Oxidative stress level in patients with chronic kidney disease

Kronik böbrek hastalığı olan hastalarda oksidatif stress düzeyi

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Geliş Tarihi: 24.04.2017 Kabul Tarihi: 25.04.2017 Doi:10.21601/ortadogutipdergisi.308443

Abstract

Aim: In this study, we aimed to evaluate the presence of oxidative stress in chronic kidney disease (CKD) by measuring dynamic thiol/disulphide homeostasis.

Material and Method: A hundred fifty patients (57 men, 93 women) followed with a diagnosis of CKD (stages 1-5, not on dialysis) and 76 healthy, disease-free individuals (30 men, 46 women) were included in the study. Blood thiol/disulphide homeostasis was measured by newly developed automatic and colorimetric method by Erel and Neselioglu.

Results: The mean serum native thiol (p=0.001), total thiol (p=0.001) and disulphide (p=0.041) levels were lower in CKD than in the control group. No significant difference was found between the CKD and control groups in terms of disulphide/thiol, disulphide/total thiol and thiol/total thiol ratios (p>0.05). Serum native thiol and total thiol levels were correlated negatively with both BMI and serum phosphorous level while both the parameters were correlated positively with glomerular filtration rate, total protein level and albumin level. A negative correlation was found between total thiol and age. Disulphide levels were correlated positively with albumin and total protein and negatively with uric acid levels.

Conclusion: Our study shows that low serum thiol levels in CKD is due to a decrease in total thiol reserve of the body and not due to conversion of thiol groups into disulphides.

Keywords: Mercaptans, oxidative stress, sulfhydryl, thiol oxidation, uremia

Öz

Amaç: Bu çalışmada kronik böbrek hastalığı olan hastalarda dinamik tiyol/disülfid dengesinin araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmaya evre 1-5 (non diyaliz) 150 (57 erkek, 93 kadın) kronik böbrek hastası ve 76 (30 erkek, 46 kadın) sağlıklı kontrol grubu alındı. Kan tiyol/disülfid dengesi yeni geliştirilen otomatik ve kalorimetrik bir yöntemle ölçüldü.

Bulgular: Tüm popülasyonun serum tiyol düzeyi ortalaması 320.8±57.8 µmol/L, total tiyol düzeyi ortalaması 352.0±61.5 µmol/L, ortalama disülfid düzeyi 15.6±5.3 µmol/L idi. Kronik böbrek hastalarında ortalama serum tiyol ($p<0.001$), total tiyol ($p<0.001$) ve disülfid düzeyleri ($p<0.001$) kontrol grubuna kıyasla anlamlı olarak daha düşüktü. Kronik böbrek hastaları ve kontrol grubu arasında disülfid/tiyol, disülfid/total tiyol ve tiyol/total tiyol oranları açısından olarak anlamlı farklılık saptanmadı.

Sonuç: Çalışmamızda hem tiyol hem disülfid düzeyinde azalma olması Kronik böbrek hastalarında serum tiyol düşüklüğünün sadece tiyol gruplarının disülfide çevrimine değil, vücuttaki total tiyol rezervinin azalmasına da bağlı olduğunu göstermektedir. Bu nedenle kronik böbrek hastalığında tiyol rezervini attırmaya yönelik nütrisyonel destek sağlanması önerilebilir.

Anahtar Kelimeler: Merkaptanlar, oksidatif stres, sülfidril, tiyol oksidasyonu, üremi

Introduction

Chronic kidney disease (CKD) is characterized by chronic and progressive decrease in renal function as a result of reduced glomerular filtration rate. Its prevalence is gradually increasing and it leads to increased morbidity and mortality, thus making it a significant disease [1].

Oxidative stress is one of the most important mechanisms of injury in CKD. Oxidative stress is present in early stages of CKD and it rises progressively with increase in the stage of the disease. Oxidative stress plays a central role in the pathophysiology of uremia and cardiovascular complications of CKD. However, the stage at which oxidative stress starts to occur during CKD progression is not known [2,3].

