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Original Article

Evaluation of dynamic thiol-disulfide balance in children with stage 3-5 chronic kidney disease

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Abstract

Aim: We aimed to examine dynamic thiol-disulfide balance, an indicator of oxidative stress, in children with stage 3-5 chronic kidney disease (CKD).

Material and Methods: Native thiol (Nt), total thiol (Tt) and disulfide (DS) levels were determined spectrophotometrically in 27 children with CKD (14 non-dialysis, 13 hemodialysis) and 40 healthy controls.

Results: Albumin, Nt, Tt and DS levels were markedly lower in CKD patients than in controls. However, the DS/Nt values were not statistically significant between the two groups. When Nt, Tt, DS and DS/Nt values were adjusted for albumin levels, Tt and Nt levels were lower in CKD patients than controls. In the CKD group, Nt and Tt levels were positively correlated with the albumin and estimated glomerular filtration rate and negatively with urea and creatinine.

Conclusion: According to these results, decreased Nt and Tt levels in patients with CKD stages 3–5 may be evidence of exposure to oxidative stress.

Keywords: Albumin; chronic kidney disease; thiol oxidation; thiol-disulfide balance.

INTRODUCTION

Beyond the classical risk factors such as hypertension, dyslipidemia, and hyperglycemia, uremia-related risk factors may also contribute to the pathogenesis of cardiovascular diseases in chronic kidney disease (CKD) patients. Oxidative stress is considered as one of the uremia-related risk factors for cardiovascular disease in CKD patients (1). Growing evidence suggests that an increase in oxidative stress characterizes advanced stages of the disease (2,3). Oxidative stress is widely defined as a disturbance in the balance between oxidants and antioxidants, which can be quantified as the redox state of thiol/disulfide in subjects (4). Thiols, which contain sulfhydryl (–SH) groups in their structure, are important antioxidants active against free radicals and other oxidants. The mechanisms of action of thiols include metal chelation, trapping of free radicals, and acting as components of a thiol/disulfide redox buffer (5). Thiol oxidation in response to oxidative stress yields reversible disulfide bonds and thus the potential for renewed thiol reduction. The thiol-disulfide exchange via redox (oxidation-reduction) reactions is important in maintaining the dynamic thiol/disulfide balance (DTDB) (6). This dynamic balance plays a primary role in some biological processes, including regulation of cellular events (i.e., gene transcription, cellular growth and proliferation, apoptosis, signaling and stabilization of protein structures) and especially antioxidant defense (7,8).

Since 1979, only the thiol side of the DTDB has been measured. In 2014, thiol and disulfide levels can be measured separately by Erel's novel metod (9). Recent studies have suggested that abnormal DTDB may be related to the pathogenesis of many diseases including thalassemia (10), cancer (11), familial Mediterranean fever (12), and diabetes mellitus (13). However, to our knowledge, DTDB in children with CKD has not been examined using the new method. In this study, we aimed

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to analyze the DTDB as an index of oxidative stress in children with stages 3 to 5 CKD.

MATERIAL and METHODS

Participants

This study was performed in Sanliurfa Education and Research Hospital, Turkey. The study involved 14 non-dialysis CKD (ND-CKD) patients, 13 hemodialysis (HD) patients, and 40 healthy controls. The ND-CKD group comprised patients with CKD stages 3 and 4 [estimated glomerular filtration rates (eGFR) of 15-59 ml/min per 1.73 m^2 as calculated using the Schwartz formula (14)]. Within the ND-CKD group, 10 patients had CKD stage 3, and 4 had CKD stage 4. Patients with stages 1 and 2 CKD, acute infection, malignancies, or other chronic diseases were not included in the study. Patients receiving immunosuppressive therapy or antioxidant agents such as thiols were also excluded from the study.

Specimen collection and analysis

Fasting blood specimens were obtained from all CKD patients and controls. In HD patients, blood specimens were collected in the middle of the dialysis week. K_2EDTA containing tubes for DTDB tests and hemoglobin, and tubes with gel separator for routine biochemistry including albumin, creatinine, urea, calcium and phosphorus were used. Blood specimens were centrifuged at 3500 rpm for 15 min to obtain plasma and serum specimens. They were then stored as aliquots at –80°C until used in the study.

Baseline laboratory tests such as hemoglobin, albumin, creatinine, urea, calcium and phosphorus of all participants were tested with standard methods. Plasma Total thiol (Tt), and Native thiol (Nt) concentrations were assayed with novel method of Erel and Neselioglu (9). According to this method, reducible disulfide (DS) bonds were first reduced to free functional thiol groups using sodium borohydride (NaBH4). Secondly, the remaining NaBH4 was removed with formaldehyde, after which, modified Ellman's reagent was used to measured the total thiol content. Dynamic DS (-S-S-) concentrations are determined using the (Tt-Nt/2) formula and the % DS/Nt ratios were calculated (9).

Statistical analysis

Statistical calculations were performed using SPSS 20 software. Student's t-test or Mann-Whitney U test was used to compare the variables between the two groups. The Spearman and Pearson correlation tests were used to assess the relationship between DTDB values and other variables (age, albumin, creatinine, urea, calcium, phosphorus, eGFR and hemoglobin). Differences between DTDB parameters were assessed by analysis of covariance (ANCOVA) after albumin adjustment for the patient and control groups.

RESULTS

The demographic characteristics of the study subjects are shown in Table 1. There were no significant differences between the CKD patients and controls in terms of age and sex distribution. Hemoglobin, albumin, calcium and eGFR levels were significantly lower in patients with stages 3 to 5 CKD than in controls, whereas urea and creatinine levels were significantly higher.

As shown in Table 2, Nt, Tt and DS levels were significantly lower in patients with CKD than in controls. However, the DS/Nt values were similar in two groups. When DTDB tests were adjusted for albumin levels, Tt and Nt levels were lower in CKD patients than controls.

In the CKD group, Nt, Tt, and DS levels were positively correlated with the albumin level and negatively with the urea level. Nt and Tt levels were positively correlated with the eGFR and negatively with creatinine (Table 3).

Table 1. Demographic characteristics and laboratory values of the study populations

	CKD stages 3-5 (n:27)	Controls (n:40)	P values
Age, years	10.26±4.33	9.60±3.79	0.512
Gender, male/female	15/12	20/20	0.655
Albumin, g/dL	4.16 (2.75-4.63)	4.63 (4.41-4.88)	<0.001
Creatinine, mg/dL	2.87 (0.8-10.47)	0.44 (0.3-0.69)	<0.001
Urea, mg/dL	101.3±41.4	23.9±5.1	<0.001
Calcium, mg/dL	9.34±0.83	9.96±0.44	0.001
Phosphorus, mg/dL	4.80±1.19	4.76±0.44	0.879
Hemoglobin, g/dL	10.79±2.12	13.99±0.97	<0.001
eGFR (mL/min/1.73 m^2)	21.6 (5.5-58.8)	112.5 (98.8-158.7)	<0.001

Values expressed as numbers or median (min-max) or median±SD. CKD= Chronic kidney disease. eGFR= estimated glomerular filtration rate

Table 2. Thiol disulfide levels of the study populations

	CKD stages 3-5 (n:27)	Controls (n:40)	Unadjusted p values	Adjusted-for-albumin p values
Native thiol (Nt), µmol/L	422.6±78.9	478.2±25.3	0.001	0.009
Total thiol (Tt) µmol/L	458.9±83.2	518.9±25.3	0.001	0.009
Disulfide (DS), µmol/L	18.2±4.7	20.3±2.2	0.031	0.825
Disulfide/Native thiol (DS/Nt),%	4.36±1.06	4.27±0.53	0.671	0.429

