of CAT, SOD and GSH-Px enzymes which are enzymatic indicators of the reactive antioxidant defense. Increased enzyme activity at 72nd hour in the control group to which aspirin was not given can be explained by this hypothesis. Statistically insignificant increase in the CAT activity at the 48th and 72nd hours in the cells to which 1500 μM aspirin given can be explained by toxic effect of this dose which was expected to be protective aspirin dose.

In control groups, the increase in enzyme activities over time suggests that the presence of protective effect of aspirin dose which is less than 1500 μM given to endothelial cells.

The basic mechanism of aspirin is inactivation of a key enzyme cyclooxygenase resulting in inhibition of prostaglandin synthesis. This explains the analgesic, anti-inflammatory, antipyretic and inhibition of platelet aggregation (Vargaftig, 1978). Our study showing the potential beneficial effects of aspirin on oxidative processes at cellular level is supported by a large number of clinical investigations associated with this process. Grosser and Schröder (2003) reported that aspirin showed a cell protective effect by protecting the endothelial cells from oxidative damage in the presence of NO and also Taubert et al. (2004) stated the aspirin caused an increased NO secretion from endothelial cells and this effect increased the activity of SOD

enzyme which catalyzes the dismutation of superoxide radicals.

In addition to the classical platelet inhibition effect of aspirin, it provides the protection of vascular endothelium and therefore, it was shown that it is quite effective in the prevention of occlusive cardiovascular and cerebrovascular diseases and on the damages occurred in the beginning of atherosclerosis (Watala and Gwozdzinski, 1993).

Podhaisky et al. (1997) in their study showed that oxidative stress is a cardiovascular risk factor and an important source of endothelial damage during atherogenesis and oxygen radicals released by neutrophils and monocytes had direct damage to the endothelium. Also they stated that aspirin provides endothelial integrity and thus protecting the antithrombotic and anti-atherogenic functions of endothelium under oxidative stress. Clinical studies showed that low-dose aspirin caused endothelium-dependent arterial relaxation which is the basis of vascular homeostasis (Taubert et al., 2004).

Metal binding property of aspirin is also determined. With this feature, it can take the iron ions from the area where oxygen radicals formed. Therefore; compared to the hydrogen peroxide-dependent cell damage, aspirin had been suggested to pretend to be 10 times more potent cell protector (Podhaisky et al., 1997).

There are evidences about the reduction in living endothelial cells which exposed to hydrogen peroxide. Studies performed with aspirin demonstrated that, aspirin (3-30 mol/L) protects cells from cytotoxicity in a concentration-dependent manner. This suggested that the aspirin has a capacity to protect the endothelial cells from harmful effect hydrogen peroxide and also has a property of free radical scavenger (Grosser and Schröder, 2003).

The oxygen radicals show reactions with unsaturated lipids and initiate the lipid peroxidation reactions on membranes. Beside this, free radicals cause oxidation of sulfhydryl groups of proteins and the nucleic acids strands. Interaction of membrane lipids by free oxygen radicals initiates lipid peroxidation chain reactions and causes deterioration of the membrane structure, increase in the permeability of cell, loss of cellular ion gradient and leads to tissue damage (Jackson et al., 2002). Lipid peroxidation starts as a chain reaction form and generates an uninterrupted source for the free radicals that further initiate peroxidation. This self-perpetuating chain reactions cause irreversible damage to the cell membrane. Lipid peroxidation in cell membranes causes membrane lipoproteins oxidation and a loss of structural integrity and with the entry of abnormal ion causes cell death. When this event cannot be controlled, chain reactions occur and it causes spread of cellular death (Srivastava et al., 1989). As a result, cell membranes losses semi-permeable properties. The enzyme systems located in the cell membrane fail to do transport function. Thus, the intra-and extracellular densities of various compounds, inorganic substances and electrolytes which enter and exit the cell by using the active transport and other transport systems change. All these events bring about damage to the cells that are difficult to reverse back and this can result in the death of the cell (Srivastava et al., 1989).

The aspirin's antioxidant effects which reduce vascular tone and suppress lipid peroxidation were shown at the clinical level (Grosser and Schröder, 2003). Associated to this condition, according to the data of our study at the cellular level, there was no difference in lipid peroxidase activity measured at 0, 24th, 48th and 72nd hours between control group and the cells to which various doses of aspirin given. Moreover, there was no change in lipid peroxidase activity over time in both the control group and cells to which the different doses of aspirin given.

NO and oxygen radicals production occur simultaneously, or a radical type production can be accelerated by the effects caused by other radical type in pathological conditions (Tabima et al., 2012). Immediate removal of H_2O_2 produced in biological systems is required because of the oxidizing properties. This task is carried by CAT and GSH-Px which are important antioxidant enzymes in cells (Mohazzab-H et al., 1999).

NO synthesized by endothelial cells passes through the smooth muscle cells by diffusion and activates guanylate cyclase and, increases the level of cGMP, causes the smooth muscle relaxation. Because the activation of eNOS ends by reduction in the concentration of cytoplasmic calcium by calcium pumps, eNOS provides a low concentration of NO synthesis in a short period of time (Tabima et al., 2012). Endothelial cells in culture spontaneously release NO which is responsible for a physical and chemical stimulation. However, the NO-release is not against to acetylcholine, and it is thought that this is caused by either loss of acetylcholine receptors or loss of receptor-effector effect in cell culture (Calver et al., 1993).

Superoxide radicals in blood vessels are synthesized continuously as a constitutive. Thus, superoxide by controlling the concentration of NO in blood vessels regulates its vasodilator effect. In the case of inhibition of SOD enzyme by diethyldithiocarbamate in blood vessels, the concentration of superoxide increases ten times and NO-dependent relaxation is inhibited. In various vascular diseases, by the increased production of superoxide, the capacity of SOD enzyme can be exceeded and nitric oxide is inactivated by superoxide. Thus, the superoxide produced at normal levels regulates concentration of NO and its effect, the high concentration in pathological conditions both prevents functions of NO, and causes oxidative damage by

formation of an oxidative type (peroxynitrite) (Wolin, 1991).

Endothelium-derived NO plays a role in the physiological regulation of blood pressure, blood flow and vascular tone in different organs. NO-release can be induced by various receptor dependent (acetylcholine, bradykinin, histamine, and serotonin adenine nucleotides) and independent (free fatty acids) agonists. Endothelial cells convert physical pressure of circulating blood applied to endothelium to biochemical signals (Büssemaker et al., 2007).

The acetyl group of aspirin increases the eNOS synthesis and the usefulness (Bulckaen et al., 2008). This also indicates that it provides the protection of the cell caused by NO. NO like aspirin decreases the sensitivity of endothelial cells to H_2O_2 and other oxidizing agents. Both aspirin and NO have long-term protective effect on endothelial cells (Grosser and Schröder, 2003).

In this study, the analysis of quantitative measurement of NO activity could not be performed in some samples from the control cells and cells to which aspirin given due to the lack of activity. There was no significant difference between the NO levels measured at 0, 24th, 48th and 72nd hours from the control wells and the wells to which different doses of aspirin given. The analysis to show the change in NO activity at different time intervals in the control samples and the samples to which different drug doses given could not be performed due to lack of data.

The most important limitation of this study is that especially the scarcity of examples in different doses of aspirin prevented the evaluation of the data such as NO and to reach the expected level of significance.

In conclusion, increase in the activity of antioxidant enzymes is caused by production of free radicals in the environment due to increased cell number over time (after 48 hours) in endothelial cells. This average result might lead us to think that administration of aspirin doses of 1000 μM or less might have a cell protective effect and administration of aspirin doses over 1000 μM would result in a toxic effect causing reduction in the number of cells.

REFERENCES

Antiplatelet Trialists Collaboration, Collaborative overview of randomised trials of antiplatelet therapy I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients, 1994. 308, 81-106.

Bulckaen, H., Prévost, G., Boulanger, E., Robitaille, G., Roquet, V., Gaxatte, C., Garçon, G., Corman, B., Gosset, P., Shirali, P., Creusy, C., Puisieux, F., 2008. Low-dose aspirin prevents age-related endothelial dysfunction in a mouse model of physiological aging. Am. J. Physiol. Heart Circ. Physiol. 294, 1562-1570.

Büssemaker, E., Pistrosch, F., Förster, S., Herbrig, K., Gross, P., Passauer, J., Brandes, R.P. 2007. Rho kinase contributes to basal vascular tone in humans: Role of endothelium-derived nitric oxide. Am. J. Physiol. Heart Circ. Physiol. 293, 541-547.

Calver, A., Collier, J., Vallance, P., 1993. Nitric oxide and cardiovascular control. Exp. Physiol. 78, 303-326.

Channon, K.M., Guzik, T.J., 2002. Mechanisms of superoxide production in human blood vessels: Relationship to endothelial dysfunction, clinical and genetic risk factors. Physiol. Pharmacol. 53, 515-524.

Grosser, N., Schröder, H., 2003. Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway. Arterioscler Thromb. Vasc. Biol. 23, 1345-1351.

Hess, M.L., Krause, S., Kontos. H.A., 1983. Mediation of sarcoplasmic reticulum disruption in the ischemic myocardium: Proposed mechanism by the interaction of hydrogen ions and oxygen free radicals. Adv. Exp. Med. Biol. 161, 377-389.

Hink, U., Li. H., Mollnau, H., Oelze, M., Matheis, E., Hartmann, M., Skatchkov, M., Thaiss, F., Stahl, R.A., Warnholtz, A., Meinertz, T., Griendling, K., Harrison, D.G., Forstermann, U., Munzel, T., 2001. Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ. Res. 88, 14-22.

Irani, K., 2000. Oxidant signaling in vascular cell growth, death, and survival: A review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. Circ. Res. 87, 179-183.

Jackson, M.J., Papa, S., Bolaños, J., Bruckdorfer, R., Carlsen, H., Elliott, R.M., Flier, J., Griffiths, H.R., Heales, S., Holst, B., Lorusso, M., Lund, E., Øivind Moskaug, J., Moser, U., Di Paola, M., Polidori, M.C., Signorile, A., Stahl, W., Viña-Ribes, J., Astley, S.B., 2002. Antioxidants, reactive oxygen and nitrogen species, gene induction and mitochondrial function. Mol. Aspects. Med. 23, 209-285.

Kunsch, C., Medford, R.M., 1999. Oxidative stress as a regulator of gene expression in the vasculature. Circ. Res. 85, 753-766.

Matés, J.M., Pérez-Gómez, C., Núñez de Castro, I., 1999. Antioxidant enzymes and human diseases. Clin. Biochem. 32, 595-603.

Matés, J.M., Pérez-Gómez, C., Olalla, L., Segura, J.M., Blanca, M., 2000. Allergy to drugs: Antioxidant enzymic activities, lipid peroxidation and protein oxidative damage in human blood. Cell Biochem. Funct. 18, 77-84.

McGahon, A.J., Martin, S.J., Bissonnette, R.P., Mahboubi, A., Shi, Y., Mogil, R.J., Nishioka, W.K., Green, D.R. 1995. The end of the (cell) line: Methods for the study of apoptosis in vitro. Methods Cell Biol. 46, 153-185.

Mohazzab-H, K.M., Agarwal, R., Wolin, M.S., 1999. Influence of glutathione peroxidase on coronary artery responses to alterations in PO_2 and H_2O_2. Am. J. Physiol. 276, 235-241.

Nedeljkovic, Z.S., Gokce. N., Loscalzo, J., 2003. Mechanisms of oxidative stress and vascular dysfunction. Postgrad. Med. J. 79, 195-199.

Podhaisky, H.P., Abate, A., Polte, T., Oberle, S., Schröder, H., 1997. Aspirin protects endothelial cells from oxidative stresspossible synergism with vitamin E. FEBS Lett. 417, 349-351.

Singal, P.K., Beamish, R.E., Dhalla, N.S., 1983. Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. Adv. Exp. Med. Biol. 161, 391-401.

Singal, P.K., Khaper, N., Palace, V., Kumar, D., 1998. The role of oxidative stress in the genesis of heart disease. Cardiovasc. Res. 40, 426-432.

Singh, U., Jialal, I., 2006. Oxidative stress and atherosclerosis. Pathophysiology. 13, 129-142.

Srivastava, S.K., Ansari, N.H., Liu, S., Izban, A., Das, B., Szabo, G., Bhatnagar, A., 1989. The effect of oxidants on biomembranes and cellular metabolism. Mol. Cell Biochem. 91, 149-157.

Tabima, D.M., Frizzell, S., Gladwin, M.T., 2012. Reactive oxygen and nitrogen species in pulmonary hypertension. Free Radic. Biol. Med. 52, 1970-1986.

Taubert, D., Berkels, R., Grosser, N., Schröder, H., Gründemann, D., Schömig, E., 2004. Aspirin induces nitric oxide release from vascular endothelium: A novel mechanism of action. Br. J. Pharmacol. 143, 159-165.

Valko, M., Leibfritz, D., Moncol, J., Cronin M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44-84.

Vargaftig, B.B., 1978. The inhibition of cyclo-oxygenase of rabbit platelets by aspirin is prevented by salicylic acid and by phenanthrolines. Eur. J. Pharmacol. 50, 231-241.

Varshney, R., Kale, R.K., 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol. 58, 733-743.

Vogiatzi, G., Tousoulis, D., Stefanadis, C., 2009. The role of oxidative stress in atherosclerosis. Hellenic. J. Cardiol. 50, 402-409.

Watała, C., Gwoździński, K., 1993. Effect of aspirin on conformation and dynamics of membrane proteins in platelets and erythrocytes. Biochem. Pharmacol. 45, 1343-1349.

Wolin, M.S., 1991 Activated oxygen metabolites as regulators of vascular tone. Klin. Wochenschr. 69, 1046-1049.

Yapislar, H., Taskin, E., Ozdas, S., Akin, D., Sonmez, E., 2016. Counteraction of apoptotic and ınflammatory effects of adriamycin in the liver cell culture by clinopitolite. Biol. Trace Elem. Res. 170, 373-381.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2016; 33(3):139-143
doi: 10.5835/jecm.omu.33.03.003

Assessment of atrial electromechanical delay and left atrial mechanical functions in chronic kidney disease

Gokay Nar[a*], Aydin Guclu[b], Sinan Inci[c], Gokhan Aksan[d], Atilla Icli[a], Rukiye Nar[e]

[a] *Department of Cardiology, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey*
[b] *Department of Nephrology, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey*
[c] *Department of Cardiology, Aksaray State Hospital, Aksaray, Turkey*
[d] *Department of Cardiology, Sisli Etfal Education and Research Hospital, Istanbul, Turkey*
[e] *Department of Biochemistry, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey*

ARTICLE INFO

Article History
Received 01 / 05 / 2016
Accepted 18 / 05 / 2016

*** Correspondence to:**
Gokay Nar
Department of Cardiology,
Faculty of Medicine,
Ahi Evran University,
Kirsehir, Turkey
e-mail: gokay_nar@yahoo.com

Keywords:
Atrial electromechanical delay
Atrial fibrillation
Echocardiography
End-stage renal disease

ABSTRACT

The risk of atrial fibrillation (AF) development was revealed to be increased in patients with end-stage renal disease (ESRD) Elongation of the time of atrial electromechanical delay (AEMD) is a famous typical of the atrium. AEMD is a risk factor for AF development and it could be associated with chronic kidney disease (CKD). The aim of our study is to examine mechanical functions of the left atrium (LA) and AEMD times in ESRD. A total of 86 participant, 46 with ESRD and 40 as the control group, were included in the study. The demographical and laboratory information were documented. Echocardiographic dimensions were achieved in all patients. Left atrial mechanical functions and AEMD durations were calculated. Demographic and laboratory characteristics of the groups were similar except the mean diastolic blood pressure, hemoglobin, creatinine, glucose, uric acid, calcium and potassium levels. The echocardiographic assessment exposed that the ventricular septal thickness (12.7±1.5 vs. 10.4±1.5, $p<0.001$), posterior wall thickness (12.6±1.6 vs. 10.1±1.9, $p<0.001$), LA dimension (40.9±5.3 vs.34.6±2.6, $p<0.001$) and diastolic parameters decreased in the ESRD group when compared to the control group; also, LA volumes, mechanical functions, inter atrial EMD (33.2±9.1 vs. 22.7±7.7, $p<0.001$), intra-right-EMD (18.5±7.7 vs. 13.2±6.4, $p=0.001$) and intra-left-EMD (18.5±7.7 vs. 13.7±5.7, $p=0.002$) were also different between groups. ($p<0.005$) The correlation analysis showed that serum ferritin levels were correlated with AEMD. We found deteriorated LA functions and elongation in the times of AEMD in the ESRD group compared with the control group. Additionally, we found positive correlation between ferritin levels and AEMD. This result show that AEMD might be used to predict the risk of development of AF in patients with ESRD.

1. Introduction

Atrial fibrillation (AF) is the most often faced arrhythmia in clinical practice and is a major cause of ischemic stroke (Go et al., 2001). In some studies, the risk of AF development was revealed to be increased in patients with end-stage renal disease (ESRD) who are in a haemodialysis program (Korantzopoulos and Goundevenos, 2009). Also AF was shown to increase cardiovascular mortality by complicating patients with chronic kidney disease (CKD). Increased

atrial electromechanical delay (AEMD) reproduces heterogeneity of inter and intra-atrial conductivity and is a risk factor for AF development. At this time, AEMD may be measured during echocardiography non-invasively, which produce similar results to invasive electrophysiological study (Deniz et al., 2012).

Inter-atrial and intra-atrial conduction durations were shown to be increased by age and many systemic sicknesses such as diabetes and hypertension which were exposed to be conventional risk factors for AF (Emiroglu et al., 2011); however, effects of ESRD on atrial electromechanical functions were not investigated adequately. The aim of our study was to evaluate AEMD durations measured by echocardiography in patients with ESRD.

2. Methods

Study population

A total of 46 patients with Group 1, aged over 18 years, who were on a regular haemodialysis program at the nephrology clinic of Kırşehir Ahi Evran University. Healthy people who were admitted to the cardiology outpatient due to any symptom matched for age and sex were involved in this study as Group 2. ESRD patients getting haemodialysis treatment 3 times a week for at least 1 year in our institution. These patients had been experiencing an average haemodialysis program (500 mL/min dialysate flow; 250 mL/min blood flow; 4 hours of per treatment session). The study was shown in agreement with the ethical principles described by the Declaration of Helsinki (Williams, 2008)

Exclusion criteria of this study were as follows: patients having diabetes mellitus (DM), documented coronary artery disease (CAD), valvular heart disease of moderate severity, myocarditis, pericarditis, any cardiovascular drug use, rhythms other than sinus, significant valvular heart disease, chronic obstructive pulmonary disease, hepatic dysfunction, hyperthyroidism.

Investigation planning and measurements:

Firstly, demographic data of patients who were entitled to be involved in the study and signed an knowledgeable agreement form were noted. Group 1 examinations were conducted on the days when they did not receive haemodialysis to get volume standardization.

Echocardiographic evaluation

Echocardiography was achieved by a GE VingMed Vivid 7 (GE VingMed Ultrasound, Horten, Norway) Parasternal long-axis, short-axis, and apical four-chamber and two-chamber images were occupied and the calculation was completed using M-mode, 2D, continuous wave Doppler, pulsed wave Doppler, and tissue Doppler methods (Quinones et al., 2002). Posterior wall (PW) thickness, interventricular septum (IVS) thickness, left ventricular end-diastolic diameter (LVEDD) and left ventricular diameter (LVESD) were considered using M-mode method. The modified Simpson method was used to calculate left ventricular ejection fraction (EF)

Evaluating left atrial (LA) mechanical functions by echocardiography

The LA volumes were intended from the four- and two-chamber views, using Simpson's rule. LA maximum volume (Vmax) was noted just when the mitral valve was opened and LA minimum volume (Vmin) was noted just when the mitral valve was closed; LA presystolic volume (Vp) was noted at the opening of atrial systole p wave on ECG. All LA volumes were modified for body surface area (BSA).

LA emptying functions were intended as follows:

LA passive emptying volume (LAPEV): Vmax-Vp

LA passive emptying fraction (LAPEF): LAPEV/ Vmax

LA active emptying volume (LAAEV) Vp-Vmin

LA active emptying fraction (LAAEF): LAPEF / Vp

LA total emptying volume (LATEV)=Vmax-Vmin

(Aydın et al., 2004).

Interatrial and intraatrial electromechanical delay

All EMD times used to measure interatrial and intraatrial electromechanical delay was determined by the tissue Doppler imaging (TDI) method and simultaneous electrocardiographic rhythm traces. Atrial systole was measured to be the A wave (A), which was the second negative deviation at diastole. The time interval between the start of the P wave in the superficial ECG and the highest of late diastolic wave (Am wave) was distinct as atrial electromechanical coupling (PA), although the measurements were occupied from the lateral mitral annulus (lateral PA), septal annulus (septal PA), and right ventricular tricuspid annulus (tricuspid PA). Interatrial and intraatrial electromechanical delays were calculated (Dabrowska-Kugacka et al., 2009):

- Interatrial electromechanical delay (Interatrial EMD): Time difference between lateral PA and tricuspid PA; and
- Intraatrial electromechanical delay (Intraatrial EMD): Time difference between septal PA and tricuspid PA.

Statistical analysis

All data were analysed using SPSS for Windows version 15.0 software (Chicago, IL, USA). Categorical variables were obtainable as frequencies and percentages; continuous variables were expressed as means and SD. The normal distribution of continuous variables was tested with the Kolmogorov-Smirnov test. Continuous variable differences between groups were examined by the Mann-Whitney U test. Correlation

analyses were performed using Spearman's coefficient of correlation. The comparison of categorical values was carried out with the chi-square test. p<0.05 was considered significant.

3. Results

Clinical and laboratory findings are revealed in table 1. There were important differences in mean diastolic blood pressure, haemoglobin, creatinine, serum glucose, uric acid, phosphor, potassium between the groups (p<0.05) (Table 1). Systolic and diastolic blood pressures were significantly higher in the ESRD group. Echocardiographic dimensions, there were significant differences IVS thickness(12.7±1.5mm vs. 10.4±1.5mm, p<0.001) PW thickness (12.6±1.6mm vs. 10.1±1.9mm, p<0.001), LA dimension (40.9±5.3 vs. 34.6±2.6, p<0.001), DT (222.5±44.8 vs. 199.9±28.9, p=0.008), IVRT (116.1±18.8 vs. 105.8±12.8, p=0.004), E/A ratio (1.0±0.4 vs. 1.2±0.3, p=0.04) and tissue Doppler early diastolic flow (0.7±0.2 vs. 0.9±0.2, p=0.001) between groups (p>0.05) (Table 2).

Table 1. Baseline clinical and laboratory characteristics of study population and comparison between groups

	Group 1 (n=46)	Group 2 (n=40)	*p* value
Age (years)	57.6 ± 13.6	52.8 ± 8.9	0.061
Male sex % (n)	21	22	0.387
Mean systolic blood pressure (mmHg)	121.7 ± 20.0	117.3 ± 12.2	0.221
Mean diastolic blood pressure (mmHg)	77.3 ± 13.4	70.9 ± 9.9	0.015
Hemoglobin (g/L)	10.9 ± 1.2	14.1 ± 1.4	<0.001
White blood cell (10^3 µL)	7.4 ± 2.8	6.6 ± 1.8	0.146
Creatinine (mg/dL)	7.9 ± 2.5	0.8 ± 0.1	<0.001
Serum glucose (mg/dL)	106.2 ± 30.8	87.2 ± 8.7	<0.001
Uric acid (mg/dL)	6.4 ± 1.1	4.7 ± 1.1	<0.001
Phosphor (mg/dL)	5.1 ± 1.3	3.5 ± 0.6	<0.001
Calcium (mg/dL)	8.9 ± 0.7	9.1 ± 0.3	0.278
Potassium (mmol/IL)	4.8 ± 0.6	4.3 ± 0.3	<0.001

The LA volume indices are shown in Table 3. There were no significant differences in LATEV, LAAEF, LAAEV, LAPEV between the ESRD and Group 2 (p>0.05). Atrial electromechanical coupling parameters at different sites measured via tissue Doppler imaging are shown in Table 4. PA lateral (144.1±13.5 vs. 126.3±10.6, p<0.001), PA septal (129.4±13.1 vs. 116.8±9.7, p<0.001), PA tricuspid (110.9±10.9 vs. 103.1±9.1, p=0.001), IA-EMD (33.2±9.1 vs. 22.7±7.7, p<0.001), IRight-EMD (18.5±7.7 vs. 13.2±6.4, p=0.001) and ILeft-EMD (18.5±7.7 vs. 13.7±5.7, p=0.002) durations were significantly longer in the ESRD group than in the control group There was a significant correlation between ferritin levels and AEMD durations (Fig. 1).

Table 2. Conventional echocardiographic parameters and Comparison Between Groups

	Group 1 (n=46)	Group 2 (n=40)	*p* value
IVS thickness (mm)	12.7 ± 1.5	10.4 ± 1.5	<0.001
PW thickness (mm)	12.6 ± 1.6	10.1 ± 1.9	<0.001
LVEDD (mm)	46.1 ± 4.9	46.9 ± 4.6	0.436
LVESD (mm)	30.7 ± 4.8	31.6 ± 4.3	0.476
LA dimension (mm)	40.9 ± 5.3	34.6 ± 2.6	<0.001
LVEF (%)	58.0 ± 9.0	59.9 ± 5.8	0.256
DT (ms)	222.5 ± 44.8	199.9 ± 28.9	0.008
IVRT (ms)	116.1 ± 18.8	105.8 ± 12.8	0.004
E (m s–1)	0.71 ± 0.2	0.79 ± 0.18	0.121
A (m s–1)	0.76 ± 0.22	0.69 ± 0.11	0.076
E/A	1.0 ± 0.4	1.2 ± 0.3	0.040
E'	0.7 ± 0.2	0.9 ± 0.2	0.001

IVS: Ventricular septal thickness; **PW:** Posterior wall thickness; **LVEDD:** Left ventricular end-diastolic dimension; **LVESD:** Left ventricular end-systolic dimensions; **LA:** Left atrium; **LVEF**: Left ventricular ejection fraction; **E:** Early diastolic flow; **A:** Atrial contraction signal; **DT;** Deceleration time; **IVRT**: Isovolumetric relaxation time; **E':** Early tissue doppler flow

Table 3. LA Electromechanical Functions and Comparision Between Groups

	Group 1 (n=46)	Group 2 (n=40)	*p* value
LA Vmax (mL m²)	44.9 ± 14.7	34.9 ± 6.6	<0.001
LA Vmin (mL m²)	23.5 ± 8.5	15.9 ± 4.3	<0.001
LA Vp (mL m²)	33.0 ± 10.9	24.9 ± 4.5	<0.001
LA EF (%)	47.5 ± 8.1	53.8 ± 10.4	0.002
LATEV (mL m²)	21.5 ± 7.8	19 ± 5.9	0.108
LAAEF (%)	28.8 ± 8.1	36.1 ± 10.1	<0.001
LAAEV(mL m²)	9.5 ± 3.9	8.9 ± 3.0	0.505
LAPEF (%)	26.3 ± 6.9	30.7 ± 10.5	0.024
LAPEV(mL m²)	11.9 ± 5.6	10.9 ± 4.6	0.346

LA Vmax: Left atrium maximum volume; **LA Vmin:** Left atrium minimum volume; **LA Vp:** Left atrium volume before atrial systole; **LAEF:** Left atrium ejection fraction; **LATEV:** Left atrium total emptying volume; **LAAEF:** Left atrium active emptying fraction; **LAAEV:** Left atrium active emptying volume; **LAPEF:** Left atrium passive emptying fraction; **LAPEV:** Left atrium passive emptying volume

4. Discussion

In this study, we observed deterioration of LA mechanical functions and increase of AEMD durations in patients with ESRD in comparison to the Group 2. Also, we detected a correlation between AEMD durations and serum ferritin levels.

Patients with ESRD were shown to have a higher risk of cardiac arrhythmias and sudden cardiac death in previous studies. About half of cardiovascular deaths of patients with ESRD are due to cardiac arrhythmias and sudden death (Chan et al., 2010). Furthermore, to the pro-arrhythmogenic effect of HD, increasingly decreasing kidney functions cause electrolyte imbalance. Similarly, coronary artery disease, hypertension, heart failure and ventricular hypertrophy, which are seen often as co-morbidities with ESRD, contribute to development of arrhythmias. AF is the most often seen arrhythmia in daily practice,

Table 4. Electrocardiographic and tissue Doppler echocardiographic findings

	Group 1 (n=46)	Group 2 (n=40)	*p* value
PA Lateral (ms)	144.1 ± 13.5	126.3 ± 10.6	<0.001
PA Septal (ms)	129.4 ± 13.1	116.8 ± 9.7	<0.001
PA Tricuspid (ms)	110.9 ± 10.9	103.1 ± 9.1	0.001
IA-EMD (ms)	33.2 ± 9.1	22.7 ± 7.7	<0.001
IRight-EMD (ms)	18.5 ± 7.7	13.2 ± 6.4	0.001
ILeft-EMD	18.5 ± 7.7	13.7 ± 5.7	0.002

PA: Time interval from the on set of the P-wave on the surface ECG to the peak of the late diastolic wave (A wave); **IA-EMD:** Inter-atrial electromechanical delay; **IRight-EMD:** Intra-right electromechanical delay; **ILeft-EMD:** Intra-left electromechanical delay

it increases the risk of stroke five times (Hart, 2000). The risk of AF is known to be increased in patients with ESRD. Alvaro et al. (2011) have followed up 10358 patients for a mean duration of 10.1 years in their ARIC study and detected a strong association between CRD and AF, independent of other risk factors. Winkelmayer et al (2011) have found a two-fold increase in one-year mortality in patients with AF in their study on 2.5 million patients with HD (38.9% vs. 19.3%). Defining the risk factors causing atrial fibrillation (AF) are very important for decreasing the morbidity and mortality. In addition to conventional risk factors such as diabetes, hypertension, a number of echocardiographic and clinical factors were found in recent years (To et al., 2007), but some of these techniques are not suitable for clinical practice, as they are invasive. P wave dispersion at ECG and dimension of LA dilatation at echocardiography have low prognostic value. AEMD durations measured echocardiographically, which were technologically advanced recently are used in determination of AF risk and these were revealed to be correlated with invasive methods.

The effect of ESRD on AEMD was investigated in few studies. In the study by Karavelioğlu et al. (2014), atrial electromechanical coupling times were shown to be increased in haemodialysis patients, and also left atrial diameter and left ventricular end-diastolic pressures were found to be associated with AEMD durations. In the study by Tekcea et al. (2013) AEMD durations which were shown to be increased in patients with ESRD before haemodialysis were shown to decrease after dialysis, and dialysis was found to have positive effects not only on structural remodelling, but also on electrical remodelling. In a study by Turkmen et al. (2015) increased left intra-atrial EMD time in patients with HD was found to increase 2-year mortality due to combined cardiovascular events and all-cause mortality.

It was shown that ESRD affects LA mechanical functions in studies and various mechanisms were proposed. The risk of HT development is increased in patients with CKD and these patients have weaker blood pressure control (Sarnak et al., 2003). CKD likewise activates the renin, angiotensin, aldosterone system, which cause atrial fibrosis and electrical remodelling (Siragy and Carey, 2010). The cause of deteriorated LA mechanical functions and increased AEMD durations in patients with ESRD may originate from interrelations between these various mechanisms mentioned above.

Another interesting point in the present study, is the

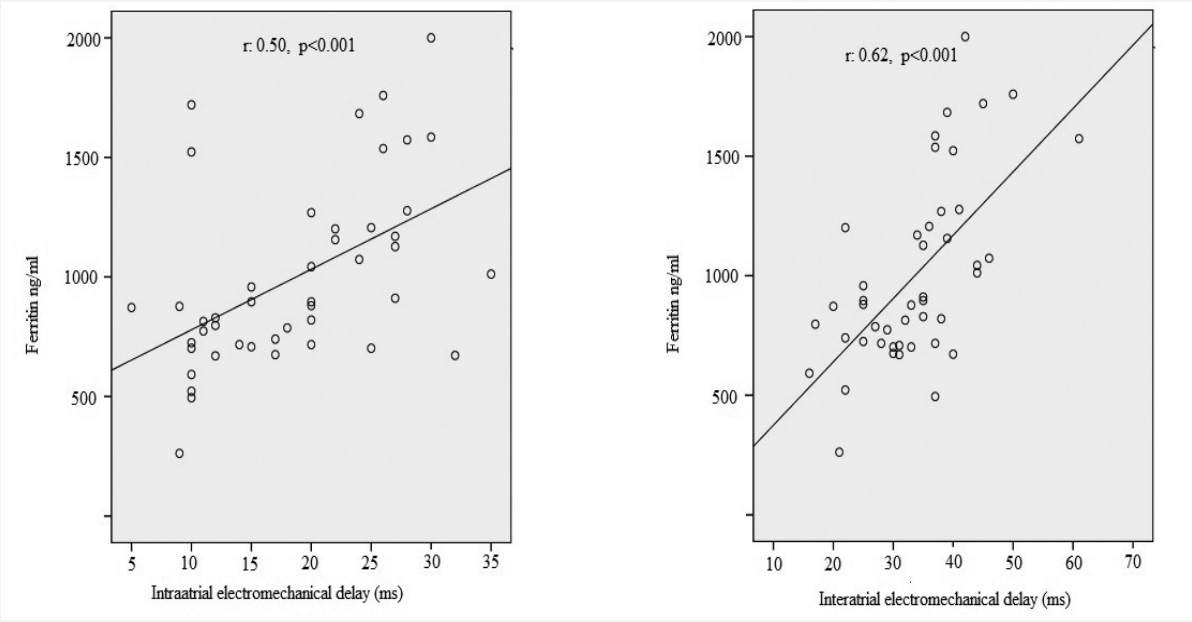


Fig. 1. Correlation graphs of atrial electromechanical delays with Ferritin levels

correlation between serum ferritin levels and AEMD durations. It is known that oral and intravenous iron treatment are given to dialysis patients for optimal response to human erythropoietin treatment. However, long-term iron treatment may cause oxidative stress, and inflammatory effects may disturb endothelial functions (Borawski et al., 2004) and iron was revealed to increase oxidative stress, thus producing deterioration in endothelial functions in disorders for example ESRD and thalassemia, in which iron load is increased (Bishu and Agarwal, 2006). The standing of inflammation in AF development was revealed in many illnesses where there is systemic inflammation. Also, iron overload in which primary and secondary hemosiderosis may also be seen, increases inflammation. The cause of the correlation between ferritin and AEMD durations may be due to increased iron levels affecting atrial conduction.

REFERENCES

Alvaro, A., Faye, L., Lopez, M., 2011. Chronic kidney disease is associated with the incidence of atrial fibrillation the atherosclerosis risk in communities (ARIC) study. Circulation. 123, 2946-2953.

Aydın, M., Ozeren, A., Bilge, M., Dursun, A., Cam, M., Elbey, M.A., 2004. Effects of dipper and non-dipper status of essential hypertension on left atrial mechanical functions. International Journal of Cardiology. 96, 419-424.

Bishu, K., Agarwal, R., 2006. Acute injury with intravenous iron and concerns regarding long-term safety. Clin. J. Am. Soc. Nephrol. 1, 19-23

Borawski, J., Gozdzikiewicz, J., Abramowicz, P., Naumnik, B., Mysliwiec, M., 2004. Endothelial injury markers with high dose intravenous iron therapy in renal failure. Clin. Appl. Thromb. Hemost. 10, 403-406.

Chan, C.T., Levin, N.W., Chertow, G.M., 2010. Determinants of cardiac autonomic dysfunction in ESRD. Clin. J. Am. Soc. Nephrol. 5, 1821-1827.

Dabrowska-Kugacka, A., Lewicka-Nowak, E., Ruciński, P., 2009. Atrial electromechanical sequence and contraction synchrony during single-and multisite atrial pacing in patients with brady- tachycardia syndrome. Pacing. Clin. Electrophysiol. 32, 591-603.

Deniz, A., Sahiner, L., Aytemir, K., 2012. Tissue Doppler echocardiography can be a useful technique to evaluate atrial conduction time. Cardiol. J. 19, 487-493.

Emiroglu, M.Y., Bulut, M., Sahin, M., 2011. Assessment of atrial conduction time in patients with essential hypertension. J. Electrocardiol. 44, 251-256.

Go, A.S., Hylek, E.M., Phillips, K.A., Chang, Y., Henault, L.E., Selby, J.V., 2001. Prevalence of diagnosed atrial fibrillation in adults: National implications for rhythm management and stroke prevention: The anticoagulation and risk factors in atrial fibrillation (ATRIA) study. JAMA. 285, 2370-2375.

Hart, R.G., 2000. Stroke prevention in atrial fibrillation. Curr. Cardiol. Rep. 2, 51-55.

Karavelioglu, Y., Karapınar, H., Ozkurt. S., 2014. Evaluation of atrial electromechanical coupling times in hemodialysis patients. Echocardiography. 31, 449-455.

Korantzopoulos, P.G., Goudevenos, J.A., 2009. Atrial fibrillation in end-stage renal disease: An emerging problem. Kidney Int. 76, 247-249.

Quinones, M.A., Otto, C.M., Stoddard, M., Waggoner, A., Zoghbi, W.A., 2002. Recommendations for quantification of Doppler echocardiography: A report from the doppler quantification task force of the nomenclature and standards committee of the American society of echocardiography. J. Am. Soc. Echocardiogr. 15, 167-184

Sarnak, M.J., Levey, A.S., Schoolwerth, A.C., Coresh, J., Culleton, B., Hamm, L.L., McCullough, P.A., Kasiske, B.L., Kelepouris, E., Klag, M.J., Parfrey, P., Pfeffer, M., Raij, L., Spinosa, D.J., Wilson, P.W., 2003. Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American heart association councils on kidney in cardiovascular disease, huigh blood pressure research, clinical cardiology, and epidemiology and prevention. Circulation. 108, 2154-2169.

Siragy, H.M., Carey, R.M., 2010. Role of the intrarenal renin-angiotensin-aldosterone system in chronic kidney disease. Am. J. Nephrol. 31, 541-550.

Tekcea, H., Ozturk, S., Aktas G., 2013. The effects of a single dialysis session on atrial electromechanical conduction times and functions. Kidney Blood Press Res. 37, 622-630.

To, A.C., Yehia, M., Collins, J.F., 2007. Atrial fibrillation in haemodialysis patients: Do the guidelines for anticoagulation apply? Nephrology. 12, 441-447.

Turkmen, K., Demirtas, L., Topa E., 2015. Predictive value of atrial electromechanical delay on long-term cardiovascular outcomes in hemodialysis patients. Am. J. Nephrol. 42, 239-249.

Williams, J.R., 2008. The Declaration of Helsinki and public health. Bulletion of the World Health Organization. 86, 650-651.

Winkelmayer, W.C., Patrick, A.R., Liu, J., 2011. The increasing prevalence of atrial fibrillation among hemodialysis patients. J. Am. Soc. Nephrol. 22, 349-357.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2016; 33(3): 145-149
doi: 10.5835/jecm.omu.33.03.004

Percutaneous transluminal angioplasty in haemodialysis patients with central or peripheral venous stenosis

Mehmet Yaman[a*], Musa Sahin[b], Hakki Simsek[b], Hasan Ali Gumrukcuoglu[b], Mustafa Tuncer[b], Yilmaz Gunes[c], Serkan Akdag[b]

[a] *Department of Cardiology, Faculty of Medicine, Ordu University Education And Research Hospital, Ordu, Turkey*
[b] *Department of Cardiology, Faculty of Medicine, Yuzunci Yil University, Van, Turkey*
[c] *Department of Cardiology, Faculty of Medicine, Hisar hospital, İstanbul, Turkey*

ARTICLE INFO

Article History
Received 21 / 04 / 2016
Accepted 06 / 06 / 2016

*** Correspondence to:**
Mehmet Yaman
Department of Cardiology,
Faculty of Medicine,
Ordu University Education And Research Hospital
Ordu, Turkey
e-mail: dr.yaman@windowslive.com

Keywords:
Arteriovenous fistulae
Edema
Haemodialysis
Percutaneous transluminal angioplasty
Venous stenosis

ABSTRACT

Dysfunction of arteriovenous fistulae (AVF), which result from peripheral or central venous occlusive illness, occurs very often in haemodialysis patients. In therapy, endovascular open procedures are prefered. Our study illustrated the clinical success of percutaneous transluminal angioplasty (PTA) for the treatment of these patient. A retrospective analysis was applied on patients presenting during a 2-years term with haemodialysis failure and ipsilateral arm swelling coherant with peripheral and/or central venous stenosis. PTA was performed as clinically and angiografically indicated. Technical success of PTA was defined less than 30% residual stenosis and clinical success was illustrated by resolution of pain and edema along with preservation of the AVF. Our study shows a subgroup of 26 patients that presented with symptomatic peripheral or central venous occlusive disease. Mean follow-up was 12.4 months (range, 3-24 months). PTA was successful in 26 patients 11 of whom were with central lesions and 15 of whom were with peripheral lesions. We were stated for central lesions PTA had a priority patency rates of 81.8%, 60%, 37.5% and supported primary patency rates of 90.9%, 70%, 62.5% at 3, 6, 12 months. For peripheral lesions, primary patency rates of 86.7%, 78.5%, 66.6% at 3, 6 and 12 months and assisted primary patency rates of 93.3%, 85.7% and 75%, separately. PTA for central and peripheral venous stenosis is be a successful and safe procedure in hemodialysis patients. In patients with lesions that are responsible for dilation, continuous functional access in the affected extremity is sustained, especially for patients with peripheral venous stenosis.

1. Introduction

In patients with end-stage renal disorder, vascular access stays the Achilles' heel of maintenance haemodialysis (Tang et al., 1998). Adequate arterial influx and venous outflow is important for appropriate function of hemodialysis access in chronic haemodialysis patients (Aytekin et al., 2004). Dysfunction of arteriovenous fistulae and grafts appears frequently in haemodialysis patients and significantly makes contribution to morbidity and hospitalization in the dialysis population (Windus, 1993). There are countless etiologies of symptomatic lesions; however, they most commonly conclude from prolonged central venous catheterization in the setting of end-stage renal illness and ipsilateral

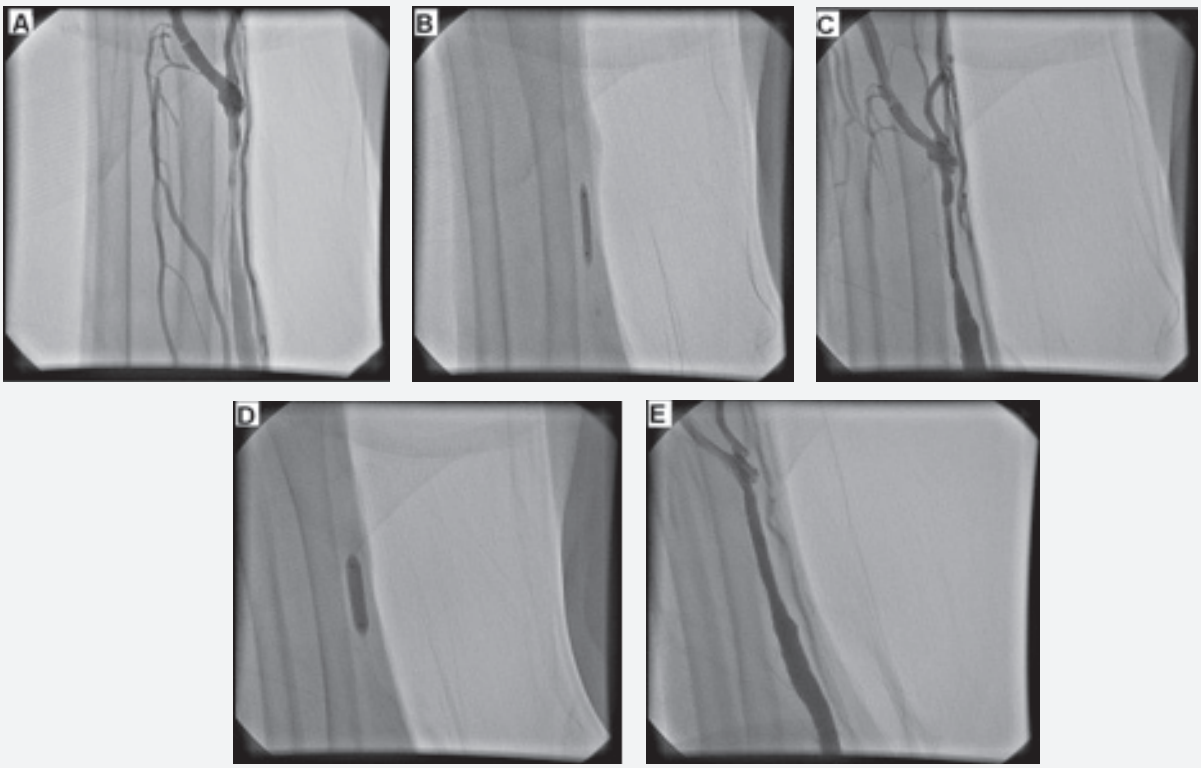


Fig. 1. Succesful PTA of Obstructed Cephalic venous. **A:** Thrombosed 95% stenosis, with marked chollaterals; **B:** First PTA procedure; **C:** Diminished chollaterals after first PTA; **D:** Second PTA procedure by bigger than peripheric ballon; **E:** Technical success; **PTA:** Percutaneous transluminal angioplasty

arteriovenous fistula (AVF). The effect of stenoses in these patients has been reported to be as high as 50% (Hernandez et al., 1998).

Stenosis or obstruction of a draining vein of a functioning vascular access can cause venous hypertension with associated pain, incapacitating swelling, and even ulceration (Mcnally et al., 1987). In therapy for symptomatic peripheral or central venous occlusive disease, endovascular open procedures are prefered. Surgical approaches need general anesthesia and has a high surgical morbidity in an end-stage renal disease setting. In addition, patency rates have not been significantly better than with endovascular techniques and extensive reconstructions might not be reasonable in a group of patients with multiple comorbidities (Wisselink et al., 1993; Bhatiai et al., 1996; Kalman et al., 1998).

At our institution, peripheral/central venography and percutaneous transluminal angioplasty (PTA) are used regularly for diagnosis and treatment of symptomatic venous stenosis or obstruction in hemodialysis patient. Our study was undertaken to describe the clinical success of PTA for the treatment of symptomatic venous stenosis or obstruction and failing AVF associated with significant and disabling upper-extremity edema.

2. Materials and methods

The study was performed under the recommendations of World Medical Association Declaration of Helsinki. A retrospective analysis was performed of patients presenting with hemodialysis for two years (between january 2014 and december 2015), prolonged posthemodialysis bleeding, difficult needle cannulation of the access, or ipsilateral arm or neck swelling appropriate with peripheral and/or central venous stenosis or occlusion. Patients underwent upper-extremity and central venography, and these patients with at least one peripheral or central venous lesion were involved.

