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Kronik Böbrek Hastalığında Serum Mg, NO ve IMA Seviyeleri

The Levels of Serum Mg, NO and IMA in Chronic Kidney Disease

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ÖZ

GİRİŞ ve AMAÇ: *Kronik böbrek hastalığı (KBH) hastalarında, serum ischemia-modified albumin (IMA) seviyeleri, normal popülasyona göre daha yüksek bulunmuştur. Oksidatif stres arttıkça, endotel disfonksiyonuna bağlı nitrik oksit (NO) üretimi azalmaktadır. Magnezyum (Mg), endotel üzerinde NO üretimini artırır. Çalışmamızın amacı, KBH (evre 1-4) hastalarında serum IMA, NO ve Mg seviyelerini birbiriyle karşılaştırmaktı.*

YÖNTEM ve GEREÇLER: *Çalışmaya evre (1-4) 29 KBH hastası dahil edildi. Ayrıca, 40 kişilik sağlıklı gönüllüden oluşan bir kontrol grubu da oluşturuldu. Her iki grupta serum IMA, NO ve Mg tayini yapıldı. Serum Mg kolorimetrik yöntemle; IMA, hızlı kolorimetrik metodla; NO, ELISA ile analiz edildi. İstatistiksel anlamlılık için $p < 0.05$ kabul edildi.*

BULGULAR: *Hasta grubunda serum IMA düzeyi kontrol grubuna göre istatiksel anlamlı daha yüksekti ($p=0.023$). Serum NO ve Mg yönünden gruplar arasında farklılık bulunmadı ($p > 0.05$). Hasta grubunda, serum IMA ve NO arasında istatistiksel anlamlı ve pozitif yönde ilişki saptandı ($p=0.023$, $r=0.421$). Serum IMA ve Mg arasında istatistiksel anlamlı ilişki saptanmadı ($p=0.96$). Serum NO ve Mg arasında istatistiksel anlamlı ve negatif ilişki bulundu ($p=0.02$, $r=-0.43$).*

TARTIŞMA ve SONUÇ: *KBH (evre 1-4) hastalarının serumunda NO ve Mg seviyelerine bakılması, oksidatif stresi öngörmede faydalı bulunmamıştır.*

Anahtar Kelimeler: *Magnezyum, nitrik oksit, ischemia-modified albumin, kronik böbrek hastalığı*

ABSTRACT

INTRODUCTION: *In chronic kidney disease (CKD) patients, serum levels of ischemia-modified albumin (IMA), are found to be higher compared to the normal population. As oxidative stress increases, nitric oxide (NO) production reduces linked to endothelial dysfunction. Magnesium (Mg) increases NO production by endothelium. The aim of the study was to compare serum IMA, NO, and Mg levels in patients with CKD (stage 1-4).*

METHODS: *The study included 29 CKD patients with stage 1-4. Additionally, a control group comprised 40 healthy volunteers. Serum IMA, NO, and Mg testing was performed in both groups. Serum Mg was analyzed with the colorimetric method, IMA was analyzed with the rapid colorimetric method, while NO was analyzed with ELISA. Statistical significance was accepted as $p < 0.05$.*

RESULTS: *Serum IMA levels were significantly higher in the patient group than in the control group. ($p=0.023$), while there were no differences between the groups in terms of serum NO and Mg ($p>0.05$). In the patient group a statistically significant and positive correlation was identified between serum IMA and NO ($p=0.023$, $r=0.421$). There was no statistically significant correlation between serum IMA and Mg ($p=0.96$). There was a statistically significant and negative correlation between serum NO and Mg ($p=0.02$, $r=-0.43$).*

DISCUSSION AND CONCLUSION: *Examination of the serum NO and Mg levels in CKD patients (stage 1-4) was not found to be beneficial to predict oxidative stress.*

Keywords: *Magnesium, nitric oxide, ischemia-modified albumin, chronic kidney disease*

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INTRODUCTION

Oxidative stress has a significant role in the pathophysiology of renal fibrosis (1). Beginning in the early stages in chronic kidney disease (CKD) patients, the balance between oxidant and antioxidant systems is disrupted and this situation reaches highest levels in end-stage renal disease (2). Ischemia-modified albumin (IMA) is accepted as a potential cardiovascular risk marker caused by increased oxidative stress (3,4). As CKD progression increases, serum IMA levels elevate (5). Nitric oxide (NO) is a molecule regulating vasodilatation, shear stress and vascular tonus, in addition to preventing thrombotic events and vascular inflammation. As oxidative stress increases, NO production reduces linked to endothelial dysfunction (6,7). Magnesium (Mg) is one of the most important cations in the body and acts as cofactor in more than 300 enzymatic reactions (8). In cells exposed to continuous free radicals, magnesium was identified to have beneficial effects against oxidative stress (9). The aim of our study is to compare serum IMA, NO and Mg levels in patients with CKD (stage 1-4).

