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The evaluation of thiol/disulphide homeostasis in diabetic nephropathy

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ABSTRACT

Aims: Thiol/disulphide homeostasis plays a critical role in antioxidant defense, and detoxification in body. Although alteration of thiol/disulfide homeostasis had been shown in patients with diabetes, the thiol/disulfide balance in patients with type 2 diabetes and nephropathy is not yet known.

Methods: Twenty-six healthy volunteers (group 1), and 17 normal albuminuric (group 2), 24 middle albuminuric (group 3), 20 severe proteinuric (group 4) patients with type 2 diabetes were included. Proteinuria was tested by measuring microalbumin/creatinine ratio in spot urine. Thiol/disulphide homeostasis concentrations were measured using method developed by Erel et al.

Results: Mean blood urea and creatinine levels were found to be significantly higher and GFR level was found to be significantly lower in group 4 than in the other groups. Native thiol levels are significantly lower in groups with diabetes than in healthy group and in groups 3 and 4, compared to group 2. Total thiol level was significantly lower in groups 3 and 4 than group 1 and 2. Disulphide/native thiol and disulfide/total thiol ratios were significantly higher in the groups with diabetes than in group 1 and in the group 4 than in the group 2.

Conclusion: The level of native and total thiols were found to be decreased significantly with the grade of nephropathy in patients with type 2 diabetes and the balance had been disrupted in favor of disulphide. We suggest that deteriorated thiol/disulphide balance may be one of the important factors in the development or progression of diabetes induced nephropathy.

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1. Introduction

Diabetes induced nephropathy is a well-known microvascular complication of diabetes with serious morbidity and mortality and leading cause of end-stage renal disease [1]. The production of reactive oxygen species (ROS) is increased during hyperglycemia and they play a significant role in the pathogenesis of diabetes induced complications [2]. Increased oxidative stress via enhanced ROS production in diabetes have been linked to vasoconstriction, vascular smooth muscle cell growth and migration, endothelial dysfunction, modification of extracellular matrix proteins, and increased renal sodium reabsorption [3].

Thiols are important antioxidant agents in the organism and have an important defensive role against ROS [4–6]. Thiols can undergo oxidation reaction via oxidants and form disulphide bonds. These bonds can be transformed to the thiols again; as a result, the thiol/disulfide homeostasis is maintained [7,8]. It has been showed that thiol levels were decreased due to its oxidation, disulphide levels were increased and thus thiol/disulphide ratios were decreased in diabetes mellitus (DM) [9,10]. However, to the best of our knowledge, it is not known the thiol/disulphide balance in the course of diabetes induced nephropathy.

Therefore, we aimed to investigate how to be affected the thiol-disulphide homeostasis in patients with type 2 DM and nephropathy.

2. Material and methods

A total of 61 outpatients with type 2 DM and 26 healthy subjects were enrolled into the study. The study protocol was approved by the local ethics committee, in accordance with the ethical principles for human investigations, as outlined by the Helsinki Declaration and written informed consent was obtained from all subjects. Patients with systematic inflammatory disease, any severe organ failure, malignancy, pregnancy and any disease caused to nephropathy except diabetes were not included. Patients who had a result of microalbumin to creatinine ratio in last six months were selected and spot urine microalbumin creatinine ratio was measured again in these subjects. Twenty-six healthy age and gender matched voluntary enrolled as control group (group 1, n = 26). Sixty-one patients with diabetes were divided to three groups according to the level of proteinuria; normal (albumin/creatinine < 30 mg/g) albuminuria group (group 2, n = 17), moderately increased (albumin/creatinine = 30–300 mg/g) albuminuria group (group 3, n = 24) and severely increased (albumin/creatinine > 300 mg/g) albuminuria group (group 4, n = 20).

Albuminuria was measured by immuno-turbidimetry method (Beckman Coulter System) in early morning urine samples. Thiol/disulphide homeostasis was measured with methods as defined by Erel et al. [11]. According to this method, reducible disulphide bonds were reduced to compose free functional thiol groups. Unused reductant sodium borohydride was used up and extracted with formaldehyde, and all thiol groups containing native and reduced ones were determined after reaction with 5, 5′-dithiobis-(2-nitrobenzoic) acid. Half of the difference between native and total thiols ensured the dynamic disulphide quantity. After detection of the amounts of native thiol and disulphide, the ratio of disulphide to native thiol was calculated [11].

The data obtained from study were analyzed using SPSS software version 20.0 (SPSS Inc., Chicago, Illinois, USA). One-way ANOVA and LSD as a post-hoc test were used for continuous variables with normal distribution, while Kruskal Wallis and Mann Whitney *U* test as a post-hoc test were used for non-normality data. Chi-square test was used to compare categorical data. Pearson correlation coefficient was used to determine correlations between thiol, disulphide and renal and metabolic parameters. Subsequently, linear multivariate analyses was performed to investigate relation between parameters of diabetes and lipid, and thiol/disulphide homeostasis. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

All groups were similar in terms of age and gender. Mean blood urea ($p < 0.001$ between group 1 and 4, $p = 0.001$ between group 2 and 4, $p = 0.002$ between group 3 and 4) and creatinine ($p < 0.001$ between group 1 and 4, 2 and 4, $p = 0.001$ between group 3 and 4) levels were significantly higher and mean GFR was lower in group 4 as compared to other groups. Mean triglyceride level was significantly higher and mean HDL level was lower in group 3 compared to group 1 ($p = 0.002$ and 0.001, respectively). Comparisons of groups in terms of clinical and biochemical parameters were shown in Table 1.

Mean native thiol levels were significantly lower in group 2 (386.2 ± 35.3 μmol/L), 3 (329.1 ± 63.3 μmol/L) and 4 (318.2 ± 53.3 μmol/L) as compared to group 1 (426.4 ± 44.3 μmol/L) ($p = 0.013$ between group 1 and 2, $p < 0.001$ for others) , in group 3 and 4 as compared to group 2 ($p = 0.001$ for both). Mean total thiol levels were significantly lower in group 3 (388.9 ± 62.6 μmol/L) and 4 (390.3 ± 71.8 μmol/L) as compared to group 1 (468.4 ± 52.4 μmol/L) and 2 (448.7 ± 51.6 μmol/L) ($p < 0.001$ between group 1 and the other two, $p = 0.002$ between group 2 and 3, $p = 0.004$ between group 2 and 4). Furthermore, mean disulphide levels were significantly higher in group 2 (31.2 ± 11.8 μmol/L), 3 (29.9 ± 12.0 μmol/L) and 4 (36.1 ± 18.9 μmol/L) than in group 1 (20.5 ± 7.9 μmol/L) ($p = 0.013$ between group 1 and 2, and $p < 0.001$ for others). Additionally, we found that mean disulphide/native thiol (4.9 ± 1.8, 8.0 ± 2.7, 9.6 ± 4.8 and 11.4 ± 6.2%, respectively) and disulphide/-total thiol (4.4 ± 1.5, 6.8 ± 2.0, 7.8 ± 3.3 and 8.9 ± 7.8%, respectively) ratios were significantly higher in groups with diabetes compared to control group ($p = 0.019$ between group 1 and 2 for disulphide/native thiol, $p = 0.007$ between group 1 and 2 for disulphide/total thiol, $p = 0.018$ between group 2 and 4 for disulphide/native thiol, $p = 0.028$ between group 2 and 4

Table 1 – Comparison of clinical and laboratory parameters between groups.

Parameter	Group 1 (n = 26)	Group 2 (n = 17)	Group 3 (n = 24)	Group 4 (n = 20)	P
Gender (male/female)	13/13	7/10	15/9	9/11	0.528[*]
Age (year)	48.7 ± 10.4	50.7 ± 10.1	54.6 ± 9.0	53.4 ± 9.1	0.139[*]
Glucose (mg/dL)	90.4 ± 8.8	155.9 ± 37.0	216.0 ± 98.4	198.0 ± 89.0	<0.001[*,b,c] 0.003[a], 0.007[d]
A1c (%)	–	7.9 ± 2.1	8.7 ± 1.6	9.5 ± 2.3	0.046[*], 0.014[e]
Urea (mg/dL)	31.1 ± 9.9	30.0 ± 5.5	33.3 ± 15.3	50.6 ± 30.0	0.001[*,e], <0.001[c], 0.002[f]
Creatinine (mg/dL)	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.1 ± 0.5	<0.001[*,c,e], 0.001[f]
GFR (mL/dk)	87.2 ± 6.8	98.1 ± 14.6	90.5 ± 16.3	72.5 ± 27.8	<0.001[*,e], 0.006[c], 0.001[f]
Total cholesterol (mg/dL)	169.9 ± 37.4	176.1 ± 43.8	189.0 ± 49.3	189.3 ± 40.9	0.328[*]
Triglyceride (mg/dL)	138.9 ± 55.4	189.4 ± 88.9	237.7 ± 176.0	189.3 ± 77.7	0.025[*], 0.002[b]
HDL cholesterol (mg/dL)	46.2 ± 9.5	40.9 ± 9.7	37.2 ± 10.5	41.5 ± 8.5	0.015[*], 0.001[b]
LDL cholesterol (mg/dL)	97.8 ± 33.3	97.4 ± 39.1	109.7 ± 28.7	109.9 ± 32.5	0.406[*]

Abbreviations. A1c: glycated hemoglobin, GFR: glomerular filtration rate, HDL: high density lipoprotein, LDL: low density lipoprotein, NS: non-significant.
[*] : between all groups.
[a] : between group 1 and 2.
[b] : between group 1 and 3.
[c] : between group 1 and 4.
[d] : between group 2 and 3.
[e] : between group 2 and 4.
[f] : between group 3 and 4.

for disulphide/total thiol, and $p < 0.001$ between group 1 and the other two for both parameters). All of these results were shown in Table 2.

In correlation analysis, native ($p = 0.003$) and total thiol ($p = 0.039$) levels were found to be negatively correlated while disulphide/native thiol ($p = 0.015$) and disulphide/total thiol ($p = 0.012$) ratios were found to be positively correlated with plasma glucose level. Disulphide/total thiol ratios were found to be positively correlated with plasma triglyceride level ($p = 0.045$). Moreover, HDL cholesterol level were significantly and negatively correlated with disulphide level, and ratios of disulphide/native thiol and disulphide/total thiol ($p = 002$ for all. The correlations of parameters were shown in Tables 3 and 4. Subsequently, none of the parameters (A1c, glucose, LDL cholesterol, HDL cholesterol and triglyceride) were correlated with any of the thiol/disulphide homeostasis parameters in linear multivariate analysis.

4. Discussion

To our best knowledge, this is the first study that investigates the thiol/disulphide homeostasis in different degrees of diabetes induced nephropathy. We found that (I) thiol/disulphide homeostasis was altered in diabetes, (II) thiol levels were gradually decreased with the progression of diabetes induced nephropathy. However, disulphide level was not affected with the presence of nephropathy.

Diabetes induced hyperglycemia can cause overproduction of ROS in mitochondria by several way; activation of polyol and hexosamine pathway, increased formation of advanced glycation end product and expression of its receptor and activation of protein kinase C isoforms [12]. Finally, this oxidant status gives rise to the tissue damage. Furthermore, several evidences suggest that increased oxidative stress may also contribute to the pathogenesis of diabetes

Table 2 – Comparison of thiol and disulphide parameters between groups.

Parameter	Group 1 (n = 26)	Group 2 (n = 17)	Group 3 (n = 24)	Group 4 (n = 20)	p
Native thiol (μmol/L)	426.4 ± 44.3	386.2 ± 35.3	329.1 ± 63.3	318.2 ± 53.3	<0.001[*,b,c], 0.013[a], 0.001[d,e]
Total thiol (μmol/L)	468.4 ± 52.4	448.7 ± 51.6	388.9 ± 62.6	390.3 ± 71.8	<0.001[*,b,c], 0.002[d] , 0.004[e]
Disulphide (μmol/L)	20.5 ± 7.9	31.2 ± 11.8	29.9 ± 12.0	36.1 ± 18.9	0.002[*], 0.013 [a] , <0.001[b,c]
Disulphide/native thiol (%)	4.9 ± 1.8	8.0 ± 2.7	9.6 ± 4.8	11.4 ± 6.2	<0.001[*,b,c], 0.019[a], 0.018[e]
Disulphide/total thiol (%)	4.4 ± 1.5	6.8 ± 2.0	7.8 ± 3.3	8.9 ± 7.8	<0.001[*,b,c], 0.007 [a], 0.028[e]

[*] : between all group.
[a] : between group 1 and 2.
[b] : between group 1 and 3.
[c] : between group 1 and 4.
[d] : between group 2 and 3.
[e] : between group 2 and 4.

Table 3 – Pearson correlation analyses of thiol/disulphide parameters with renal and diabetic parameters.

Parameter	A1c		Glucose		Urea		Cretainine		GFR	
	r	p	r	p	r	p	r	p	r	p
Native thiol (μmol/L)	−0.057	0.662	−0.310	0.003	−0.121	0.265	−0.181	0.093	0.026	0.810
Total thiol (μmol/L)	−0.076	0.562	−0.222	0.039	−0.080	0.463	−0.123	0.256	0.017	0.878
Disulphide (μmol/L)	−0.058	0.655	0.203	0.06	0.097	0.373	0.135	0.213	−0.022	0.839
Disulphide/native thiol (%)	−0.040	0.761	0.261	0.015	0.088	0.416	0.143	0.187	0.002	0.985
Disulphide/total thiol (%)	−0.049	0.706	0.269	0.012	0.103	0.343	0.154	0.154	−0.005	0.966

Abbreviations. A1c: glycated hemoglobin, GFR: glomerular filtration rate, NS: non-significant.

Table 4 – Pearson correlation analyses of thiol/disulphide parameters with lipid parameters.

Parameter	LDL-C		Triglyceride		HDL-C	
	r	p	r	p	r	p
Native thiol (μmol/L)	0.066	0.543	−0.132	0.223	0.156	0.149
Total thiol (μmol/L)	0.003	0.976	−0.056	0.606	0.023	0.836
Disulphide (μmol/L)	−0.153	0.157	0.182	0.092	−0.324	0.002
Disulphide/native thiol (%)	−0.154	0.154	0.203	0.059	−0.322	0.002
Disulphide/total thiol (%)	−0.150	0.164	0.215	0.045	−0.333	0.002

Abbreviations. HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol.

induced nephropathy and its progression to end-stage renal disease [2,3,13].

Status of dynamic thiol/disulfide homeostasis has crucial role in antioxidant protection, detoxification, apoptosis and regulation of enzymatic activity [14,15]. The measurement of oxidative stress through thiol/disulfide parameters by using novel method of Erel et al. has recently growing interest in research since it reflects the total thiol capacity and oxidation in the body [11]. This fact means that previous studies did not reflect the total amount of thiol and disulphide levels of body. Ates et al. examined the dynamic thiol/disulfide homeostasis in prediabetes and type 1 DM by this novel method and they found that thiol oxidation increased in patients with prediabetes and type 1 DM [9,16]. Ozler et al. showed that native thiol levels decreased at 24–28 weeks of pregnancy in women with gestational DM [17]. