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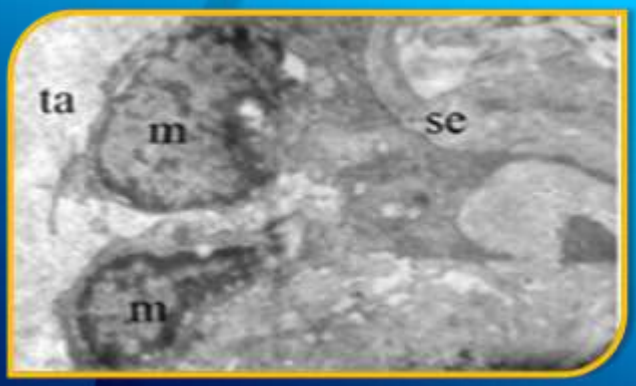
EXPERIMENTAL BIOMEDICAL RESEARCH



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1-World Medical Association.Declaration of Helsinki: ethical principles for medical research involving human subjects. http://www.wma.net/en/ 30publications/10policies/b3/index.html. Accessed October 14, 2010.

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Original Article

Evaluation of hemogram parameters in diabetic patients with coronary artery ectasia

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ABSTRACT

Aim: To compare the importance of hemogram parameters in predicting the disease in diabetic patients with coronary artery ectasia (CAE) and normal coronary artery.
Methods: The records of 7287 patients who underwent coronary angiography between January 2017 and October 2019 were reviewed. After appropriate exclusions, diabetic patients were divided into coronary artery ectasia and normal coronary artery groups. A total of 248 patients were included in the study and hemogram parameters of these two groups were compared.
Results: Compared to control group white blood count (WBC) *[8 (4-13) vs. 7 (5-12) u/mm3, p=0.023]*, hemoglobin *[13 (10-16) vs. 14 (10-20) gr/dL, p=0.015]*, red cell distribution width (RDW) *[16 (14-20) vs. 15 (12-19) %, p=0.026]*, neutrophil *[4.5 (2.1-11.4) vs. 4.0 (0.2-7.5) u/mm3, p=0.003*], platelet counts (Plt) *[266 (196-450) vs. 236 (163-362) k/mm3 p<0.001]*, platelet distribution width (PDW) *(17.9 (16.2-20.4) vs. 17.7 (15.9-19.7) % p=0.011)*, mean platelet volume (MPV) *[8.4 (6.4-11.2) vs. 7.9 (6.6-10.1) Fl, p=0.015*], plateletcrit (PCT) *[0.20 (0.14-0.32) vs. 0.19 (0.13-0.26), p<0.001]*, and neutrophil lymphocyte ratio (NLR) *[2.1 (1.0-9.7) vs. 1.6 (0.2-5.7), p=0.002]* were significantly higher in CAE patients.
Conclusion: The results of this study suggest that the increased some hemogram parameters may be useful in predicting disease in diabetic patients with CAE.
Keywords: Coronary artery ectasia, diabetes mellitus, hemogram parameters.

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Introduction

Coronary artery ectasia (CAE) is defined as the dilation of the coronary artery lumen. The term “ectasia" is defined as the widespread expansion of a coronary artery, whereas focal coronary expansion is called “coronary aneurysm” [1]. CAE is a disease of the coronary arteries with abnormal dilatation of the coronary arteries [2]. The diameter of the dilated segment is 1.5 times the diameter of the normal adjacent segment [3]. CAE is a form of coronary atherosclerosis characterized by internal and external elastic lamina disorder [4, 5]. The incidence of CAE ranges from 0.3 to 4.990 [6, 7]. CAE can be seen due to genetics, Kawasaki disease, mycotic or septic emboli, Marfan syndrome, polyarthritis nodosa arthritis, Takayasu disease, systemic lupus erythematosus, hypertension, smoking, cocaine use, percutaneous transluminal coronary

angioplasty, stent, and directional coronary atherectomy. CAE is also related to apical hypertrophic cardiomyopathy [8].

The etiopathogenesis of CAE is not fully understood [9, 10]. Although the main cause of coronary artery ectasia is unknown, atherosclerosis has been the most accused pathogenesis [11]. One of the most important indicators of atherosclerotic processes is endothelial dysfunction [12, 13]. CAE has commonly been evaluated as a variant of atherosclerotic heart disease. However; more intense inflammation has been detected in CAE than obstructive coronary artery disease (CAD) [14]

Histology is usually due to chronic vascular inflammation that shows thickened fibrotic intima with lipid accumulation [15]. The thinning of the tunica media environment associated with chronic inflammation is considered to be the main pathogenesis of extensive remodeling [10]. Ectasia can lead to slow flow in the coronary arteries, dissection, thrombus formation, and vasospasm [16, 17]. The primary symptom of CAE is chest pain. Ectasia can cause the acute coronary syndrome, ventricular arrhythmias and sudden cardiac death without severe coronary artery stenosis [7]. Coronary angiography is the gold standard test for the diagnosis of coronary artery ectasia [8].

Diabetes mellitus (DM) is an important public health problem due to high morbidity and mortality from microvascular and macrovascular complications [18]. Endothelial dysfunction is the basis for the development of long-term complications of diabetes [19]. The activity and aggregation of platelets are important in terms of thrombus during the atherogenesis process [20, 21]. Therefore, in this study, we aimed to evaluate the hemogram parameters of diabetic CAE patients.

Materials and Methods

We reviewed 7287 angiograms performed between January 2017 and October 2019, from Bolu Abant Izzet Baysal University Medical Faculty Hospital. "The Siemens Axiom Artis diagnostic device (Siemens Healthcare GmbH, Forchheim, Germany)" was used to perform coronary angiography. Coronary angiography (CAG) was performed to investigate ischemic heart diseases based on clinical indications. The study was conducted in accordance with the ethical approval of the University Ethics Committee. (Date: 24/10/2019; Decision number: 2019/217) Data about patients were obtained from the institution's database and patient files. CAG images recorded in digital format were evaluated visually by two blind cardiologists and patients diagnosed as CAE were included in the study. Patients included in the study were selected from patients with chronic coronary syndrome (CCS). Patients with clear CAE evidence were selected. The baseline demographic data and clinical cardiovascular risk factors; hypertension, diabetes mellitus, smoking or ex-smoking, family history of CAD, dyslipidemia weight and height were determined from hospital records. There was no significant difference in demographic parameters between CAE patients and the control group (normal coronary angiography). Subjects with a history of chronic diseases such as heart failure (ejection fraction <50%), acute coronary syndrome (ACS), previous coronary artery bypass grafting, percutaneous coronary intervention, significant valve disease, patients under 18 years of age, atrial fibrillation, hypertension, smoking, autoimmune diseases, pregnancy, iatrogenic ectasia, myocarditis, pericarditis, acute and chronic lung disease, obstructive sleep apnea, chronic inflammation, active infection, cancer, immunosuppressive therapy,

hypo/hyperthyroidism, stroke, mental retardation, delirium, dementia, any hematological abnormality (sickle cell anemia, thrombocytopenia etc.), and antiplatelet / anticoagulant agents and steroid users, liver or kidney failure and electrolyte imbalance were excluded from the study.

Statistical analysis

Statistical analysis was conducted with SPSS software (SPSS 22.0 for Windows, IBM Co, Chicago, IL, USA). Kolmogorov Smirnov test was used to determine distribution normality. Normal variables were compared with the T-test and expressed as mean ± standard deviation. Mann Whitney U test was used for variables showing the abnormal distribution and expressed as median (IQR: interquartile interval). A chi-square test was used for comparison of nonparametric variables. A p-value lower than 0.05 was considered statistically significant.

Results

We enrolled 248 individuals including 124 CAE patients (mean age: 61.4±10.9 years) and 124 control persons (mean age: 59.3±10.3 years). The mean age was 61.4±10.9 and 59.3±10.3 in the patient and the control groups, respectively. Frequencies of sex, and body mass index (BMI) were not significantly different between the patient and the control groups (Table 1). Compared to control group white blood count (WBC) [8 (4-13) vs. 7 (5-12) u/mm3, *p=0.023*], hemoglobin [13 (10-16) vs. 14 (10-20) gr/dL, *p=0.015*], red cell distribution width (RDW) [16 (14-20) vs. 15 (12-19) %, *p=0.026*], neutrophil [4.5 (2.1-11.4) vs. 4.0 (0.2-7.5) u/mm3, *p=0.003*], platelet counts (Plt) [266 (196-450) vs. 236 (163-362) k/mm3 *p<0.001*], platelet distribution width (PDW) (17.9 (16.2-20.4) vs. 17.7 (15.9-19.7) % *p=0.011*), mean platelet volume (MPV) [8.4 (6.4-11.2) vs. 7.9 (6.6-10.1) Fl, *p=0.015*], plateletcrit (PCT) [0.20 (0.14-0.32) vs. 0.19 (0.13-0.26), *p=0.001*], and neutrophil lymphocyte ratio (NLR) [2.1 (1.0-9.7) vs. 1.6 (0.2-5.7), *p=0.002*] were significantly higher in CAE patients. There was no significant difference between the two groups in terms of other biochemical and hemogram values (Table 2).

Table 1. General characteristics of the study groups.

Baseline characteristics	Diabetic patients with CAE (n=124)	Diabetic patients with NCA (n=124)	*P value*
Age (years)	61.4±10.9	59.3±10.3	*0.134*
Male/female	70/54	42/82	*0.447*
LVEF (%)	58.48±4.34	59.07±4.61	*0.297*
Heart rate	73,5 (50-100)	76 (57-107)	*0.352*
SBP (mmHg)	120 (100-150)	120 (90-158)	*0.577*
DBP (mmHg)	70 (63-94)	80 (60-100)	*0.210*
HbA1c (%)	7.3 (4.4-12.0)	6.9 (5.6-12.8)	*0.489*
BMI	32.0 (18.7-41.1)	31.2 (20.1-41.9)	*0.628*

CAE: Coronary artery ectasia, NCA: Normal coronary artery, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HbA1c (%): Hemoglobin A1c, BMI: Body mass index.

Table 2. Laboratory data of the study groups.

Parameters	Diabetic patients with CAE (n=124)	Diabetic patients with NCA (n=124)	*p*
LDL-cholesterol (mg/dL)	112 (49-271)	122 (46-211)	*0.336*
Triglyceride (mg/dL)	162 (75-631)	166 (70-548)	*0.076*
Total cholesterol (mg/dL)	189 (126-586)	208 (95-294)	*0.637*
HDL-cholesterol (mg/dL)	44 (24-75)	46 (29-86)	*0.392*
Glomerular filtration rate (%)	84 (32-110)	87 (42-125)	*0.573*
ALT (u/l)	17 (9-50)	20 (9-132)	*0.277*
AST (u/l)	19 (12-42)	21 (7-48)	*0.256*
TSH	1.4 (0.3-4.5)	1.5 (0.3-4.2)	*0.054*
CRP (mg/L)	1 (0.10-18)	1 (0.01-11.2)	*0.012*
WBC, (u/mm^3)	8 (4-13)	7 (5-12)	*0.023*
Hemoglobin (gr/dL)	13 (10-16)	14 (10-20)	*0.015*
MCV	86 (64-99)	87 (79-97)	*0.918*
RDW (%)	16 (14-20)	15 (12-19)	*0.026*
Neutrophil, (u/mm^3)	4.5 (2.1-11.4)	4.0 (0.2-7.5)	*0.003*
Lymphocyte, (u/mm^3)	2.2 (1.2-3.2)	2.5 (0.1-3.6)	*0.102*
Monocyte, (u/mm^3)	0.5 (0.2-1)	0,5 (0.2-4.3)	*0.589*
Basophils, (u/mm^3)	0.06 (0.001-0.1)	0.07 (0.001-0.2)	*0.888*
Eosinophil, (u/mm^3)	0.143 (0.009-0.658)	0.138 (0.033-0.815)	*0.846*
Platelet counts (Plt) (k/mm^3)	266 (196-450)	236 (163-362)	*0.001*
PDW (%)	17.9 (16.2-20.4)	17.7 (15.9-19.7)	*0.011*
MPV (Fl)	8.4 (6.4-11.2)	7.9 (6.6-10.1)	*0.015*
PCT	0.20 (0.14-0.32)	0.19 (0.13-0.26)	*0.001*
Neutrophil lymphocyte ratio (NLR)	2.1 (1.0-9.7)	1.6 (0.2-5.7)	*0.002*
Platelet lymphocyte rate (PLR)	132.5 (71.2-371.9)	97.6 (64.8-4085.7)	*0.516*

CAE: Coronary artery ectasia, NCA: Normal coronary artery, GFR: glomerular filtration rate, ALT: alanine aminotransferase, AST: aspartate aminotransferase, PDW: Platelet distribution width; RDW: Red cell distribution width; MPV: Mean platelet volume; HDL: high-density lipoprotein; LDL: low-density lipoprotein; WBC: White blood count; PCT: plateletcrit.

Discussion

This study showed that hemogram parameter levels were different between CAE patients and controls. To the best of our knowledge, this is the first study to evaluate hemogram parameters in patients with diabetic coronary artery ectasia.

CAE has been related to rising morbidity and

mortality [15]. Angiographies performed to investigate ischemic heart disease indicate an average of 1-5% CAE [4]. CAE is thought an atypical variant of coronary atherosclerosis, that characterized by disruption of the elastic lamina [4, 5]. The key role of inflammation in the initiation and progression of atherosclerosis is well known [22, 23].

Inflammation plays a major role in the development of atherosclerosis and in all stages of CAD [24]. Chronic inflammation is considered to play a role in the etiology of CAE [25, 26]. In our study, as in previous studies[27], the level of inflammatory biomarker CRP was found to be high.

Circulating white blood cell count (WBC) and its subtypes and their relationship to cardiovascular outcomes have been evaluated in previous studies [28]. Leukocyte, monocyte, and neutrophil levels have found to be high in patients with isolated CAE [29]. Neutrophil lymphocyte ratio (NLR) is being evaluated as a new marker of inflammation. Recently, it has been suggested that the NLR rate is a new biomarker for cardiovascular events and prognosis [30]. Balta et al [31] investigated the relationship of NLR in isolated CAE patients and found it to be high. In our study, this rate was high in CAE patients.

Increase in Mean platelet volume (MPV) and platelet distribution width (PDW) in diabetic patients is thought to be related to diabetic vascular complications [32]. MPV and PDW levels have been found to be high in diabetic patients with macrovascular complications [33]. MPV and PDW were higher in diabetic patients with thromboembolic complications [34]. This confirms that CAE is an atherosclerotic disease. MPV, an indicator of platelet activation, has an independent effect on the pathophysiology of atherosclerosis. MPV levels were high in patients with acute myocardial infarction, unstable angina pectoris, and congestive heart failure [35]. As in our study, MPV levels were found to be high in CAE patients in previous studies [36]. In contrast to this study, in our study, all CAE patients were diabetic. In our study, patients with coronary artery ectasia had higher MPV than normal coronary arteries.

Platelets have an important role in the pathogenesis of homeostasis and thrombosis [37]. It has been shown in previous studies that platelet indices are increased in diabetic patients [38]. We also found these indexes high in our study.

Conclusions

Routine hematological analyzes are important, simple, effortless and cost-effective tests. These tests may be predictive of CAE, which requires prospective large-scale randomized control trials.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *The study was conducted in accordance with the ethical approval of the University Ethics Committee. (Date: 24/10/2019; Decision number: 2019/217).*

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Original Article

Early graft survival after renal transplantation, single center experience

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ABSTRACT

Aim: The best treatment for patient with end stage kidney disease is kidney transplantation which improve their quality of life and survival rate. The aim of our study is to determine the factors that affect the results of early outcomes of graft function.

Method: Twenty-eight adult patients who underwent renal transplantation from 2016 to 2017 were included in our university.

Results: The median age of the recipients was 38.5 (range: 19-65) and 68% (19 patients) were male. Acute rejection was detected in 8 patients. Patients who developed rejection were found to have higher panel reactive antibody positivity and higher parathyroid hormone levels. Panel reactive antibody positivity was found to be 25% in patients who developed rejection and 0% in patients who did not develop rejection *($p = 0.02$)*. The parathyroid hormone level was calculated as 963.2 ± 587 in the rejection group and 378 ± 227 in the rejection group *($p = 0.003$)*. It was observed that 37.5% of DM patients had rejection and 10% in non-diabetic patients. The difference was statistically significant *($p = 0.08$)*.

Conclusion: Panel reactive antibody positivity and parathyroid hormone levels increased the likelihood of rejection. The effect of the presence of diabetes mellitus in the patient on the development of rejection was observed to be limited. Our findings were consistent with the literature. Because of the number of patients and the short follow-up period, further studies are needed.

Keywords: Renal transplantation, acute rejection, graft survival, graft failure.

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Introduction

Renal transplantation has been preferred for better survival and quality of life in patients with end-stage renal diseases [1]. Studies have shown that many donor and recipient factors affect patient survival after transplantation. Some of these factors include age, gender, body weight, number of human leukocyte antigen (HLA) mismatches, duration of warm ischemia, development of acute rejection, delayed graft function, general health status, proteinuria, albumin level, Panel Reactive Antibody (PRA), new onset hypertension (HT), diabetes mellitus (DM), hyperlipidemia, cytomegalovirus, hepatitis B, hepatitis C infections, serum uric acid level, gene polymorphisms (Caveolin 1,

chemokine receptor 5 polymorphism, etc.) and serum homocysteine levels [2-10].

Studies on early graft survival after renal transplantation are important in terms of contributing to long-term outcomes. Pre-transplant blood product transfusion history may result in HLA antigen sensitization. The highest risk for sensitization occurred in multipara women, multiple transfusions and failed organ transplants. Frequent transfusion also caused an increase in PRA. The pro-inflammatory condition adversely affects graft survival [11,12].

In the preoperative era, imaging of renal vascular system is important for surgical success and graft survival. Transplantation using multiple renal artery grafts is as safe as single-artery grafts when evaluated for urologic complications [13]. Another factor related to the survival of the transplanted kidney is the age of the recipient and donor. Renal recipients over 60 years of age have an increased risk of graft failure [14]. Graft failure and long-term mortality have also increased in recipients of older donors compared to younger kidney donation cases [15]. The incidence of early graft failure in obese recipients is probably increased because of vascular suture problems [16,17]. Previous or active smoking is associated with decreased patient and graft survival and increased rejection rate [18]. Although diabetes is a common cause of end-stage renal disease, new-onset diabetes mellitus after transplantation can be an important complication of renal transplantation via affecting the survival of allograft by increasing cardiovascular risk [19-22]. Low post-dialysis systolic blood pressure and low pre-dialysis diastolic blood pressure were associated with decreased risk of death, whereas post-dialysis high diastolic blood pressure was associated with increased risk of death. Low blood pressure before transplantation was also associated with decreased risk of graft failure [23]. Ischemia reperfusion injury after renal transplantation affects short-term and long-term graft outcomes. Ischemia reperfusion injury is associated with delayed graft function, graft rejection, chronic rejection and chronic graft dysfunction [24].

Studies based on protocol biopsies have shown that acute rejection of both cellular and humoral type may lead to long-term changes due to reduced graft survival [25-28]. Compared with patients without acute rejection, those with acute rejection in the first year were observed more frequently in patients with HLA mismatches [29]. Decreased early graft function after kidney donation from live donors was found to reduce graft survival without rejection. However, the effect on graft survival in the long term is uncertain [30]. Therefore, we conducted a retrospective study to show the factors affecting early graft survival after renal transplantation in our institution.

Materials and Methods

The study was enrolled 28 sequential adult cases who underwent kidney transplantation from the living donor between 2016 and 2017 at transplantation clinic of our University Hospital. We also enrolled data of 28 donors. The study was designed retrospectively. The study was conducted in accordance with the ethical approval of the University Ethics Committee (Decision number: 29032017-4). The data were obtained from the hospital database and patients' files. Age, gender, additional diseases, weight, height and laboratory test results were recorded.

For the statistical analyzes, IBM SPSS (Statistical Package for the Social Sciences) Version 16.0 software was used. In the study, numerical data are given as median (range).

Categorical and non-parametric variables were analyzed with Chi-square test and Mann Whitney U test, respectively. Statistical first type error margin (α) was taken as 0.05 for this study. Therefore, the results for p <α were considered statistically significant at 95% confidence level.

Results

The median age of the recipients was 38.5 (range: 19-65) years and 68% of the subjects (19 patients) were male. Fifty percent (14 patients) had a history of cigarette smoking. The median body mass index of the patients was 23 (range: 16-34) kg/m^2. Hypertension and diabetes mellitus were in 46% and 18% of the patients. Glomerulonephritis was the etiologic factor of end stage renal disease in the remaining subjects. When ABO blood groups were examined, it was observed that 54% have A, 21% have B, and 25% have 0 group type. Besides, 89% of the patients were Rh + and remaining were Rh-.

Median creatinine and GFR values of graft kidneys at 0, 3 and 6 months were given in Table 1.

Table 1. The creatinine and GFR values of the grafts at 0, 3 and 6 months after transplantation.

Parameters	0 months	3 months	6 months
Creatinine (mg / dl)	1.26	1.29	1.19
GFR (ml / min / 1.73 m2)	65	64	70

The median age of the donors was 48.5 (Range: 25-72) years and 57% were women. 57% of the donors had a history of smoking. The median body mass index of the donors was calculated as 26 (Range: 18-33) kg/m^2. When ABO blood groups were examined, it was observed that 43% were A, 11% were B and 46% were 0. There was no donor from the AB blood group. Also, 86% of the donors were Rh +. Two donors (7%) had polar arteries. In terms of compliance of donors and patients, 11 (39%) patients were the same gender with donor, 22 (79%) were have the same ABO blood group, and 23 (82%) were have similar Rh type with donor. Twenty-two (79%) of the donors were relatives, and there was no relation between the 6 of the donors.

Table 2. Recipient factors that may affect rejection.

Parameters	Rejected	Unrejected	*P*
Recipient age Year ± standard deviation	35,8±16	41,9±12,4	*0,30*
Gender of the recipient • male • female	5 (63) 3 (37)	14 (70) 6 (30)	*0.70*
Smoking • smoked • non-smoked	4 (50) 4 (50)	10 (50) 10 (50)	*1*
DM history • yes • no	3(37,5) 5(62,5)	2(10) 18(90)	*0,08**
HT history • yes • no	5(62,5) 3(37,5)	8(40) 12(60)	*0,41*
Pregnancy history • yes • no	2 2	2 4	*0,34*
History of blood transfusion • yes • no	2(28,6) 5(71,4)	6(30) 14(70)	*0,94*
Previous transplantation history • yes • no	1(12,5) 7(87,5)	1(5) 19(95)	*0,48*
Presence of Class II Panel Reactive Antibody • positive • negative	2(25) 6(75)	0 20(100)	*0,02**
Induction therapy • given • none	2(25) 6(75)	8(40) 12(60)	*0,45*
Patient body mass index (kg / m2)	21,9±5,3	24,4±4,8	*0,15*
Parathormone level (mg/dl)	963,2±587	378±227	*0,003**
Dialysis time median (range), (weeks)	0 (0-104)	1,5 (0-78)	*0.50*

A total of 8 (28.5%) acute rejections were seen. When the patients with and without rejection were compared, there was no difference in urine output before transplantation, duration of dialysis before transplantation, patient age, gender, body mass index, smoking history, ejection fraction, history of hypertension, blood transfusion and previous renal transplantation (Table 2).

The presence of panel reactive antibodies and parathyroid hormone levels were different in patients with acute graft rejection. Both of the 2 patients with positive panel reactive antibody had rejection, but only 6 of the 26 patients with negative PRA had rejection *(p= 0.02)* (Table 2). In addition, patients with acute rejection had higher parathyroid hormone levels than patients without rejection. The mean parathyroid hormone level was 963.2 ± 587 in the rejection group and 378 ± 227 in the rejection group *(p = 0.003)* (Table 2).

It was observed that patients with a history of DM developed rejection with a frequency of 37.5% and this rate was observed as 10% in patients without a history of DM. The difference was close to statistical significance, yet, insignificant *(p =0.08)* (Table 3).

When the patients with and without rejection were examined, it was found that the age, sex, smoking status, CMV status, warm ischemia time, donor glomerular filtration rate, donor body mass index and presence of polar artery did not affect the development of rejection (Table 3). Similarly, it was found that gender accordance between donor and recipient, having the same ABO blood type, having the same Rh blood type, and the number of incompatible HLA mismatch did not affect the presence of rejection (Table 3). When the effect of the relationship between the donor and the recipient on the rejection was investigated, it was seen that in all patients having rejection,

Table 3. The factors related to donor and recipient/ donor compatibility that may affect rejection.

Parameters	Rejected	Unrejected	*P*
Donor age	42.8±10,6	49.3±11.7	*0.14*
Donor gender • **male** • **female**	3(37,5) 5(62,5)	9(45) 11(55)	*0,70*
Donor body mass index (kg / m2)	24,9±4,9	26,5±3,5	*0,37*
Donor smoking • **smoked** • **non smoked**	4 (50) 4 (50)	12 (60) 8 (40)	*0,63*
Number of HLA mismatches	2,9±1,3	3±1,5	*0,91*
Donor glomerular filtration rate (ml / min / 1.73 m2)	111,7±21,4	103±13,5	*0,18*
Polar artery • **yes** • **no**	1(12,5) 7(87,5)	1(5) 19(95)	*0,48*
Donor CMV IgG • **positive** • **negative**	8(100) 0	19(95) 15	*0,52*
Warm ischemia time, min	14,4±0,8	14.5±1.1	*0,83*
Relation • **relative** • **unrelated**	8(100) 0	14(70) 6(30)	*0,08**
Gender match • **incompatible** • **compatible**	4(50) 4(50)	7(35) 13(65)	*0,46*
ABO match • **incompatible** • **compatible**	1(12,5) 7(87,5)	5(25) 15(75)	*0,46*
Rh match • **incompatible** • **compatible**	1(12,5) 7(87,5)	4(20) 16(80)	*0,64*

the transplantation was from the relative donor. No rejection was observed in any of the 6 patients who received transplantation from non-relative donors. The results were close to statistical significance, yet, insignificant *(p = 0.08)* (Table 3).

Discussion

Renal transplantation has recently been preferred for better survival and quality of life in patients with end-stage renal disease [1]. Studies on early graft survival after renal transplantation are important in terms of

contributing to long-term outcomes. In a study with a follow-up of 122 months after renal transplantation, a correlation was found between proteinuria (calculated with protein creatinine ratio) and poor graft function in the first 3 months. The advantage of this study is long follow-up period and especially values higher than 0.5% of protein creatinine ratio have been found to be observed more frequently in vascular events [31]. No correlation was found in our study between rejection and micro-albumin/creatinine ratio in spot urine at 0, 3 and 6 months. The lack of correlation with respect to graft survival may be due to our lower follow-up.

One of the issues that can be important in the anamnesis is the history of blood product transfusion before transplantation. Erythrocyte transfusion may result in HLA antigen sensitization. The highest risk for sensitization occurred in multipara women, multiple transfusions, and failed organ transplants, but previous data have shown equal or greater risk for men. As a result, an increase in PRA is associated with poor graft survival [11, 12]. In our study, no correlation was found between the recipient's blood product transfusion history and the number of incompatible HLAs between the recipient and the donor, while rejection developed in 2 of the 2 patients with panel reactive antibody positivity. The ratio of PRA positivity was 25% among patients who developed rejection and 0% in patients who did not develop rejection. Our findings are compatible with the literature.

Another factor related to the survival of the transplanted kidney is the age of the recipient and donor. Renal recipients have an increased risk of graft failure, especially with age greater than 60 years [14]. When kidney donors are examined, it is seen that graft failure and long-term mortality are increased in the recipients of older donors compared to younger donor cases. However, these recipients appear to be better or more accomplished than those of the kidneys of donors with standard or extended criteria [15]. When we examined our patients with and without rejection, it was found that the age of the donor did not affect the development of rejection. This may be due to younger age of our patients and donors.

Early results up to one year after kidney transplantation may also be affected by the nutritional status of the recipients. In our study, we found significantly higher rates of early graft failure in both thinner and overweighed recipients. The incidence of early graft loss increases in those recipients and may be due to the more frequent technical problems of the operation in obese patients [16, 17]. In our study, we could not show any relationship between increase in body mass index and rejection rate probably due to no obese patients was present in our cohort.

When we examine the effects of smoking on endothelial damage and its effect on delaying or even preventing the healing process; previously or active smoking is associated with decreased patient and graft survival and increased rejection rate [18]. It was shown that the smoking of the donor and the recipient was not significant between the patients with and without rejection. This may be due to the short follow-up period.

Although diabetes is a common cause of end-stage renal disease, new-onset diabetes after transplantation can be an important complication of renal transplantation [19]. New-onset diabetes influences allograft survival and has an impact on renal function and increased cardiovascular risk, leading to patient survival [20-22]. In a prospective study, the 12-year graft survival rate was 70% in the non-diabetic control group and 48% in those

developing new-onset diabetes [32]. In our study, diabetes mellitus, which was present before renal transplantation, was found to have a trend for association with rejection.

In a study about 13881 primary renal transplant recipients, low after dialysis diastolic blood pressure and low pre dialysis diastolic blood pressure were associated with decreased risk of death, whereas high diastolic blood pressure after dialysis was associated with increased risk of death. Low blood pressure before transplantation was also associated with decreased risk of graft failure [23]. In our patients, it was shown that the factors affecting the rejection of the graft kidney did not include the pre-transplant hypertension. This may be due to the small number of patients.

Acute rejection of both cellular and humoral type may lead to long-term changes due to reduced graft survival [25-28]. Acute rejection findings of both cellular and humoral types were observed in our patients. Humoral antibody was positive in 2 of 8 patients with acute rejection.

Compared with patients without acute rejection, those with acute rejection at 1 year were more frequently observed in patients with a greater number of HLA mismatch [29]. In our study, no correlation was found between HLA compliance and rejection rate. However, we found that PRA positivity is correlated with acute graft rejection.

It has been found that decreased early graft function after kidney transplantation from live donors reduces graft survival without rejection. However, its effect on graft survival in the long term is not clear. Decreased early graft function is defined as delayed or slow graft function. Weight gain, pre-transplantation dialysis treatment and warm ischemia have been identified as risk factors for the emergence of decreased early graft function. Decreased early graft function also showed negative effects on long-term graft survival [30]. In our patients, BMI of the recipient, pre-transplantation dialysis treatment and duration of warm ischemia were not significantly different in terms of rejection.

High serum PTH levels in both pre-transplantation and post-transplantation were associated with decreased graft function. Roodnat and colleagues in the study of 407 renal recipients in terms of total graft survival when evaluated in terms of high pre-transplantation PTH level was found to be a linear relationship between graft failures [33]. In our study, a positive correlation was found with higher level of PTH in the pre-transplantation period with acute graft rejection.

In conclusion, we found that pre-transplantation PRA positivity and post-transplantation parathyroid hormone levels increased the probability of rejection. The effect of the presence of diabetes mellitus on the development of rejection was limited. Therefore, we suggest that strictly following-up PRA and parathyroid hormone levels in renal transplant recipients.

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Ethical statement: *The study was conducted in accordance with the ethical approval of the University Ethics Committee (Decision number: 29032017-4).*

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Original Article

A prospective study of serum concentrations of leptin, homocysteine and insulin resistance in children with steroid-sensitive nephrotic syndrome

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ABSTRACT

Aim: To measure serum leptin, homocysteine concentrations and insulin resistance in active and remission stages of children with nephrotic syndrome (NS) and to investigate their role in NS pathogenesis.

Methods: A total of 70 children were included in the study, 40 patients who had been diagnosed with NS and 30 healthy patients were control. Changes in plasma concentration of the serum homocysteine, leptin, and insulin were measured and compared with the other parameters in the groups.

Results: Serum leptin concentrations in active phase were lower than the remission phase *(1.48 ± 0.09 ng/dl, 1.84 ± 1.64 ng/ml, p<0.05)*. Also, serum homocysteine concentrations in NS group during the active phase were lower than the remission phase and the control group *(6.45±2.54 ng/dl, 9.35±2.99 ng/ml, 7.76± 1.97 ng/ml, p<0.05*). The serum fasting insulin concentrations and homeostatic model assessment for insulin resistance (HOMA-IR) values of remission phase were significantly higher than those of active phase *(p<0.05)*. A positive relationship was found between the homocysteine concentrations and the body mass index of the patient; whereas, a negative relationship was detected between erythrocyte sedimentation rate (ESR), and the LDL-cholesterol concentrations *(p<0.05)*. ESR was found as the only factor associated with lower concentrations of homocysteine during the active phase *(r:-0.592, p<0.05)*.

Conclusion: In this study, we demonstrated that serum leptin and homocysteine concentrations decreased in active phase and increased in remission phase in children with NS. Insulin resistance could also develop as a result of steroid use in a short period of time in these patients.

Keywords: Nephrotic syndrome, leptin, homocysteine, insulin resistance, proteinuria.

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Introduction

Nephrotic syndrome (NS) is a clinical condition characterized by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 80% of the nephrotic

syndromes seen in children present as minimal lesions that respond to steroid treatment [1]. Though NS is frequently encountered, its etiopathogenesis is not precisely clarified; however, genetic factors and immunology are suspected [2]. Today, genetic and metabolic studies aimed at explaining the development of the disease have been vigorously undertaken. It is known that patients given steroid treatment have a tendency of insulin resistance, obesity and endothelial dysfunction particularly in children [3].

Leptin, which is a 167-amino acid polypeptide, is synthesized in tissues like the adipose tissue, the placenta, the gastrointestinal tract and neuronal tissues, but the activity mainly occurs in the adipose tissue [4,5]. Body fat and serum leptin concentrations are directly proportional therefore, leptin concentrations are increased for obese person. Insulin resistant is determined in rodents which has leptin resistance and deficiency [6]. Leptin effects the 24-hour urinary protein amounts in children and is associated with the child's body weight and the severity of the disease [7].

Homocysteine, a thiol-containing amino acid, is generated by intracellular demethylation of dietary methionine, which is catabolized to form either cystathionine or cysteine [8]. Hyperhomocysteinemia is an independent risk factor for coronary heart disease, and display endothelial dysfunction. [9]. Renal function influences plasma homocysteine concentrations, and various reports have shown that plasma homocysteine concentrations may be lowered or elevated in children with NS compared to healthy controls [10].

We hypothesized that leptin and homocysteine metabolism is impaired in children with idiopathic nephrotic syndrome and during steroid treatment. The present study was designed to assess the changes in plasma concentration of serum homocysteine, leptin and insulin in patients with nephrotic syndrome in active and remission phase after steroid treatment.

Materials and Methods

This study was conducted in children for the first time diagnosed with NS at our Pediatric Nephrology. This study was approved by the Medical Ethics Committee of University of Health Sciences, Istanbul Haseki Teaching Hospital (Approval date and number: 29.05.09/43). Furthermore, study was conducted in accordance with the revised Helsinki Declaration. The parents of the children in the patient and the control groups, as well as all children over 12 years of age, were informed in detail about the study, and informed consent with signature was received.

The power calculation for the present study based on an effect size of 0.5 for leptin (ng/ml), a standard deviation of 2 (ng/ml) and an alpha level set at 0.05. Required sample size to get a power of 0.8 according to these assumptions was 30 patients for each group. Number of 10 patients were added as extra cases in case of withdrawal or drop out possibility. Therefore, at the end of study number of patients were 40 while control were 30.

The inclusion criteria to for the study were as follows: patients who were first diagnosed with NS between the ages of two and sixteen, responded to steroid treatment, did not have an additional disease. Blood samples were obtained first on admission as active phase (proteinuria), second at the end of treatment as remission phase (non-proteinuria). In the first step of the study, patients who had proteinuria above 40 mg/m2/hour, serum albumin concentrations below 2.5 gr/dl and hyperlipidemia were primarily evaluated during the active phase, or proteinuria phase,

prior to the start of steroid treatment. Then, these patients were given 2 mg/kg/day prednisolone as stated in the steroid treatment protocol. To assess the responsiveness to the steroid treatment, it was accepted that proteinuria in urine measured with a dipstick should be found in trace amounts, negative or under 4 mg/m2/hour, and that serum albumin concentrations should be over 3.5 g/l for three consecutive days for four weeks. Afterward, the same dosage of steroids was kept on every other day for the following four weeks. The steroid treatment was then gradually decreased and carried on fulfilling five months. Remission phase blood samples were obtained at the end of treatment. The systemic examinations of patients were carried out after their detailed anamneses and medical histories were obtained in both phases. As control group, age-sex matched thirty healthy children were chosen to determine normal leptin and homocysteine concentrations.

Blood samples were obtained after 12 hours fasting from patients who were included in the study and centrifuged at 1300 rpm for half an hour. Serum samples were taken into fine tubes and stored at -20°C. Routine biochemical examinations were practiced by Abbott C-16000 chemistry analyzer, and standard conventional methods were performed. Leptin concentrations were determined by enzyme-linked quantitative immunological measurement technique, using a Leptin ELISA kit (BioSource LEPTİN EASIA®, Nivelles Belgium). Homocysteine concentrations, however, were determined by use of an IMMULITE 2000® homocysteine kit and a competitive immunity measurement method. Insulin concentrations was measured by a chemiluminescent immunoassay method (ADVIA Centaur analyzer; Bayer Diagnostics) on fasting blood samples. HOMA-IR was calculated as [fasting glucose (mg/dl) x fasting insulin (lU/ml)/405] [11]. Intra assay and inter-assay variations for the concentrations of leptin, homocysteine and insulin variables were calculated with the formula (CV: Standard Deviation/Mean).

Statistical analysis

Statistical analyses of the data were performed using the Statistical Package for the Social Sciences (SPSS), version 19, program (SPSS Inc., Chicago, IL, USA). All continuous values were presented as mean ± standard deviation, where suitable. The categorical values were presented as the frequency and percentage. Categorical variables were compared using Pearson's chi-squared test. Independent samples t-test was used for comparing two groups. Paired data were analyzed using paired samples t-test when data were normally distributed. General linear model was used for adjusting the effect of BMI confounder. The Spearman correlation coefficient was calculated to evaluate the correlation between the continuous variables. The values determined as $p<0.05$ were accepted as statistically significant

Results

Demographics, anthropometric variables and arterial blood pressures of the study groups are reported in Table 1. When the biochemical parameters of the patients with nephrotic syndromes in their active (proteinuria) phases and remission phases (non-proteinuria) were compared, as expected, total protein, albumin, cholesterol, LDL-cholesterol, triglyceride and IgM concentrations of patients in their active phases were found to be significantly different than those concentrations found during their in remission phases (Table 2). The serum fasting insulin concentrations and HOMA-IR values of

Table 1. Demographic, anthropometric variables and arterial blood pressures of the study groups.

Parameters	Active phase (n=40)	Remission (n=40)	Control (n=30)	*P*
Age (year)	7.45 ± 4.86	7.73 ± 4.58	8.28 ± 3.25	*NS*
Gender (M/F)	28/12	28/12	21/9	*NS*
BMI (kg/m²)	18.9 ± 3.4	17.8 ± 3.9	15.9 ± 1.8	*< 0.001*
SBP (mmHg)	101.2 ± 10.6	96.2 ± 8.2	100.2 ± 8.8	*NS*
DBP (mmHg)	64.2 ± 8.7	60.2 ± 6.9	63.8 ± 6.9	*NS*

Data are reported as mean ± standard deviation (SD) or number and percentage. BMI: Body mass index, SBP: Sistolic blood pressure, DBP: Diastolic blood pressure, NS: Not significant.

Table 2. Biochemical values of the patients with nephrotic syndrome (active phase and remission) and healthy children.

Parameters	Active phase (n=40)	Remission (n=40)	Control (n=30)	P^*	P^{**}	P^{***}
Glucose(mg/dl)	85.9 ± 14.1	86.5 ± 6.6	89.7 ± 10.9	*NS*	*NS*	*NS*
Urea (mg/dl)	23.4 ± 9.2	23.7 ± 6.3	27.6 ± 7.5	*NS*	*0.04*	*NS*
Creatinine (mg/dl)	0.42 ± 0.13	0.44 ± 0.13	0.53 ± 0.09	*NS*	*0.002*	*0.01*
Total protein (g/dl)	4.25 ± 0.75	6.54 ± 0.63	7.16 ± 0.51	*< 0.001*	*< 0.001*	*0.001*
Albumin (g/dl)	2.00 ± 0.72	4.08 ± 0.51	4.46 ± 0.37	*< 0.001*	*< 0.001*	*0.003*
Leucocyte count (/mm³)	8155 ± 4937	7990 ± 2588	8120 ± 2475	*NS*	*NS*	*NS*
Hemoglobin (g/dl)	12.7 ± 1.6	12.5 ± 0.8	12.0 ± 0.8	*NS*	*0.03*	*NS*
Thrombocyte (x1000/mm³)	438 ± 249	328 ± 100	305 ± 86	*NS*	*0.01*	*NS*
CRP (mg/l)	0.10 ± 0.12	0.12 ± 0.15	0.24 ± 0.38	*NS*	*NS*	*NS*
Cholesterol (mg/dl)	368.7 ± 168.2	196.9 ± 82.3	150.2 ± 19.7	*< 0.001*	*< 0.001*	*0.02*
LDL (mg/dl)	254.0 ± 143.4	111.6 ± 54.4	80.6 ± 19.9	*< 0.001*	*< 0.001*	*0.02*
HDL (mg/dl)	51.7 ± 21.4	52.0 ± 13.8	45.1 ± 10.2	*NS*	*NS*	*NS*
Triglyceride (mg/dl)	295.7 ± 216.3	111.7 ± 55.6	118.1 ± 65.6	*< 0.001*	*< 0.001*	*NS*
Insulin (µIU/ml)	7.09 ± 3.36	12.8 ± 8.8	9.69 ± 6.91	*0.006*	*NS*	*NS*
HOMA-IR	1.50 ± 0.75	2.76 ± 1.90	2.20 ± 1.64	*0.007*	*NS*	*NS*
ESR (mm/h)	59.4 ± 32.8	18.0 ± 13.4	15.5 ± 9.2	*NS*	*NS*	*NS*
IgG (g/dl)	403.1 ± 290.2	830.0 ± 251.9	1026 ± 317	*NS*	*NS*	*0.006*
IgA (g/dl)	112.4 ± 45.1	115.4 ± 53.9	114.5 ± 55.8	*NS*	*NS*	*NS*
IgM (g/dl)	255.5 ± 208.1	149.3 ± 159.8	114.8 ± 49.2	*0.046*	*0.05*	*NS*
IgE (g/dl)	380.4 ± 926.5	205.0 ± 299.4	129.8 ± 243.1	*NS*	*NS*	*NS*
C3 (g/l)	124.7 ± 25.1	116.4 ± 24.4	123.8 ± 55.9	*NS*	*NS*	*NS*
C4 (g/l)	24.9 ± 5.5	24.4 ± 7.2	19.8 ± 5.1	*NS*	*0.001*	*0.01*

Data are reported as mean ± standard deviation (SD) or number and percentage. CRP=C-reactive protein, HOMA-IR= Homeostatic model assessment of insulin resistance. P^*: Active phase vs remission of the patients with nephrotic syndrome, P^{**}: Active phase vs control, P^{***}: Remission vs control, NS: Not significant.

remission phase were significantly higher than those of active phase *(p<0.05).*

The active and remission phases of the patients and the leptin and homocysteine values of the control group are shown in Table 3. When we analysed leptin and homocysteine concentrations, we considered BMI as a confounder which was a significant different between the study and the control group. The serum leptin concentrations in active phase were lower than the remission phase *(1.48 ± 0.09 ng/dl, 1.84 ± 1.64 ng/ml, p<0.05)*. Also, serum homocysteine concentrations in NS group during the active phase were lower than the remission phase and the control group *(6.45±2.54 ng/dl, 9.35±2.99 ng/ml, 7.76± 1.97 ng/ml, p<0.05)*. In brief, the average homocysteine concentrations of the study groups can be formulated in ascending order as "active phase < control group < remission phase" (Table 3). Effective demographical, clinical and laboratory values on the reduction of serum homocysteine concentrations during the active phases of patients with NS were assessed.

A positive relationship was determined between homocysteine concentrations, the body mass index (BMI) of patients and their serum IgG concentrations; whereas, a negative relationship was detected between the erythrocyte sedimentation rate, and the total cholesterol and LDL-cholesterol concentrations (Table 4).

Table 3. Comparison of serum concentrations of leptin and homocysteine concentrations in study groups and BMI as a confounder.

Parameters	**Active phase** (n=40)	**Remission** (n=40)	**Control** (n=30)	***P****	***P***	***P****
Leptin (ng/ml)	1.48 ± 0.09	1.84 ± 1.64	1.57 ± 0.90	*0.004*	*NS*	*NS*
Homocysteine (µmol/l)	6.45 ± 2.54	9.35 ± 2.99	7.76± 1.97	*0.045*	*0.001*	*0.014*

Data are reported as mean ± standard deviation (SD) or number and percentage. P*: Active phase vs remission of the patients with nephrotic syndrome, P**: Active phase vs control, P***: Remission vs control, NS: Not significant.

Table 4. The factors correlated with homocysteine concentrations of the patients in the active phase of the nephrotic syndrome (only significant correlations show).

Homocysteine					
	BMI	**ESR**	**Cholesterol**	**LDL-cholesterol**	**IgG**
r	0,38	-0,59	-0,42	-0,47	0,39
P	0,049	0,003	0,032	0,017	0,044

BMI= body mass index; ESR: erythrocyte sedimentation rate.

The factors causing low concentrations of homocysteine that were revealed via the Spearman correlation analysis were then tested to determine the independent predictor by implementing a Stepwise Linear Logistic Regression analysis. BMI, serum IgG concentrations, ESR, cholesterol, LDL-

cholesterol concentrations, which are some factors that may affect homocysteine concentrations, were included in the model. As a result of the administration of this model, an increase in sedimentation rate was found as the only factor associated with lower concentrations of homocysteine during the active phase of patients with NS.

Discussion

The etiopathogenesis of steroid-sensitive NS has not been clearly identified. In the literature, it has been revealed that there are many factors stimulating the disease, and as a consequence of these factors, the illness commences after a series of immunological events [2,3,10]. Today, there are studies proceeding that will be able to shed light on NS's etiopathogenesis. It is not yet clearly known whether the changes in leptin and homocysteine concentrations occur as a result of protein loss due via urine, the main cause of the illness, or whether there are other factors that contribute to the formation of NS [12-16]. We targeted to determine the leptin and serum homocysteine concentrations and the related factors in our patient group. In addition, we aimed to observe the effect of steroids on the insulin metabolism in the treatment of NS.

Glomerular dysfunction develops in NS, and heavy proteinuria is thus observed. Some studies have shown that leptin excretion through urine increases in children with NS during the proteinuria phase. Parallel to this, the serum leptin concentrations of these children were reduced and both urine and serum leptin concentrations returned to normal during the remission phase of the disease [12,13]. In other studies, however, it has been noted that though patients' leptin excretion increased during the proteinuria phase, there was no alteration in their serum leptin concentrations [12-14]. Therefore, the condition of the serum leptin concentrations in children with NS and the relationship between these concentrations and the disease's pathogenesis remain unclear and controversial. Although the serum leptin concentrations of our patients during the proteinuria phase were determined to be lower than during the non-proteinuria phase and lower than the concentrations in the healthy children of the control group. The systemic elimination of leptin that is circulating in the blood happens through the kidneys. Leptin is not metabolized by the kidneys and is thus excreted as unimpaired proteins [17]. Therefore, in parallel with the daily urine decrease in children with chronic renal failure, the amount of leptin excreted via urine also diminishes and the serum leptin concentrations increase [3,15,16]. In NS, however, increased protein filtration causes leptin excretion via urine to increase. Though urinary leptin concentrations were not studied in our study, it was determined that leptin excretion with urine increased and the serum leptin concentrations decrease significantly. In a similar study, urinary leptin excretion is found as increased while serum leptin concentration is decreased. Serum leptin concentration plays an important role in the pathophysiology of NS (18)

Homocysteine, not involved in the 20 amino acids among the structural elements of proteins, is an amino acid involving thiol. Homocysteine, synthesized in the liver, muscle and other tissues, is excreted via urine from the kidneys after being metabolized with remethylation and transsulphuration reactions [19]. It has been shown that plasma homocysteine concentrations are inversely correlated with creatinine clearance, and hyperhomocysteinemia is often seen in patients with renal failure [20]. Moreover, homocysteine plays a significant role in endothelial damage and in the formation of

atherosclerosis developed as a consequence of this damage. It has been reported that homocysteine prominently increases in patients with chronic renal failure, diabetes, obesity, hypertension and metabolic syndrome prominently and leads to endothelial dysfunction [21,22]. Our study found that the homocysteine concentrations of patients during their acute phases were low, attributed to the increase in its excretion through urine. Consequently, this suggested that homocysteine alteration occurred in patients as a result of NS and was not responsible for the pathogenesis of the disease.

In the literature, homocysteine concentrations during the proteinuria phase were found to be high compared to the concentrations in healthy children; however, the reason for this could not be clarified [23,24]. In other most studies, though, it has been reported that serum homocysteine concentrations decreased depending on urinary excretion during the proteinuria phase and returned to normal during remission [25]. Homocysteine levels predicted damage accrual independently and is considered as proinflammatory marker in SLE patients [26,27]. In our patients, the decreased serum homocysteine concentrations during the proteinuria phase were higher than the values of those in the control group. Our findings were compatible with the recent study conducted by Tkaczyk et al. [28]. Their study further revealed that while the serum homocysteine concentrations were low during the proteinuria phase, they began to increase two weeks later and were considerably higher than those of the control group after eight weeks. Tkaczyk et al. [29] also found that the administration of cyclosporine A caused a significant increase in homocysteine and cysteine concentrations. However, we can infer that this abnormal increase may have been a reactional increase related to steroid use to treat NS, similar to the treatment of polymyalgia rheumatica [30].

After the use of vitamin B6, B12 and folic acid in the same patient group, the homocysteine concentrations decreased again [30,31]. Although another study showed that homocysteine concentrations were low during the proteinuria phase, they normalized during remission at 12 weeks and 1 year. [32]. Therefore, when patients went into remission, these vitamin concentrations could be seen to decline and the homocysteine concentrations to increase. Elevated homocysteine concentrations during steroid treatment were associated with endothelium dysfunction and atherosclerosis; although vitamin concentrations were not measured in our study, vitamin supplementation is suggested based on estimation of low vitamin concentrations. Modulation of endothelial dysfunction in children with NS may be considered a therapeutic strategy to decrease the risk of future adverse cardiovascular events [33]. Indeed, if serial homocysteine concentrations had been determined at certain intervals after steroid treatment was ceased, this hypothesis would have been supported.

A positive relationship was determined between the homocysteine concentrations, the body mass index (BMI) and the serum total IgG concentrations alongside an established negative relationship between the erythrocyte sedimentation rates (ESR) and the total cholesterol and LDL-cholesterol concentrations. As BMI can be deceptive owing to the edematous period in NS, the relationship between the homocysteine concentrations and BMI was ignored. Nevertheless, when other data was analyzed carefully, they were all observed to be associated with the activity of the disease. In order to determine the most important independent predictor that could lead

to low concentrations of homocysteine, multiple logistic regression was applied. In our study, elevated ESR was observed as single independent predictor when stepwise linear logistic regression was applied to factors that causes low homocysteine concentration. ESR is the index of inflammatory activity and gives an indication regarding the progress of the disease and response to treatment. Therefore, this finding states that low homocysteine concentration could be another parameter in addition to ESR which indicates severity and response to treatment as a surrogate marker. The long-term effects of alterations on the homocysteine concentrations in patients will be possible only through randomized prospective studies. The long-term effects of homocysteine in NS are unexplored issues for future studies.

Another important finding of our study was being significant difference between serum insulin concentrations of patients and homeostasis model assessment of insulin resistance (HOMA-IR) values during the active and remission phases. Although only two patients (5%) had a high HOMA-IR value (≥2.5) during the active phase, HOMA-IR values were found as ≥2.5 in sixteen out of forty patients (40%) after steroid treatment in remission phase. Furthermore, the serum fasting insulin concentrations of remission phase were significantly higher than those of active phase. Thus, discovering both insulin concentrations and HOMA-IR values at high concentrations in patients receiving steroid treatment indicated the development of insulin resistance. As the therapeutic benefits of glucocorticoids continue to expand across medical specialties, the incidence of steroid-induced insulin resistance will continue to rise [34]. If these patients do not have to use steroids again, over time these values will partly or totally return to normal.

The major limitation of this study was the lack of urinary analyses. Unfortunately, we did not study the urinary homocysteine and leptin concentrations of the patients. Another important limitation was the lack of frequent measurement of the serum homocysteine, leptin concentrations and vitamin levels during the steroid treatment. Last limitation of the study was difference of BMI between the study and the control group, which was adjusted by considering as a confounder.

Conclusion

In this study, we demonstrated that serum leptin and homocysteine concentrations decreased in active phase and increased in remission phase in children with NS. This decrease was likely caused by excretion via urine and is an important parameter revealing the activity of the illness. In addition, temporary or permanent insulin resistance could develop as a result of steroid use in a short period of time for this patient group. These and future studies will be crucial for understanding the pathogenesis of NS and determining treatment approaches.

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Original Article

Ultrastructural examination of left internal mammary artery under electron microscopy in patients with chronic kidney disease who underwent coronary bypass surgery

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ABSTRACT

Aim: To investigate the vascular damage of internal mammary artery graft with electron microscope secondary to chronic renal failure transmission in patients who underwent coronary artery bypass grafting surgery.

Method: A total of 30 patients (10 patients with chronic renal failure and 20 patients without chronic renal failure) who underwent coronary artery bypass graft surgery were included in this prospective study. Left internal mammary artery graft was harvested as conventional fashion with no touch technique. Samples were prepared and then examined with the transmission electron microscope. Every arterial sample was individually examined ultrastructurally, and the changes were recorded. Then the samples of the control group and chronic renal failure group were compared.

Results: There were no significant differences between chronic renal failure group and the control group in terms of demographics, comorbidities, intraoperative data and postoperative outcomes, and the groups were statistically similar *($p<0.05$)*. Moreover, no statistically significance was detected in terms of structure and ultrastructure between the groups.

Conclusion: The results of our study revealed that no ultrastructural changes were observed in the structure of IMA, suggesting that this graft would provide a good graft patency.

Keywords: Coronary artery bypass grafting, chronic renal failure, internal mammary artery, ultrastructural study, electron microscope.

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Introduction

Cardiovascular diseases and associated complications are the most important factors in morbidity and mortality in patients who have developed chronic kidney disease (CKD) and continue their lives with dialysis therapy [1,2]. Cardiovascular diseases account for about one third of the causes of hospitalization in these uremic patients [3]. The rate of mortality due to the cardiovascular diseases is approximately 8 to 20 folds higher in dialysis patients compared to general population [4]. Ischemic heart disease is usually resulted from coronary artery disease, but it occurs with non-atherosclerotic

reasons in 27% of hemodialysis patients. Ischemic heart disease occurring due to non-atherosclerotic reasons is associated with underlying cardiomyopathy, small vessel disease (caused by hypertension, diabetes mellitus or calcium-phosphate accumulation), decreased capillary density and abnormal myocyte bioenergy [5]. Myocardial infarction has been remarkable during autopsy in about 25% of dialysis patients [6]. In an angiographic study on patients with end stage renal disease and who were planned to receive hemodialysis treatment, significant coronary stenosis was found in over than 75% of the patients [7].

Capability to widely and successfully perform topic that these operations could also be performed in patients with accompanying high-risk group diseases of other systems. Today, in patients with chronic kidney disease, coronary artery bypass grafting (CABG) surgery has been gradually increased and successfully performed [8-11].

Structural and functional properties of conduits during the surgical myocardial revascularization is of important issue which affects the success of the surgery [12,13]. It is possible that examination of histologic and morphologic features of coronary bypass grafts used in patients with CKD may provide information about graft patency in CABG. However, in the existing literature, the studies investigating the quality of coronary bypass grafts are very limited for patients with CKD.

The aim of this study was to examine the endothelial functions and vascular damage resulted from CKD in internal mammary artery (IMA) that is a coronary bypass grafts, in patients with CKD who underwent CABG, under transmission electron microscope. Therefore, we investigated whether the use of IMA, a very valuable graft, for CABG in patients with CKD is safe or not.

Materials and Methods

Preoperative features of patients

A total of 30 patients scheduled for CABG operation were included in this prospective study. Of the patients scheduled for CABG, 10 had CKD and 20 had no CKD. This study was conducted after receiving approval from the institutional ethics committee (Date: 16.03.2009; Decision no.: 2009/03/05) and written informed consents were obtained from all participants. The principles of the Helsinki Declaration were completely complied in the study. Patients were divided into two groups; as CKD and non-CKD (control) groups. Patients undergoing reoperation, non-renal organ failure and with an IMA which was not suitable for use were excluded from the study. The control group was randomly selected among the patients scheduled for CABG who had no organ failure and reoperation.

Operative procedure

After standard anesthesia protocol, all patients were underwent CABG with standard fashion with sternotomy. The operations were performed as off-pump CABG in 4 and on-pump CABG in 6 patients in the patient group while off-pump operations were performed in 3 and on-pump operations in 17 patients in the control group. In this study, all samples were obtained from the left internal mammary artery (LIMA) grafts. LIMA grafts were harvested as conventional fashion with no touch technique, by using an electrocautery from the subclavian artery to the site where it was branched as superior epigastric artery and musculophrenic artery. Side branches were hemoclipped. Systemic heparinization was applied with unfractionated heparin of 200 IU/kg in patients who underwent off-pump CABG, and 350 IU/kg in those who underwent on-pump CABG with cardiopulmonary bypass.

Preparation and examination of IMA samples

For the analysis, approximately 1 cm terminal segment of LIMA graft was removed before LIMA-left anterior descending (LAD) artery anastomosis. The samples were fixed in 2.5% glutaraldehyde solution for 24 hours. The samples were then rinsed with pH 7.4 SBP (Sorenson's Phosphate Buffer) buffer solution, and subjected to post-fixation treatment with 0.1% osmium tetroxide solution. The samples were rinsed again with SBP buffer solution, and dehydrated. Propylene oxide and epoxy resin material were mixed at 1:1 ratio and the samples were kept in this solution for 1 hour. At the end of one hour, same amount of epoxy resin material was added on this mixture and the mix ratio was raised to 1:3. The samples were kept at the rotator for one night, embedded into the epoxy material using plastic capsules and kept at 60ºC for 48 hours. Sections of 2 µm was cut and stained with methylene blue and examined under the light microscopy to determine the sites where thin sections would be cut. Thin sections of about 60 nanometers were obtained with the same ultramicrotome. These thin sections were stained with uranyl acetate and lead citrate with double contrast method, and examined under the transmission microscope (Jeol JEM 1200 EX, Japan) and the images were obtained. Each arterial samples were ultrastructurally examined and the changes were recorded. Samples of the CKD and control groups were compared.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 software. Numerical parameters were expressed as mean ± standard deviation, minimum and maximum values, while categorical variables were expressed as frequency and percentage. Normality of the variables was tested using Kolmogorov-Smirnov test. Independent Sample t test (student t test) among the parametric tests was used in comparison of the normally distributed variables, and Mann-Whitney U test among the non-parametric test was used in comparison of the skewed data. Chi-square, Fisher's and Mantel Haenszel tests were used in comparison of the categorical variables. A $p<0.05$ value was considered as statistically significant.

Results

The mean age was 54.2±9.7 years in CKD group, and 60.9±12.1 in the control group. Nine of patients with CKD were male and 1 was female, while 16 of patients in the control group were male and 4 were female.

Table 1. Sociodemographic features and comorbidities of the groups.

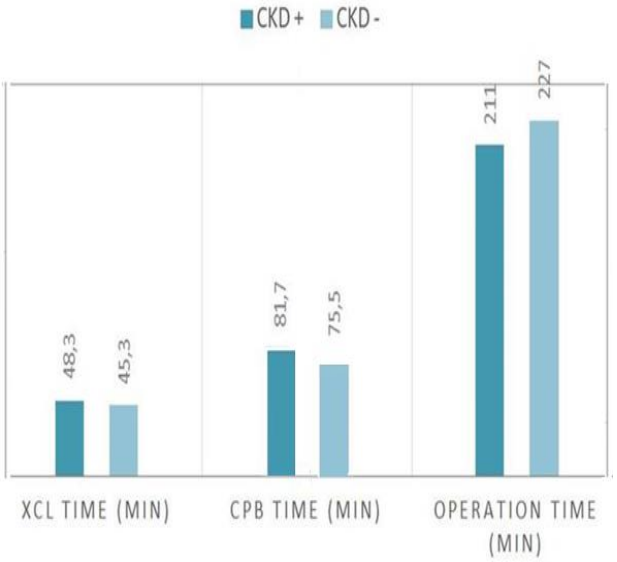
Parameters	CKD +		CKD -		*P value*
	N	%	N	%	
Patient number	10	33	20	67	
Age (year)	54.2 ± 9,7		60.9 ± 12,1		*0.141*
Gender (M/F)					
Male	9	90	16	80	*0.488*
Female	1	10	4	20	
Smoking					
Current	0	0	9	45	***0.017***
Former	3	30	1	5	
Never	7	70	10	50	
Unstable AP	4	40%	6	30	*0.270*
Stable AP	4	60%	16	80	*0.375*
NYHA					
Class 2	6	60	8	40	*0.442*
Class 3	4	40	12	60	
Hypertension	8	80	10	50	*0.235*
Diabetes mellitus	4	40	4	20	*0.243*
Previous CVE	0	0	1	5	*0.472*
Carotid artery disease	3	30	1	5	*0.095*
COPD	7	30	3	15	*0.330*
PVD	1	10	2	10	*1.000*

CKD: Chronic kidney disease; AP: Angina pectoris; COPD: Chronic obstructive pulmonary disease; CVE: Cerebrovascular event; PVD: Peripheral vascular disease; NYHA: New York Heart Association.

There were no statistically significant differences between the groups in terms of baseline clinical characteristics *(p>0.05)*, except for smoking *(p=0.017)*. Sociodemographic features and comorbidities of 10 patients with CKD and 20 patients without CKD are presented in Table 1.

When operative data were considered, there were no statistically significant differences between both groups in terms of aortic cross clamp (XCL), cardiopulmonary bypass (CPB) and operation times (p>0.05) (Figure 1).

Figure 1. Distribution of the cross clamp (XCL), cardiopulmonary bypass (CPB) and operation times of patients.



Preoperative and postoperative blood urea and creatinine values were statistically significantly higher in CKD group compared to the control group (p<0.001). No statistically significant difference was found between the groups in terms of pre- and postoperative sodium and potassium levels (p>0.05). Postoperative blood urea and sodium values were statistically significantly increased compared to the preoperative values in CKD group (p<0.05). Postoperative creatinine and potassium values were higher than the preoperative values, although the difference was not statistically significant (p>0.05). There was no significant difference between pre- and postoperative biochemical values in the control group (p>0.05). Preoperative and postoperative biochemical values of the groups are given in Table 2.

Table 2. Preoperative and postoperative biochemical values of the groups.

Parameters		CKD +	CKD -	*P value*
Preoperative	Urea	91.3 ± 32,9	39.75 ± 9,0	***<0.001***
	Creatinine	4.6 ± 2,3	1.2 ± 0,9	***<0.001***
	Na	136.9 ± 4,4	139.1 ± 2,8	*0.107*
	K	4.3 ± 0,7	4.2 ± 0,4	*0.424*
Postoperative	Urea	112.7 ± 29.7[a]	43.2 ± 11,4	***<0.001***
	Creatinine	4.9 ± 2,3	1.2 ± 0,3	***<0.001***
	Na	141 ± 5.3[a]	141.0 ± 6,2	*0.864*
	K	4.6 ± 0,9	4.4 ± 0,3	*0.382*

There were no significant differences between the groups in terms of IMA flow and early postoperative outcomes including revision due to severe bleeding, atrial fibrillation and the necessity of intraaortic balloon pump (p>0.05) (Table 3).

Table 3. Comparison of IMA flow, IABP, revision, and postoperative AF status between the groups.

Parameters	CKD +		CKD -		*P value*
	N	%	N	%	
IMA flow					
Good	10	100	19	95	*0.472*
Poor	0	0	1	5	
IABP requirement	1	10	2	10	*1.000*
Revision due to bleeding	1	10	1	5	*0.605*
Postoperative AF	0	0	5	25	*0.083*

AF: Atrial fibrillation; CKD: Chronic kidney disease; IABP: Intraaortic balloon pump; IMA: Internal mammary artery.

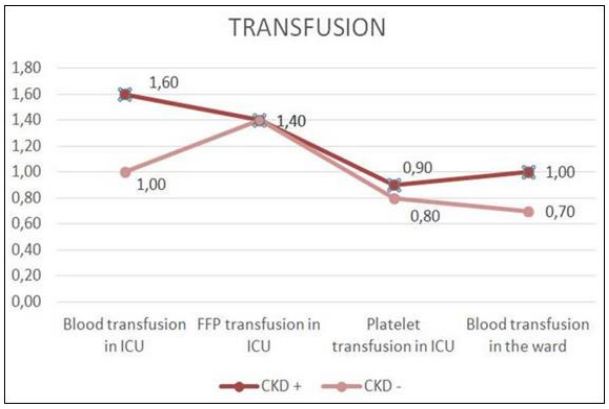
Although higher amounts of blood products were transfused in the intensive care unit and ward in CKD group, the differences between the groups were not statistically significant (p>0.05). Comparison of the groups in terms of transfusion amounts is shown in Table 4 and Figure 2.

Table 4. Blood product transfusions in the groups.

Parameters	CKD +	CKD -	*P value*
ES transfusion in ICU	1.6 ± 0.96	1.0 ± 0.92	*0.108*
FFP transfusion in ICU	1.4 ± 1.89	1.4 ± 1.50	*1.000*
Platelet transfusion in ICU	0.9 ±1.52	0.8 ± 1.77	*0.821*
ES transfusion in ward	1.0 ± 0.47	0.7 ± 0.57	*0.163*

CKD: Chronic kidney disease; ES: Erythrocyte suspension; FFP: Fresh frozen plasma; ICU: Intensive care unit.

Figure 2. Comparison of the chronic kidney disease (CKD) + and CKD - groups in terms of transfusion.



In the electron microscopic evaluation of the tunica intima layer, it was found that endothelial cells lining inner surface of the vessel were ultrastructurally normal. No any ultrastructural pathologic finding was observed in cell membranes, nuclei, intracytoplasmic organelles and basal membranes of the endothelial cells. Whereas subendothelial layer in the tunica intima was observed to consist of a loose connective tissue. Free collagen fibers and connective tissue cells in this layer were ultrastructurally normal. In the examination of smooth muscle cells found in the tunica media layer, no ultrastructurally pathologic finding was found in the cell membranes, nuclei, intracytoplasmic organelles and basal membranes of these cells. Finally, in the electron microscopic examination tunica adventitia of the vessels, abundant collagen fibers and connective tissue cells were in a normal structure. As a consequence, entire IMA samples collected from the patients with CKD were ultrastructurally normal, and there was no significant ultrastructural difference between these samples and the samples collected from the control group. Although the patients had chronic kidney failure, ultrastructurally they had a completely normal vessel structure (Figure 3, 4).

Discussion

The remarkable finding of our study was that no any ultrastructural changes in IMA grafts was detected in patients with CKD who underwent CABG, and there were no any ultrastructural differences compared to the control group. Unchanged structure of IMA suggests that long term patency of this graft will be satisfactory.

Cardiac surgery which has shown a rapid development with invention of the cardiopulmonary bypass machine in the second half of the 20th century, is still continuing to develop and has been successfully performed in many centers. The aim of CABG operation is to provide sufficient blood flow to ischemic heart site, to improve quality of life of the patient, and to prolong lifetime [14-16]. Patency rate of the autogenous grafts used for this operation determines patients' quality of life and the success of CABG operations. Therefore, the target is to choose the grafts with a good patency rates [17]. Structural and functional properties of CABG conduits used in the

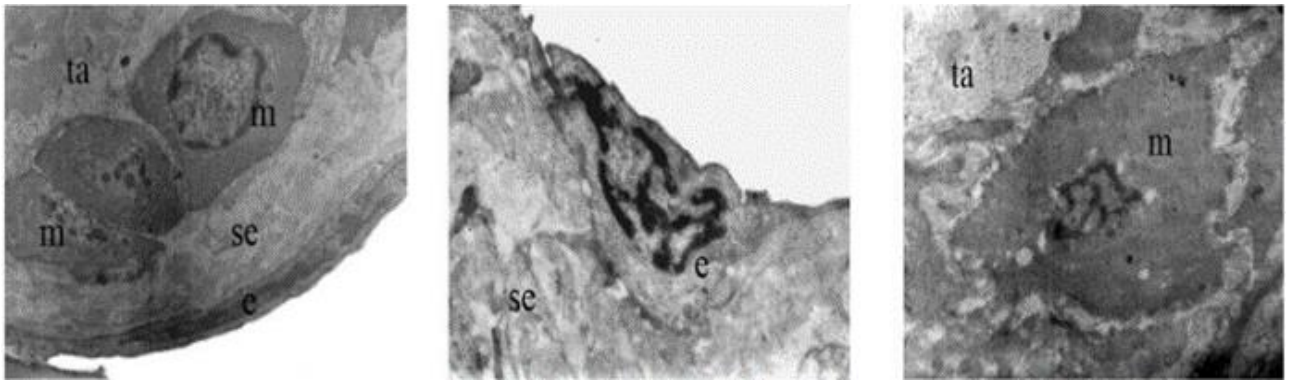


Figure 3. Examples of electron misroscopy images in the study group. ta: tunica adventitia; m: tunica media; e: endotelium; se: subendotelium.

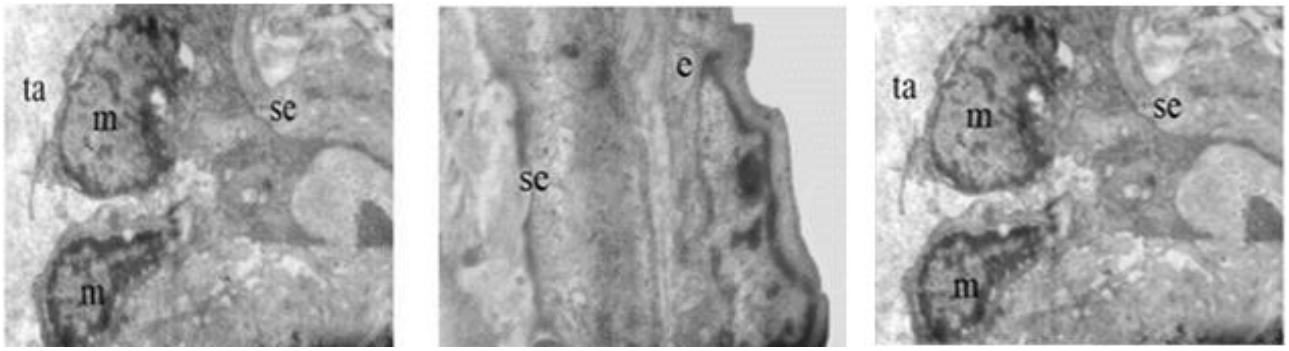


Figure 4. Examples of electron misroscopy images in the control group. ta: tunica adventitia; m: tunica media; e: endotelium; se: subendotelium.

myocardial revascularization of patients undergoing coronary bypass are important factors affecting outcome of the operation [12,13,18]. Patient's age, clinical status, vessel to be bypassed, utility of the graft, comorbidities and surgeon's experience are determinants of the graft choice. In the present study, IMA was used as a graft in all patients, and IMA-LAD anastomosis was applied as a standard procedure.

Internal elastic lamina plays a critical role in the arterial wall structure. Presence of fenestrations in the internal elastic lamina stimulates early and progressive intimal hyperplasia. It can be expressed that a damage to the internal elastic lamina is less prone to the proliferation of smooth muscle cells in the media, and thus to intimal hyperplasia than the muscular arteries. In addition, numerous elastic lamellae and internal elastic lamina forms a barrier against the incision of smooth muscle cells [19]. It is known that there is an association between the absence of elastic lamellae in the media and number of fenestrations in the internal elastic lamina, and potential result of this was development of more intimal hyperplasia. Consequently, the presence of elastic lamellae in the media shows a protective effect against the occurrence of fenestrations in the internal elastic lamina and intimal hyperplasia [20].

It is thought that histologic structure in the arterial grafts used in CABG may be affected by atherosclerosis, influencing rate of patency. Choosing the segments to be anastomosed

according to the histological structure and lumen diameter will positively affect rates of patency [13,17,18]. Main part of the IMA, which is the middle part consisting of 60% of the total length is less reactive than the distal and proximal sections [21].

An important factor determining long-term patency of IMA graft is the feature of the graft itself. Biological integrity of the graft and compliance to its new position is closely associated with the rates of long term patency and development of cardiac events. IMA graft has become an indispensable graft because of its superior long-term results up to 20 years [22]. It has been proven that the rate of patency is much higher in IMA compared to saphenous vein grafts, and while atherosclerotic lesions are developed in venous grafts, IMA graft is more resistant [23].

Another issue studied in coronary artery bypass is vasomotor features of IMA grafts in diabetic patients. Considering that vasoactive responses of coronary artery bypass grafts of diabetic patients who underwent myocardial revascularization would affect postoperative outcomes; Wendler et al. investigated vasomotor features of IMA grafts, and reported that biologic integrity of these grafts was preserved even in the presence of impaired glucose metabolism [24]. Similarly, in our study biologic and morphologic integrity was protected even in patients with advanced chronic kidney failure. However, there are several studies in the literature reporting endothelial dysfunction, which resulted in decrease or loss of the antithrombotic and vasodilator functions of IMA in diabetic patients [25,26]. On the other hand, in the present study conducted on IMA grafts of patients with CKD, no ultrastructural pathology was found in the structure of IMA wall and organelles. Therefore, we concluded that functions will not be impaired in an endothelium with protected cell structure. In a study by Pompilio et al. [25], the authors examined endothelium dependent functions of IMA in normotensive diabetic men who had normal cholesterol values, and found that nitric oxide and Prostaglandin 12 homeostasis was disrupted in this group of patients compared with the patients without known cardiovascular risk factors. They also examined surface features with a scanning microscope and reported that endothelium was intact and no atherosclerotic sign was observed.

Although mortality rates are higher in CKD patients compared to normal population, satisfactory results can be achieved if these patients are operated with correct indications when diagnosed with coronary artery disease. Today CABG operations can be performed with very low morbidity and mortality rates [8-11]. Positive results were obtained from all studies conducted on patency rates on IMA and examination of its histomorphological features. The main limitations of our study were relatively small number of participants and its single-centered design.

Conclusions

IMA grafts of patients with CKD were ultrastructurally examined in our study, and no pathology was observed. In addition, no pathology was found also in the samples collected from the control group, and there was no significant difference between these two groups. Our results reflected that the use of IMA graft in CKD patients undergoing coronary bypass is the most appropriate option among the other revascularization option. In addition, we think that IMA is superior over the other autogenous and artifact grafts used in coronary bypass operations in terms of graft patency. However, further studies with a larger

series of patients about coronary revascularization options and results in CKD patients and the strategy to be followed are warranted.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *This study was conducted after receiving approval from the institutional ethics committee (Date: 16.03.2009; Decision no.: 2009/03/05) and written informed consents were obtained from all participants.*

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Original Article

Role of uric acid and other parameters in sudden sensorineural hearing loss

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ABSTRACT

Aim: To investigate the levels of metabolites (predominantly uric acid) effective on biochemical, and coagulation parameters and evaluate their effects on the onset, and course of the disease.

Methods: In this retrospective study, files of 92 patients hospitalized between January 2007, and December 2013, in our clinic with established diagnosis of sudden hearing loss were screened. The biochemical (predominantly uric acid), and hematological parameters were compared with those of the control group. In addition, the patient group was divided into two groups according to uric acid levels and the difference between the groups was investigated in terms of the onset or course of the disease.

Results: A significant difference was not detected between the patient, and the control groups regarding mean uric acid levels. Among biochemical parameters glucose, creatinine, and international normalized ratio (INR) were significantly higher ($p<0.05$) while a significant intergroup difference was not detected as for other parameters. A significant intergroup difference was not detected in mean pure- tone averages, and mean hearing gain at admission between two groups formed based on uric acid levels, while post-treatment pure-tone average was significantly better in patients with higher serum uric acid levels. In the patient group, uric acid levels were significantly higher in patients with partial hearing loss relative to those with total loss.

Conclusion: In our study, we could not find a significant difference between the patient and the control groups as for uric acid levels. However, we have encountered evidence supporting the possible role of serum uric acid levels in the prognosis of sudden hearing loss.

Keywords: Sudden hearing loss, uric acid, oxidative stress, vascular injury.

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Introduction

"Sudden Hearing Loss" (SHL) is defined as development of sensorineural hearing loss (SNHL) of at least 30 dB at three contiguous frequencies within less than 72 hours [1]. Although its etiology has not been clarified definitively, vascular theory is the most

accepted one [2]. Cochlear perfusion with terminal arteries, and its higher sensitivity to hypoxia tend to support vascular injury as an etiologic factor. Available evidence indicates that metabolic diseases with microvascular effects such as diabetes, and hyperlipidemia are effective in the development of SHL [3]. Still, SHL secondary to neurological damage due to viral, neurotoxic, traumatic etiologies can be also seen [1].

Uric acid is the end-product of purine metabolism, and its levels increase in line with oxidative stress. In various studies performed, increased levels of uric acid have been indicated in conditions developed in association with vascular pathologies as myocardial infarction, and cerebrovascular events, and also its close association with endothelial dysfunction has been reported [4]. However in a few studies performed in recent years, correlations between lower uric acid levels, and increasing prevalence, and deteriorated course of some neurological diseases as Parkinson's disease, and Alzheimer's disease have been detected [5].

This trial aims to investigate the role of uric acid in the etiology, and prognosis of SHL. Together with uric acid, other metabolites and blood level parameters that might affect plasma have been analyzed.

Materials and Methods

The study was performed after obtaining approval of the ethics committee of the faculty (Decision #17522305/678). File numbers of the inpatients treated in our ENT service between January 2007 and December 2013 were acquired from informatics department of otorhinolaryngology department of our tertiary care center. The cases coded as "Sudden idiopathic hearing loss" according to International Classification of Diseases (ICD) were taken into account. Consequently, medical files of 56 female, and 82 male patients who were hospitalized, and treated with the diagnosis of sudden hearing loss were obtained. Fifteen female and 12 male patients whose laboratory data at admission could not be obtained were excluded from the study. Among the remaining cases, one female patient with chronic kidney disease, 2 patients (1 F and 1 M) diagnosed as schwannoma on MRI, and 2 female patients with presumed diagnosis of fistula were not included in the study. Besides 14 patients with diabetes (12 M and 2F) were also excluded from the study. As a result, a total of 92 patients (35 F and 57 M) were included in the study (Table 1).

Table 1. Distribution of the patients included in, and excluded from the study based on causative factors.

Parameters	Female	Male	Total
Patients with the diagnosis of sudden hearing loss	56	82	138
Laboratory data at admission not obtained	15	12	27
Chronic kidney disease	1	0	1
Schwannoma	1	1	2
Fistula	2	0	2
Diabetes and hypertension	2	12	14
Included in the study	35	57	92

Ninety-two (35 F and 57 M) patients with similar age, and gender-distribution relative to the patient group who applied to our outpatient clinic within the first 3 months of the year 2014 without any degree of hearing loss, and chronic disease and also underwent complete routine biochemical tests constituted the control group. In this study the following biochemical, and hematological parameters were evaluated: uric

acid, FBG, blood urea nitrogen (BUN), creatinine, hemoglobin (Hb), platelet, mean platelet volume (MPV), platelet distribution width (PDW), platelet count or plateletcrit (PTC), prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR). These hematologic parameters of the patient, and the control groups were compared, and any significant intergroup difference (if any) was evaluated.

Average, and median uric acid values were 4.6 mg/dl, and 4.5 mg/dl, respectively. Based on the median uric acid value, the patients were divided into 2 groups as those with uric acid levels of ≤ 4.5 mg/dL (n=47), and > 45 mg/dL (n=45). Pure-tone averages at the onset of the disease, and after the treatment, and also mean hearing gains of these groups were compared. In groups of patients formed based on the degree of the hearing loss, and audiometric pattern at admission, any significant difference (if any) as for mean uric acid levels, and a difference (if any) in the intragroup distributions of the groups constructed based on uric acid levels, and hearing gains were investigated. The patients were divided into 2 groups based on pure-tone averages at admission as partial (<90 dB), and total hearing loss (≥90 dB). Based on the median value, the patients were divided into 2 groups as those with uric acid levels of ≤ 4.5 mg/dL (n=47), and > 45 mg/dL (n=45). Pure-tone averages at the onset of the disease, and after the treatment, and also mean hearing gains of these groups were compared. In groups of patients formed based on the degree of the hearing loss, types of audiograms recorded at admission, any significant difference (if any) as for mean uric acid levels, and a difference (if any) in the intragroup distributions of the groups constructed based on uric acid levels, and hearing gains were investigated.

The patients were divided into 2 groups based on pure-tone averages at admission as partial (<90 dB), and total hearing loss (≥90 dB). When categorized in types of audiograms, upward-sloping, and flat-type audiograms which have been associated with better prognosis in literature studies [1] were included in Group 1, while flat type audiograms, and those indicating total hearing loss were categorized in Group 2.

Treatment response was evaluated using Siegel classification [6], and the patients were divided into groups based on baseline pure tone averages as calculated in post-treatment audiometric tests as follows: hearing gain > 15 dB (no improvement); ≥ 15 dB, and mean hearing acuity > 25 dB (partial improvement) pure-tone average in audiometry was ≥ 25 dB (total recovery).

Statistical analysis

For statistical analysis SPSS (Statistical Package for Social Sciences) for Windows 13.0 program was used. In the comparison of data of the patient, and the control groups which demonstrated normal distribution as age, FBG, uric acid, platelet counts, MPV, and PTT values, Student –T test was used. For the comparison of variables with non-normal distribution such as BUN, creatinine, hemoglobin, platelet, PDW, INR, and PT values, Mann -Whitney U (MWU) test was employed.

Results

Demographic and biochemical comparisons

Mean age of 35 female, and 57 male patients enrolled in the study was 43.6±16.84 (10-79) years. Mean age of the control group consisting of equal number of male and female patients was 42.8±16.5 (19-90) years. Any significant

intergroup difference was not detected regarding mean age of the patients (p=0.525).

Mean uric acid levels of the patient, and control groups were 4.65 (n=92), and 4.63 mg/dL (n=92), respectively without any statistically significant intergroup difference (p=0.871). Mean uric acid levels of the female study participants in the patient, and the control groups were 4.00 (n=36), and 3.789 mg/dL (n=36), respectively without any statistically significant difference (p=0. 270). Mean uric acid levels of the male study participants of the patient, and the control groups were 5.06 mg/dL (n=56), and 5.16 mg/dL, respectively (n=56) without statistically significant intergroup difference (p=0,619).

Among other parameters studied, creatinine, glucose, INR, and PT values were significantly higher in the patient group. BUN, Hb, platelet, MPV, plateletcrit, PDW, and PTT were not statistically significantly different between both groups. (**Table 2**)

Demographic and biochemical data of the patient group

Mean, and median uric acid levels of the patient group were 4.65, and 4.5 mg/dl, respectively. The patients were divided into two groups based on median uric acid value as Group 1 (n=47; uric acid ≤ 4.5 mg/dL) and Group 2, (n=45; uric acid > 4.5 mg/dL). Among biochemical metabolites, and coagulation parameters Hb, BUN, and creatinine were significantly higher in Group 2 ($p < 0.01$), while other parameters did not differ significantly between groups. These two groups were compared as for treatment onset, and prognosis. In Group 1, 46, and in Group 2, 38 patients had their audiometric records. Pure-tone averages of the study participants estimated at admission were compared, and any statistically significant difference could not be found between groups (Group 1, 83. 23 dB, and Group 2, 74.11 dB) (p=0.072). Post-treatment audiometric test records were available for 46 individuals in

Table 2. Comparison of laboratory parameters between the patient, and control groups.

Parameters	Patient	Control	Range	*p Value*	Test
Age (years)	43,6	42,0	-	*0,525*	St. T
Uric acid (mg/dl9	4,65	4,63	2,6-7,0	*0,871*	St. T
BUN (mg/dl9	15,3	14,2	6-23	*0,079*	MWU
Creatinine (mg/dl)	0,76	0,75	0,5-1,2	*0,008**	MWU
Glucose (mg/dl)	95,4	87,9	55-105	*<0,01**	St. T
Hemoglobin (gr/dl)	13,9	14,0	12-17	*0,665*	MWU
Platelet (x10^3/µl)	261	260	130-400	*0,885*	St. T
MPV (fl)	8,3	8,48	7,4-11	*0,346*	St. T
PLT (%)	0,21	0,21	0-0,99	*0,298*	MWU
PDW (%)	16,7	16,7	0-99,9	*0,848*	MWU
PTT (sec)	28,1	29,05	22-40	*0,085*	St. T
INR	1,06	1,01	-	*<0,01**	MWU
PT (sec)	13,3	12,9	11-14	*0,013**	MWU

BUN: blood urea nitrogen; MPV: mean platelet volüme; PDW: platelet distribution width; PTC: platelet count or plateletcrit; PT: prothrombin time; PTT: partial thromboplastin time; INR: international normalized.

Group 1 and 36 participants in Group 2. Posttreatment pure-tone averages were 70.16 dB in Group 1, and 54.58 dB in Group 2, with a statistically significant intergroup difference (p=0.034). Whereas mean hearing gain in Groups 1, and 2 were 12.98, and 20.78 dB, respectively, without any statistically significant intergroup difference (p=0.067). In summary, even a statistically significant difference could not be found, in the group with higher uric acid level milder hearing loss was detected at the onset of the disease with acquisition of higher hearing gain. Besides post treatment pure- tone averages were significantly better (Table 3).

hearing loss were 4.8 mg/dl, and 4.1 mg/dl, respectively with a statistically significant intergroup difference (p=0.008). In other words as uric acid levels increased, onset of sudden hearing loss was less aggressive.

Audiometric records of 80 patients could be accessible. Patients with upward-sloping, and flat audiograms had a better prognosis (Group 1, n=46) while patients with audiograms demonstrating a downward-sloping pattern and total loss were evaluated in Group 2 (n=34). Mean uric acid value of the group with upward-sloping, and flat –type audiograms, and hence better prognosis was 4.7 mg/dL, while that of the patients with poor prognosis, who had

Table 3. Comparison of admission, and post-treatment pure-tone average, and gain values of the patients grouped based on their uric acid levels expressed in decibels.

Parameters	**Uric acid range (mg/dl)**		
	Uric acid ≤ 4,5 (n=47)	**Uric acid > 4,5 (n=45)**	***p Value***
PTA at admission (dB)	83,23 (n=46)	74,11 (n=38)	*0,072*
Posttreatment PTA (dB)	70,26 (n=46)	54,58 (n=36)	*0,034**
Hearing gain (dB)	12,98 (n=46)	20,78 (n=36)	*0,067*

PTA: Pure-tone average.

Table 4. Distribution of patients grouped based on uric acid levels according to gain groups.

Parameters	**Uric acid range (mg/dl)**		
	Uric acid ≤ 4,5 (n=47)	**Uric acid > 4,5 (n=45)**	**Total**
No hearing gain	33	18	51
Partial hearing gain	9	10	19
Complete hearing gain	4	8	12
Total	46	36	82

Hearing test records of 8 out of 92 patients at admission were not available, and the remaining 84 patients were divided into groups with partial, and total hearing loss (Group 1, n=53, and Group 2, n=29). Average uric acid levels of the patients with partial, and total

downward-sloping, and total loss type audiograms was 4.3 mg/dL, without any significant intergroup difference (p=0.149).

The patients were divided into three groups based on their hearing gains they retrieved from the treatment using modified Siegel

classification [6]. The patients were divided into three groups. Mean hearing gain of the patients in Group 1 was less than 15 dB after treatment (no improvement) while of Group 2 was less than 25 dB after treatment, and their hearing acuity was still less than 25 dB (partial improvement). In Group 3 mean hearing gain was above 25 dB (total improvement group).

Posttreatment pure-tone averages of 82 out of 92 patients were available. These patients were in the groups of no hearing gain (n=51; 62.2 %), partial gain (n=19; 23.2 %), and total gain (n=12; 14.6 %). When these groups were compared according to 2 groups divided based on uric acid values as ≤ 4.5 mg/dL, and > 4.5 mg/dL p was equal to 0.098 according to *chi*-square test, and distribution of the groups were statistically significantly different. However, when distributions into groups were analyzed numerically, the number of patients with hearing gain was higher in the group with increased uric acid levels, while lesser number of patients in this group had not achieve any hearing gain from the treatment (Table 4).

Discussion

In approximately 80 % of SHL patients an identifiable etiology is not found, and in its etiology mostly vascular theory has been emphasized [2]. Accordingly, hearing loss can arise from a sudden vascular bleeding in the cochlea [7], an occlusion caused by an embolus *etc.* [8], vasospasm [9] or alteration of blood viscosity [10].

In this study our target was essentially to analyze levels of uric acid whose role in vascular diseases, and oxidative stress have gained prominence in recent years in patients with SHL, compare its levels with those of the control group, and investigate its impact on prognosis. In our study, starting from vascular theory, a group of 92 patients with SHL were investigated as for uric acid levels, hematologic parameters which will effect blood viscosity, and coagulation, and biochemical variables which might exert an effect on plasma levels of uric acid, and these variables were compared with those of the control group

In a study performed in the year 2012, a group of 147 patients with SHL was compared with a group of 103 control subjects with respect to similar parameters, and any significant intergroup difference could not be found regarding BUN, and creatinine values [11]. In our study we also investigated uric acid values, renal functions, and hence creatinine, and BUN values which reflect uric acid clearance. Mean creatinine value in the patient group was 0.01 mg/dL higher than that of the control group that was statistically significant. Mean creatinine value in the patient group was 0.01 mg/dL higher than that of the control group which was statistically significant. Though higher mean BUN, and uric acid values were measured in the patient group, a significant difference was not found relative to the control group.

In a study performed in 2014, all patients hospitalized with the diagnosis of SHL had undergone 75 gr oral glucose tolerance test, and more improved prognosis was detected in the normo-glycemic group relative to the group with impaired glucose tolerance test, and diabetes [12]. Even though diabetic patients were not included in our study, mean FBG level in the patient group was significantly higher when compared with the control group. These data may signify that though not at diabetic levels, higher fasting blood glucose (FBG) levels may be potentially effective factors in vascular etiopathogenesis of SHL.

Myeloprolipherative disorders as essential thrombocytosis, and polycythemia can predispose to vascular pathologies by increasing blood viscosity. In a study where 147

SHL cases were compared as treatment – responsive (n=102), and refractory (n=45) patients, hemoglobin levels were found to be significantly higher in the treatment-refractory group [11]. In our study, mean hemoglobin value in the control group was 0.1 mg /dL higher without any statistically significant intergroup difference.

Platelets are the smallest cells in the peripheral blood, and they are responsible from the release of mediators involving in coagulation, inflammation, thrombosis, and atherosclerosis [13]. In a study performed in the year 2006, any significant difference could not be found between the control, and SHL groups regarding PT, PTT, and platelet counts [14]. We did not come across a significant difference between the patient, and the control groups when compared in terms of platelet counts, and plateletcrit values. Although any difference was not found between both groups as for PTT, INR, and PT -albeit within their normal limits- the levels of these parameters were significantly higher in the patient group, and higher levels of these parameters lead to a delay in coagulation with resultant increase in the risk of bleeding, and on the contrary a decrease in the risk of thrombus formation.

Mean platelet volume (MPV) is the average of all platelet volumes, and it is used as a biomarker in the evaluation of production, and function of platelets. Large platelets are more active both from metabolic, and enzymatic aspects. Hence when compared with small platelets, they have a higher tendency to precipitate, and so an increased coagulation potential. In cases with vascular occlusion, acute, and chronic syndromes, MPV levels increase, but decrease in infections, autoimmune diseases or inflammatory conditions. In our study any significant difference was not found between the patient, and the control groups as for MPV, and PDW values.

In studies performed in recent years the relationship between uric acid, and vascular injury of vascular, and renal tissues has been advocated. Pro-inflammatory, complement, platelet, and coagulation cascade activating, neutrophil, and macrophage stimulating, protease, and oxidant synthetizing effects of urate crystals are already known [4]. Deterioration of endothelial dysfunction has been detected in patients who were given uric acid preparations, and also an association between endogenous uric acid concentration and severity of endothelial dysfunction has been revealed [15]. Similarly, incidence rates of atherosclerosis, arteriosclerosis, glomerulosclerosis, vestibular ataxia, and renovascular pathologies increase in gout patients [16].

In the literature, higher uric acid levels have been reported to increase risks of coronary artery disease, and cerebrovascular disease. Increased uric acid levels have been thought to contribute to the atherosclerotic process by effecting endothelial functions, oxidative metabolism, adhesion, and aggregation of platelets [4]. As reported in the literature studies, after excluding other factors, each 1 mg/dL increase in serum uric acid levels both in men, and women induces significant increases in cardiovascular, and coronary artery disease-related mortality rates [17]. Ding et al. advocated possible association between hyperuricemia, and thrombotic complications [18].

However, uric acid provides more than 50 % of antioxidant capacity of the blood, and exerts stabilizing effects on vitamins C, and E. Therefore, potential increase in uric acid levels has been suggestively associated with an antioxidant response to oxidative stress. It has

been thought that acute increase in uric acid levels is especially a response to oxidative stress, while its chronic increase is thought to be a risk factor for coronary, and cardiovascular diseases [19]. Even if its harmful pro-oxidant effect prevails over its beneficial antioxidant effect, its beneficial antioxidant effects seem to be more effective in the central nervous system [5]. In our study a significant difference was not detected in uric acid levels between the patient, and the control groups. In the literature we haven't encountered any study which investigated the relationship between uric acid, and SHL. In accessible literature studies the effects of uric acid, and other risk factors on SHL has been investigated. In a study by Friedrich et al. the frequency of vascular risk factors as hyperuricemia, hyperglycemia, and cigarette smoking in 264 patients with SHL had been investigated, and the authors had detected higher rates of hyperuricemia, and hyperglycemia in patients with SHL relative to healthy population. In the same study, an inverse correlation was detected between recovery of hearing function, and number of risk factors [20]. In another study 163 patients with SHL were analyzed as for risk factors including hypertension, hyperlipidemia, smoking, hyperuricemia, and obesity, and significantly greater number of vascular risk factors were detected in the patient group relative to the control group [21].

In our study we evaluated the impact of uric acid levels on the onset, and prognosis of SHL, and 91 of 92 patients had uric acid levels within normal range, and only one patient had an uric acid level above 7.5 mg/dL (8,4 mg/dl). This patient applied with a hearing loss of 70 dB, and did not recover at all after treatment When the patient population were divided into groups with median uric acid levels of ≤ 4.5 mg/dl, and > 4.5 mg/dl, and pure-tone averages at admission were compared, pure-tone averages were found to be 83.23 dB, and 74.11 dB in Groups 1, and 2, respectively without any statistically significant intergroup difference (p=0.072). Posttreatment pure-tone averages of Groups 1, and 2 were 70.26, and 54.58 dB, respectively with a statistically significant intergroup difference (p=0.034). Hearing gains of Groups 1, and 2 were 12.98, and 20.78 dB without any statistically significant intergroup difference (p=0.067). In conclusion, although a statistically significant intergroup difference was not found, in the group with higher uric acid level at the onset of the disease hearing loss was less severe, and higher hearing gain was obtained.

The patient population was divided into groups with partial, and total hearing loss (Group 1, n=53, and Group 2, n=29) with median serum uric acid levels as 4.8, and 4.1 mg/dL, respectively with a statistically significant intergroup difference *(p=0.008)*. Similarly, the patients with better prognosis, and upward-sloping and flat-type audiograms (n=46), and those with downward- sloping type , and total hearing loss (n=34) were compared as for mean serum uric acid levels (Group 1, 4.7 mg/dL, and Group 2, 4.3 mg/dL) without any significant intergroup difference *(p=0.149)*. In other words, onset of sudden hearing loss was less aggressive as serum uric acid levels increased.

Distribution of two groups constructed based on uric acid levels was separated into 3 groups as for hearing gains, and still any significant difference was not detected between these two groups (p=0.098). Though any intergroup difference was not detected, when distribution of patients was examined greater number of patients were seen in the group with lower uric acid levels, while in the group with higher uric acid levels the patients with partial, and complete recovery were more numerous.

As we already mentioned, most of the studies cited in the literature, have correlated uric acid levels, and sudden vascular diseases, worse disease prognosis, and thrombosis. However, in some studies deteriorated course of some neurological diseases such as multiple sclerosis [22], Parkinson's disease [23], Alzheimer's disease [24] in patients with lower uric acid levels have been reported [5]. Based on our data, the group with higher uric acid levels had better pre-and post-treatment pure-tone averages, hence treatment gain, and improved prognosis. Starting from the hypothesis that acute increase in uric acid levels occurs in response to oxidative stress, and uric acid ameliorates neuronal damage thanks to its antioxidant, and immunoregulatory effects [5, 19] increased uric acid level can be suggested as a positive prognostic factor in SHL. In our study, scarce number of patients with supranormal uric acid levels, lack of any prominent difference between groups determined based on median uric acid value, unequal number of male, and female patients in groups precluded accurate interpretation of the results. In the years to come, if a larger-scale patient population with uric acid levels higher than normal range can be investigated, then it will be possible to achieve more accurate results.

This is a retrospective study whose data set was constructed by screening the files of patients with SHL who applied to our clinic within the previous 6 years. Laboratory values of the patients with SHL were derived from the measurements made within 5 years, and for the control group, data were harvested among the measurements made for the patients applied for preoperative preparation for anesthesia within the previous year. Since medical files were screened to obtain data about symptoms, use of medication, and audiogram results, and physicians who prepared the files changed during 5 years, some omissions may be found in patient information. In the future, suspicious data stemming from various factors including concomitant diseases, and timing of laboratory tests can be minimized by designing prospective studies.

Conclusion

In conclusion, in this study we evaluated uric acid that is thought to be effective in endothelial dysfunction, at the onset, and progression of SHL, but we couldn't find a significant difference between the patient, and the control groups as for uric acid levels. However, a significant difference was found between the patient, and the control groups with respect to other hematological, and biochemical parameters including creatinine, INR, and PT. Besides, we found that supported better disease onset, and prognosis in patients with higher uric acid levels. In conclusion, well-planned prospective studies with larger sample size should be performed to obtain more accurate data about vascular etiology of the sudden hearing loss, and the role of other biochemical parameters in the etiopathogenesis of SHL.

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Original Article

Extraspinal findings on routine lumbar spinal MR imaging: Prevalence and etiologies in 4012 patients

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ABSTRACT

Aim: To investigate the prevalence and reporting rates of incidental findings (IF) in the routine magnetic resonance imaging (MRI) of the lumbar spine, and to emphasize their clinical importance.

Methods: A total of 4012 lumbar MRI taken between January 2014 and December 2016 were reevaluated. The low back pain and sciatalgia those suspected for lumbar spinal pathology were chosen for this study. Extra-spinal abnormalities were classified according to a modified CT Colonography Reporting and Data System (C-RADS) and analyzed.

Results The mean age of patients was 49, 83 (range 17-87) years. Of the cases, 2472 were women and 1540 were men. In 3834 cases, disk pathology was observed. In 1282 cases extraspinal pathology was detected. The largest group in the study consisted of C-RADS E2 with 1048 patients (82.5%). There were 195 patients (28.3%) in the C-RADS E3 group and 23 (1.8%) patients in the C-RADS E4 group, potentially important.

Conclusion: Our results show that random extra-spinal abnormalities in the lumbar spine MRI, are very common and systematic evaluation and proper reporting of MRI are crucial.

Keywords: Low back pain, sciatalgia, magnetic resonance imaging, extraspinal pathologies, incidental findings.

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Introduction

Since the widespread use of picture archiving and communication system (PACS) for image evaluation in most clinics, Incidental findings (IF) which are unrelated to the primary symptoms of the patient, have been observed more frequently in routine lumbar spine magnetic resonance imaging (MRI) [1-5]. Most of IF (>95%) had no clinical significance but sometimes clinically important and life-threating conditions like aneurysms, malignancies of other intraabdominal organs can be detected if imaging carefully evaluated for other organs inside the field of view [1-3]. The detection of these extra findings also brings variety of practical and ethical issues related to clinical management of the patient [3]. There are some studies in the literature about the frequencies of these IF, legal and cost issues of the additional examinations for the determined

pathology [1-6]. In addition, Quattrocchi et al. [3] used the modified CT colonography reporting and data system (C-RADS) for the first time in this area, which reported a wide range of random extraspinal pathologies found during lumbar magnetic resonance (MR) exams.

The aim of this study is to investigate the prevalence and reporting rates of incidental findings in the routine lumbar MRI, and to emphasize their clinical importance.

Materials and Methods

Study design

Lumbar MRI examinations, which were performed due to the preliminary diagnosis of lumbar disc herniation between January 2014 and December 2016, were retrospectively analyzed from the PACS of our radiology department to determine extraspinal pathologies. These were patients admitted to the hospital due to back and leg pain and suspected lumbar spinal pathology. The study was reviewed and approved by the local ethics committee (Decision no: 128/2017-10-04). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patients with a known history of malignancy and multiple lumbar MRI examinations and children under 17 years of age were excluded from this study. In addition, examinations with contrast medium administration were excluded. After excluding repeated MRI examinations of the same patient, a total of 4059 patients were examined. In addition, 47 patients were excluded from the study. These are: 23 patients under the age of 17, images of 4 patients are of poor quality and 20 patients have malignancy. As a result, the demographic findings and extraspinal pathologies of 4012 patients were investigated.

Magnetic resonance imaging

All lumbar MR imaging examinations performed in the supine position were done with a 1.5T (Symphony TIM, Siemens, Erlangen) magnet, and our study protocol was sagittal T1- and T2-weighted sequences, axial T2-weighted sequences, and a sagittal counting image covering the entire vertebral column to evaluate the transitional vertebrae. The detailed MR imaging protocol included sagittal plane turbo spin echo T2-weighted sequences (slice thickness: 4.0 mm; field of view: 32 × 32 cm; TR/TE: 594/13 ms) and axial turbo spin echo T2-weighted sequences (slice thickness: 3.0 mm; field of view: 28×23 cm; TR/TE: 5280/94 ms).

Data analysis

All MR images were evaluated in different sessions by at least two radiologists who are experts in this field. Generally, incidental extraspinal pathologies include anatomical anomalies (variants such as retroaortic renal vein and horseshoe kidneys, cysts of solid organs such as liver kidney), reproductive system pathologies (ovarian cysts, uterine fibrosis, endometrial thickening…), tumors of the abdomen and pelvic organs and other findings such as hematosalpinx, hydronephrosis, aortic aneurysms, gallstones, intestinal diverticulosis.

Extra-spinal abnormalities were classified according to a modified CT Colonography Reporting and Data System (C-RADS) [3]. During the review of the MR imaging reports clinically significant findings (E3 and E4 according to modified C-RADS classification),

benign conditions (C-RADS E2) and anatomic variations were noted. C-RADS E1 category included only anatomic variants, within the C-RADS E2 category were clinically unimportant findings for which no further work-up or assessment was indicated (e.g renal cyst, diverticulosis), the C-RADS E3 category included incompletely defined, indeterminate and most likely benign findings (e.g minimally complex renal cyst, hydronephrosis) for that further investigation(s) is indicated by clinical correlation, the C-RADS E4 category designated for potentially important findings which requires further investigations and communication with the referring physician (e.g. solid renal mass, abdominal aortic aneurysm). If there were multiple extraspinal findings in the MR imaging examination, the study was categorized according to most important clinical abnormality.

All measurable results of patients such as demographic data, MR findings and adapted CRADS classifiers were uploaded to the database and descriptive statistics were made.

Results

Extraspinal pathologies were investigated in 4012 patients, 1540 of the patients were men and 2472 were women. In our study the mean age of patients was 49, 83 (range 17-87) years. In 3834 cases, disc pathology was observed.

In 1282 cases extraspinal pathology was detected. 16 cases with anatomical variations were included in the C-RADS E1 category. Table 1 shows the distribution of pathologies in the C-RADS E2, E3 and E4 groups. The largest group in the study consisted of C-RADS E2 with 1048 patients (82.5%). There were 195 patients (28.3%) in the C-RADS E3 group and 23 (1.8%) patients in the C-RADS E4 group.

Significant vascular extraspinal abnormalities such as aortic aneurysm and retroaortic renal vein were found (Figure 1). The presence of aortic aneurysm (C-RADS E4) has a potentially serious clinical condition. Retroaortic left renal vein can cause urological symptoms such as inguinal or flank pain and hematuria (C-RADS E3). In Table 1, very different potentially important (C-RADS E4) and likely unimportant (C-RADS E3) extraspinal findings of the genitourinary system are presented. Recurrence of renal cell carcinoma was detected in one case (C-RADS E4) (Figure 2). Potentially important various uterine findings like endometrial hyperplasia, endometrium carcinoma, cervix carcinoma and hematosalpinx were found (Figure 3). Uterine leiomyoma (fibroid) commonly seen as a mural, subserozal or submucosal mass (Figure 4).

Various gastrointestinal extraspinal findings such as diverticulosis, liver metastasis (Figure 5) and cholelithiasis were found, matching the C-RADS E4 and C-RADS E3 classification (Table 1). Iliac benign bone cysts were found as an extraspinal findings on lumbar MR images (Table 1).

Discussion

Many extraspinal pathologies may be found in the images of patients who underwent lumbar MRI research for low back and leg pain [7]. Sometimes these coincidental findings may be more important than spinal pathologies, so the management of the patient might change and cause medicolegal implications for the radiologists. [1]. Evaluation of the images in the PACS had offered additional information and higher detection of these incidental extraspinal findings, including the region out of interest and sagittal T1-weighted localizer sequence for the vertebral body counting [6]. Therefore, radiologists should try to review all information in PACS in order to detect potentially important incidental findings [6].

Table 1. Summary of IF, classified according to the modified C-RADS classification.

Organ/system	Finding	Number	Rate (%)	Men	Women
C-RADS E 2: clinically unimportant findings—no further work-up indicated					
Kidney	Cystic lesion	668	52.7	410	258
	Horseshoe kidney	6	0.47	3	3
	Staghorn stone	3	0.23	2	1
Uterus	Solid benign lesion	156	12.32	0	156
	Adenomyosis	74	5.8	0	74
Ovaries	Cystic lesion	75	5.9	0	75
	PCOS	6	0.47	0	6
	Endometrioma	4	0.31	0	4
Prostate	Hyperplasia	1	0.07	1	0
Bladder	Bladder diverticulosis	3	0.23	3	0
	Stone	2	0.15	2	0
Bowel	Diverticulosis	17	1.34	7	10
	Duplication cyst	1	0.07	0	1
Gall bladder	Cholelithiasis	12	0.94	4	8
Diaphragm	Hiatal hernia	13	1.02	6	7
Incisional hernia		2	0.15	1	1
Abdominal LAP		2	0.15	2	0
Subcutaneous lipoma		3	0.23	1	2
Total E 2		**1048**	**82.55**	**442**	**606**
C-RADS E 3: likely unimportant findings, incompletely characterized					
Vascular system	Aortic dilatation	50	3.9	31	19
	Retroaortic left renal vein	3	0.23	1	2
Kidney	Hydronephrosis	64	18.1	34	30
	Neurogenic bladder	2	0.15	1	1
	Renal atrophy	16	1.26	7	9
	Polycystic kidney disease	1	0.07	1	0
Uterus	Endometrial hyperplasia	31	2.44	0	31
Liver	T2W hyperintense lesion	23	1.81	10	13
Iliac bone cyst		4	0.31	1	3
Pelvic lenfangioma		1	0.07	0	1
Total E 3		**195**	**28.34**	**86**	**109**
C-RADS E 4: potentially important findings					
Aortic aneurysms		8	0.63	5	3
Recurrent renal cell carcinoma		1	0.07	0	1
Endometrium carcinoma		11	0.86	0	11
Cervical carcinoma		1	0.07	0	1
Hematosalpinx		1	0.07	0	1
Liver metastasis		1	0.07	0	1
Total E 4		**23**	**1.81**	**5**	**18**
Total		**1266**	**100.0**	**533**	**733**

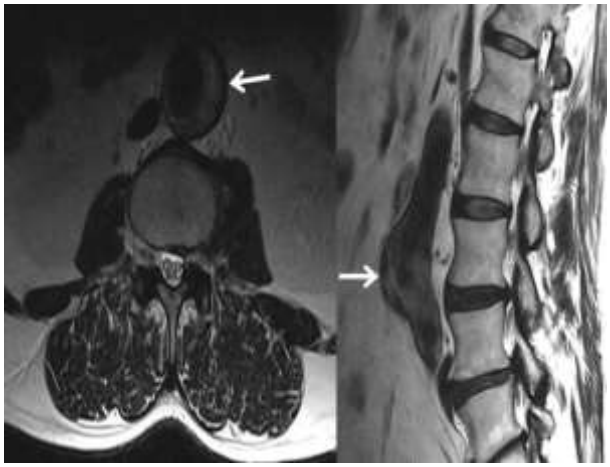

Figure 1. Fusiform aneurysm with T2-weighted axial sagittal MRI with a thrombus thickness of 17 mm, starting from the infrarenal level in a 70-year-old male patient.

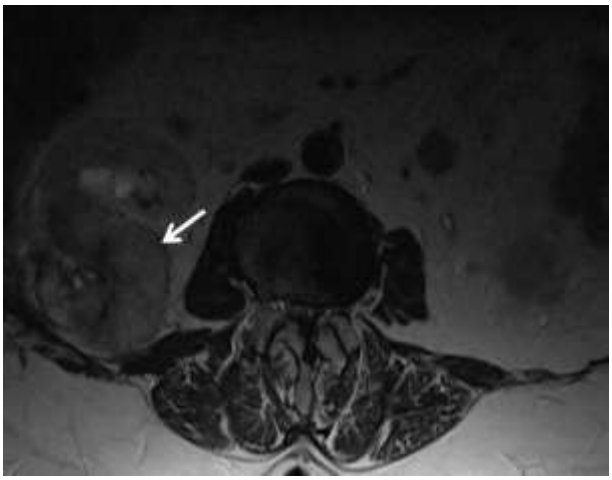

Figure 2. Recurrent mass on axial T2-weighted image in a 66-year-old woman with operated RCC.

Due to the widespread use of picture archiving communication systems for the last two decades, a large increase has been recorded in the number of incidental findings identified in lumbar MRI [8,9]. As expected, with the advent of gradually advanced imaging techniques, it is understood that incidental findings are increasingly detected in other anatomical regions in addition to the lumbar spine. A similar trend has also been described in brain imaging like in the article by Vernoij et al [9]. Lee et al. [10] reported that 4.6% of IF was clinically significant in lumbar computed tomography (CT) scans, such as renal mass, aortic aneurysm, and lymphadenopathy. In the study of Zidan et al. [11], in 90 (23.7%) of 379 patients examined, the incidental findings were detected in the MRI scans of the lumbar spine. They argued that some of these findings were not clinically relevant because they were not associated with diseases or causes that initiated the diagnostic imaging test, other findings were important, and their early detection played an important role in associated treatment and prevention, potentially reduced morbidity and mortality rates. Tuncel et al [12] re-evaluated totally 1278 lumbar MRI. Among them, 34 (2.2%) clinically important incidental findings

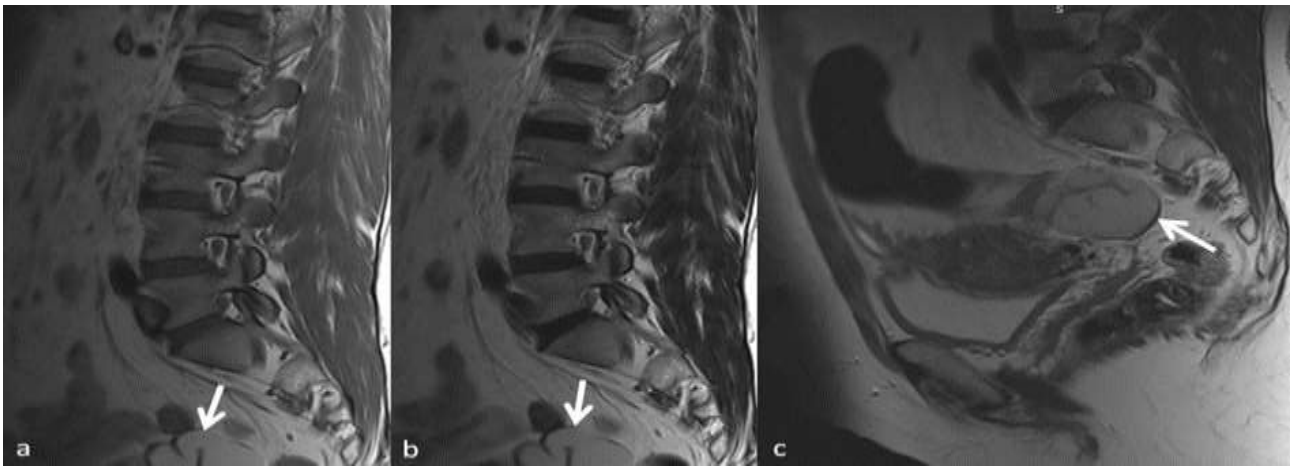

Figure 3. Hyperintense tortuous tubular structures in sagittal T1A (a) and T2A lumbar MR images of a 45 year old female patient with low back pain. Lumber MRI will not reveal pain herniation or degeneration findings. In the pelvic MRI taken on the passage of pain, (c) both tubs are full of dilate, tortuosity and blood.

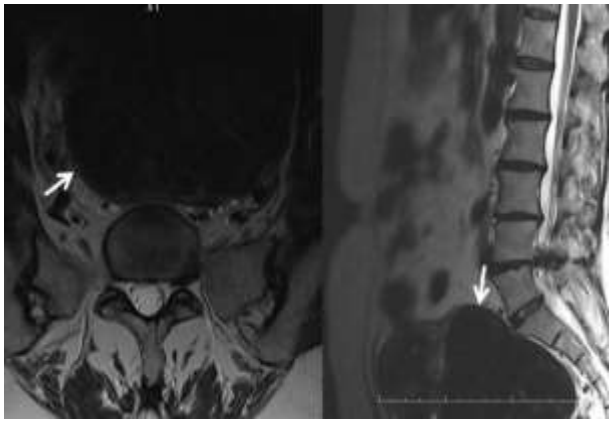

Figure 4. A hypointense mass in the uterine corpus in axial and sagittal T2A-weighted images in a 51-year-old woman.

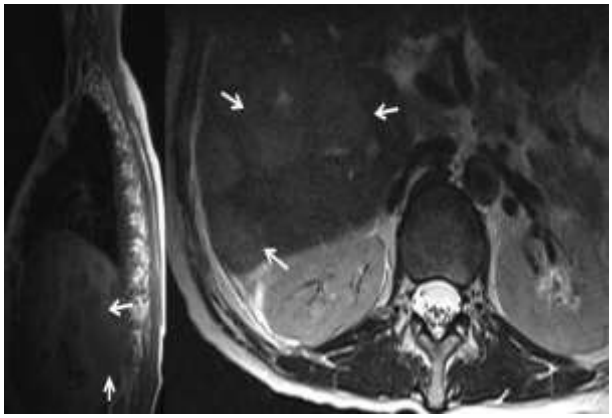

Figure 5. Liver metastatic solid lesions in sagittal localization and axial T2-weighted images in the liver of a 55-year-old woman.

were reported. They suggested that incidental findings which are clinically important occasionally omitted from routine lumbar MRI reports. Therefore, detailed examination of the lumbar MRI and extraspinal structures can be important for patient's clinical evaluation in daily practice. Fu et al. [6] screened 5104 patients who experienced low back pain or sciatica and patients with extraspinal malignancies seen in both CT and MRI were enrolled and analyzed. The prevalence of newly diagnosed extraspinal malignancies were 0.5%. The possible reason may be due to these lesions that induce low back and/or leg pain like degenerative disc disease. Quattrocchi et al [3]. 3.000 lumbar spine MRI examination was analyzed retrospectively. In their studies, extraspinal findings were found in 2,060 (68.6%) of 3,000 lumbar spine MRI examinations; In 362 (17.6%) patients had indeterminate or clinically important findings (E3 and E4) requiring clinical correlation or further evaluation. After reviewing the original archived radiological reports, potentially significant C-RADS E3 and E4 extra spinal IF were reported in 47 of 265 (17.7%) and 8 of 74 patients (10.8%). We screened 4012 patients who experienced low back and leg pain who underwent routine non-enhanced MRI examinations and, extraspinal findings were detected in 840 (21%) patients. 358 (9%) of the patients had indeterminate or clinically signs (C-RADS E3/E4) which requires clinical evaluation or further investigation. Among these incidental extraspinal findings, 39 were important; 12 aortic aneurysms (1.4%), 1(0.1%) relapsed renal cell carcinoma, 18 (2%) lymphadenopathies, 6 (0.7%) cases of cervix or endometrial thickening, 1 (0.1%) hematosalpinx and 1 (0.1%) liver metastasis.

Our study has some limitations. First, our study is a retrospective research. Second, follow-up examinations of the patients with clinical significance in the classification of E3 and E4 are missed. However, the fact that our study is a large cohort study and the C-RADS classification system offers useful results in this area.

Conclusion

Extraspinal findings are frequently encountered in lumbar MRI examinations. Although most of the findings are not clinically important, some of them are important due to the fact that it might affect the life quality of the patient or might be life threatening. Therefore, proper reporting of MRI scans both identifies

clinically important IF and can also prevent medico-legal consequences for the radiologist. In addition, the radiologist should add the examinations of the organs outside from the spinal region to the systemic evaluation in order to prevent overlooking the malignancies of the surrounding tissues which might be asymptomatic.

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Ethical statement: *The study was reviewed and approved by the local ethics committee (Decision no: 128/2017-10-04).*

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Original Article

Millennium pandemic: A review of coronavirus disease (COVID-19)

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ABSTRACT

Coronaviruses, a large family of single-stranded RNA viruses, can infect humans and animals, and can cause neurological, gastrointestinal and hepatic diseases as well as causing various lung diseases, including pneumonia, with shortness of breath, cough and fever. At the end of December 2019, a group of health authorities reported unidentified cases of pneumonia in a seafood market in Wuhan, China. The World Health Organization (WHO) used term 2019 novel coronavirus (COVID-19) to refer to a coronavirus that affected the lower respiratory tract of patients with pneumonia in Wuhan, China on 29 December and the WHO announced that the official name of the 2019 novel coronavirus was coronavirus disease (COVID-19). COVID-19 is seen in many countries around the World and has been accepted as a pandemic by WHO. It is defined as a suspicious case with fever, sore throat, cough, and people with a history of traveling to China or some parts of the country, or someone who contact with a patient who has a history of travel in China or contact with a confirmed COVID-19 infection patient. Currently, there is no proven vaccine or antiviral therapy that can be used against animal or human coronavirus. To control the outbreak, the drugs must be developed as soon as possible. Various drugs have been used in the treatment of COVID-19 and the main ones are chloroquine, remdesivir, lopinavir/ritonavir, oseltamivir, favipiravir. Since the virus affects the whole World, vaccines and/or new curative antiviral drugs are needed to end the pandemic. For this purpose, large-scale observational studies are needed.

Keywords: 2019-nCoV, COVID-19, coronavirus, epidemiology, pneumonia, treatment, pandemic.

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Introduction

Coronaviruses, a large family of single-stranded RNA viruses, can infect humans and animals, and can cause neurological, gastrointestinal and hepatic diseases as well as causing various lung diseases, including pneumonia, with shortness of breath, cough and fever [1, 2]. Four coronavirus species called HKU1, NL63, 229E, OC43 are in circulation in humans and are generally cause mild respiratory diseases [3].

In the last 20 years, 2 violent events occurred with the transition of betacoronaviruses from animals to humans. The first incident was in

2002. A new genus of beta coronavirus passed on to people using palm civet cats as hosts and bats as intermediate hosts in Guangdong province in China. This virus, called severe acute respiratory syndrome (SARS) coronavirus, affected 8422 people in China and Hong Kong and 916 people died (mortality rate was 11%) [4]. Almost 10 years later, in 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV) appeared in Saudi Arabia, using dromedary camels as hosts, bats as intermediate hosts, 2494 people affected and 858 people died (mortality rate was 34%) [5].

At the end of December 2019, a group of health authorities reported unidentified cases of pneumonia in a seafood market in Wuhan, China [6]. Although most of the early infected patients were seen in the Huanan seafood market in Wuhan, China, 13 of 141 cases were not affiliated with the market [7]. Although it started in the first patient on 1 December 2019, its relationship with seafood market was not reported. There was no epidemiological link between the first case and the next ones. Probably the first virus came to the market and spread from there [8].

The World Health Organization (WHO) used term 2019 novel coronavirus (COVID-19) to refer to a coronavirus that affected the lower respiratory tract of patients with pneumonia in Wuhan, China on 29 December and the WHO announced that the official name of the 2019 novel coronavirus was coronavirus disease (COVID-19) [9]. The current reference name for the virus is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and at the end of December 2019, this virus was blamed as the cause of pneumonia cases occurring in Wuhan, China [6]. COVID-19 has been declared as a Public Health Emergency of International concern by the WHO [10].

COVID-19 is seen in many countries around the World and has been accepted as a pandemic by WHO. With this review, we aim to explain general information about COVID-19, its diagnosis, laboratory and radiological findings, diagnosis and treatment methods.

Clinical findings and symptoms

Examining the current epidemiological study, most individuals had a close contact history with a patient with COVID-19 or a recent travel history to Wuhan or Hubei province in China [11]. The symptoms of COVID-19 infection were not specific. The most common symptoms were fever, weakness, fatigue and dry cough. Some patients also had headache and/or muscle pain, but no upper respiratory tract symptoms [11]. Diarrhea was reported with a frequency of 10.6% in SARS and 30% in MERS [12]. More than half of the patients developed shortness of breath. The median duration of the appearance of dyspnea from the beginning of the disease was 8 days [7]. If the disease could not controlled, patients with COVID-19 may develop acute respiratory distress syndrome (ARDS), followed by septic shock, metabolic acidosis and coagulation dysfunction [11]. Although pneumonia was also seen in patients infected with COVID-19, few had pleuritic chest pain [13].

Patients were divided into 3 groups as mild, severe and critical, depending on the severity of the symptoms (Table-1). Mild patients had non-pneumonia or mild pneumonia. Severe patients had several clinical findings, including dyspnea, respiratory frequency $\geq$ 30/min, blood oxygen saturation $\leq$ 93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300, and/or lung infiltrates >50% within 24 to 48 hours. Critical patients had severe conditions, such as respiratory failure, septic

shock, and/or multiple organ dysfunction or failure [14].

Table 1. Clinical symptoms associated with COVID-19.

Clinical Types	Symptoms
Mild	Nonpneumonia or mild pneumonia
Severe	Dyspnea, respiratory frequency ≥ 30/min, blood oxygen saturation ≤ 93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300, and/or lung infiltrates >50% within 24 to 48 hours
Critical	Respiratory failure, septic shock, and/or multiple organ dysfunction or failure

Laboratory findings

Among the laboratory findings, leukopenia and lymphopenia were the most common [7, 15, 16]. Lymphopenia was the most important laboratory finding in COVID-19 infection. While lactate dehydrogenase (LDH) and creatine kinase (CPK) increased in all patients, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was found to be high in half of the patients. In most patients, myocardial tests were abnormal, with elevated CPK and LDH. In most patients, procalcitonine was normal, while C - reactive protein (CRP) was above the normal range. D-dimer was elevated in one third of the patients [7, 13, 15, 16].

In a study investigating cytokines in the serum of COVID-19 patients. Interleukin (IL)1B, IL1RA, IL7, IL8, IL9, IL10, basic in plasma fibroblast growth factor (FGF), granulocyte colony stimulating factor (GCSF), granulocyte-macrophage colony stimulating factor (GMCSF), interferon-gamma (IFNγ), induced protein-10 (IP10), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein-1A (MIP1A), macrophage inflammatory protein-1B (MIP1B), platelet-derived growth factor (PDGF), tumor necrotic factor alpha (TNFα) and vascular endothelial growth factor (VEGF) concentrations were higher than healthy adults. Plasma levels of IL5, IL12 p70, IL15, Eotaxin and RANTES (regulated upon activation, normal T cell expressed and secreted [also known as CCL5]) were similar to that of healthy individuals and patients. When the serum plasma of patients with and without intensive care unit is compared, IL2, IL7, IL10, GCSF, IP10, mitochondrial pyruvate carrier 1 (MPC1), MIP1A, and TNFα levels were higher in intensive care patients than in non-intensive care patients [7].

Radiological findings

Radiological images of patients infected with COVID-19 are quite diverse and can be rapidly progressed [17-19]. At least two-thirds of patients had at least two lobes affected. At least 5 lobes were effected in half of the patients. The most common manifestations in computerize tomography (CT) images are irregular ground glass opacities (GGO) and patchy consolidations, especially in the middle and outer zones of the lung [19, 20].

In one study, radiological findings was divided into 4 stages according to CT images of patients infected with COVID-19. In the early stage, unilateral or bilateral GGO in the lower lobes of the lung was the main radiological findings. In the progressive stage, diffuse and bilateral GGO and consolidation in more than 2 lobes

were the main findings. In the peak stage, diffuse GGO and consolidation began to become more evident. In the absorption stage, wide GGO could be observed and consolidation was gradually absorbed [20].

Pathological findings

Pathological findings of COVID-19 were rare. In one study, primary finding in biopsy of a 50-year-old man with cardiac arrest due to respiratory failure died after 14 days were cellular fibromyxoid exudates, and bilateral diffuse alveolar hemorrhage areas and infiltrated areas where lymphocytes predominate [21]. Multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were identified in the intraalveolar spaces, showing viral cytopathic-like changes. No obvious intranuclear or intracytoplasmic viral inclusions were identified. These pathological features show great similarities to SARS-CoV and MERS-CoV infection [22-24].

Diagnosis

It is defined as a suspicious case with fever, sore throat, cough, people with a history of traveling to China or some parts of the country, or someone who contact with a patient who has a history of travel in China or contact with a confirmed COVID-19 infection patient [25].

1. Physical examination

Positive results may not be achieved in patients with mild patients. Severe patients may have shortness of breath, rales in lungs, weakened breath sounds, dullness in percussion, and increased or decreased tactile speech tumor [12, 13].

2. Laboratory tests

According to Koch's proposals, the gold standard is virus isolation in the laboratory diagnosis of the virus [26]. Viral nucleic acids can be useful in the early diagnosis of the disease, which is the most important thing. The nucleic acid in the RNA sequence of SARS-CoV-2 was aimed to be detected and the full gene sequence of SARS-CoV-2 was obtained. For this purpose, samples were taken from the upper respiratory tract (throat stick / nasopharyngeal rod / sputum sample / endotracheal sample / aspirates and bronchoalveolar lavage) and the diagnosis of SARS-CoV-2 infection was made by real-time PCR [25-27].

Other laboratory examinations are generally not specific. The white cell count is usually low or normal. There may be lymphopenia, and a lymphocyte count of < 1000 can be a sign of serious disease. Platelet count is usually low or normal. CRP and erythrocyte sedimentation rate (ESR) is generally high, but procalcitonin levels are usually normal. AST, ALT, creatinine, CPK, LDH, D-Dimer, prothrombin time may increase and these values are associated with severe disease [25].

3. CT examination

The chest X-ray usually shows infiltrated areas in the lung, but the x-ray may be normal in the early stages of the disease. CT is more sensitive and specific in demonstrating GGO, infiltrated areas and lower lobe consolidations in the lung. CT is also normal in asymptomatic patients / patients without lower respiratory tract involvement. In fact, in COVID-19 patients whose molecular tests are negative, abnormal findings can be detected in CT. Also, positivity can be detected in these patients by repeating molecular tests [25].

Treatment

Currently, there is no proven vaccine or antiviral therapy that can be used against animal or human coronaviruses. To control the

outbreak, the drugs must be developed as soon as possible. WHO stated that a vaccine for SARS-CoV-2 could be available within 18 months [28]. The most important step in the clinical management of the disease is largely symptomatic therapy. Intensive care unit may be required for severe patients and those with organ involvement [29].

Various drugs have been used in the treatment of COVID-19 and the main ones are as follows:

Chloroquine

Chloroquine is a drug with high potential to treat COVID-19 infection. Chloroquine has been used for many years in malaria treatment [30]. Several possible mechanisms for chloroquine treatment have been investigated. Chloroquine can replace intracellular pH by following the replication steps of various viruses. And with this mechanism, chloroquine can have potential positive effects in treating SARS-CoV-2 infection [31, 32]. One study found that chloroquine prevents replication of the new type of coronavirus [33]. Gao et al. found that chloroquine prevents pneumonia exacerbation, improves lung images and allows negative transformation of the virus [34].

Remdesivir

Remdesivir (GS-5734) is a 1′-cyano-substituted adenosine nucleotide analog prodrug [35]. Remdesivir has been reported to treat a first case of COVID-19 in America [36]. A study have shown that remdesivir gives good results in COVID-19 patients and treats the disease [33]. In addition, another study stated that remdesivir may be the best treatment option in COVID-19 [37].

Lopinavir/Ritonavir

Lopinavir is a protease inhibitor used in HIV treatment, with ritonavir as a booster. Lopinavir or lopinavir/ritonavir showed anti-coronavirus activity in in-vitro studies. IFN-α has broad-spectrum antiviral effect, it is an agent used in the treatment of hepatitis-B virus. IFN-α (5 million U bid inh) and lopinavir/ritonavir (400 mg/100 mg bid po) combine therapy are recommended as antiviral therapy. This treatment used to treat SARS [38]. This treatment is also recommended for SARS-CoV-2 [39].

Oseltamivir

Oseltamivir is a neuraminidase enzyme inhibitor and is used to treat influenza [40]. In a 42-year old male patient, COVID-19 pneumonia was detected in January 2020 in Wuhan province, China. CT images were improved after treatment with ganciclovir and oseltamivir [41]. In many studies, oseltamivir was used in combination with various antiviral drugs in patients infected with COVID-19, no positive results have been encountered [7, 13, 16].

Favipiravir

Favipiravir is a new type of RNA-dependent RNA polymerase inhibitor. In addition to its anti-influenza virus activity, favipiravir is capable of blocking the replication of flavi-, alpha-, filo-, bunya-, arena-, noro-, and other RNA viruses [42]. A study conducted in China in February 2020 provided positive results about favipiravir. One group was given favipiravir and the other group was given lopinavir/ritonavir. The third group was the control group. Compared to the groups, better antiviral efficacy was observed in the group treated with favipiravir, and no significant adverse effects were observed in this group. Significantly more negative effects were encountered in the group treated with lopinavir/ritonavir [43].

In another study, in COVID-19 patients who did not receive antiviral therapy before, favipiravir was found to be an effective treatment when anti-viral side effects were excluded, since it provided improvement in the

clinic in 7 days, effectively reducing fever and cough [44].

Comments

As a result, 2019 novel coronavirus is a new virus and its mechanism and treatment is not fully known. Since the disease especially affects the airways and lungs, patients complained of cough and shortness of breath and some patients died from the ARDS. Although the disease occurs in China, it affects the whole world, the pandemic table is heavy in many countries, and people, countries are negatively affected both socially and economically and their health policies are insufficient. In studies conducted, patients with older age, chronic diseases such as diabetes mellitus, hypertension, chronic obstructive pulmonary disease (COPD), cancer, kidney failure, heart failure were evaluated as risk groups [45-47]. That's why people over 65 years old, people with chronic diseases such as diabetes mellitus, hypertension, cancer, heart failure, kidney failure and those who use immunosuppressive drugs need to protect themselves more.

As of 29.03.2020, the number of cases worldwide has reached 665.616, the number of deaths has reached 30.857, and the number of patients recovering from the disease has reached 141.746. The number of cases in China has reached 82.061, the number of dead has reached 3182. The number of cases in Italy has reached 92.472 and the number of dead has reached 10.023. The number of cases in United States has reached 124.686 and the number of dead has reached 2.192. The number of cases in Spain has reached 73.235 and the number of dead has reached 5.982. The number of cases in Turkey has reached 7402 and the number of dead has reached 108 people [48]. According to these data, as of 29 March 2020 date, SARS-CoV-2 mortality is 4.63% all over the World, %4.03 in China, 10.84% in Italy, 8.17% in Spain, 1.76% in United States and 1.46% in Turkey (Table 2). Although the virus first appeared in China [7] Italy has become the new center of the virus due to the dramatic increase in the number of cases and deaths in Italy.

Table 2. As of March 29, 2020, SARS-CoV-2 confirmed cases, number of deaths, mortality rate (%) in some countries.

Countries	Confirmed cases	Number of deaths	Death rate (%)
Italy	92,472	10,023	10.84
Indonesia	1,155	102	8.83
Spain	73,235	5,982	8.17
Iran	35,408	2,517	7.11
Netherlands	9,819	639	6.51
Philippines	1,075	68	6.33
France	38,105	2,314	6.07
United Kingdom	17,312	1,019	5.89
China	82,061	3,304	4.03
Belgium	9,134	353	3.86
Japan	1,693	52	3.07
Sweden	3,447	105	3.05
Brazil	3,904	114	2.92
Denmark	2,366	65	2.75
Ecuador	1,823	48	2.63
Romania	1,452	37	2.55
Portugal	5,170	100	1.93
Switzerland	14,076	264	1.88
United States	124,686	2,192	1.76
South Korea	9,583	152	1.59
Ireland	2,415	36	1.49
Turkey	7,402	108	1.46
Malaysia	2,320	27	1.16
Poland	1,638	18	1.1
Canada	5,655	61	1.08

In conclusion, since the virus affects the whole World, vaccines and/or new curative antiviral drugs are needed to end the pandemic. For this purpose, large-scale observational studies are needed.

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