Oxidative stress is defined as molecular and cellular dysfunction caused by an imbalance between production of free radicals or reactive oxygen species and the antioxidant system [4]. Reactive oxygen species are thought to be the primary cause of tissue injury because they are oxidizing agents. Many enzymatic and non-enzymatic antioxidant molecules that act as defense mechanism against reactive oxygen metabolites have been defined. Thiols are one of the antioxidant molecules that can react with free radicals, thereby preventing tissue and cellular injury [5]. Thiols, also known as mercaptans, are sulfhydryl-group (-SH) containing compounds bound to carbon atom. Thiol groups of proteins, low molecular weight compounds, and cysteine residues, and other thiol groups are oxidized by oxidant molecules in the biological system. The so formed disulphide bonds can again be reduced to thiol groups and in this way thiol/ disulphide homeostasis is maintained. Thiol/ disulphide homeostasis is damaged in conditions of oxidative stress. This imbalance may disrupt function of proteins containing thiol group and this condition causes increased sensitivity of cysteine-rich proteins to oxidization [6,7].

Various parameters are used to detect oxidative stress and evaluate its severity. Thiol/ disulphide homeostasis is one of these parameters. Since 1979, this two-way balance has been measured only in one direction; however, with the method developed by Erel et al. both variables can be measured separately and additively and thus can be evaluated individually and as a whole [8]. Studies have shown that serum thiol levels decrease in patients with CKD [7,9-11]. This decrease may be due to two reasons: first, due to reduction in protein intake and loss of amino acids via proteinuria, and the other due to conversion of thiols to disulphide via oxidation. However, sole measurement of serum thiol levels does not give us any clue about the mechanism of this decrease. With the thiol/ disulphide homeostasis tests we used in this study, it is possible to demonstrate the homeostasis between thiol and disulphide production, and it is also possible to calculate the total body thiol reserve by measuring total serum thiol.

In this study, we aimed to evaluate the presence of oxidative stress in CKD by measuring thiol/disulphide homeostasis.

Material and method

Study population

This cross-sectional study was conducted at the Nephrology Clinics of Ankara Numune Training and Research Hospital. 150 patients (57 men, 93 women) followed with a diagnosis of CKD (stages 1-5, not on dialysis) were included in the study. 76 healthy, disease-free individuals (30 men, 46 women) presenting to the hospital for check-up and who were proven not to have renal disease via laboratory tests, were included in the control group.

Patients with diabetes mellitus, hepatic dysfunction, acute/chronic infection, heart failure, acute kidney disease, thyroid disease, vasculitis, connective tissue disease, and malignancy, and smokers, those taking alcohol, immunosuppressive medication, statins, antioxidant medication or vitamins were excluded from the study. CKD was diagnosed and staged according to the National Kidney Foundation Disease Outcomes Quality Initiative (KDOQI) criteria [12].

Biochemical parameters

Blood samples obtained in the morning after 12 h of fasting were immediately sent for biochemical evaluation. Samples were centrifuged at 4000 rpm for 10 min to separate plasma and serum, and serum samples were stored at –80°C. Then, all biochemical parameters were measured in the same serum sample.

Fasting blood glucose and creatinine were measured by the colorimetric method using a Hitachi Modular P800 (Roche Diagnostics Corp. Indiana, Indianapolis, USA) analyzer while uric acid was measured by the colorimetric enzymatic method.

Spot urine samples were collected from all patients in the morning to measure urinary creatinine and protein levels. Urine creatinine was measured using spectrophotometric method. Urine protein was measured by microalbumin turbidimetric method using a Hitachi Modular P800 (Roche Diagnostics Corp. Indiana, Indianapolis, USA) auto-analyzer.

The glomerular filtration rate (GFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: $GFR = 141 \times \min(Scr/\kappa,1)\alpha \times \max(Scr/\kappa,1)-1.209 \times 0.993^{age} \times 1.018$ [if female] × 1.159 [13].

Serum thiol/disulphide homeostasis

Blood thiol/disulphide homeostasis was measured by newly developed automatic method [8]. Thiol/disulphide homeostasis tests were performed as described previously. Briefly, reducible disulphide bonds were first reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde and all of the thiol groups including reduced and native thiol groups were determined after reaction with DTNB (5,5'-dithiobis-2-nitrobenzoic acid). Half of the difference between total thiol and native thiol gave the dynamic disulphide amount. After determination of native thiol and disulphide amounts, total thiol amount, native thiol / total thiol ratio, disulphide / total thiol ratio and disulphide / native thiol ratio were calculated.

Statistical Analysis

Statistical analysis was performed using SPSS v.20.0 for Windows (IBM Inc., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine the normality of the distribution of data. Continuous variables with normal distribution were expressed as mean ± SD, and continuous variables without normal distribution were expressed as median (minimum-maximum). Categorical variables were presented as number and percentage. Continuous variables with normal distribution were compared using the independent samples t-test; continuous variables without normal distribution were compared using the Mann-Whitney U test. The relationship between numeric parameters was analyzed using Pearson's and Spearman's correlation analysis. The level of statistical significance was set at $p<0.05$.