All medical records were revised for demographic and clinical data. Details of the interference were gained by a review of the operative reports and angiograms (Fig. 1). Central veins were defined as the axillary vein, subclavian vein, brachiocephalic vein, or superior vena cava. The location of the lesion and severity of stenosis were noted in Table 1.

Our actual standard is two or more inflations for 40 to 60 seconds with systemic heparinization (5000 IU heparin, intravenously) prior to balloon inflation. Technical success of PTA was defined by completion venography in two views from different angles demonstrating less than 30% residual stenosis and clinical success was illustrated by resolution of

Table 1. Location of lesions and severity of stenosis

Location	n=26	Mean % Stenosis Range
Brachiocephalic vein	2 (7.6%)	83 ± 10 (75-90)
Subclavian vein	6 (23.0%)	84 ± 7 (75-95)
Axillary vein	3 (11.5%)	90 ± 5 (85-95)
Cephalic vein	7 (26.9%)	86 ± 7 (80-100)
Basilica vein	5 (19.2%)	86 ± 10 (75-100)
Median cubital vein	3 (11.5%)	85 ± 5 (80-90)

pain and edema along with preservation of the AVF. Primary patency was defined as the interval from the time of intervention until thrombosis or the time of measurement of patency. Assisted primary patency was defined as the lack of occlusion of the vessel after the primary intervention but containing any interventions to treat restenosis.

Patients were evaluated during routine follow-up with clinical success defined by an improvement in symptoms. The duration of the symptom-free period was decided from patient history and physical examination. In cases of an ipsilateral AVF, the status of the fistula was taken note at each time point. Failure was defined by recurrence of symptoms that lead to repeat venography and PTA. Generally, patients were released on the day of the procedure after clinical evaluation. Long-term anticoagulation or antiplatelet therapy was not prescribed.

3. Results

Seventy-seven patients underwent venography during the study term. The current study shows a subgroup of 26 patients presented with symptomatic peripheral or central venous occlusive illness defined by hemodialysis failure, prolonged posthemodialysis bleeding, difficult needle cannulation of the access, or ipsilateral arm or neck swelling. Follow-up was available for each treated patient with an average of 12.4 months (range, 3-24 months). There were 10 men and 16 women with an average age of 54.1 (range 20-75) years. In all patients, there was an ipsilateral arteriovenous fistula. No patient in the current study had a past of pacemaker insertion.

All procedures were performed percutaneously. There were 11 lesions on the right and 15 on the left. The position of the lesion and severity of stenosis, eleven of which central and fifteen of which peripheral in venous, were noted in Table 1. The degree of stenosis was greater than 75% in all cases.

Initial percutaneous angioplasty was technically successful in 26 patients, 11 of whom were with central lesions and 15 of whom were with peripheral lesions. After PTA, we repeated the same process with a bigger size ballon in eight cases of which resudial stenous was more than 30%. In these eight cases, resudial stenosus decreased below 30%. There were no procedure-related complications at the access site, venous perforations, or deaths.

In all of the patients with succesful PTA, edema in arm and increased venous pressure were improved. PTA procedure was performed to patients with repeated semptoms during follow-up. We were reported for central lesions PTA and peripheral lesions PTA in table 2.

Table 1. Patency rates of central and peripheral lesions

	3 month	6 month	12 month
Central Lesions PTA			
Priority patency rate	81.8%	60%	37.5%
Assisted priority patency rates	90.9%	70%	62.5%
Peripheral Lesions PTA			
Priority patency rates	86.5%	78.5%	66.6%
Assisted priority patency rates	93.3%	85.7%	75%

PTA: Percutaneous transluminal angioplasty

4. Discussion

In chronic hemodialysis patients with AVF, stenosis or occlusion of the central and/or peripheral veins that concludes in considerable edema of the arm is a formidable problem because it very often necessitates closing the vascular access, which is sometimes the last one available (Moriniere et al., 1997). The primary aim in this setting is to assure symptomatic relief for the patient while maintaining function of the associated AVF when present (Bhatiai et al., 1996).

Percutaneous interventional technique can be done under local anesthesia, is well-tolerated by the patient, and is associated with shorter hospitalization time than surgery. As long as surgical repair techniques result in significant morbidity, percutaneous interventional techniques have been widely used in the management of stenosis or occlusion of the symptomatic central and/or peripheral veins. PTA has seen to be an effective, safe, and comparatively inexpensive procedure with a high technical success rate (Glanz et al., 1987; Ingram et al., 1988). Stent solely should be deployed when in response to failed PTA, or when there is quick restenosis or vessel perforation but never as an initial therapy.

In our study, we present 77 consecutive patients with eleven of which central and fifteen of which peripheral venous stenosis or occlusion and an average follow-up of 12.4 months. Most studies in literature are concerned with the treatment of central venous lesions in haemodialysis patient. Different from these studies, our study consisted of two groups one of them with central venous lesions and other group with peripheral venous. For central lesions PTA had a primary patency rate of 81.8% at three months, 60% at six months, and 37.5% at 12 months. Assisted primary patency rates were better, with an improvement to 90.9% patency at three months, 70% at six months and 62.5% at 12 months. And for peripheral lesions PTA had primary patency rates of 86.7%, 78.5% and 66.6% at three, six and 12 months and assisted primary patency rates of 93.3%, 85.7% and 75%, respectively. However, to

achieve better assisted primary patency rates, multiple interventions were needed. In our study, primary patency rates and assisted primary patency rates of peripheral lesions were better than of central lesions. Because of the high frequency of elastic recoil, the recurrence rate for central venous lesions after PTA was higher than that for peripheral lesions (Scott et al. 2004). So, during follow-up central venous lesions needs more interventions after initial PTA.

Oderich et al. (2000), Haage et al. (1999), used PTA + stent as an initial treatment of central venous stenosis or occlusions. Oderich et al. (2000), presented 40 central venous obstructions that were treated with 50 stents. Over a mean follow-up of 16 months, primary patency rates of 27% and 9% one and two years and secondary patency rates of 71% and 39%, respectively were reported. Another series by Haage et al. (1999), evaluated 50 patients who experienced stent placement as the primary cure for central venous obstruction. They reported primary patency rates of 56% and 28% at 1 and 2 years, respectively.

But we are agree with Scott et al. (2004) used PTA treatment of central venous stenosis or occlusions and Glanz et al. (1987) used percutaneous trans venos angioplasty (PTVA) treatment of central and peripheral venous lesions. Scott et al. (2004) presented 35 central venous stenoses that were treated with PTA. Transvenous angioplasty had a primary patency rate of 85% at 30 days, 55% at six months, and 43% at one year. Assisted primary patency rates were better, with an improvement to 80% patency at one year and 64% at two years. Glanz et al. (1987) performed PTA to hemodialysis patients with stenosis or occlusion of the central and/or peripheral veins that concludes in considerable edema of the arm, reported an initial success rate of 82% and six, 12-month primary patency rates were 57%, 45%, respectively.

The results of our study, like Scott and Glanz studies are similar to the Oderich and Haage studies, without the use of stents. Disadvantages of stent placement consist of potential collateral vein obstruction, shortening and migration of the stent (Verstanding et al., 2003), infection (Pruitt et al., 2002), and the loss of outflow in the extremity if the stent should fail. The SIR guidelines do not recommend the routine use of stents to avoid restenosis and state that the role of stents has yet to be fully defined (Aruny et al., 1999). Three prospective randomized studies have found that stent placement does not provide an advantage over successful angioplasty (Beathard, 1993; Quinn et al, 1995; Hoffer et al., 1997).

Owing to the negative consequences of central and peripheral venous stenosis, catheterization of the central and peripheral veins should be avoided when at all possible. Even when stents were placed for stenosis after failed balloon angioplasty, the primary patency was never the same as that for successful balloon angioplasty alone. Thus, we reccommend improving the success rate of balloon angioplasty and reducing the use of stents. Furthermore, more reinterventions may be needed to maintain secondary patency compared with balloon angioplasty alone.

The present study had some study limitations. This article lie inherently in the design of the study and the present study was limited by the small patient population, particularly in the subgroups, and by its retrospective design. Further studies with large group of patients are needed to make correct decision.

Finally PTA for central and peripheral venous stenoses appears to be a successful and safe procedure that is effective and enhances in hemodialysis patients.Succesfuly intervention is achieved only with surveillance and repetitive interventions, but it seem to be devoid of major morbidity. In patients who had successfully intervention, especially for patients with peripheral venous stenosis is maintained continuous functional access in the affected extremity.

REFERENCES

Aruny, J.E., Lewis, C.A., Cardella, J.F., Cole, P.E., Davis, A., Drooz, A.T., Grassi, C.J., Gray, R.J., Husted, J.W., Jones, M.T., McCowan, T.C., Meranze, S.G., Van Moore, A., Neithamer, C.D., Oglevie, S.B., Omary, R.A., Patel, N.H., Rholl, K.S., Roberts, A.C., Sacks, D., Sanchez, O., Silverstein, M.I., Singh, H., Swan, T.L., Towbin, R.B., Trerotola, S.O., Bakal, C.W.; Society of Interventional Radiology Standards of Practice Committee. 1999. Quality improvement guidelines for percutaneous management of the thrombosed or dysfunctional dialysis access. Standards of Practice Committee of the Society of Cardiovascular and Interventional Radiology. J. Vasc. Interv. Radiol. 10, 491-498.

Aytekin,C., Boyvat, F., Yagmurdur M.C., Moray, G., Haberal, M., 2004. Endovascular stent placement in the treatment of upper extremity central venous obstruction in hemodialysis patients. Eur. J. Radiol. 49, 81-85.

Beathard, G.A., 1993. Gianturco self-expanding stent in the treatment of stenosis in dialysis access grafts. Kidney Int. 43, 872-877.

Bhatiai, D.S., Money, S.R., Ochsner, J.L., Crockett, D.E., Chatman, D., Dharamsey, S.A., Mulingtapang, R.F., Shaw, D., Ramee S.R., 1996. Comparison of surgical bypass and percutaneous balloon dilation with primary stent placement in the treatment of central venous obstruction in the dialysis patient: One-year follow-up. Ann. Vasc. Surg. 10, 452-455.

Glanz, S., Gordon, D.H., Butt, K., Hong, J., Lipkowitz, G., 1987. The role of percutaneous angioplasty in the management of chronic hemodialysis fistulas. Ann. Surg. 206, 777-781.

Haage, P., Vorwerk, D., Piroth, W., Schuermann, K., Guenther, R.W., 1999. Treatment of hemodialysis-related central venous stenosis or occlusion: Results of primary Wallstent placement and follow-up in 50 patients. Radiology. 212, 175-180.

Hernandez, D., Diaz, F., Rufino, M., Lorenzo, V., Pérez, T., Rodríguez, A., De Bonis, E., Losada, M., González-Posada, J.M., Torres, A., 1998. Subclavian vascular access stenosis in dialysis patients: Nature, history and risk factors. J. Am. Soc. Nephrol. 9, 1507-1510.

Hoffer, E.K., Sultan, S., Herskowitz, M.M., Daniels, I.D., Sclafani, S.J., 1997. Prospective randomized trial of a metallic intravascular stent in hemodialysis graft maintenance. J. Vasc. Interv. Radiol. 8, 965-973.

Huang, H.L., Chen, C.C., Chang, S.H., Hung, K.C., Hsieh, I.C., Chang, H.J., Wen, M.S., Fang, J.T., 2005. Combination of duplex ultrasound-guided manual declotting and percutaneous transluminal angioplasty in thrombosed native dialysis fistulas. Ren Fail. 27, 713-719.

Ingram, T.L., Reid, S.H., Tisnado, J., Cho, S.R., Posner, M.P., 1988. Percutaneous transluminal angioplasty of brachiocephalic vein stenosis in patients with dialysis shunts. Radiology. 166, 45-47.

Kalman, P.G., Lindsay, T.F., Clarke, K., Sniderman, K.W., Vanderburgh, L., 1998. Management of upper extremity central venous obstruction using interventional radiology. Ann. Vasc. Surg. 12, 202-206.

Mcnally, P.G., Brown, C.B., Moorhead, P.J., Raftery, A.T. 1987. Unmasking of subclavian vein obstruction following creation of arteriovenous fistulae for heamodialysis. A problem following subclavian line dialysis? Nephrol. Dial. Transplant. 1, 258-260.

Moriniere, P., Rodary-Vautier, R., Fillioux-Morfaux, V., Dehouck, B., el Remond, A., Fournier, A., 1997. Percutaneous recanalization of occlusion of central and proximal veins in chronic hemodialysis. Technical note. Kidney Int. 52, 1406-1411

Oderich, G.S., Treiman, G.S., Schneider, P., Bhirangi, K., 2000. Stent placement for treatment of central and peripheral venous obstruction: A long-term multi-institutional experience. J. Vasc. Surg. 32, 760-769.

Pruitt, A., Dodson, T.F., Najibi, S., Thourani, V., Sherman, A., Cloft, H., Caliendo, A., Smith, R.B., 2002. Distal septic emboli and fatal brachiocephalic artery mycotic pseudoaneurysm as a complication of stenting. J. Vasc. Surg. 36, 625-628.

Quinn, S.F., Schuman, E.S., Demlow, T.A., Standage, B.A., Ragsdale, J.W., Green, G.S., Sheley, R.C., 1995. Percutaneous transluminal angioplasty versus endovascular stent placement in the treatment of venous stenoses in patients undergoing hemodialysis: intermediate results. J. Vasc. Interv. Radiol. 6, 851-855.

Scfild, A.F., 2010. Maintaining vascular access: The management of hemodialysis arteriovenous grafts. J. Vasc. Access. 11, 92-99.

Scott, M., Surowiec, Allison, J., Fegley, William, J., Tanski, et all., 2004. Endovascular management of central venous stenoses in the hemodialysis patient: Results of percutaneous therapy. Vasc. Endovascular. Surg. 38, 349-354.

Tang, S., Lo, C.Y., Tso, W.K., Lo, W.K., Li, F.K., Chan, T.M., 1998. Percutaneous transluminal angioplasty for stenosis of arteriovenous fistulae: A review of local experience. HKMJ. 4, 36-41.

Verstandig A.G., Bloom, A.I., Sasson, T., Haviv, Y.S., Rubinger, D., 2003. Shortening and migration of Wallstents after stenting of central venous stenoses in hemodialysis patients. Cardiovasc. Intervent. Radiol. 26, 58-64.

Windus, D., 1993. Permanent vascular access: A nephrologist's view. Am. J. Kidney Dis. 21, 457-471.

Wisselink, W., Money, S.R., Becker, M.O., Rice, K.L., Ramee, S.R., White, C.J., Kazmier, F.J., Hollier, L.H., 1993. Comparison of operative reconstruction and percutaneous balloon dilation for central venous obstruction. Am. J. Surg. 166, 200.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Clinical Research

J. Exp. Clin. Med., 2016; 33(3): 151-155
doi: 10.5835/jecm.omu.33.03.005

Using of three dimensional volume rendering angiography in the determination of vessel-free areas of the scalp in the patients underwent intracranial aneurysm surgery during the placement of three-pins metallic head fixation device

Ahmet Gokyar[a], Cengiz Cokluk[b*], Abdullah Marangoz[b]

[a] *Department of Neurosurgery, Faculty of Medicine, Amasya University, Amasya, Turkey*
[b] *Department of Neurosurgery, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*

ARTICLE INFO

Article History
Received 19 / 04 / 2016
Accepted 13 / 06 / 2016

*** Correspondence to:**
Cengiz Cokluk
Department of Neurosurgery,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun, Turkey
e-mail: cengizcokluk@yahoo.com

Keywords:
Mayfield-Kees head holder device
Pins placement
Three-dimensional angiography

ABSTRACT

Three-pins head holder device has been safely used for many years in many neurosurgical procedures for providing 3-point rigid cranial fixation. Equal impingement of pins ensures firm skull positioning and fixation after carefully positioning of skull pins around the vessel free areas. The raw data of the three-dimensional computerized tomography angiography (3D-CTA) obtained from the patients with subarachnoid hemorrhage were transferred to computer and recorded in a software program. This software program created three-dimensional images of skull using previously transferred raw data with volume rendering technique. Safe areas for pins placement, in terms of vessel-free areas, were determined using three-dimensional volume rendering angiography of the skull. The study group consists of 53 (27 female and 26 male) patients. The mean age of the patients was estimated as 57.9±9.7 years. Branches, distribution and critical anastomosis of the superficial temporal artery were also determined. In the other way, the course of the posterior auricular and occipital artery ascending along the external surface of the mastoid bone was also detected in all cases. In the patients underwent surgery for intracranial aneurysm, using of three-dimensional volume rendering angiography may be used in the determination of vessel-free areas of the external surface of the scalp during the placement of three-metallic pins of head holder device.

1. Introduction

Three-point metallic head fixation device has been safely used by neurosurgeons for many years in many neurosurgical procedures including cerebral, cervical and upper thoracic regions in the stabilization and fixation of the head (Yasargil, 1994; Lee and Lin, 2010; Kuruoglu et al., 2015a; Kuruoglu et al., 2015b; Kuruoglu et al., 2015c). It was originally invented and developed by FH Mayfield in collaboration with G Kees, a talented medical artist (Tew, 1982).

Neurological surgeon should fix the three-pins device with accurate positioning and proper pressure in order to prevent neurovascular injury during the surgical procedures. The pins of the device should be placed to the vessel-free areas in the skull. Moreover surgeons repeatedly check and confirm the immobilization of the connection parts in the avoiding of potential complications (Lee et al., 2009).

In this study, the feasibility of the using of three-dimensional volume rendering angiography in the

determination of vessel-free areas of the scalp in the patients underwent intracranial aneurysm surgery during the placement of three pins metallic head fixation device was evaluated and literature was reviewed.

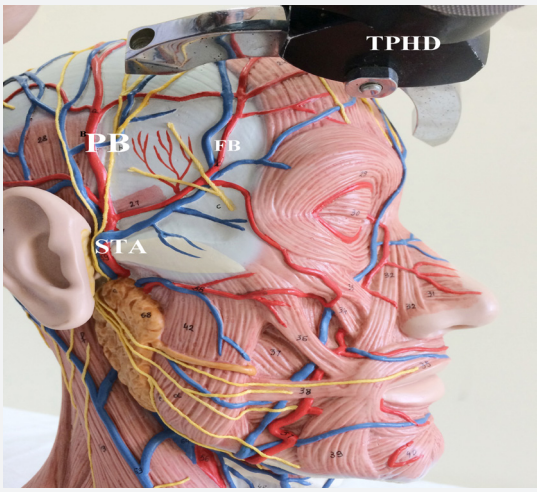


Fig. 1. This figure shows the application of three-pins head holder to the frontal area in a model before the operation (**PB:** Parietal branch; **STA:** Superficial temporal artery, **FB:** Frontal branch; **TPHD:** Three-pins head holder device)

2. Materials and methods

Any additional radiological examination providing a drug was performed to the patients for this study. The patient population included the cases that were brought to our neurosurgery department because of subarachnoid hemorrhage, and further decided to perform a 3D-CTA for cerebral aneurysm evaluation. The raw data of the 3D-CTA were transferred and recorded to a computational software database. The purpose of performing 3D-CTA was only the examination of the intracranial vascular pathology after the insult of subarachnoid hemorrhage. Some part of these raw data were used for examination of the three dimensional vascular anatomy of the scalp by using three-dimensional volume rendering angiography. Imaging data were stored in digital imaging and communications in medicine (DICOM) format and subsequent analyzed with imaging software to convert into the three-dimensional volume rendered neurovascular images.

Three-dimensional images of the head were evaluated in terms of general shape, distribution, location, critical anastomosis, and variations of the scalp vasculature in the determination of vessel-free areas for three-pins placement before the aneurysm surgery. The distance of the frontal and parietal branches of the STA, posterior auricular and occipital artery was estimated in according to medial epicanthus and the pinna. The types of the variations of the superficial temporal artery including frontal and parietal branches were also evaluated. According to measured values, vessel-free areas were marked on the model testing with three-pins head fixation device (Fig. 1, 2). The distance of the safe zone from the pinna and the medial epicanthus were estimated and marked on the patient's skull before applying of the three-pins head holder (Fig. 3, 4, 5). The distance of the safe zone from mastoid tip and pinna was estimated in the occipital region (Fig. 6).

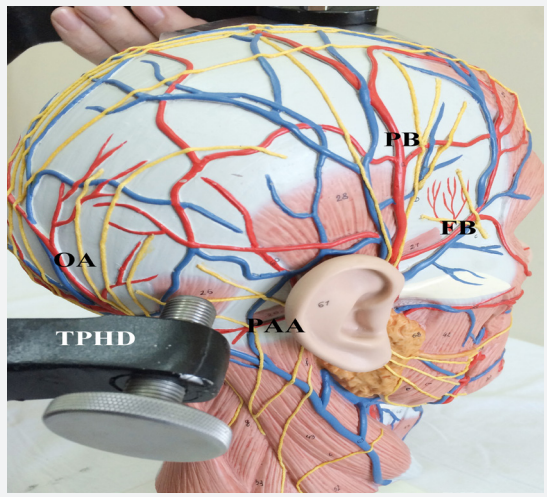


Fig. 2. This figure shows the application of three-pins head holder to the occipital area in a model before the operation (**TPHD:** Three-pins head holder device, **PAA:** Posterior auricular artery, **OA:** Occipital artery; **PB:** Parietal branch; **FB:** Frontal branch)

3. Results

The study group consisted of 53 (27 female and 26 male) patients. The mean age of the patients was estimated as 57.9±9.7 years. Any additional radiological procedure and/or drug were performed to any patient for this study. The purpose of the radiological examination of the patients was only diagnosing and examining of their own disease. The radiological images from this examination were retrospectively transferred to a computer. Three-dimensional imaging of scalp vasculature was created with 3D-Volume Rendering Technique by using OsiriX MD software program.

Normal arterial vascular anatomy was found in 28 (52.83%) of the cases. Remaining 23 (47.17%) of the cases had some variations. The common variations were found as frontal branch duplication, parietal branch duplication, and frontal and/of parietal branch re-bifurcations. Distorted vascular anatomy was found in 23 (47.17%) of the patients. The most frequent distortion was the fusiform dilatation of STA in the cases with intracranial aneurysm.

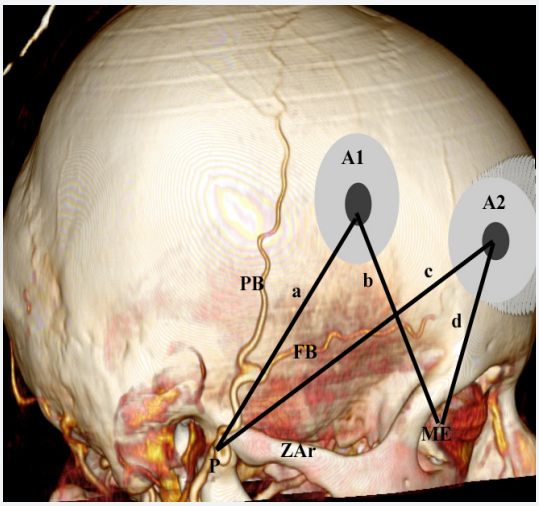


Fig. 3. This figure shows the safe areas in a patient with normal vascular anatomy (**A1:** Safe area on the parietal area; **A2:** Safe zone on the frontal area; **PB:** Parietal branch; **FB:** Frontal branch; **P:** Pinna; **ZAr:** Zygomatic arch; ME: Medial epicanthus; **a:** The distance from pinna to parietal safe zone; **b:** The distance from medial epicanthus to parietal safe zone; **c:** The distance from pinna to frontal safe zone; **d:** The distance from medial epicanthus to frontal safe zone)

4. Discussion

Mayfield-Kees three-point head fixation device has been used successfully and safely in neurosurgical operating theatre for many years. It is simple to use and offer safe fixation of the head during surgery (Yasargil, 1994). Slipping of the device pins, infection, air embolism, penetration through the cranium, and epidural hematoma puncturing major scalp vessels, were reported in seldom cases (Yasargil, 1994; Lee et al., 2009; Lee and Lin, 2010). It consists of a basic unit, swivel adapter, and the three-point head holder. The pins should always be positioned on the cranium in areas not covered by muscle.

Regardless whether neurosurgeon uses the Mayfield, Gardner, Sugita, or any variations, several methods should be taken into account in the avoiding or reducing the complications related with the pins (Yasargil, 1994). Using sterile technique, cleaning of the area with shampoo before procedure, application of large amount of betadine, adjuvant antibiotic treatment, using sterile pins will reduce the infection rates. The tension of the pins should be checked by hand, and should insert at approximately 90 degrees to the scalp in order to avoiding slippage. If the patient is positioned in supine, the single prong should be placed just above the mastoid, and double arm should be positioned at the temporalis insertion line. For the avoiding of large scalp vessel puncturing, in the patients underwent aneurysm surgery, the images obtained from three-dimensional volume rendering angiography may be used to detect the vessels.

The volume rendering technique may be used in the three-dimensional evaluation of some anatomical structures such as the superficial temporal and artery, arterial branching and vascular variations, the extension of the temporal muscle, and the thickness of the skull. Volume rendering technique is a group of modalities in the converting of two-dimensional images to the three-dimensional images (Drebin et al., 1988; Calboun et al., 1999; Tomandi et al., 2006). The two-dimensional images acquired by a computerized tomography and magnetic resonance imaging are used to create the volume rendered images (Drebin et al., 1988; Calboun et al., 1999; Hwang et al., 2011). In this study, we used OsiriX software program for volume rendering technique to create three-dimensional images of the feeder arteries of the scalp.

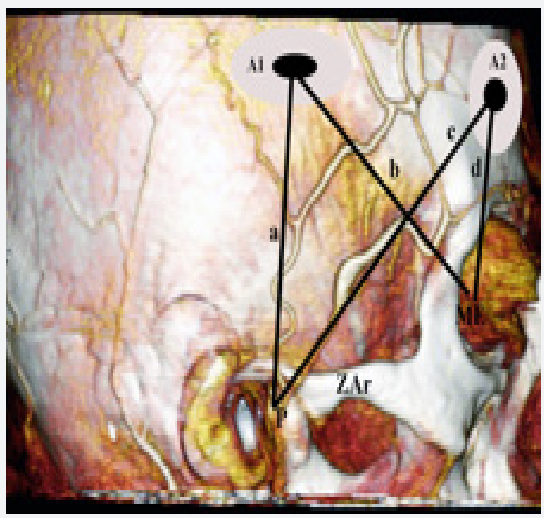


Fig. 4. This figure shows the safe zones in a patient with the variations of the scalp vasculature (**A1:** Safe area on the parietal area; **A2:** Safe zone on the frontal area; **PB:** Parietal branch, FB: Frontal branch; **P:** Pinna; **ZAr:** Zygomatic arch; **ME:** Medial epicanthus; **a:** The distance from pinna to parietal safe zone; **b:** The distance from medial epicanthus to parietal safe zone; **c:** The distance from pinna to frontal safe zone; **d:** The distance from medial epicanthus to frontal safe zone)

Three-dimensional viewer provides modern rendering modes such as multiplanar reconstruction, surface rendering, volume rendering, and maximum intensity projection. In the present study, we used OsiriX software in the processing of DICOM images. This software may show the basal cerebral arteries and skin feeders together with the bone muscle structures

of the head. Using of this technique in the cases with aneurysmal subarachnoid hemorrhage can give useful knowledge about the shape, distributions, branching, diameter and location of the extra-cranial skull arteries. Location of the arteries can be used in the determination of the pins places for the preservation of the arteries. Preserving of the arteries may prevent the occurrence of the complications related with the arterial origin.

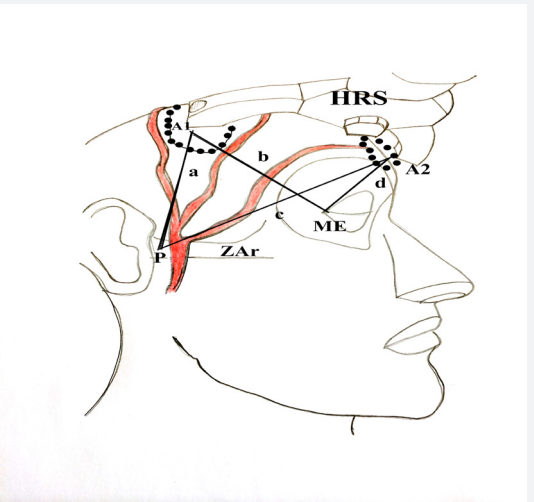


Fig. 5. This schematic figure shows the application of head resting device to the safe zone after measuring of the safe zones from the pinna and medial epicanthus (**A1:** Safe area on the parietal area; **A2:** Safe zone on the frontal area; **PB:** Parietal branch, **FB:** Frontal branch; **P:** Pinna; **ZAr:** Zygomatic arch; **ME:** Medial epicanthus; **a:** The distance from pinna to parietal safe zone; **b:** The distance from medial epicanthus to parietal safe zone; **c:** The distance from pinna to frontal safe zone; **d:** the distance from medial epicanthus to frontal safe zone; **HRS:** Head resting device).

In the present study, it was found that 47.17% of the cases showed some type of variations. Duplication of frontal and parietal branch of superficial artery may be seen in the frontal and parietal region in where double arm of the head-resting device is applied. On the other hand, fusiform dilatation of the vessels may

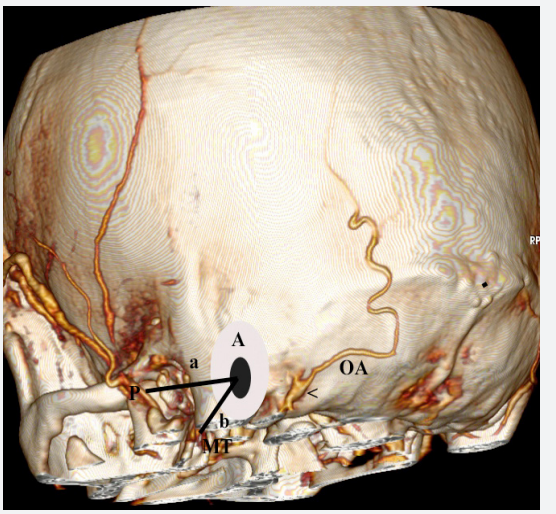


Fig. 6. This figure shows the safe zone on the mastoid part of the temporal bone, and measuring of the distance from the mastoid tip and the pinna (**A:** Occipital safe zone; **P:** Pinna; **a:** The distance from the pinna to the occipital safe zone; **b:** The distance from the mastoid tip to the occipital safe zone; **OA:** Occipital artery; arrow shows the enlargement of the occipital artery)

be present. In order to reduce vessel puncturing and possible venous air embolism the tips of the pins should be positioned on the relatively avascular regions. Using of this technique vascular anatomy of the scalp may be determined in terms of pins placement.

In conclusion, superficial temporal and occipital artery and their branches may be imagined with three-dimensional volume rendering technique intended for imagination of basal cerebral arteries. These images may be used in the determination of the localization of the pins of Mayfield-Kees head holder. The position and branching of the arterial vessels feeding to the scalp, and extension of the temporal muscle in relation with the external auditory meatus and epicanthus may be estimated using this software program and marked on the skin surface. The using of 3D-CTA in the cases with aneurysm is useful in the determination of pins places in the head to avoid the vessel injury related to pins.

REFERENCES

Calboun, P.S., Kuszyk, B.S., Heath, D.G., Carley, J.C., Fishman, E.K., 1999. Three-dimentional volume rendering of spiral CT data: Theory and method. RadioGraphics. 19, 745-763.

Drebin, R.A., Carpenter, L., Hanrahan, P., 1988. Volume rendering. Comput. Graph. 22, 65-74.

Hwang, S.B., Kwak, H.S., Han, Y.M., Chung, G.H., 2011. Detection of intracranial aneurysms using three dimensional multidetector-row CT angiography: Is bone substraction necessary? Eur. J. Radiol. 79, 18-23.

Kuruoglu, E., Cokluk, C., Marangoz, A.H., Aydin, K., 2015a. Fusiform enlargement of the superficial temporal artery in the cases with intracranial aneurysm. Turk Neurosurg. 25, 737-741.

Kuruoglu, E., Cokluk, C., Marangoz, A.H., Aydin, K., 2015b. Application of three-dimentional computerized tomographic angiography in the planning of pterional scalp incision to preserve the superficial temporal artery. Turk Neurosurg. 25, 350-352.

Kuruoglu, E., Cokluk, C., Marangoz, A.H., Aydin, K., 2015c. The evaluation of three-dimensional anatomy of the superficial temporal artery using the volume rendering technique. Turk Neurosurg. 25, 285-288.

Lee, M.J., Lin, E.L., 2010. The use of the tree-pronged Mayfield head clamp resulting in an intracranial epidural hematoma in an adult patient. Eur. Spine Journal. 19, 8187-8189.

Lee, T.H., Kim, S.J., Cho, D.S., 2009. Broken Mayfield head clamp. J. Korean Neurosurg. Soc. 45, 306-308.

Tew, J.M., 1982. Neurosurgeon of the year; Frank Henderson Mayfield. Surg. Neurology. 17, 1.

Tomandi, B.F., Hammen, T., Klotz, E., Ditt, H., Stemper, B., Lell, M., 2006. Bone-substraction CT angiography for the evaluation of intracranial aneurysms. Am. J. Neuroradiol. 27, 55-59.

Yasargil, M.G., 1994. Microneurosurgery. Verlag, G. T., (Ed). Volume 4. Thieme Medical Publishers Inc., New York.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Experimental Research

J. Exp. Clin. Med., 2016; 33(3): 157-161
doi: 10.5835/jecm.omu.33.03.006

The outbreak of *Acinetobacter baumannii* producing OXA-23 and OXA-51 type carbapenemases in a state hospital

Tulin Guven Gokmen[a*], Beril Akcimen[b], Begum Kayar[c], Mahdi Marzi[c], Fatih Koksal[c]

[a] *Department of Microbiology, Ceyhan Veterinary Faculty, Cukurova University, Adana, Turkey*
[b] *Laboratory of Microbiology, Kadirli State Hospital, Osmaniye, Turkey*
[c] *Department of Microbiology, Medical Faculty, Cukurova University, Adana, Turkey*

ARTICLE INFO

Article History
Received 05 / 04 / 2016
Accepted 24 / 05 / 2016

*** Correspondence to:**
Tulin Guven Gokmen
Department of Microbiology,
Ceyhan Veterinary Faculty,
Cukurova University,
Adana, Turkey
e-mail: tulinguven01@hotmail.com

Keywords:
A. baumannii
Outbreak
OXA-23
OXA-51

ABSTRACT

Acinetobacter baumannii is non-fermentative gram-negative bacilli which plays an important pathogen especially in intensive care units causing infections and epidemics. Carbapenem resistance often is consisted of OXA-type carbapenemase. In this study, we aimed to determine carbapenem resistance and clonal relationships of *A. baumannii* isolated from patient and enviromental samples by phenotypic and genotypic methods in 10-bed intensive care unit. Multiplex-PCR method was used to determine the genes of OXA type beta-lactamases (blaOXA) and clonal relations between strains were investigated by pulsed-field gel electrophoresis (PFGE) method. All of the isolates were found to be carbapenem resistant and had the blaOXA-51-like and blaOXA-23-like gene. Also, all of isolates were seen to be 100 % related by PFGE method. As a result, isolates of patients with ventilator-associated pneumonia and isolates survived on ventilator of Intensive Care Unit were found to be 100% clonal associated with PFGE and had same MIC values for imipenem and meropenem. blaOXA-23 and blaOXA-51 genes has been determined all of the isolates. It can be accepted a short-term and small outbreak.

1. Introduction

A. baumannii, have been increasingly reported as significant microorganisms involved in various nosocomial infections and several hospital outbreaks especially in intensive care units (ICU) (Bergogne-Bérézin and Towner, 1996). Because of its ability to rapidly acquire antimicrobial resistance and its propensity to persist in the environment, *A. baumannii* is difficult to control in the hospital setting (Kohlenberg et al., 2009). Health-associated *A. baumannii* infections are difficult to treat due to the presence of multidrug-resistant (MDR) organisms, which includes resistance to β-lactams, aminoglycosides, fluoroquinolones and more recently, carbapenems (Moniri et al., 2010).

Carbapenem resistance often is consisted of OXA type carbapenemase. OXA-type enzymes are collected in four subgroups (OXA-51, OXA-58, OXA-23 and OXA-24). The beta-lactamases (blaOXA) genes encoding enzymes are determined by PCR method using specific primers. While the blaOXA-51 is

intrinsic to A. baumannii, blaOXA-58, blaOXA-23 and blaOXA-24 may be part of different mobile elements (Naas et al., 2006). PCR products are confirmed by applying the sequence analysis. Also, Multiplex-PCR methods have been used for the determination of these genes rapidly.

Molecular epidemiology of nosocomial *A. baumannii* infections is essential to develop effective strategies to control their spread (Nasr and Attalah, 2012). The pulsed-field gel electrophoresis (PFGE) has shown to be suitable for the investigation of hospital outbreaks. PFGE has been validated as a useful epidemiologic tool to study *A. baumannii* outbreak and is considered as the gold standard of epidemiological typing (Sabat et al., 2013).

Antimicrobial susceptibility test

Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method in accordance with EUCAST guidelines. The antibiotics tested were amikacin, gentamicin, ampicillin/sulbactam, piperacillin/tazobactam, ceftazidim, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, colistin and tetracycline. Also, minimal inhibitory concentration values (MIC) of imipenem and meropenem were determined by agar dilution method ranged from 4 to 256 µg/ml according to EUCAST 2014 guideline. The phenotypic detection of carbapenamases was done by the modified Hodge Test. *E.coli* ATCC 25922 was used as control strain.

Table 1. Antibiotic resistance profile of patients and enviromental *A.baumannii* isolates

	Patient 1 Tracheal aspirate	Patient 2 Tracheal aspirate	Patient 3 Tracheal aspirate	Patient 1 Out of ventilator hoses	Patient 1 Inside of ventilator hoses	Patient 1 Ventilator Surface	Patient 1 Ventilator Keyboard
Amikacin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Ciprofloxacin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Tetracycline	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Sulbactam/Ampicillin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Trimethoprim Sulfamethoxazole	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Ceftazidime	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Gentamicin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Levofloxacin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Imipenem	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml	Resistant MIC:16 µg/ml
Meropenem	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml	Resistant MIC:32 µg/ml
Piperacillin/Tazobactam	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Cefepime	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Colistin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

In this study, we aimed to determine carbapenem resistance and clonal relationships of *A. baumannii* isolated from patients and enviromental samples by phenotypic and genotypic methods in 10-bed ICU.

2. Materials and methods

Bacterial isolates

In March-2014, three tracheal aspirates and 30 environmental samples were collected from a 10-bed ICU admitted to our department with suspected outbreak in Adana Kadirli State Hospital. Patient cabinets, patient tables, hospital beds, ventilator surface, ventilator hoses inside-out, monitor surfaces were taken with swap and incubated Brain-Heart Infusion Broth (Merck, Germany) overnight at 37^0C. Incubated samples were inoculated Blood and Endo agar (Merck, Germany). Gram-negative, oxidase-negative cocobacillus was identified by the commercial identification kit which BBL Crystal System (Becton Dickinson Microbiology Systems, USA).

Detection of carbapenem resistance genes

Multiplex PCR was done for the detection of the four families of OXA-type carbapenamases found in *A. baumannii* (Woodford et al., 2006). The PCR conditions were as follows: Initial denaturation at 94°C for 5 min, 33 cycles of 94°C for 25 s, 53°C for 40 s and 72°C for 50 s, followed by an elongation step at 72°C for 6 min. The PCR products were visualized by agarose gel electrophoresis (blaOXA-23-like: 501 bp, blaOXA-51-like: 353 bp, blaOXA-24- like: 246 bp and blaOXA-58-like: 599 bp). After that, products were purified by using the PCR DNA purification kit (QIA Quick Gel Extraction Kit; Qiagen, Valencia, CA, USA) and subjected to automated DNA sequencing (ABI 310, Genetic Analyser; Applied Biosystems, USA). Analysis results were performed with the BLAST program (http://www.ncbi.nlm.nih.gov).

PCR was done for the detection of the ISAba1 segment (548 bp) located in the upstream of blaoxa genes (Turton et al., 2006a).

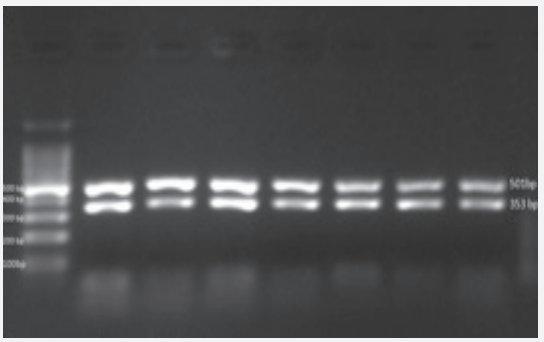


Fig. 1. PCR products obtained using bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-24} and bla_{OXA-23} primers in patients and enviromental *A.baumannii* isolates

Pulsed field gel electrophoresis

PFGE method detected total genom polymorphism is accepted as a "gold standard" for genotyping (Ertürk et al., 2014). Macrorestriction analysis of chromosomal DNA with ApaI (New England Biolabs, Boston, Mass.) was done by PFGE method (Durmaz et al., 2009). PFGE was run in a CHEF-DR II apparatus (Bio-Rad, USA), with pulses ranging from 5 to 30s at a voltage of 6 V/cm at 12°C for 20 h. Products were detected after staining with ethidium bromide (50 µg/ml) and photographed. Gel images were exported to Gelcompar II software (version 3.0; Applied Maths, SintMartens-Latem, Belgium) for analysis. Comparisons were made by using the band-based Dice coefficient. Dendrograms were generated by using the unweighted pair group method by arithmetic averaging method with 1% position tolerance. Isolates were considered to be genetically related if the Dice coefficient correlation was 80% or greater.

3. Results

Strain identification and characterization

A total of seven carbapenem-resistant isolates were studied. The isolates were cultured from three tracheal aspirate and four enviromental samples (ventilator surface, inside and out of ventilator hoses, ventilator keys of one patient). All were identified as *A.baumannii* by the BBL Crystal System (Becton Dickinson Microbiology Systems, USA).

Antimicrobial susceptibility test

The MICs of all antimicrobial agents tested exceeded the EUCAST resistance breakpoints. Imipenem ve meropenem MIC value of all isolates was determined 16 and 32 µg/ml, respectively, by the agar dilution method according to EUCAST guidelines (Table 1). Presence of carbapenamases was corrected by the modified Hodge Test.

Detection of β-lactamase genes and ISAbaI

PCR products of the appropriate size were obtained from seven isolates using primers for blaOXA-23-like (501 bp), blaOXA-51-like (353 bp), but no amplicons were obtained with primers targeting blaOXA-24-like or blaOXA-58-like genes. DNA sequencing was applied to genes encoding the OXA-23 and OXA-51 carbapenemase. Also, ISAba1 element was found in seven *A.baumannii* isolates.

Determination of the clonal relationship by PFGE

The strains of samples from clinical patients and environmental samples which were isolated from ICU, showed similarity of 100% in PFGE patterns (Fig. 2, Fig. 3).

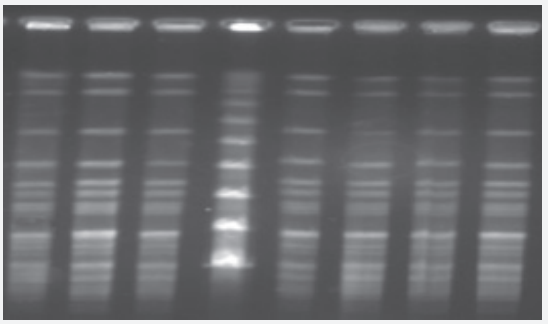


Fig. 2. PFGE profile of patients and enviromental *A.baumannii* isolates

4. Discussion

In recent years, the carbapenem-resistant A. baumannii isolates has become one of the most important agents, particularly ventilator-associated pneumonia (VAP) cases, in intensive care units (Nhu et al., 2014). Also, some studies showed that the same strain has been responsible for infection and contamination in ICU (Ertürk et al., 2014).

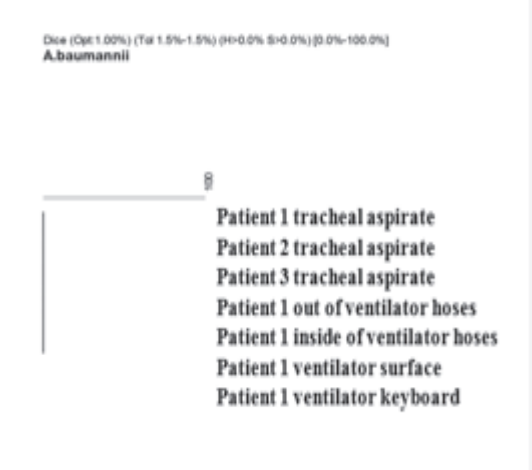


Fig. 3. The dendrograme of patients and enviromental *A.baumannii* isolates

Carbapenem is often used as "last-line agents" or "antibiotics of last resort" when patients with infections become gravely ill or are suspected of harboring resistant bacteria. Unfortunately, the recent emergence of multidrug-resistant (MDR) pathogens seriously threatens this class of lifesaving drugs. Several recent studies clearly show that resistance to carbapenems is increasing throughout the world (Papp-Wallace et al., 2011). The various studies were performed for carbapenem resistance rate of *A. baumannii*. A study conducted in our region shows that imipenem resistance rate of *A. baumannii* was 15.1% (Taşova et al., 1999). In Ankara, imipenem resistance of Acinetobacter species was found to be 53.6% (Arıkan, 2003). In 2010, imipenem and meropenem resistance was determined as 49% and 63% respectively (Balcı et al., 2010). In the 2011 report of national hospital infections surveillance network, carbapenem resistance have been reported as 74% *A. baumannii* strains on hospital infection (Gözütok et al., 2013). Carbapenem resistance rates shows a rising curve over the years. In our study, all of the *A. baumannii* isolates were resistant to imipenem and meropenem.

Carbapenem resistance mechanisms of *A. baumannii* are hydrolysis of beta-lactam antibiotics by beta-lactamase enzymes, changes on outer membrane proteins and penicillin-binding proteins and efflux pump (Peleg et al., 2008). The most common mechanism of resistance are the OXA-enzymes (Queenan and Bush, 2007).

OXA-type beta-lactamases continue to spread rapidly moving to dangerous levels carbapenem resistance among *A. baumannii* strains. OXA-type carbapenemases show a global distribution. The movement of blaOXA genes are performed by the presence of the insertion sequences and transposons in some cases, and therefore, they have the potential to spread very rapidly. OXA-type carbapenemases are responsible for a significant rate of the carbapenem resistance from all over the world. Especially, OXA-23 and OXA-51 are widely all over the world. In our country, the carbapenemases epidemiology of Acinetobacter studies have been performed and OXA-23 and OXA-58 outbreaks have been reported (Arslan, 2014).

In our region, in *A. baumannii* strains isolated a burn unit, blaOXA-24 and blaOXA-51 have been identified (Gökmen et al., 2012). In addition to, the OXA-24/40 has been reported in our country (Sarı et al., 2013).

In various studies, the insertion sequence ISAba1 has been determined to located upstream of the blaOXA-23, blaOXA-51, blaOXA-58 carbapenemase genes and cephalosporinase blaampC genes in many *A. baumannii* isolates and increased expression of these genes (Heritier et al., 2006; Poirel and Nordmann, 2006; Turton et al., 2006). In our study, isolates containing blaOXA-23 and blaOXA-51 have carried ISAba1segment. Consequently, they were resistant to imipenem and meropenem.

The mechanical ventilation is one of major risk factor in outbreaks of carbapenem resistant *A. baumannii* (Karabay et al., 2012). ICU mortality rate of VAP ranged from 45.6% to 60.9% and has been found to be as high as 84.3% when VAP was caused by Extreme Drug Resistant *A. baumannii* (Inchai et al., 2014).

Our study showed that three ventilator-associated pneumoniae (VAP) cases were caused by carbapenem resistant *A. baumannii* survived on ventilator of ICU. Isolates were found to be 100% clonal associated with PFGE and had same MIC values for imipenem and meropenem. blaOXA-23 and blaOXA-51 genes has been determined all of the isolates. Similarly, in patients with VAP of ICU, one pulsotype (similarity coefficient 95%) has contained 21 out of 23 isolates harbouring both the blaOXA-51 and blaOXA-23 genes (Nhu et al., 2014).

The limitation of our study is that, the number of isolates inadequate to say the outbreak. However, this outbreak occured in two week and ten-bed ICU. In addition, the detection of the infection source and clonal relationship of the isolates was evidence that a small-scale outbreak.

As a result, resistance profile and DNA band profile were same in seven strains. Thus, it was considered that there was a small and short-term outbreak in our study.

After this study, effective disinfection applications were applied to ICU and enviromental samples were taken. *A.baumannii* could not growth on culture. It showed that molecular epidemiology studies and effective disinfection process ended oubreak of carbapenem resistant *A.baumannii* strains in ICU.

REFERENCES

Arıkan, A.Ö., 2003. Acinetobacter baumannii izolatlarında antibiyotik direnci: 2002 yılı İbni Sina Hastanesi verileri, Mikrobiyol. Bült. 37, 241-246.

Arslan, H., 2014. Acinetobacter moleküler epidemiyolojisi. ANKEM. 28, 71-72.

Balcı, M., Bitirgen, M., Kandemir, B., Arıbaş, E.T., Erayman, I., 2010. Antibiotic susceptibility of nosocomial acinetobacter baumannii strains. 24, 28-33.

Bergogne-Bérézin, E., Towner, K.J., 1996. Acinetobacter spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. 9, 148-165.

Durmaz, R., Otlu, B., Koksal, F., Hosoglu, S., Ozturk, R., Ersoy, Y., Aktas, E., Gursoy, N.C., Caliskan, A., 2009. The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of Acinetobacter baumannii, Escherichia coli and Klebsiella spp. Jpn. J. Infect. Dis. 62, 372-327.

Ertürk, A., Çiçek, A.Ç., Gümüş, A, Cüre, E., Şen, A., Kurt, A., Karagöz, A., Aydoğan, N., Sandallı, C., Durmaz, R., 2014. Molecular characterisation and control of Acinetobacter baumannii isolates resistant to multi-drugs emerging in inter-intensive care units. Ann. Clinic. Microbiol. Antimicrob. 13, 36. doi:10.1186/s12941-014-0036-2.

European Commitee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Versions3.1.Available:http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf Accessed 10 May 2014.

Gözütok, F., Sarıgüzel, F.M., Çelik, İ., Berk, E., Aydın, B., Güzel, D., 2013. Hastane infeksiyonu etkeni acinetobacter baumannii suşlarının antimikrobiyal direnç oranlarının araştırılması. ANKEM. 27, 7-12.

Gökmen, T.G., Güran, M., Benk, I.G., Kızılyıldırım, S., Köksal, F., 2012. Yanık ünitesinde kısa-dönem/uzun-dönem acinetobacter baumannii salgını. Ankem. 26, 120-125.

Héritier, C., Poirel, L., Nordmann, P., 2006. Cephalosporinase overexpression as a result of insertion of ISAba1 in Acinetobacter baumannii. Clin. Microbiol. Infect. 12, 123-130. doi: 10.1111/j.1469-0691.2005.01320.x.

Inchai, J., Pothirat, C., Bumroongkit, C., Limsukon, A., Khositsakulchai, W., Liwsrisakun, C., 2014. Prognostic factors associated with mortality of drug-resistant Acinetobacter baumannii ventilator-associated pneumonia. J. Inten. Care. 3, 9. doi: 10.1186/s40560-015-0077-4.

Karabay, O., Yahyaoğlu, M., Öğütlü, A., Sandıkçı O, Tuna N, Ceylan S., 2012. Factors associated with mortality in acinetobacter baumannii infected intensive care unit patients. Mikrobiyol. Bul. 46, 335-337.

Kohlenberg, A., Brümmer, S., Higgins, P.G., Sohr, D., Piening, B.C., De Grahl., C., Halle, E., Rüden, H., Seifert, H., 2009. Outbreak of carbapenem-resistant Acinetobacter baumannii carrying the carbapenemase OXA-23 in a German university medical centre. J. Med. Microbiol. 58, 1499-1507. doi: 10.1099/jmm.0.012302-0.

Moniri, R., Kheltabadi, Farahani, R., Shajari, G.H., Nazem, Shirazi, M.H., Ghasemi, A., 2010. Molecular epidemiology of aminoglycosides resistance in acinetobacter spp. with emergence of multidrug-resistant strains. Iran. J. Public. Health. 39, 63-68.

Naas, T., Poirel, L., Nordmann, P., 2006. Pyrosequencing for rapid identification of carbapenem-hydrolysing OXA-type β-lactamases in Acinetobacter baumannii. Clinic. Microbiol. Infect. 12, 1236-1240. doi:10.1111/j.1469-0691.2006.01563.x.

Nasr, R.A., Attalah, M.F., 2012. Molecular epidemiology of nosocomial acinetobacter baumannii isolates. Nat. Sci. 10, 76-82.

Nhu, N.T.K., Lan, N.P.H., Campbell, J.I, Parry, C.M., Thompson, C., Tuyen, H.T., et al., 2014. Emergence of carbapenem-resistant Acinetobacter baumannii as the major cause of ventilator-associated pneumonia in intensive care unit patients at an infectious disease hospital in southern Vietnam. J. Med. Microbiol. 63, 1386-1394. doi:10.1099/jmm.0.076646-0.

Papp-Wallace, K.M., Endimiani, A., Taracila, M.A, Bonomo, R.A., 2011. Carbapenems: Past, present, and future. Antimicrob. Agents. Chemother. 55, 4943-4960. doi: 10.1128/AAC.00296-11.

Peleg, A.Y., Seifert, H., Paterson, D.L., 2008. Acinetobacter baumannii: Emergence of a successful pathogen. Clin. Microbiol. Rev. 21, 538-82. doi: 10.1128/CMR.00058-07.

Poirel, L., Nordmann, P., 2006. Carbapenem resistance in Acinetobacter baumannii: Mechanisms and epidemiology. Clin. Microbiol. Infect. 12, 826-836. doi: 10.1111/j.1469-0691.2006.01456.x.

Queenan, A,M., Bush, K., 2007. Carbapenemases: the versatile β-lactamases. Clin. Microbiol. Rev. 20, 440-458. doi: 10.1128/CMR.00001-07 doi: 10.1128/CMR.00001-07.

Sabat, A.J., Budimir, A., Nashev, D., Sá-Leão, R., van Dijl, J.M., Laurent, F., Grundmann, H., Friedrich, A.W., 2013. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Eurosurveill. 18, 1-14.

Sarı, A.N., Biçmen, M., Gülay, Z., 2013. The first report on the outbreak of OXA-24/40-like carbapenemase-producing Acinetobacter baumannii in Turkey. Jpn. J. Infec.t Dis. 66, 439-442.

Taşova, Y., Akgün, Y., Saltoğlu, N., Yılmaz, G., Kara, O., Dündar, I.H., 1999. Nozokomiyal Acinetobacter infeksiyonları. Flora. 4, 170-176.

Turton, J.F., Woodford, N., Glover, J., Yarde, S., Kaufmann, M.E., Pitt, T.L., 2006a. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. 44, 2974-2976. doi: 10.1128/JCM.01021-06.

Turton, J.F., Ward, M.E., Woodford, N., Kaufmann, M.E., Pike, R., Livermore, D.M, Pitt, T.L., 2006b. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS. Microbiol. Lett. 258, 72-77. doi: 10.1111/j.1574-6968.2006.00195.x.

Woodford, N., Ellington, M.J., Coelho, J.M., Turton, J.F., Ward, M.E., Brown, S., Amyes, S.G., Livermore, D.M., 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int. J. Antimicrob. Agents. 27, 351-353. doi: DOI: 10.1016/j.ijantimicag.2006.01.004.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Case Report

J. Exp. Clin. Med., 2016; 33(2): 163-165
doi: 10.5835/jecm.omu.33.03.007

Is phenylketonuria causes bronchospasm during general anesthesia?

Aysun Caglar Torun*, Mehmet Ziya Yilmaz

Department of Pedodontia, Faculty of Dentistry, Ondokuz Mayis University, Samsun, Turkey

ARTICLE INFO

Article History
Received 10 / 02 / 2015
Accepted 12 / 04 / 2015

*** Correspondence to:**
Aysun Caglar Torun
Department of Pedodontia,
Faculty of Dentistry,
Ondokuz Mayis University,
Samsun, Turkey
e-mail: aysunct@hotmail.com

Keywords:
Catecholamines
Phephenylketonuria
Phenylalanine
Bronchospasm

ABSTRACT

Phephenylketonuria (PKU) is caused by an accumulation of phenylalanine in blood and tissue due to phenylalaninehydroxylase enzyme deficiency, which means that phenylalanine, an essential amino acid, cannot be converted to tyrosine. The accumulation of phenylalanine in nonbrain tissues and the decreased production of tyrosine can cause various clinical symptoms. Catecholamines are synthesized via a series of reactions initiated by tyrosine 3,4-dihydroxyphenylalanine hydroxylation. And in the absence of tyrosine, the synthesis of epinephrine can be reduced. There appear to be no studies in the present literature on non-neurological symptoms associated with decreased catecholamine synthesis in patients with PKU. In the present case, we described a severe bronchospasm in a child with PKU during general anesthesia. Further research is needed to confirm whether the bronchospasm that occurred in this case was due to a lack of catecholamine induced by PKU. A link between a deficiency of catecholamines, which are required for neuronal and hormonal control, and pulmonary findings in PKU can be established with clinical and experimental studies.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive inherited metabolic disease.The prevalence of PKU is 1:10.000-30.000 worldwide and 1:2600 in Turkey (Williams et al., 2008). This high incidence of PKU in Turkey has been linked to the common practice of consanguineous marriages. The disease is caused by an accumulation of phenylalanine in blood and tissue due to phenylalanine hydroxylase enzyme deficiency, which means that phenylalanine, an essential amino acid, cannot be converted to tyrosine (Al Hafid and Christodoulou, 2015). Dysfunction of tetrahydrobiopterin, the cofactor of phenylalanine hydroxylase, may also play a role in PKU. The accumulation of phenylalanine and a lack of tyrosine form the clinical basis of the disease. Although babies with PKU appear healthy at birth, symptoms begin to develop once they start feeding (Ney et al., 2014). The disease may not be recognized until after the occurrence of irreversible severe mental and motor retardation. A phenylalanine-restricted diet is the most important treatment to prevent further brain damage (Vockley et al., 2014).

Many different clinical findings have been described in patients with PKU, with microcephaly, hypertonia, hyper-reflexia, autism, seizures, and seborrheic and eczematous skin rashes the most common clinical findings. In the present case, we describe pulmonary side effects following the induction of general anesthesia in a child with PKU.

2. Case

A 10- year-old male diagnosed with PKU in the neonatal period was admitted to our center for dental treatment under general anesthesia. The patient was receiving dietary treatment for PKU, and his blood phenylalanine levels were within normal limits. His general condition was good, but he showed a lack of awareness of his environment. Due to an inability to cooperate with the dentist, general anesthesia was required for the dental treatment. No pathology was detected in the preoperative evaluation. The patient had been anesthetized twice under deep sedation (tooth extraction and circumcision) and had not experienced any problems. General anesthesia was induced with propofol (2 mg/kg), fentanyl (1 mg/kg), and vecuronium (0.1 mg/kg), administered intravenously. For maintenance of anesthesia, 2% sevoflurane and a mixture of 50% oxygen and 50% air were used. The patient's vital signs were stable. Immediately after intubation, his airway pressure (45-50 mmHg) increased, and breath sounds decreased. A bronchospasm was diagnosed. The inhaled bronchodilator salbutamol was administered, in addition to methylprednisolone (2 mg/kg), which was administered intravenously to the patient. Thirty minutes after intubation, the patient's airway pressure and ventilation improved. Oxygen saturation remained in the range of 98-99%, and no further decrease was observed. The patient was extubated without any problem, and the postoperative follow-up revealed no problem.

3. Discussion

Phenylalanine, which cannot be converted to tyrosine in PKU, undergoes transamination to pyruvate and conversion to phenylpyruvate. Phenylpyruvate accumulates in the blood and tissues and is excreted by urine. The metabolite phenylpyruvate results in urine and body fluids having a musty smell. In excessive amounts, the accumulated phenylalanine in the blood competes with other amino acids to pass the blood-brain barrier and can lead to a reduction of metabolites in the brain. This can result in impaired brain development and defective myelination, which can cause epileptic seizures. Phenylalanine inhibits the enzymatic synthesis of serotonin in the brain. The resulting low levels of serotonin may be the cause of the mental retardation in PKU patients. In addition, it has been suggested that a chronic lack of glutamine due to the excessive use of glutamine in the formation of phenylglutamine may be a direct cause of brain injury in PKU (Ney et al., 2014).

The accumulation of phenylalanine in non brain tissues and the decreased production of tyrosine can cause other clinical symptoms. Increased levels of phenylalanine in body fluids may reduce the amount of tyrosine, as well as that of other amino acids, in body fluids by inhibiting the absorption of these amino acids from the gastrointestinal tract and their reabsorption from the kidneys (Giovannini et al., 2007).

Catecholamines and melanin are synthesized via a series of reactions initiated by tyrosine 3,4-dihydroxyphenylalanine hydroxylation. Thyroxine, which is synthesized by iodination of tyrosine residues, serves as the precursor of thyroid hormone (Scriver, 2007). Thus, in the absence of tyrosine, the synthesis of epinephrine, melanin and thyroxine can be reduced.

Clinical symptoms associated with deficiency of epinephrine, thyroxine and melanin can be expected in PKU. According to the literature, psychiatric symptoms are particularly common in PKU patients due to a lack of serotonin and catecholamine (Bilder et al., 2013). We thought it might be a catecholamine deficiency due to a lack of tyrosine in this case. We ephasized the hypothesis that the low level of catecholamine would be cause of experienced bronchospasm. But there are no studies in the present literature on non-neurological symptoms associated with decreased catecholamine synthesis in patients with PKU. In the present case, we described a severe bronchospasm in a child with PKU during general anesthesia. A review of the literature did not reveal any other studies on lung problems in patients with PKU.

Some peptides are known to be effective especially in airway inflammation and hyper-responsiveness resulting from infections and allergies. Dinh et al. (2005) showed that the expression of tachykinin peptides was increased in a mouse model of allergic airway inflammation. They reported that these peptides may play a role in the pathogenesis of airway diseases. There are very few studies on sympathetic neurons and their transmitters in allergic airway inflammation. In one recent study of a mouse model of allergic airway inflammation, the authors reported that the neuropeptides catecholamine and tyrosine were involved in sympathetic-adrenergic control and that the expression of these neuropeptides did not increase after allergen exposure (Dinh et al., 2004). They stated that it was not possible to completely exclude a role for these proteins in the pathogenesis of allergic airway inflammation (de Jongste et al., 1991). New studies are needed to confirm whether the bronchospasm that occurred in this case was due to a lack of catecholamine induced by PKU.

4. Conclusion

A link between a deficiency of catecholamines, which are required for neuronal and hormonal control, and pulmonary findings in PKU can be established with clinical and experimental studies. Further researchs are needed on the potential effects of tyrosine deficiency in patients with PKU in the different organs of the body.

REFERENCES

Al Hafid, N., Christodoulou, J., 2015. Phenylketonuria: A review of current and future treatments. Transl. Pediatr. 4, 304-317.

Bilder, D.A., Burton, B.K., Coon, H., Leviton, L., Ashworth, J., Lundy, B.D., Vespa, H., Bakian, A.V., Longo, N., 2013. Psychiatric symptoms in adults with phenylketonuria. Mol. Genet. Metab. 108, 155-160.

Dinh, Q.T., Groneberg, D.A., Witt, C., Peiser, C., Cifuentes, L.B., Frossard, N., Klapp, B.F., Fischer, A., 2004. Expression of tyrosine hydroxylase and neuropeptid etyrosine in Mouse sympatheticairway-specificneuronsunder normal situation and allergic airway inflammation. Clin. Exp. Allergy. 34, 1934-1941.

Dinh, Q.T., Mingomataj, E., Quarcoo, D., Groneberg, D.A., Witt, C., Klapp, B.F., Braun, A., Fischer, A., 2005. Allergic airway inflammation induce stachykin in peptides expression in vagal sensory neurons innervating mouse airways. Clin. Exp. Allergy. 35, 820-825.

Giovannini, M.,Verduci, E., Salvatici, E., Fiori, L., Riva, E., 2007. Phenylketonuria: Dietary and therapeutic challenges. J. Inherit. Metab. Dis. 30, 145-152.

de Jongste, J.C., Jongejan, R.C., Kerrebijn, K.F., 1991. Control of airway caliberbyautonomic nerves in asthma and in chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 143, 1421-1426.

Ney, D.M., Blank, R.D., Hansen, K.E., 2014. Advances in the nutritional and pharmacological management of phenylketonuria. Curr. Opin. Clin. Nutr. Metab. Care. 17, 61-68.

Scriver, C.R., 2007. The PAH gene, phenylketonuria, and a paradigmshift. Hum. Mutat. 28, 831-845.

Williams, R.A., Mamotte, C.D., Burnett, J.R., 2008. Phenylketonuria: An inborn error of phenylalanin emetabolism. Clin. Biochem. Rev. 29, 31-41.

Vockley, J., Andersson, H.C., Antshel, K.M., Braverman, N.E., Burton, B.K., Frazier, D.M., Mitchell, J., Smith, W.E., Thompson, B.H., Berry, S.A., 2014. Phenylalanin ehydroxylase deficiency: Diagnosis and management guideline. Genet. Med. 16, 188-200.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Case Report

J. Exp. Clin. Med., 2016; 33(3): 167-169
doi: 10.5835/jecm.omu.33.03.008

Paterson-Kelly syndrome in a patient with celiac disease

Piltan Buyukkaya[a*], Nevin Alayvaz Aslan[a], Sude Hatun Aktimur[a], Serdar Turan[b], Hilmi Atay[a], Seda Gün[c], Engin Kelkitli[a], Ahmet Bektas[d], Mehmet Turgut[a]

[a] *Department of Internal Medicine, Division of Hematology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[b] *Department of Internal Medicine, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[c] *Department of Pathology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[d] *Department of Gastroenterology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*

ARTICLE INFO

Article History
Received 30 / 12 / 2014
Accepted 22 / 04 / 2015

*** Correspondence to:**
Piltan Buyukkaya
Department of Internal Medicine
Division of Hematology,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun, Turkey
e-mail: pitan79@hotmail.com

Keywords:
Bougie dilation
Celiac disease
Dysphagia
Iron-deficiency anemia

ABSTRACT

Paterson-Kelly syndrome is characterized with iron deficiency anemia and esophagial web. Association between Paterson-Kelly and Celiac disease is not well-known. Especially in our country, there is insufficient data about these two diseases. We report a case with a complaint of dysphagia and diagnosed as Paterson-Kelly syndrome with celiac disease. Dysphagia was resolved with bougie dilation, oral iron supplement and gluten free diet. We want to emphasize the importance of screening for celiac disease in patient with dysphagia and iron-deficieny anemia.

1. Introduction

Paterson Brown Kelly syndrome (Plummer Vinson syndrome) is described with triad of dysphagia, iron-deficiency anemia and esophagial webs. Loss of iron dependent enzyme in esophagial mucosa cause impaired peristaltism. So web formation occurs in esophagus. So there is a direct association with iron deficiency anemia and esophagial web. It has been known that treatment of iron deficiency resulted with improvement of dysphagia (Klifto et al., 1983). In celiac disease, iron deficiency anemia was devoloped due to impaired absorption of iron from duodenum in the 12-18% of patients (Ackerman et al., 1996). There are very few case reports suggesting association between celiac disease and Plummer Vilson syndrome (Dickey and McConnell, 1999; Malhotra et al., 2000, Sood et al., 2005; Sinha et al., 2008). We want to report a case with severe dysphagia and celiac disease who was succesfully treated with iron supplements.

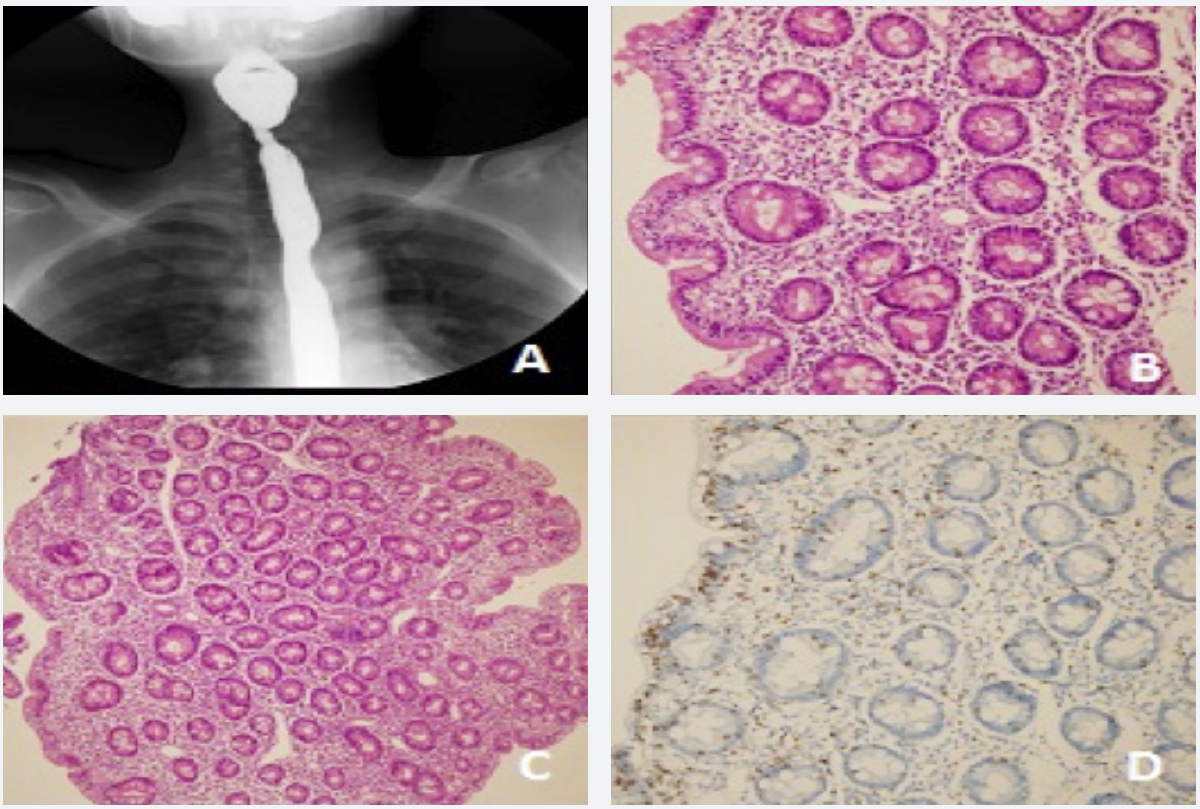


Fig. 1. A. Pharyngoesaphagography shows 1,5 cm lenght web on prowimal esaphagus. **B-C:** Pathologic examination of duodenal biopsy with high field **(B)** and low **(C)** power view shows villous atrophy and blunting; **D**: Lymphocytic infiltration with CD3 positive lymphocytes in surface epithelium of duodenum

2. Case report

A fourty-two year old woman came to hematology clinic with complaints of exhaustion, dizziness and palpitation. In her story, she had difficulties in swallowing especially with the solid foods for ten years. She have had nousea and vomiting for 15 days. Her difficulty in swallowing was increased recently. She lost 6 kg in the past six months. She has taken oral iron treatment discontinuously for ten years because of anemia. On physical examination she looked pale and angular stomatitis was present. Laboratory examination of the patient showed hemoglobin:7.9 g/dL, platelets (PLT): 1302 000/uL, iron saturation: 2,64%, serum iron level: 8 µg/dL (37-145), ferritin: 2.28 ng/mL (21.8-274), folic acid: 3,7 ng/mL (4.6-18.7) and vitamine B12:419 pg/dL (197-866) with normal liver and renal function. Examination of peripheral blood smear showed microcytic hypochromic anemia and excess thrombocytes with no atypical nucleated cells. For further investigation of iron deficiency anemia serologic test was done for celiac disease and antigliadin Ig A was 53.1 U/mL(<12), antigladin IgG was 62 U/mL(<12) and anti-tissue transglutaminase level was 69 U/L (0-10).

In gastroscopic examination there was narrow part that could not be passed with endoscope in the upper esophagus sphincter localization. In pharyngoesaphogography, after the pharyngoesophageal junction there was a web approximately 1.5 cm length on proximal esophagus (Fig. 1A). In another session, dilatation was done with 9-11-13 mm-bougies. On the second part of duodenum there was a ‘’cracked earth view ‘’and biopsy was taken from there. Villus atrophy and blunting were observed via microscopic examination (Fig. 1B,C). There was a lymphocytic infiltration with CD3 positive lymphocytes in surface epithelium (Fig. 1D).

Patient was diagnosed as gluten enteropathy and gluten free diet was started and intravenous ferric hydroxide was given with a dose of 1500mg. After one month of treatment her Hbg was raised to 11.2 gr/dL and her complaints were disappeared.

3. Discussion

There is a well-known relationship with iron deficiency anemia and celiac disease (Corazza et al. 1995; Ackerman et al., 1996). In celiac disease, 12-18% of patients have iron-deficieny anemia (Corazza et al., 1995) because of iron malabsorption.

Also iron deficienc anemia is one of the components of Paterson Kelly syndrome. Strong relationship with these two disease was shown in two case series with 72 and 63 patients (Chisholm et al., 1971; Bredenkamp et al., 1990). But there were few case reports and one prospective study regarding association of esophagial web and celiac disease. In a prospective study from India includes 21 patients with esophagial web and dysphagia. Eighteen of them had iron-deficiency anemia and five (23.8%) of them diagnosed as celiac disease with serology and endoscopic biopsy (Sinha et al., 2008). Duration of dysphagia before diagnosis of celiac disease ranged from six months to 15 years (Sinha et al., 2008). Sood et al. (2003) retrospectively investigated 96 cases with celiac disease and they found that three of them had esophagial web also. In most of case reports and study, none of the patients had chronic diarrhea at presentation (Sood et al., 2005; Sinha et al., 2008).

Celiac disease is misdiagnosed in case with esophagial web because serologic test and endoscopic biopsy is not routinely done. In our case, there was no symptom of diarrhea or skin lesions that indicate celiac disease. But patient had iron deficiency anemia and dysphagia since ten years and symptoms were resistant to iron replacement. Dickey and McConnell (1999) reported two cases with celiac disease and esophagial web and they were diagnosed as celiac disease after 13 and 9 years from dysphagia begun.

In many patients with Paterson-Kelly syndrome dysphagia is resolved after iron replacement (Hoffman and Jaffe, 1995). But in celiac disease in the absence of gluten free diet, iron deficiency anemia persist. In our patient bougie dilation was done for dysphagia and iron supplements were given orally. Also the importance of gluten free diet was emphasized to the patient. During a follow-up for two months dysphagia did not recur and iron deficiency anemia was improved. In other case reports (Dickey and McConnell, 1999; Malhotra et al., 2000; Sood et al., 2005) and prospective study (Sinha et al., 2008) dysphagia was resolved after bougie dilation and iron replacement with gluten free diet.

Patients with Paterson-Kelly syndrome have a risk of malignancy-carcinoma of the esophagus and postcricoid carcinoma (Novacek, 2006; Ben Gamra et al., 2007). So regular follow up these patients is important and necessary.

4. Conclusions

Celiac disease is usually misdiagnosed in patient with Paterson-Kelly syndrome. So patients with esophagial web and iron deficiency anemia should be investigated for celiac disease even if there is no diarrhea or skin manifestation of celiac disease.

REFERENCES

Ackerman, Z., Eliakim, R., Stalnikowicz, R., Rachmilewitz, D., 1996. Role of small bowel biopsy in the endoscopic evaluation of adults with iron deficiency anemia. Am. J. Gastroenterol. 91, 2099-2102.

Ben Gamra, O., Mbarek, C., Mouna, C., Zribi, S., Zainine, R., Hariga, I., El Khedim, A., 2007. Plummer vinson syndrome. Tunis Med. 85, 402-404.

Bredenkamp, J.K., Castro, D.J., Mickel, R. A., 1990. Importance of iron repletion in the management of Plummer-Vinson syndrome. Ann. Otol. Rhinol. Laryngol. 99, 51-54.

Chisholm, M., Ardran, G.M., Callender, S.T., Wright, R., 1971. A follow-up study of patients with post-cricoid webs. Q. J. Med. 40, 409-420.

Corazza, G.R., Valentini, R.A., Andreani, M.L., D'Anchino, M., Leva, M.T., Ginaldi, L., De Feudis, L., Quaglino, D., Gasbarrini, G., 1995. Subclinical coeliac disease is a frequent cause of iron-deficiency anaemia. Scand. J. Gastroenterol. 30, 153-156.

Dantas, R.O., 1999. Iron deficiency and dysphagia. Am. J. Gastroenterol. 94, 3072-3073.

Dickey, W., McConnell, B., 1999. Celiac disease presenting as the Paterson-Brown Kelly (Plummer-Vinson) syndrome. Am. J. Gastroenterol. 94, 527-529.

Hoffman, R.M., Jaffe, P.E., 1995. Plummer-Vinson syndrome. A case report and literature review. Arch. Intern. Med. 155, 2008-2011.

Klifto, E.J., Allen, S.K., Metzman, M., Chase P.J., Kupersmit, M., 1983. Plummer-Vinson syndrome: Report of a case and review of the literature. J. Am. Osteopath. Assoc. 83, 56-59.

Malhotra, P., Kochhar, R., Varma, N., Kumari, S., Jain, S., Varma, S., 2000. Paterson-Kelly syndrome and celiac disease a rare combination. Indian J. Gastroenterol 19, 191-192.

Novacek, G., 2006. Plummer-Vinson syndrome. Orphanet. J. Rare. Dis. 1, 36.

Sinha, S.K., Nain, C.K., Udawat, H.P., Prasad, K K., Das, R., Nagi, B., Singh, K., 2008. Cervical esophageal web and celiac disease. J. Gastroenterol. Hepatol. 23, 1149-1152.

Sood, A., Midha, V., Sood, N., Bansal, M., 2005. Paterson Kelly syndrome in celiac disease. J. Assoc. Physicians. India. 53, 991-992.

Sood, A., Midha, V., Sood, N., Malhotra, V., 2003. Adult celiac disease in northern India. Indian J. Gastroenterol. 22, 124-126.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Case Report

J. Exp. Clin. Med., 2016; 33(3): 171-173
doi: 10.5835/jecm.omu.33.03.009

Pregnancy in a patient with univentricular circulation: A case report and literature review

Ayse Zehra Ozdemir[a], Hasan Ulubasoglu[b], Davut Guven[a*]

[a] *Department of Obstetrics and Gynecology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[b] *Department of Obstetrics and Gynecology, Dr. Munif Islamoglu State Hospital, Kastamonu, Turkey*

ARTICLE INFO

Article History
Received 13 / 01 / 2015
Accepted 23 / 04 / 2015

*** Correspondence to:**
Davut Guven
Department of Obstetrics and Gynecology,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun, Turkey
e-mail: dguven@omu.edu.tr

Keywords:
Congenital heart disease
Pregnancy
Univentriculer circulation
Univentriculer heart

ABSTRACT

Univentricular heart is a complex congenital heart disease. With pregnancy, the complexity of this disease increases and it has many risks for fetus and mother, including maternal mortality. A pregnant woman has univentricular heart has a healthy pregnancie and delivery period which is a rare medical event.

1. Introduction

In pregnancy, congenital heart diseases are more common than acquired one in developed countries (Pitkin et al., 1990). With the progress in surgical and medical management of pediatric congenital heart disease, patients can reach the childbearing age. Univentricular heart is a rare congenital heart disease mixing of pulmonary and systemic circulation in ventricle level. Hemodynamic changes during pregnancy makes a full term pregnancy rare and threats these patients' life (Buckland and Pickett, 2000). We herein report a case of pregnancy in a patient with univentricular circulation.

2. Case report

We present a 27 year old primigravida who has an univentricular circulation (Fig. 1). In her past medical history, at the second month of her life her parents noticed her voice fall at the time of crying, cardiac catheterization showed an univentriculer heart and transposition of great vessels with pulmoner stenosis. She regularly visited her pediatric cardiologist and any cardiac surgery wasn't considered to this case and transplantation was excluded, she was generally asymptomatic and her exercise tolerance is well but she sometimes troubled with dyspnea, cardiac pain, syncope and arrhythmia episodes, and sometimes she needed

hospitalization and medication for these problems. Her doctor strongly warned her about pregnancy and when the pregnancy was confirmed the doctor repeated the danger of this pregnancy in her own life, and related obstetric problems such as: Misscariage or preterm delivery. The patient chose to continue the pregnancy. She was regularly seen by an obstetrician and a cardiologist. She was anticoagulated with enoxaparine during her pregnancy. She sometimes troubled with arrhythmia but didn't need any medication.

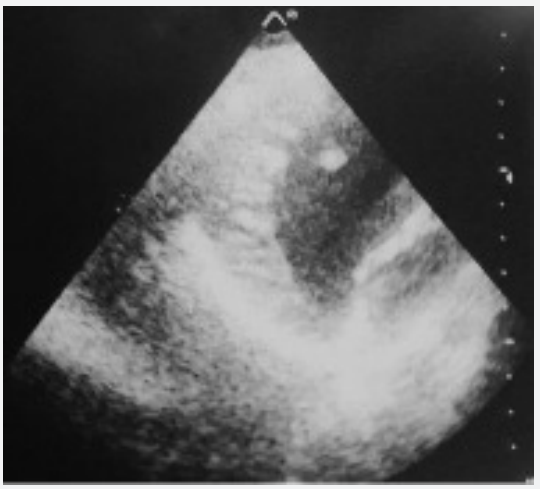


Fig. 1. Univentriculer which is not separated by a septum: Echocardiographic imagine

The fetus was assessed regularly with growth measurements and flow velocity of umblical artery and amniotic fluid. A detailed fetal ultrasonographic scan was performed to exclude any cardiac anomaly at 20th week. At 31th week of gestation the abdominal circumference of fetus is 0.1 percentil, biparietal diameter, and femur length was consistent with 31th week, so that an asymetric intrauterine-growth restriction (IUGR) was confirmed and 24 hours after administration of second dose of celestone, an elective caesarean section performed, anesthesiologist were informed about this, and finally she gave born a female 1460 gram infant with Apgar score 7-8.

The patient didn't need intensive care unit at the end of the surgery, but the baby because of respiratory distress sendrome (RDS) transferred neonatal intensive care unit, postoperative anticoagulation continued and the patient was discharged successfully six days after her surgery, the baby stayed in neonatal intensive care unit because of RDS but no congenital heart disease was detected.

3. Discussion

Univentricular heart accounts for 3.2% of congenital cardiac abnormalities (Theodoridis et al., 2005). It is a circulation that systemic and pulmonary venous return mix in single ventricle (Bernstein, 2008). If the patient maintains balanced systemic and pulmonary circulations, the patient survives without any operation.

Pregnant women with congenital heart disease have some maternal and fetal risks and it is very important to predict which patient has increased risk for maternal and fetal complications.

A study by Presbitero et al. (1994) examining the outcomes of 96 pregnancies in 44 women with a variety of cyanotic congenital heart disease (CHD) reported that the arterial oxygen concentration at rest (>85%) and the hemoglobin concentration at the beginning of pregnancy (<20 g/dL) are the main determinants of live birth. Values of our case were the arterial oxygen concentration at rest (90-95%) and the hemoglobin concentration at the beginning of pregnancy (15-16 g/dL).

They noticed that women with CHD can continue their pregnancy with low risk for them but there is a high incidence of miscarriage, premature births, and low birth weights (Presbitero et al.,1994). Values in our case were compatible with the literature and birth has achieved with multidisipliınary approach.

Risk of any congenital heart disease in fetus is 3%-6% up to a 10-fold increase over the general population (Siu and Colman, 2001). So a detailed fetal ultrasonographic scan is very important.

In a prospective study of pregnancy outcomes in women with heart disease; no association between the type of delivery and peripartum cardiac event rate was found (Burn et al., 1998).

Vaginal delivery is recommended for patient who has univentricular heart (Cunningham et al., 2010). But some obstetric indications make cesaerean more common in these pregnancies, in our patient an asymmetric IUGR and unfavorable cervix are the indications.

Congenital heart disease patients may survive relatively long times so it is very important to learn more about a pregnancy complicated with maternal congenital heart disease.

If our knowledge about these pregnancies increase, a multidisiplinary management including a cardiologist, obstetrician and obstetric anesthesiologist will encourage this patient for pregnancy.

REFERENCES

Bernstein, D., In: Behrman, R.E., Kliegman, R.M., Jenson, H.B., Stanton, F.B., 2008. Nelson textbook of pediatrics. 18th ed. WB Saunders; Cyanotic congenital heart disease, Philadelphia, p.1918-1930.

Buckland, R., Pickett, J.A., 2000. Pregnancy and univentricular heart: Case report and literature review. Int. J. Obstet. Anesth. 9, 55-63. doi: 10.1054/ijoa.1999.0341.

Burn, J., Brennan, P., Little, J., 1998. Recurrence risks in offspring of adults with major heart defects. Lancet. 351, 311-316. doi:10.1016/S0140-6736(97)06486-6.

Cunningham, F.G., Leveno, K.J., Bloom, S.L., Hauth J.C., Rouse D.J., Spong C.Y., 2010. Williams Obstetrics. 23rd ed. Cardiovascular disease, United States of America, p.958-982.

Pitkin, R.M., Perloff, J.K., Koos, B.J., 1990. Pregnancy and congenital heart disease. Ann. Intern. Med. 112, 445-454. doi:10.7326/0003-4819-76-3-112-6-445.

Presbitero, P., Somerville, J., Stone, S., 1994. Pregnancy in cyanotic congenital heart disease. Circulation. 89, 2673-2676. doi: 10.1161/01.CIR.89.6.2673.

Siu, S.C., Colman, J.M., 2001. Heart disease an d pregnancy. Heart. 85,710-7155. doi:10.1136/heart.85.6.710.

Theodoridis, T.D., Anagnostou, E., Zepiridis, L., 2005. Successful pregnancy and caeserean section delivery in a patient with single ventricle and transposition of the great arteries. J. Obstet Gynaecol. 25, 69-70. doi:10.1080/01443610400025911.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Case Report

J. Exp. Clin. Med., 2016; 33(3): 175-177
doi: 10.5835/jecm.omu.33.03.010

Delayed diagnosis of selective immunoglobulin deficiency: A case report

Veysel Kars[a], Tahsin Celepkolu[a], Ahmet Yilmaz[a], Fatih Esmer[b], Sevdegul Karadas[c*]

[a] *Department of Family Physician, Faculty of Medicine, Dicle University, Diyarbakir, Turkey*
[b] *Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey*
[c] *Department of Emergency Medicine, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey*

ARTICLE INFO

Article History
Received 08 / 02 / 2016
Accepted 03 / 04 / 2016

*** Correspondence to:**
Sevdegul Karadas
Department of Emergency Medicine,
Faculty of Medicine,
Yuzuncu Yil University,
Van, Turkey
e-mail: sevdegulkaradas@yahoo.com.tr

Keywords:
Pruritus
Recurrent infections
Selective immunoglobulin a deficiency
Sinopulmonary infections

ABSTRACT

Selective immunoglobulin A deficiency (SIgAD) is the most common of all primary immunodeficiency diseases; however, the pathogenesis has not been fully understood. Although most people with SIgAD are asymptomatic, some may present with recurrent infections; such as respiratory disorders, gastrointestinal tracts disorders, and allergic disorders. Herein, we report a case with SIgAD who presented with the complaints of pruritus, dental caries and chronic sinopulmonary infections.

1. Introduction

Selective immunoglobulin A deficiency (SIgAD) is defined as a serum Ig A level at less than 5 mg/dl. This ilness is the most common one which occurs in primary immuno deficiency but pathogenesis is not defined clearly (Phankingthongkum et al., 2002).

First described in serum in 1953, SIgAD has a worldwide prevalence that differs from one region to another; 1:143 in the Arabian peninsula, 1:163 in Spain, 1:252 in Nigeria, 1:875 in England and 1:965 in Brazil (Yel, 2010). A lower incidence of SIgAD has been reported from Asian countries; such as Japan, China, India, and Saudi Arabia (Phankingthongkum et al., 2002). In the USA, the prevalence of SIgAD is estimated to range from 1:223 to 1:1.000 in community studies and from 1:333 to 1:3.000 among healthy blood donors (Cunningham, 2001).

Although most people with SIgAD are asymptomatic, some may present with recurrent infections of respiratory and gastrointestinal tracts, allergic disorders and autoimmune manifestations (Yel, 2010).

Herein, we report a case with SIgAD who has presented to outpatient clinic with pruritus, dental caries and chronic sinopulmonary infections.

2. Case report

A 26-year-old female who admitted to our outpatient clinic with the complaint of itching who had suffered from it 15 years. In the past she had been diagnosed with acute gingivitis, pneumonia and a seasonal allergic rhinitis, and had been prescribed corticosteroids, antihistamines, analgesics and antibiotics. However, the itching proved to be persistent, her other symptoms recurred, and she developed several dental cavities. Information about the patient's family history, history of allergy, weight loss, occupation and hobbies was obtained. She had no prior illnesses. Her vital parameters were stable. Except for several scracth marks all over her limbs due to itching, there ısn't any physical examınaton (Fig. 1).

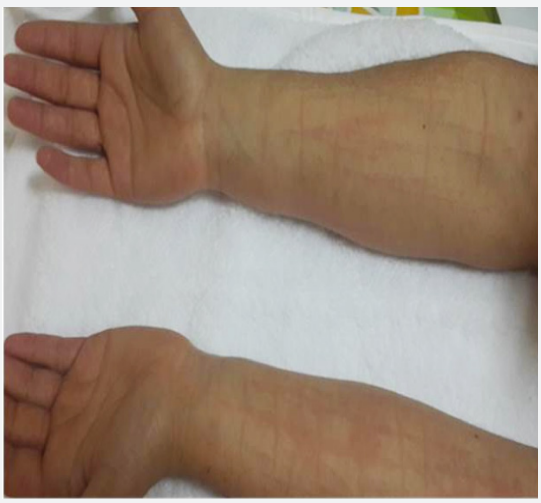


Fig. 1. There were skin abrasions on both arms, due to prolong period of scratching

A pulmonary function test was performed. Forced expiratory volume in 1 (FEV1) was measured as 85% and FEV1/forced vital capacity (FVC) was measured as 84%. Table 1 shows the laboratory parameters. Blood IgA level was measured to be 2.5 mg/dl, and IgG and IgM were normal. A preliminary diagnosis of IgA deficiency was made.

3. Discussion

There are two classified types of IgA deficiency: complete IgA deficiency and partial IgA deficiency. If blood IgA level is lower than 5mg/dL but IgG and IgM levels are normal, this condition is identified as a complete IgA deficiency. If blood IgA level is 5 mg/dl or higher, but the standard deviation of the age-specific mean value is below 2, then it is classified as a partial IgA deficiency (Conley and Nortarangeld, 1999).

Some patients with IgA deficiency frequently develop recurrent sinopulmonary infections, allergies, autoimmune conditions, and malignancies. Other

Table 1. Laboratory parameters of the patient

Variables	Level of laboratory parameters	Normal ranges
Immunoglobulin G	1480 mg/dL	650-1600 mg/dL
Immunoglobulin A	2.5 mg/dL	3.5-250 mg/dL
Immunoglobulin M	191 mg/dL	50-300 mg/dL
Immunoglobulin E	1060 mg/dL	0-100 mg/dL
Antinuclear antibodies	Negative	Negative
C3 (Complement)	124 mg/dL	79-152
C4 (Complement)	27.9	16-38
Salmonella	Negative	Negative
Brucella	Negative	Negative
C-RF	Negative	Negative
ASO	52.4 ıu/mL	0-200
Anti-ds DNA antibodies	Negative	Negative
HBsAg*	Negative	Negative
Anti HBs**	Negative	Negative
Anti HCV***	Negative	Negative
Aspartate aminotransferase	26 u/L	0-40 u/L
Alanine aminotransferase	13 u/L	0-41 u/L
Thyroid stimulating hormone	2 µıu/mL	0.2-4.2 µıu/mL
Free throxin	1.1 ng/dL	0.9-1.7 ng/dL
Carcino-embryogenic antigen	1.9 ng/dL	0-5.2 ng/dL
Anti thyroglobulin antibody	19.3 ıu/mL	0-155 µıu/mL
Anti TPO****	10.1 ıu/mL	0-34 µıu/mL
Carcinoma antigen-15-3	20.6 mg/dL	0-25 mg/dL
Carcinoma antigen-125	18.1 mg/dL	0-35 mg/dL
Carcinoma antigen-19-9	11.9 mg/dL	0-39 mg/dL

*: Hepatitis B virus-surface antigen; **: Anti hepatitis B virus-surface antigen; ***: Anti hepatitis C Virus antibody; ****: Antithyroidperoxidase antibodies; **ASO:** Antistreptolizin-O

associated diseases that are commonly seen in patients with IgA are gastrointestinal infections; such as giardiasis, malabsorption, lactose intolerance, celiac disease, ulcerative colitis, nodular lymphoid hyperplasia, and malign proliferation (Yel, 2010).

SIgAD can also be related with the increased frequency of allergic disorders. In one study, atopy was found in 58% of pediatric and adult patients with IgA deficiency (Buckley, 1975). Another study reported a history of allergy and asthma in 13% of patients with IgA-deficiency, which is probably not higher than the percentage of people with atopy in general population (Edwards et al., 2004).

In 2008, (Jacob et al., 2008), investigated IgA deficiency in 126 patients and found that 48% had respiratory allergies and atopic dermatitis. In a prospective study, IgA-deficient pediatric patients were found to be at an increased risk of pseudocroup at year one and parentally reported to have had food hypersensitivity at year four, both of which were possibly not IgE-mediated, as compared to children with normal serum levels of IgA (Janzi et al., 2009). In another study, 40.5% of the patients presented with the

symptoms of allergy. It was found that 25% of patients with IgA deficiency were diagnosed during screening for allergic disorders (Cunningham, 2001).

There are many factors involved in the formation of dental cavities such as; age, education level, eating habits, oral hygiene, frequency of dentist visits and fluoride intake. In addition, long-term antibiotic treatments for recurrent infections and immunoglobulin replacement therapy may also suppress microorganisms in dental plaque (Fernandes et al., 2012).

Legler et al. (1981), found that patients with immunodeficiency had a higher incidence of dental caries than the general population. In the case we presented, the patient had several dental caries and had undergone recurrent dental operations.

In conclusion, in patients with long term pruritus, tooth decay and sinopulmonary infections, oral examination should be performed at regular intervals in addition to the normal physical examination. In patients with recurrent dental caries, IgA deficiency should be considered and an early diagnose should be made.

REFERENCES

Buckley, R.H., 1975. Clinical and immunologic features of selective IgA deficiency. Birth Defects Orig. Artic. Ser. 11, 134-142.

Conley, Me., Nortarangelod, E. A., 1999. Diagnostic criteria for primary immuno deficiencies. Clin. Immunol. 93, 190-197.

Cunningham, R.C., 2001. Physiology of IgA and IgA deficiency. J. Clin. Immunol. 21, 303-309.

Edwards, E., Razvi, S., Cunningham-Rundles, C., 2004. IgA deficiency: Clinical correlates and responses to pneumococcal vaccine. Clin. Immunol. 111, 93-97.

Fernandes, K.S., Kokron, C.M., Barros, M.T., Kalil, J., Gallottini, M., 2012. Oral manifestations in patients with hypogammaglobulinemia. Oral Surg., Oral Med., Oral Pathol. and Oral Radiol. 114, 19-24. doi: 10.1016/j.oooo.2012.02.006.

Jacob, C.M., Pastorino, A.C., Fahl, K., Carneiro-Sampaio, M., Monteiro, R.C., 2008. Autoimmunity in IgA deficiency: Revisiting the role of IgA as a silent housekeeper. J. Clin. Immunol. 28, 56-61.

Janzi, M., Kull, I., Sjöberg, R., Wan, J., Melén, E., Bayat, N., Ostblom, E., Pan-Hammarström, Q., Nilsson, P., Hammarström, L., 2009. Selective IgA deficiency in early life: Association to infections and allergic diseases during childhood. Clin. Immunol. 133, 78-85.

Legler, D.W., McGhee, J.R., Lynch, D.P., Mestecky, J.F., Schaefer, M.E., Carson, J., Bradley, E.L., Jr. 1981. Jr. Immunodeficiency disease and dental caries in man. Arch. Oral Biol. 26, 905-910.

Phankingthongkum, S., Visitsunthorn, N., Vichyanond, P., 2002. IgA deficiency: A report of three cases from Thailand. Asian Pac. J. Allergy. Immunol. Sep. 20, 203-207.

Yel, L., 2010.Selective IgA deficiency. J. Clin. Immunol. 30, 10-16.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Case Report

J. Exp. Clin. Med., 2016; 33(3): 179-182
doi: 10.5835/jecm.omu.33.03.011

Unusual cause of refractory infection in head and neck surgery; Retained surgical sponges

Ismail Kucuker[a*], Onur Cukurluoglu[b], Tolga Eryilmaz[c], Serhan Tuncer[d], Selahattin Ozmen[e]

[a] *Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[b] *Private Practice, Istanbul, Turkey*
[c] *Department of Plastic Reconstructive and Aesthetic Surgery, Dışkapı Research and Trainig Hospital, Ankara, Turkey*
[d] *Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Gazi University, Ankara, Turkey*
[e] *Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Koç University, Istanbul, Turkey*

ARTICLE INFO

Article History
Received 19 / 01 / 2015
Accepted 04 / 05 / 2015

*** Correspondence to:**
Ismail Kucuker
Department of Plastic Reconstructive and Aesthetic Surgery,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun, Turkey
e-mail: drismailkucuker@yahoo.com.tr

Keywords:
Facial surgery
Gossypiboma
Maxilla surgery
Neck dissection
Retained surgical sponge
Spangioma

ABSTRACT

Refractory infections are not common in head and neck region. As gossypiboma (retained surgical sponges) is also rare in head and neck surgeries, it is generally ignored as a potential diagnosis. In this article we aimed to call attention to gossypiboma cases in refractory maxillary infections. We present three cases of retained surgical sponges after head and neck surgery occurred in between 2003 and 2011. We also discussed the possible causes and prevention strategies for them.

1. Introduction

Blood supply of head and neck region is very rich and bleeding is common during surgeries. Short incision or if possible intraoral incisions are preferred in this region, illumination of surgical zones are limited and viewing angles are narrow. When intensive bleeding from deeper zones is overloped, it becomes very hard to detect the exact point of the bleeding. In this situation sponges (cotton textiles) are generally cluttered to the bleeding area and surgery continues in a different region. Although all surgeons are aware of the importance of removing these sponges. Sometimes they can be forgotten due to combination of multiple risk factors like size of the sponge, massive bleeding

that hides the sponge or decreased attention of the surgeon.

Depending on the settlement position, these foreign bodies can stay asymptomatic for years. But in head and neck region according to small gaps and thin soft tissue coverage, these masses become symptomatic shortly after the surgery. Leak of a bad smelling fluid with fistula formation and antibiotic resistant infection are the major signs. In most of the cases the foreign body is not palpable due to edema that hides the sponge. Although treatment after diagnosis is easy in this region, as retained gauze (gossypiboma) is an unexpected diagnosis, patients usually receive long term antibiotic treatments before radiologic examinations or explorative surgery.

In this article we tried to call attention to gossypibomas in refractory maxillofacial infections. We present three cases of retained surgical sponges after head and neck surgery occurred in between 2003 and 2011. We also reviewed the literature, discussed the possible causes and prevention strategies for them.

2. Case reports

Case 1

A fiftyfive years old male was operated for right maxilla anterior wall, zygoma and orbital floor fractures in a different center. The reconstruction was done with rigid fixation using titanium implants. One month after surgery he complained about swelling and erythema on his right cheek. A fistula formation was present in right gingivobuccal mucosa. Antibiotic therapy with broad spectrum antibiotic was given for a week. Despite the therapy, clinical findings got worse and a diagnostic computerized tomography (CT) was planned. On CT scans, 3x3 cm abscess formation was found out over right maxillary sinus wall. In explorative surgery an unexpected mass; retained surgical sponge was found out as the cause of infection (Fig.1). Clinical findings recovered after the removal of the sponge.

Case 2

A nineteen year old male patient underwent Le-Fort 1 osteotomy for maxillary retrusion. Two hours after the recovery a massive bleeding occurred from the gingivobuccal insicions and the patient underwent a second surgery. In the operation exact bleeding zone was not detectable. Therefore small pieces of sponges were cluttered over the bleeding. After hemostasis, the operation ended and he was discharged two days following the second surgery. Two months after the surgery he admitted with swelling on the right cheek and a fluid leakage from right gingivobuccal sulcus with a bad odor. From our previous experience exploration was done under local anesthesia and a small piece of sponge was found over the right buccal fat pad. (Fig. 2) His complaints resolved after the explorative surgery.

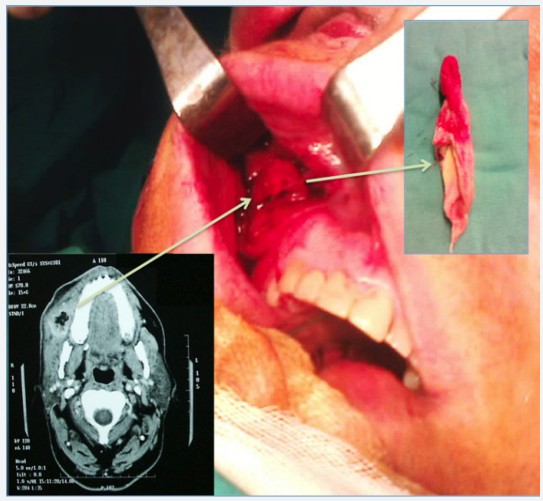


Fig. 1. 55 years old male with refractory maxillary infection. Computerized tomographyscans yields a 3*3 cm abscess formation and a small piece of surgical sponge was found out to be the reason of refractory infection on exploration

Case 3

A sixtyseven years old women underwent left unilateral neck dissection for squamous cell carcinoma on the left upper lip. On the fifteenth day of operation a firm mass was palpated over the left sternocleidomastoid (SCM) muscle with a fistula formation over the area (Fig. 3). Exploration yielded a surgical sponge settled lateral to SCM muscle under the lateral skin flap (Fig. 3). Fistula healed after the removal of the foreign body.

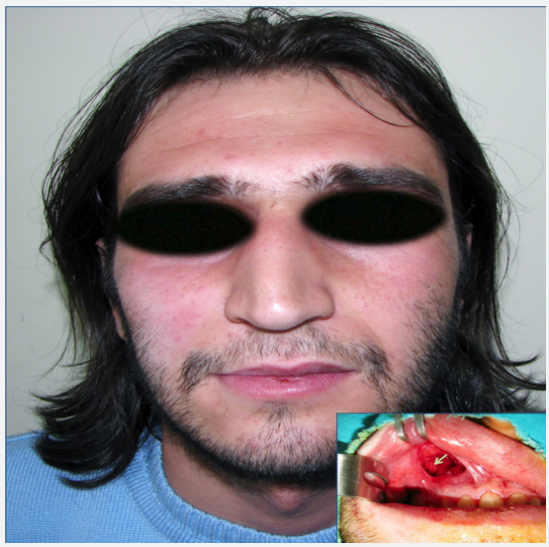


Fig. 2. 19 years old male patient with retained surgical sponge after LeFort-1 osteotomy advancement surgery

3. Discussion

The importance of retained surgical instruments was first published by Wilson in 1884. Since then over 160 articles and 300 cases were published related with retained surgical instruments (Wan et al., 2009). Estimated risk for retained surgical instrument is one in 5500 surgeries (Cima et al., 2008). Retained surgical sponge (gossypiboma); is the most common material (69%) forgotten in surgical areas (Gawande et al., 2003).

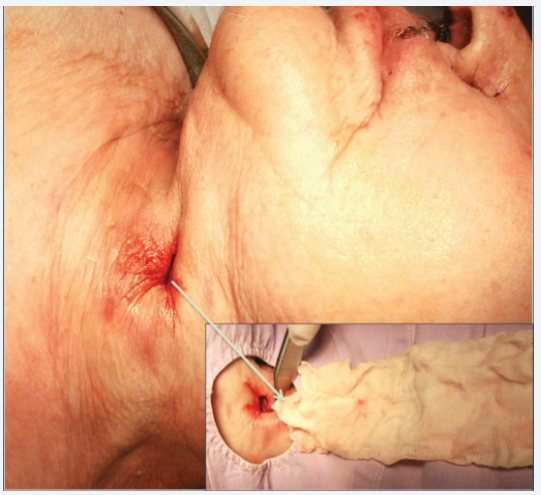


Fig. 3. 66 years old female patient with retained surgical sponge after left unilateral neck dissection done for the treatment of squamous cell carcinoma of the lower lip

Gossypiboma is more common in abdominal, pelvic and thoracic surgeries. The percentage of gossypibomas in head and neck region covers only 4% of all cases (Wan et al., 2009). Most of these cases are intracranial textilomas or muslinomas that forms secondary to vascular wrapping of muslin material in anevrysm surgery (Prabhu et al., 1994; Berger et al., 2003) Other reported rare gossypiboma cases of head and neck region are related to mandible contouring surgery (Song et al., 2009), submandibular gland excision (Amr, 2009), adenoidectomy (Ozer et al., 2007) and endoscopic sinus surgery (Tan and Sethi, 2011). Gossybipomas related to maxilla surgery and neck dissection are very rare and commonly is not assumed as a possible diagnosis.

Surgical sponges are fibrous, absorbable materials composed of sterile cotton or synthetic fabrics. As their size decreases and they are covered with blood, they resemble normal tissues and it becomes harder to distinguish them (Yıldırım et al., 2006; Dossett et al., 2008; Wan et al., 2009). In the literature average time of diagnosis for retained surgical sponges is six to nine months and only 37% of cases were diagnosed in the first year. But in craniofacial surgery diagnosis is possible in a few months because of small gaps and reliably thinner soft tissue coverage over the sponges compared to abdomen and thorax. In all cases retained sponges were diagnosed within two months after the primary surgery.

Before the gossypiboma diagnosis first we must suspect about retained surgical sponge. Swelling and erythema are the most common symptoms. In all our three cases a fistula was formed from the incision site and a fluid leak with a bad odor was present. Palpable mass was only present in the third case, possibly because of the thin soft tissue coverage over the neck. Computerized tomography was only applied in the first case. In the other two cases clinical evaluations were sufficient to decide exploration of the surgical area. Retaining a surgical material inside the patient is a shame for the surgeon. When we also consider the legal issues prevention of this situation has a great importance. In 2003 Gawande et al. investigated many factors as a potential risk factors for retained surgical materials. These factors were; age, sex and body mass index of the patients, absence of sponge count, operation time, estimated blood loss during operation, emergency operations, unexpected changes in the operation, more than one surgical team in the operation, more than one major operation in a single session, change in nursing staff and absence of primary surgeon on skin closure. He found out that only emergency procedures and unexpected changes in the operation caused statistically significant changes. In this study it has been shown that, the risk of retained surgical sponges increases by nine times in emergency procedures. Also it has been mentioned that the risk even increases four times more when an unexpected condition like excessive bleeding occurs during the surgery.

When we examine our cases, we cannot comment on the first case because the operation was done in a different center. But the sponge piece cluttered over the right buccal mucosa was too small which may be the potential risk factor. Second case was an surgical emergency and sponges were cluttered on the hemorrhage area because exact bleeding source was not found. So possibly a small piece of sponge was left behind over the right buccal fat pad. On the third cases the primary surgeon was not present at closure and sponges were left behind.

Although no risk factors can explain retaining a surgical instrument in surgical area, some factors can ease the potential risks. These factors are thought to be; deep and dark surgical areas with limited incisions, emergency procedures, unexpected changes during surgery like intensive bleeding, operation duration (surgeon exhaustion), change in surgeon or nursing team, use of small sponge pieces for cluttering, using non-visible sponges on x-ray and ignoring sponge count especially in maxillofacial surgeries. Potential risk factors were summarized in Table 1.

Table 1. Potential risk factors for retained surgical sponges in craniofacial surgery

Dark and deep surgical zones
Emergency procedures
Unexpected conditions during surgery like massive bleeding
Time and duration of surgery (surgeon exhaustion)
Change in surgeon or nursing team
Using small pieces of sponges for cluttering
Using non-visible sponges on x-ray
Ignoring sponge count

Some measures can be taken to prevent this uneventful situation. In abdominal or thoracic surgeries sponge counts alone or with radiologic analysis is recommended in all cases (Rappaport and Haynes, 1990; Gawande et al., 2003; Lincourt et al., 2007). But sponge count or radiography is not routinely done in craniofacial surgery. Maybe rather than sponge count noting the cluttered sponges and its location can be noted by helping staff and these notes can be reminded to surgeon before skin closure. Second precaution should be using big sponge pieces for cluttering and suspending one edge of the sponge outside the incision.

Also using radiopaque sponges will increase detection rates when there is suspicion. Exhausted surgeons will be more likely to lose their attention and concentration during surgery. With less attention potential risk of retaining the cluttered sponge in surgical area should increase. So if possible, exhausted surgeons should delay surgical procedures or let a secondary surgeon to enter the operation. For better visualization of the surgical area the incision must be big enough to see the whole area. Light can be increased by wearing a head light. Prevention of bleeding as much as possible will decrease the necessity of sponge clutters. Before skin closure primary surgeon must control the surgical area. Preventive measures are summarize in table 2.

Table 2. Measures for prevention of retained surgical sponges in craniofacial surgery

The number and place of sponges can be noted by helping staff
Using big and radiopaque sponges will increase detection rates
For a better visualization; incision must be big enough and light can be increased by a head light
Surgeon exhaustion should be prevented (Less mistakes)
Change in surgeon or nursing team
Excessive bleeding should be prevented (Less need to sponge clutters)
Primary surgeon must check the surgical area before closure

In conclusion, retained surgical sponge is an uneventful situation for all surgeons. When we consider no surgeon makes this mistake twice (Gawande et al., 2003), every surgeon should understand the potential risks of retained surgical sponges and must take basic precautions for the prevention of it.

REFERENCES

Amr, A.E., 2009. A submandibular gossypiboma mimicking a salivary fistula: A case report. Cases J. 2, 6413.

Berger, C., Hartmann, M., Wildemann, B., 2003. Progressive visual loss due to a muslinoma-report of a case and review of the literature. Eur. J. Neurol. 153, 8.

Cima, R.R., Kollengode, A., Garnatz, J., Storsveen, A., Weisbrod, C., Deschamps, C., 2008. Incidence and characteristics of potential and actual retained foreign object events in surgical patients. J. Am. Coll. Surg. 207, 80-87.

Dossett, L.A., Dittus, R.S., Speroff, T., May, A.K., Cotton, B.A., 2008. Cost-effectiveness of routine radio-graphs after emergent open cavity operations. Surgery. 144, 317-321.

Gawande, A.A., Studdert, D.M., Orav, E.J., Brennan, T.A., Zinner, M.J., 2003. Risk factors for retained instruments and sponges after surgery. N. Engl. J. Med. 348, 229-235.

Lincourt, A.E., Harrell, A., Cristiano, J., Sechrist, C., Kercher, K., Heniford, B.T., 2007. Retained foreign bodies after surgery. J. Surg. Res. 138, 170-174.

Ozer, C., Ozer, F., Sener, M., Yavuz, H., 2007. A forgotten sponge pack in the nasopharynx: An unfortunate complication of adenotonsillectomy. Am. J. Otolaryngol. 28, 191-193.

Prabhu, S.S., Keogh, A.J., Parekh, H.C., Perera, S., 1994. Optochiasmal arachnoiditis induced by muslin wrapping of intracranial aneurysms. A report of two cases and a review of the literature. Br. J. Neurosurg. 8, 471-876.

Rappaport, W., Haynes, K., 1990. The retained surgical sponge following intra-abdominal surgery. A continuing problem. Arch. Surg. 125, 405-407.

Song, S.Y., Hong, J.W., Yoo, W.M., Tark, K.C., 2009. Gossypiboma after mandibular contouring surgery. J. Craniofac. Surg. 20, 1607-1610.

Tan, V.E., Sethi, D.S., 2011. Gossypiboma: An unusual intracranial complication of endoscopic sinus surgery. Laryngoscope. 121, 879-881.

Wilson, C., 1884 Foreign bodies left in the abdomen after laparotomy. Gynecol. Tr. 9, 109-112.

Wan, W., Le, T., Riskin, L., Macario, A., 2009. Improving safety in the operating room: A systematic literature review of retained surgical sponges. Curr. Opin. Anaesthesiol. 22, 207-214.

Yildirim, S., Tarim, A., Nursal, T.Z., Yildirim, T., Caliskan, K., Torer, N., Karagulle, E., Noyan, T., Moray, G., Haberal, M., 2006. Retained surgical sponge (gossypiboma) after intraabdominal or retroperitoneal surgery: 14 cases treated at a single center. Langenbecks Arch. Surg. 391, 390-395.

Journal of Experimental and Clinical Medicine
http://dergipark.ulakbim.gov.tr/omujecm

Case Report

J. Exp. Clin. Med., 2016; 33(3): 183-186
doi: 10.5835/jecm.omu.33.03.012

Respiratory epithelial adenomatoid hamartoma with inflammatory nasal polyposis

Emre Gunbey[a], Esra Kavaz[a], Bilge Can Meydan[b], Hediye Pinar Gunbey[c], Hulya Savas Mutlu[b], Recep Unal[a]

[a] *Department of Otorhinolaryngology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[b] *Department of Pathology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*
[c] *Department of Radiology, Faculty of Medicine Ondokuz Mayıs University, Samsun, Turkey*

ARTICLE INFO

Article History
Received 02 / 01 / 2016
Accepted 10 / 05 / 2016

*** Correspondence to:**
Emre Gunbey
Department of Otorhinolaryngology,
Faculty of Medicine,
Ondokuz Mayıs University,
Samsun, Turkey
e-mail: emregunbeymd@hotmail.com

Keywords:
Hamartoma
Nasal polyp
Olfactory cleft
Paranasal computed tomography
Respiratory epithelial adenomatoid

ABSTRACT

Respiratory Epithelial Adenomatoid Hamartoma (REAH) is a type of hamartoma characterized by prominent glandular proliferations lined with ciliated respiratory epithelium originating from the surface epithelium. REAH should be differentiated from inflammatory nasal polyp, inverted papilloma and adenocarcinoma to avoid from more aggressive surgery then is needed for the REAH and to avoid from unnecessary long-term follow-up with medical treatments. In this report, we present a case of REAH who was followed up with topical and systemic steroids for years with the misdiagnosis of inflamatory nasal polyposis and discuss the clinical, radiological and histopathological features of the disease at increasing importance in recent years.

1. Introduction

Hamartomas are abnormal mixture of tissues with a localized excessive overgrowth in the cells and tissues of an organ. Hamartomas often occur in lung, liver, kidney and intestinal tract (Liang et al., 2007). Respiratory epithelial adenomatoid hamartoma (REAH) is a subtype of hamartoma, first described in 1995 (Wenig and Heffner, 1995). Until the last few years, REAH of the nose and paranasal sinuses was considered very rare and since Wenig and Heffner's cases in 1995 to last few years, about 10 REAH cases of nose have been reported (Himi et al., 2002; Metselaar et al., 2005; Ingram et al., 2006; Fitzhugh and Mirani, 2008; Cao et al., 2010; Braun et al., 2013). On the contrary, recent studies showed that REAH is much more common than expected and a significant proportion of patients with REAH were misdiagnosed and managed or treated with a diagnosis of inflamatory

nasal polyp, in the past (Gauchotte et al., 2013; Lee et al., 2013; Nguyen et al., 2014). In this article we present a case of REAH who was followed up with topical and systemic steroids for years with the misdiagnosis of inflamatory nasal polyposis and discuss the clinical, radiological and histopathological features of the disease of increasing importance in recent years.

2. Case report

An 82-year-old male presented at our otolaryngology outpatient clinic with major complaints of nasal obstruction and hyposmia. He had a nasal operation history at another center for similar complaints ten years ago but there was no information about the content of previous operation and diagnosis. These complaints resumed after a short period postoperatively and the patient was long term managed by topical and systemic steroids with the diagnosis of inflamatory nasal polyposis. He denied any benefit from these treatments. His symptoms have worsened over a three-month period despite oral steroid and antibiotic medications. He had no history of systemic disease, allergy, malignancy, smoking or drug use. Endoscopic nasal examination revealed an anterior septal perforation about 3 mm in diameter and well-circumscribed, soft polypoid tissues that fills bilateral middle meatus and posterior nasal cavities. There was no mass lesion near the perforation and the perforation was thought to be due to the previous surgery or prolonged use of nasal steroids. Physical examination of the oral cavity, oropharynx, ears and neck was normal. An axial and coronal section computed tomography (CT) of the nose and paranasal sinuses was then performed to evaluate the extent of the disease. CT showed the presence of bilateral soft tissue densities at bilateral ethmoid and maxillary sinuses, olfactory clefts and posterior nasal cavities and also a defect at the anterior nasal septum was detected. There was no destruction or invasion at the surrounding structures such as orbita, skull base or maxillary sinus walls (Fig. 1). The patient underwent an endoscopic sinus surgery. The polypoid tissues filling the bilateral middle meatus, olfactory cleft and nasal cavities were cleaned. The remnants of the middle turbinates was encountered secondary to previous surgery. Bilateral anterior and posterior ethmoidectomy and middle meatal antrostomy was performed and the pathologic tissues at the maxillary sinuses were removed. The operation was completed uneventfully. There were no complications during the postoperative period. Microscopic examination showed a large number of proliferated glands lined by ciliated respiratory epithelium and the surface of the lesion was found to comprise pseudostratified, ciliated columnar epithelium. There was no evidence of atypical cells or metaplasia (Fig. 2). The final histopathologic diagnosis was REAH. At 6-month follow-up, there was no evidence of recurrence.

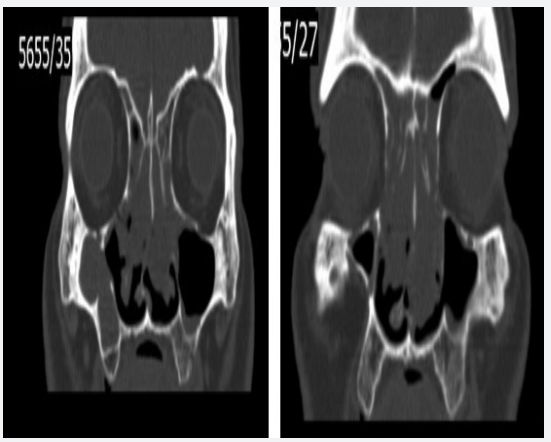


Fig. 1. Paranasal sinus computed tomography showed the presence of bilateral soft tissue densities at bilateral ethmoid and maxillary sinuses, olfactory clefts and nasal cavities, also a defect at the anterior nasal septum was detected

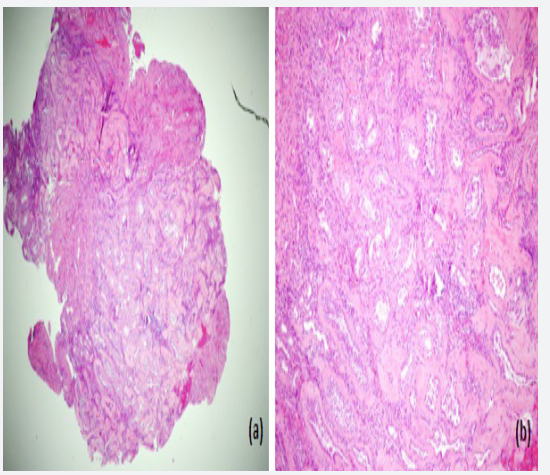


Fig. 2. Microscopic examination showed a large number of proliferated glands lined by ciliated respiratory epithelium and the surface of the lesion was foung to comprise pseudostratified, ciliated columnar epithelium (original magnification **a:**x40, **b:** x200, Hematoksylin&Eozin)

3. Discussion

Our traditional knowledge was requiring us to evaluate the bilateral bright gray or off-white color polypoid nasal masses as inflammatory nasal polyposis and to evaluate the topical or systemic steroids and endoscopic sinus surgery as treatment alternatives. In 1995, Wenig and Heffner have reviewed again the histopathological findings of patients who were operated because of nasal polyposis and described a type of hamartoma characterized by prominent glandular proliferations lined with ciliated respiratory epithelium originating

from the surface epithelium, called REAH, in 31 patients. The last few years, about ten cases of REAH were reported and REAH emphasized as a very rare clinical condition in the mentioned articles (Himi et al., 2002; Metselaar et al., 2005; Ingram et al., 2006; Fitzhugh and Mirani, 2008; Cao et al., 2010; Braun et al., 2013). However, an awareness occured at this issue on recent years and many researchers reviewed histological findings of patients especially operated for inflammatory nasal polyps retrospectively and they realized that a significant portion of those patients the real diagnosis was REAH and actually thay began to publish them. Lorentz et al. (2012), Vira et al. (2011), Lee et al. (2013) and Mühlmeier et al. (2014) published the largest series ranging from 25 to 50 cases. Studies revealed that REAH is a benign lesion predominantly affecting men after their third decades of life, with a mean age of 50-55 years. The most important complaints of REAH patients are nasal obstruction, loss of smell, headache and runny nose. REAH has been reported to cause more a loss of odor than nasal polyps. The most important reason is excess incidence of REAH at the olfactory cleft (Liang et al., 2007; Cao et al., 2010; Lee et al., 2013). Two form of REAH have been described as isolated and inflammatory with nasal polyposis. In published series approximately 70% of patients are reported to be with nasal polyps and 30% are isolated (Vira et al., 2011; Lorentz et al., 2012; Lee et al., 2013). Vira et al. (2011) found that 44% of cases of non-isolated REAH develop on allergic chronic sinusitis and 17% on nasal polyposis background. Isolated form is more seen in the olfactory cleft. The most distinguishing feature of our case was that both the olfactory cleft was affected and REAH was with extensive nasal polyposis. Lee et al. (2013) mentioned the affinity of REAH to the olfactory cleft and identified increased mean maximum olfactory cleft length in these cases. The studies on REAH found no etiologic role of Ebstein Bar virus (EBV) and Human Papilloma virus (HPV) (Hua et al., 2014; Mühlmeier et al., 2014). The mechanisms driving the development of REAH are unknown, and its nature as a benign tumor, hamartoma, or reactive inflammatory process is still open to discussion.

Radiologically, the most common finding is an opacification of the affected sinus and connection to the posterior nasal septum. The majority of the cases reported to be bilateral. REAH must be suspected if there is opacification in CT and widening of the olfactory cleft (OC) (>10 mm), then REAH with inflamatory polyposis should be taken into consideration in the differential diagnosis to avoid overly aggressive skull-base surgery before biopsy confirmation of a benign lesion. Hawley et al. (2013) reported that when the olfactory cleft is 10 mm or more, the sensitivity and specificity for the presence of REAH are 88% and 74%, respectively CT reveales no more severe sinus disease in REAH with inflamatory polyposis, however, another important point is to evaluate that REAH can be accompanied by neoplastic lesions such as inverted papilloma, adenocarsinoma and hereditary hemorrhagic telangiectasia. Magnetic resonance imaging (MRI) of paranasal sinuses is rarely reported in REAH, reveales clearly delineated cerebriform tissue filling in the olfactory clefts. On MRI lesions are in hypo-iso intense signal characteristic compared to normal nasal septum in T1-weighted imaging and the enhancement pattern is variable T2-weighted images are observed as a hyperintense signal. In our case, there were widespread opacities in maxillary and ethmoid sinuses as well as an expansion at the olfactory cleft (Braun et al., 2013). We did not need preoperative MRI looking on CT to findings.

REAH appear as shiny polypoid masses, grey to white or yellowish in colour, with various sizes, on macroscopic evaluation. Microscopically, it is charecterized with submocosal glandular proliferation with small to medium in size with prominent dilatation, which are lined with single layer of ciliated respiratory columnar epithelium. Cribriform structure and complex glandular growth is usually absent and the stroma contains inflammatory cells including eosinophils (Liang et al., 2007). Gauchotte et al. (2013) investigated the roles of tryptase producing mast cells and the production of metalloproteinases in REAH and concluded that the likely role of mast cell in REAH development may be metalloproteinase dependent. Our case bored the classic histopathologic features of REAH. A subtype of REAH was also defined, chondrosseous REAH that contains islands of cartilage interspersed throughout the lesion (Roffman et al., 2006). Although REAH is considered a non-neoplastic entity, molecular genetics findings suggest that REAH may in fact be a benign neoplasm.

REAH must be differentiated from other paranasal masses especially from an inflammatory polyp because of the clinical and histopathological similarities. The others in the differential diagnosis are inverted papilloma and adenocarcinoma. Clinically, inverted papillomas are more aggressive lesions, having the capacity to destroy bone and invade adjacent vital structures and histologically consisting of papillary fronds with a delicate fibrovascular core, covered by multiple layers of epithelial cells and characterized by invagination of the surface epithelium in the underlying stroma. Adenocarcinoma also shows invasion into the surrounding soft tissue and bone destruction, histologically originate from the glandular epithelium and are characterized by a complex glandular growth pattern with a back-to-back or cribriform pattern lacking intervening connective tissue (Vira et al., 2011; Lee et al., 2013). Immunohistochemically adenocarcinoma

demonstrates higher reactivity for mindbomb E3 ubiquitin protein ligase 1(MIB-1) staining than REAH (Ozolek et al., 2007). Also, the lack of dysplasia, increased mitotic rate and cribriform architecture, and presence of individual glands surrounded by eosinophilic basement membranes, lined by ciliated respiratory epithelium in REAH, are criteria used to differentiate REAH from low grade adenocarcinoma (Lee et al., 2013).

As a conclusion, the differential diagnosis is very important to avoid from more aggressive surgery than is needed for the REAH and to avoid from unnecessary long-term follow-up with medical treatments such steroids. Because REAH is more resistant to topical and systemic steroids.

REFERENCES

Braun, J.J., Riehm, S., Averous, G., Billing, A., Veillon, F., 2013. MRI in respiratory epithelial adenomatoid hamartoma of nasal cavities. J. Neuroradiol. 40, 216-219. doi: 10.1016/j.neurad.2012.04.002.

Cao, Z., Gu, Z., Yang, J., Jin, M., 2010. Respiratory epithelial adenomatoid hamartoma of bilateral olfactory clefts associated with nasal polyposis: Three cases report and literature review. Auris Nasus Larynx. 37, 352-356. doi: 10.1016/j.anl.2009.10.003.

Fitzhugh, V.A., Mirani, N., 2008. Respiratory epithelial adenomatoid hamartoma: A review. Head Neck Pathol. 2, 203-220. doi: 10.1007/s12105-008-0064-3.

Gauchotte, G., Marie, B., Gallet, P., Nguyen, D.T., Grandhaye, M., Jankowski, R., Vignaud, J.M., 2013. Respiratory epithelial adenomatoid hamartoma: A poorly recognized entity with mast cell recruitment and frequently associated with nasal polyposis. Am. J. Surg. Pathol. 37, 1678-1685. doi: 10.1097/PAS.0000000000000092.

Hawley, K.A., Ahmed, M., Sindwani, R., 2013. CT findings of sinonasal respiratory epithelial adenomatoid hamartoma: A closer look at the olfactory clefts. Am. J. Neuroradiol. 34, 1086-1090.

Himi, Y., Yoshizaki, T., Sato, K., Furukawa, M., 2002. Respiratory epithelial adenomatoid hamartoma of the maxillary sinus. J. Laryngol. Otol. 116, 317-318.

Hua, X., Huang, X., Liao, Z., Xian, Q., Yu, L., 2014. Clinicopathological and EBV analysis of respiratory epithelial adenomatoid hamartoma. Diagn. Pathol. 25, 9:70. doi: 10.1186/1746-1596-9-70.

Ingram, W.F., Noone, M.C., Gillespie, M.B., 2006. Respiratory epithelial adenomatoid hamartoma: A case report. Ear Nose Throat. J. 85, 190-192.

Lee, J.T., Garg, R, Brunworth, J., Keschner, D.B., Thompson, L.D., 2013. Sinonasal respiratory epithelial adenomatoid hamartomas: Series of 51 cases and literature review. Am. J. Rhinol. Allergy. 27, 322-328. doi: 10.2500/ajra.2013.27.3905.

Liang, J., O'Malley, B.W.Jr., Feldman, M., Newman, J.G., 2007. A case of respiratory epithelial adenomatoid hamartoma. Am. J. Otolaryngol. 28, 277-279.

Lorentz, C., Marie, B., Vignaud, J.M., Jankowski, R., 2012. Respiratory epithelial adenomatoid hamartomas of the olfactory clefts Eur. Arch. Otorhinolaryngol. 269, 847-852. doi: 10.1007/s00405-011-1713-9.

Metselaar, R.M., Stel, H.V., van der Baan, S., 2005. Respiratory epithelial adenomatoid in the nasopharynx. J. Laryngol. Otol. 119, 476-478.

Mühlmeier, G., Hausch, R., Arndt, A., Kraft, K., Danz, B., Maier, H., 2014. Respiratory epithelial adenomatoid hamartoma of the nose and nasal sinuses: A rare differential diagnosis of nasal polyposis. HNO. 62, 813-817. doi: 10.1007/s00106-014-2914-5.

Nguyen, D.T., Nguyen-Thi, P.L., Gauchotte, G., Arous, F., Vignaud, J.M., Jankowski, R., 2014. Predictors of respiratory epithelial adenomatoid hamartomas of the olfactory clefts in patients with nasal polyposis. Laryngoscope. 124, 2461-2465. doi: 10.1002/lary.24778.

Ozolek, J.A., Barnes, E.L., Hunt, J.L., 2007. Basal/myoepithelial cells in chronic sinusitis, respiratory epithelial adenomatoid hamartoma, inverted papilloma, and intestinal-type and nonintestinal-type sinonasal adenocarcinoma: An immunohistochemical study. Arch. Pathol. Lab. Med. 131, 530-537.

Roffman, E., Baredes, S., Mirani, N., 2006. Respiratory epithelial adenomatoid hamartomas and chondroosseous respiratory epithelial hamartomas of the sinonasal tract: A case series and literature review. Am. J. Rhinol. 20, 586-590.

Vira, D., Bhuta, S., Wang, M.B., 2011. Respiratory epithelial adenomatoid hamartomas. Laryngoscope. 121, 2706-2709

Wenig, B.M., Heffner, D.K., 1995. Respiratory epithelial adenomatoid hamartomas of the sinonasal tract and nasopharynx: A clinicopathologic study of 31 cases. Ann. Otol. Rhinol. Laryngol. 104, 639-645

ORGANIZATION OF THE ARTICLE

Manuscripts should be prepared electronically using an appropriate MS Word compatible word-processing package, formatted for A4 or letter page size, double-spaced throughout with 3 cm margins on all sides, and using 12 point font. Text should not be justified, but flush left. Words should not be hyphenated to fit on a line. Pages should be numbered sequentially.

Title page: The title page should contain the following items: (1) complete title; (2) full names of all authors; (3) complete affiliations of all authors; (4) the number of text pages of the whole manuscript and the number of figures and tables; (5) the name and complete address of the corresponding Author that includes telephone number, facsimile number and E-mail address to whom correspondence and proofs should be sent.

Abstract: This should provide a concise description of the purpose of the report or summary of the review and should not exceed 300 words.

Keywords: Provide at least **4-6 keywords** and avoiding general and plural terms and multiple concepts. These keywords will be used for indexing purposes.

Acknowledgements: Collate acknowledgements in a separate section at the end of the article that is given before references and do not, therefore, include them on the title page, as a footnote to the title or otherwise.

Research Reports should be divided into numbered sections headed by a caption (e.g. Abstract, **1. Introduction; 2. Experimental Procedure; 3. Results; 4. Discussion; Acknowledgements, References**).

Introduction: The objectives of the research should be clearly stated in this section. Relevant background information and recent published studies should be described concisely, and be cited appropriately.

Experimental Procedure: This section should contain all the details necessary to reproduce the experiments. Avoid re-describing methods already published; only relevant modifications should be included in the text.

Experimental subjects

When human subjects are used, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject. Authors should be aware of the Code of Ethics of the World Medical Association (Declaration of Helsinki) which has been printed in the *British Medical Journal* (18 July 1964).

When experimental animals are used, the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), or with the animals for experimental procedures.

Results and Discussion: These sections should present the results and interpret them in a clear and concise manner. Results should usually be presented descriptively and be supplemented by figures. Extensive citations and discussion of published literature should be not be used.

Literature references:

In the text, references should be cited by authors' surnames and year of publication. All references cited in the text (and only those cited in the text) should be included. One or two authors should be cited by surname; for three or more, the first author is cited followed by et al.:

… (Kayhan 2003) …
… (Malik and Batcharov, 2001)…
… (Smith et al., 2003) …
… (Malik and Batcharov, 2001; Smith et al., 2003; Beyaz, 2009; Kayhan, 2010) …

References that are not cited by surname should be included at the end of a phrase or sentence in parentheses, in chronological order, separated by semicolons, except for two or more papers by the same authors, which should be separated by commas. References to more than one paper in the same year should be designated by letters:

... (Malik and Batcharov, 2001; Smith et al., 2003; Kaplan et al., 2005a, 2005b) …

All references cited in the text should be listed at the end of the manuscript on a separate page, arranged in alphabetical order of first author then year of publication. The accuracy of references is the responsibility of the author. The references should include only articles that are published or in press. Unpublished data, submitted manuscripts, or personal communications should be cited within the text only. Personal communications should be documented by a letter of permission. All items in the list of references should be cited in the text and, conversely, all references cited in the text must be presented in the list. The abbreviations of journal titles should conform to those adopted by the *List of Serial Title Word Abbreviations*, CIEPS/ISDS, Paris, 1985 (ISBN 2-904938-02-8). Please use the following style for references:

*Article in a periodical***:**

Baryshnikova, L.M., Von Bohlen Und Halbach, O., Kaplan, S., Von Bartheld, C.S., 2006. Two distinct events, section compression and loss of particles ("lost caps"), contribute to z-axis distortion and bias in optical disector counting. Microsc. Res. Techniq**.** 69, 738–756.

Chapter in a book (within a series):

Elsabbagh, M., Johnson, M. H., 2007. Infancy and autism: progress, prospects, and challenges. In From Action to Cognition. Progress in Brain Research, Vol. 164, C. von Hofsten and K. Rosander, eds. Elsevier, Amsterdam, pp. 355-383.

An entire book:

Cooper, J.R., Bloom, F.E., Roth, R.H. 1986, The Biochemical Basis of Neuropharmacology. Oxford University Press, New York and Oxford.

ILLUSTRATIONS AND TABLES

Illustrations:

The use of color in illustrations can enhance the effective presentation of results, and we are pleased to offer free reproduction of color illustrations in the electronic version of JECM. There is no charge for color reproduction of illustrations in the electronic version of the journal when the use of color is clearly required to further understanding and communication. It should be borne in mind that in the journal illustrations will appear either across a single column (=8.3 cm) or a whole page (=17.6 cm). The illustrations should be numbered in Arabic numerals according to the sequence of appearance in the text, where they are referred to as Fig. 1, Fig. 2, etc.

If illustrations (or other small parts) of articles or books already published elsewhere are used in papers submitted to *The Journal Experimental and Clinical Medicine*, the written permission of the authors and publisher concerned must be included with the manuscript. The original source must be indicated in the legend of the illustration in these cases.

Color reproduction:

On the Web: If you submit usable color figures with your accepted article, then these figures will appear in color on the Web, they are reproduced in black-and-white in the printed version of the article.

Tables: Tables should be so constructed together with their captions and legends. They should be prepared with minimal reference to the text. Tables of numerical data should each be typed (with one-spacing) on a separate page and numbered in sequence in Arabic numerals (Table 1, 2, etc.). They are referred to in the text as Table 1, Table 2, etc. The title of each table should appear above it. A detailed description of its contents and footnotes should be given below the body of the table.

PROOFS, OFFPRINTS, MISCELLANEOUS

Proofs

Proofs will be sent by E-mail, as a pdf. Only printer's errors may be corrected; no change in, or additions to, the edited manuscript will be allowed at this stage. It should be kept in mind that proofreading is solely the Authors' responsibility. A form with queries from the copyeditor may accompany the proofs. Please answer all queries and make any corrections or additions required. Corrections to the proofs must be returned by E-mail or Fax within 48 hours after receipt. If the Publisher receives no response from the Authors after 3 days, it will be assumed that there are no errors to correct and the article will be published.

Page charges

There are no page charges.

Offprints

A pdf file of each paper will be provided free of charge to the corresponding Author.

Authorship

To be identified as an author, the participant should have contributed to the conception and design of the project, drafted substantive portions of the paper or edited or revised same, and taken responsibility for the analysis and conclusions of the paper.

Other participants with less responsibility for example those who merely assisted in carrying out the research should be identified and acknowledged for their contributions.

Disclosure Statement

All authors must disclose any affiliations that they consider to be relevant and important with any organization that to any author's knowledge has a direct interest, particularly a financial interest, in the subject matter or materials discussed. Such affiliations include, but are not limited to, employment by an industrial concern, ownership of stock, membership on a standing advisory council or committee, a seat on the board of directors, or being publicly associated with a company or its products. Other areas of real or perceived conflict of interest would include receiving honoraria or consulting fees or receiving grants or funds from such corporations or individuals representing such corporations.

This requirement will apply to every sort of article submitted to the Journal, including original research, reviews, editorials, letters to the editor, and any others, and should be disclosed at the time of submission.

Authors are required to indicate whether there is any financial or other conflict of interest. If none, authors should make a positive statement to the effect that "The authors declare that they have no competing financial interests."

* Formerly "**University of Ondokuz Mayis Journal of Medicine**"

COPYRIGHT RELEASE FORM

JOURNAL OF EXPERIMENTAL AND CLINICAL MEDICINE
Ondokuz Mayis University, Faculty of Medicine,
Atakum, 55139, Samsun, Turkey
Tel: ++90 (362) 312 1919 Extension: 3845
Fax: ++90 (362) 457 6041
E-Mail: jecm@omu.edu.tr

Manuscript title: ----------

Full names of all authors (in order to appear on manuscript): ----------

---

Name of corresponding author: ----------

Address of corresponding author: ----------

Telephone: ----------

E-mail: ---------- Mobile phone: ----------

The author(s) warrant(s) that:

a) The submitted manuscript is his/her/their own original work;
b) All authors participated in the work in a substantive way and are prepared to take public responsibility for the work;
c) All authors have seen and approved the manuscript as submitted;
d) The manuscript has never been published before or is not at the stage of any evaluation in another journal;
e) The text, illustrations, and any other materials included in the manuscript do not infringe upon any existing copyright or other rights of anyone;
f) In consideration of my/our manuscript submitted, I/we hereby grant JOURNAL OF EXPERIMENTAL AND CLINICAL MEDICINE the unlimited, worldwide, irrevocable royalty-free, right to publish, use, distribute, publish, license, transmit, display, exhibit, record, store, translate, digitize, broadcast, reproduce and archive, in any format or medium, whether now known or hereafter developed.

However, reproduction, posting, transmission or other distribution or use of the article or any material contained therein, in any medium as permitted hereunder, requires a citation to the Journal and appropriate credit to JOURNAL OF EXPERIMENTAL AND CLINICAL MEDICINE as publisher, suitable in form and content as follows: Title of article, author(s), journal title and volume/issue, Copyright© year.

All materials related to manuscripts, accepted or rejected, including photographs, original figures etc., will be kept by JOURNAL OF EXPERIMENTAL AND CLINICAL MEDICINE for one year following the editor's decision. These materials will then be destroyed.

This copyright form must be signed by all authors. Separate copies of the form (completed in full) may be submitted by authors located at different institutions; however, all signatures must be original.

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

Full name: ----------

Signature: ---------- Date: ----------

p-ISSN 1309-4483
e-ISSN 1309-5129

1309-4483 (2016)33;2 1H