Values expressed as median (min-max) or median ± SD. CKD: Chronic kidney disease

Table 3. Correlation between variables in patients with stage 3-5 CKD

	Native thiol (Nt)		Total thiol (Tt)		Disulfide (DS)		Disulfide/Native thiol (DS/Nt)	
	r	p	r	p	r	p	r	p
Age	-0.210	0.292	-0.242	0.225	-0.372	0.056	-0.268	0.177
Albumin	0.876	<0.001*	0.905	<0.001*	0.517	0.006*	-0.203	0.310
Creatinine	-0.408	0.035*	-0.426	0.027*	-0.310	0.115	0.033	0.870
Urea	-0.394	0.051*	-0.420	0.037*	-0.434	0.030*	-0.163	0.435
Calcium	0.323	0.100	0.307	0.119	0.020	0.921	-0.152	0.449
Phosphorus	-0.147	0.465	-0.187	0.351	-0.421	0.029*	-0.352	0.072
Hemoglobin	0.309	0.117	0.319	0.105	0.230	0.248	-0.025	0.900
eGFR	0.437	0.023*	0.454	0.017*	0.240	0.228	-0.137	0.496

*Correlation is significant at p<0.05

DISCUSSION

There is growing evidence that oxidative stress is associated with the progression of CKD and its complications (1-3). Thiols are members of the antioxidant system that protects organisms against oxidative stress. In human plasma, a small proportion of the total thiol pool is composed of low molecular mass thiols (cysteine, glutathione, homocysteine…), and the largest proportion consists of albumin and protein thiols (15). Previous studies investigating the thiol component of the DTDB system in CKD patients showed that thiol levels were low in adult patients with ND-CKD and HD (16-18). Among the potential explanations for the low thiol levels are inadequate protein intake, protein loss, and the conversion of thiols to DS bonds by oxidants (19). However, none of these studies examined the DTDB status of these patients. In 2014, Erel and Neselioglu developed a method to determine DTDB levels that allowed the separate or simultaneous measurement of both components (9). Prior to the introduction of this method, only the redox status of low molecular weight compounds had been investigated in patients with CKD. The results of those studies showed that the ratios of oxidized to reduced glutathione, cysteine, and homocysteine were significantly higher in adult patients with ND-CKD and HD than in controls (20-22). In our study, albumin, Nt, Tt and DS levels were lower in patients with CKD stages 3-5 than in the control group. However, we did not find differences in terms of DS/Nt values between the two groups. Similar findings were obtained by Ates and colleagues (19). As albumin is the main source of thiols, it was expected to Nt, Tt and DS levels were lower in CKD patients of this study. In a study by Danielski et al., reduced thiol levels were lower in the plasma of patients with hypoalbuminic than in those with normoalbuminiric HD (23). Decreased levels of thiol and albumin in adults with predialysis CKD and HD compared with the control group have also been reported, together with a positive correlation beween protein thiol levels and albumin (24). Similarly, we found a positive correlation between albumin levels and Nt, Tt and DS in patients with CKD stages 3-5. When DTDB tests were adjusted for albumin levels, Tt and Nt levels were lower in patients with CKD stage 3-5 than healthy subjects. Accordingly, decreased Nt and Tt levels in patients with CKD stages 3–5 may be evidence of exposure to oxidative stress. Besides, decreased appetite due to various causes such as uremic toxins and psychosocial factors in CKD patients may lead to inadequate protein intake, which may lead to a decrease in thiol reserve (25,26). Dietary supplementation with thiol-containing compounds such as N-acetylcysteine in CKD patients reduces the risk of cardiovascular events by preventing or alleviating oxidative stress (27,28). Accordingly, compensation for the thiol deficiency with thiol-containing agents may improve treatment efficacy in CKD patients.

This study has certain limitations. First, it was conducted in a single center with small sample size. Second, the relationship between DTDB and other oxidant/antioxidant parameters was not investigated. Therefore, comprehensive studies on the role of DTDB status in the pathophysiology of patients with CKD are required.

CONCLUSION

Nt and Tt levels were lower in patients with CKD stages 3-5 compared to healthy subjects and correlated with albumin, eGFR, urea and creatinine in patients with CKD stages 3-5. These findings suggest that decreased Nt and Tt levels in patients with stage 3-5 CKD may be associated with increased oxidative stress.

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