MATERIAL AND METHODS

Study Design

The study included 29 CKD patients (13 female, 16 male) in stage 1-4 before dialysis. Patients were randomly selected from patients attending nephrology clinic from September 2017 to March 2018. Forty healthy and volunteer individuals (26 female, 14 male) comprising the control group were selected from the relatives of the study patients. Inclusion criteria for the study were age from 18-80 years, stage 1-4 CKD and volunteering to participate in the research. Exclusion criteria for the study included end stage renal failure, acute kidney injury, acute coronary syndrome, resistant hypertension or uncontrolled diabetes mellitus (HbA1c>10).

When the etiology of CKD patients is investigated, 16 patients had diabetes mellitus, 7 patients had hypertension, 2 patients had amyloidosis, 1 patient had autosomal dominant polycystic kidney disease, 1 patient had obstructive nephropathy and 2 patients had unknown causes. The study began after receiving permission from local ethics committee. In addition, informed consent was obtained from each participant. All participants had 10 ml blood samples taken in the early hours of the morning after at least 12 hours fasting which were transported to the clinical biochemistry laboratory. Serum biochemical markers (urea, creatinine, albumin, Mg, NO, IMA) were analyzed in the laboratory. For every patient, eGFR measurement was completed according to the CKD-EPI formula (10).

Biochemical Analysis

For serum urea, creatinine, albumin and Mg measurements blood was taken in vacuum gel tubes, while for NO and IMA, blood was taken in tubes containing ethyl diamine tetra acetic acid (EDTA). Samples in tubes were left at room temperature for 30 minutes and then centrifuged for 10 minutes at 4000 rpm. Urea and creatinine were measured with the enzymatic method, while albumin and Mg were measured with the colorimetric method in a Cobas c501 analyzer using Roche kits (Roche Diagnostics GmbH) on the same day.

Until IMA and NO analysis, samples were stored at -80 degrees. Serum IMA measurement was completed with the rapid colorimetric method linked to albumin binding to cobalt developed by Bar-Or et al. To 200 μL serum, 50 μL of 1 g/L cobalt chloride solution (Sigma-Aldrich, Cobalt chloride 0.1 M solution) was added and gently shaken for a few seconds. The mixture was incubated for nearly 10 minutes for cobalt-albumin binding. The color-giving agent of dithiothreitol (DTT) was added and the amount of cobalt-bound albumin was imaged (Sigma-Aldrich, 1.5 mg/ml H2O). After 2 minutes incubation at room temperature, 0.9% NaCl solution of 1.0 ml was added. The cobalt-bound albumin amount was measured spectrophotometrically (470 nm) (Hitachi U-2900 Spectrophotometer). This result was compared with serum cobalt not treated with DTT. As the darkening of the color showed high degree of absorbance, the result is shown as absorbance units (absu) (11). Serum NO was analyzed using the Griess reaction (nitrite measurement) based on the ELISA method (Nitrate/Nitrite Colorimetric Assay Kit/Cayman Chemical, USA).

Statistics

Data in the research were transferred to the SPSS 19.0 program in an electronic environment. Data checking and statistical analysis were performed

with this program. Descriptive variables are given as mean, standard deviation, median, minimum and maximum values. To determine which statistical analysis to use, continuous variables were tested for fit to normal distribution. As variables did not fit normal distribution, the Mann-Whitney U test and Spearman correlation analysis were used. Statistical significance was accepted as $p<0.05$.

RESULTS

The patient and control groups were similar to each other in terms of gender (p=0.14). The mean age in the control group (56.48 ± 10.77 years), was lower than the patient group (68.48 ± 9.83) and the difference was found to be statistically significant (p=0.001). The mean eGFR in the CKD patients was identified as 40 ± 18.38 ml/min/1.73 m2. Of patients, 1 had stage 1, 2 had stage 2, 16 had stage 3 and 10 had stage 4 CKD. Serum IMA levels were significantly higher in the patient group than in the control group (p=0.023). Serum albumin levels were significantly lower in the patient group than in the control group (p=0.001). The biochemical data from both groups are shown in Table 1.

Table 1. Biochemical data of patient and control groups

Serum markers	Patient group (n=29)		Control group (n=40)		P
	mean ± sd	median (min-max)	mean ± sd	median (min-max)	
Urea (mg/dL)	67,36 ± 35,0	65,5 (26-168)	27,45 ± 7,4	27 (15-50)	0.001
Creatinine (mg/dL)	1,77 ± 0,57	1,64 (0,7-3)	0,77 ± 0,15	0,77 (0,4-1,1)	0.001
Albumin (g/dL)	3,68 ± 0,79	3,5 (2,1-5,2)	4,37 ± 0,4	4,5 (3,2-5,2)	0.001
Mg (mg/dL)	1,86 ± 0,41	1,94 (1,2-2,6)	2,0 ± 0,2	2,04 (1,2-2,3)	0.08
NO (µmole/L)	11,36 ± 7,37	8,96 (2,8-32,8)	11,76 ± 8,05	10,0 ± (3,71-43,7)	0.79
IMA (absu)	0,39 ± 0,79	0,39 (0,24-0,6)	0,35 ± 0,11	0,35 (0,06-0,74)	0.023

sd: standard deviation, p:Mann-Whitney Test

There was a statistically significant and negative correlation identified between age and serum albumin level in the patient group (p=0,006, r=-0.51). There was no statistically significant correlation found between age and serum IMA level (p=0.179). There was a statistically significant and negative correlation between serum albumin and IMA levels (p=0.015, r=-0.454). There was a statistically significant and negative correlation between serum Mg and NO (p=0.02, r=-0.43). There was no statistically significant correlation between serum Mg and IMA (p=0.96). There was a statistically significant and positive correlation between serum NO and IMA (p=0.023, r=0.421).

DISCUSSION

The presence of oxidative stress in uremic patients may occur with both an increase in many oxidant markers causing cellular injury and with reduced antioxidant capacity developing against these (12,13). The disruption of the balance between the oxidant and antioxidant systems in CKD patients causes the addition of complications like malnutrition, anemia and atherosclerosis (12,14). In CKD, the redox status imbalance increases as renal functions are disrupted (14).

In our study, the individuals constituting the control group were selected from healthy volunteers among the relatives of the study patients. Though the groups were similar in terms of gender, the control group comprised younger individuals. Serum albumin levels are reported to be high among those in their twenties and to reduce with later aging (15). In our study, though the correlation between age and serum albumin levels was found to be significant in the patient group, significance was not identified between age and serum IMA. During interpretation of the differences found in comparing the serum albumin and IMA in the patient and control groups, it is necessary to note the age factor between the groups.

In CKD patients, albumin reduces as serum IMA levels increase (16,17). Compared to the normal population, CKD patients are reported to have higher serum IMA levels (16). In our study, we found a statistically significant and negative correlation between serum albumin and IMA levels in CKD

patients. The mean serum IMA levels in the patient group were higher compared to the control group. IMA levels increase in serum as a result of increased oxidative stress in patients with CKD (3,18). All defined cardiovascular risk factors, like hypercholesterolemia, hypertension, diabetes mellitus and smoking, increase oxidative stress and reduce endothelial NO production (19). Most of these risk factors are included in the CKD etiology (20). Mg increases NO production from endothelium (21). In our study, there was a statistically significant and positive correlation found between mean serum IMA and NO in the patient group. There was no correlation identified between mean serum IMA and Mg levels. Between mean serum Mg and NO levels there was a negative, statistically significant correlation found. These results found for serum IMA, NO and Mg levels do not comply with the literature. This situation may show that the effect of NO and magnesium in the tissue does not comply with serum levels or may indicate the presence of other factors affecting formation of oxidative stress in chronic kidney disease.

Our study has some limiting factors. The number in the patient group was low relative to the control group. If a group including dialysis patients had been added to the study, it may strengthen the interpretation power for the scientific data. Subjects did not have smoking anamnesis taken or blood pressure measurements performed. Additionally, serum HbA1c, uric acid, lipids, other markers of oxidative stress (malondialdehyde, protein carbonyls and F2-isoprostanes) and NO metabolites (nitrate, nitrite) were not examined.

In conclusion; examination of NO and Mg levels in serum of CKD (stage 1-4) patients was not found to be beneficial to predict oxidative stress. There is a need for prospective studies including more patients about this topic.

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