Results

The demographic and laboratory findings of the whole population is shown in Table 1. A hundred fifty patients (mean age 51.4±13.1 years) with a diagnosis of stage 1-5 CKD and 76 healthy controls (mean age 49.7±9.1 years) were included in the study. The two groups were similar in terms of age, gender and body mass index (BMI). Serum albumin, mean total protein, mean hemoglobin and median GFR were lower in patients with CKD when compared to those of the control group, while mean serum uric acid and phosphorous levels and median proteinuria were higher in patients with CKD. No significant difference was found between the two groups in terms of the other laboratory parameters.

Table 1. Demographic and laboratory characteristics of the study population

Variables	CKD (n=150)	Control (n=76)	p
Gender, n (%) (male)	57(38.0%)	30(39.5%)	0.885
Age (years)	51.4±13.1	49.7±9.1	0.264
BMI (kg/m2)	27.9±3.8	27.4±4.1	0.275
Hemoglobin (g/dl)	12.6±2.1	14.2±1.7	0.001*
Glucose (mg/dl)	90.9±7.6	89.6±4.6	0.115
GFR (ml/min/1.73m2)	56.5(14-98)	136(120-149)	0.001*
Calcium (mg/dl)	9.2±0,6	9.4±0.3	0.08
Phosphorous (mg/dl)	4.3±0.6	3.4±0.4	<0.001*
Uric acid (mg/dl)	5.0±0.9	3.5±1.1	<0.001*
Albumin (g/l)	3.5±0.6	4.4±0.2	0.001*
Total Protein (g/l)	6.4±1.1	8.0±0.5	0.001*
Proteinuria (mg/mg)	2.4±0.6	0.15±0.05	<0.001*

Parameters are expressed as mean±SD and median [minimum-maximum].

*p < 0.05 is considered significant for statistical analyses.

Abbreviations: CKD: Chronic kidney disease, BMI: Body mass index, GFR: Glomerular filtration rate.

The mean serum native thiol level of the whole population was 320.8±57.8 µmol/L, mean total thiol level was 352.0±61.5 µmol/L, and the mean disulphide level was 15.6±5.3 µmol/L. The mean serum native thiol, total thiol and disulphide levels were lower in CKD than in the control group. No significant difference was found between the CKD and control groups in terms of disulphide/thiol, disulphide/total thiol and thiol/total thiol ratios (Table 2).

Table 2. Thiol/disulphide homeostasis parameters of the study population

Variables	CKD (n=150)	Control (n=76)	p
Native thiol (µmol/L)	308.7±58.9	344.7±47.8	0.001*
Total thiol (µmol/L)	338.8±62.9	377.9±49.7	0.001*
Disulphide (µmol/L)	15.1±5.9	16.6±3.7	0.041*
Disulphide/Native thiol (%)	4.9±1.9	5.2±1.3	0.181
Disulphide/Total thiol (%)	4.5±1.6	4.4±1.0	0.876
Native thiol/Total thiol (%)	91.1±3.3	91.1±2.1	0.876

Parameters are expressed as mean ± SD

*p < 0.05 is considered significant for statistical analyses.

Abbreviations: CKD: Chronic kidney disease

In the whole population, serum native thiol and total thiol levels were correlated negatively with both BMI and serum phosphorous level while both the parameters were correlated positively with GFR, total protein level and albumin level. A negative correlation was found between total thiol and age. No significant relationship was found between the other variables and thiol and total thiol levels. Disulphide levels were correlated positively with albumin, and total protein and negatively with uric acid levels. No significant relationship was present between the other variables and disulphide (Table 3).

Table 3. Correlation analysis of thiol/disulphide homeostasis parameters of the study population

	Thiol		Total Thiol		Disulphide	
	r	*p*	*r*	*p*	*r*	*p*
Age	-0.117	0.079	-0.233	0.046*	-0.129	0.152
BMI	-0.242	0.032*	-0.238	0.038*	-0.024	0.719
Glucose	0.030	0.651	0.008	0.904	-0.117	0.078
GFR	0.352	0.022*	0.359	0.016*	0.118	0.154
Albumin	0.207	0.002*	0.228	0.001*	0.296	0.003*
Uric acid	0.064	0.341	0.033	0.623	-0.255	0.020*
Total Protein	0.314	0.001*	0.334	0.001*	0.293	0.004*
Phosphorus	-0.223	0.001*	-0.219	0.001*	-0.055	0.407

*p < 0.05 is considered significant for statistical analyses.

Abbreviations: BMI: Body mass index, GFR: Glomerular filtration rate.

Discussion

This study demonstrated that serum native thiol, total thiol and disulphide levels are reduced in CKD. Disulphide /total thiol, disulphide /native thiol, native thiol/total thiol ratios were not significantly different between the two groups. To the best of our knowledge, there is no study in literature evaluating dynamic thiol/ disulphide homeostasis in CKD with this novel method.

Injury due to free oxygen radicals formed as a result of oxidative stress is prevented in the body by enzyme systems such as superoxide dismutase, catalase and glutathione-S-transferase as well as by important biological thiols such as glutathione, cysteine, homocysteine, N-acetylcysteine and gama-glutamine cysteine. Oxidative stress leads to oxidation of thiol groups of proteins and formation of disulphide bonds. Loss of thiol groups is the primary molecular mechanism leading to structural and functional changes of proteins [14].

Antioxidants, and especially the thiol groups, which responsible for defense against free radicals, are incapable of maintaining their plasma and tissue levels during these interactions [5]. Last studies have shown that serum native thiol levels decrease in CKD as a result of oxidative stres [3,7,10,11]. In our study, in addition to a decrease in serum thiol level, serum total thiol and disulphide levels were also found to be low. Our study shows that low serum thiol levels in CKD is due to a decrease in total thiol reserve of the body and not due to conversion of thiol groups into disulphides.

Albumin is an important chain breaking, extracellular antioxidant which contains an exposed cysteine–SH group and provides bulk of the total serum thiols [15]. Albumin, via its thiol groups, provides the major antioxidant protection [16]. Reduced protein intake in CKD and proteinuria as a result of conditions related with CKD (diabetes mellitus, nephrotic syndrome, etc.) causes albumin levels to decrease. In one study, decrease in serum protein thiols was demonstrated in uremic patients and this decrease was correlated positively with albumin levels [7]. In our study also, low serum thiol, total thiol and disulphide levels may be due to low serum albumin levels in CKD patients. Besides, we also think that reduction of protein intake during treatment and deficient protein intake due to loss of appetite as a result of uremia causes total thiol body reserve to decrease in CKD.

In our study, there was no significant difference between the CKD and control group with regards to disulphide / native thiol, disulphide / total thiol, and native thiol / total thiol rates. The reason behind that such rates were found similar between two groups was the higher levels of serum uric acid in CKD compared to control group. In our study, uric acid level was found higher in CKD group compared to the control group in line with the literature. Uric acid may function as a powerful antioxidant, and possibly one of the most important antioxidants in plasma. Uric acid can scavenge superoxide, hydroxyl radical, and singlet oxygen. Uric acid, thanks to its antioxidant characteristic, protects the proteins against the oxidation caused by free oxygen radicals. The increased serum uric acid level in CKD might have caused the disulphide / thiol rate to be similar to the control group by means of preventing the oxidation of thiol groups in the proteins [17].

Glutathione (γ-glutamyl cysteine glycine) is an important low molecular weight tripeptide containing high concentrations of thiol group. Glutathione is necesssary for the reduction of oxidated thiol groups and for protection against oxidative injury. Glutathione, the concentration of which is decreased with oxidative stress, becomes inadequate in reducing the oxidized thiol groups [18]. Glutathione levels have been demonstrated to decrease significantly in CKD [19]. In studies performed in humans and animal models, use of exogenous thiols increase tissue thiol levels [20]. Therefore, use of compounds containing thiols can be recommended to increase total body thiol. Compounds containing thiol include glutathione and its derivatives, cysteine, and N-acetylcysteine (NAC). NAC is a pharmacological precursor of L-cysteine. In studies performed in patients with CKD, use of NAC has been shown to reduce oxidative stress [21,22].

Limitation of our study was a cross-sectional study. Therefore, further prospective studies are needed in order to fully show the effect of reduced dietary intake of thiol containing amino acids and proteinuria on the thiol/ disulphide homeostasis in patient with CKD.

Conclusion

In CKD, reduced dietary intake of thiol containing amino acids such as cysteine and methionine as well as renal loss of these amino acids as a result of proteinuria causes the antioxidant system to be inefficient. As a result, nutrients rich in thiol containing amino acids can be recommended in patients with CKD in order to increase serum thiol levels.

Declaration of interest statement: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

Funding sources: This study was not supported financially in the form of grants, equipment, drugs, or other.

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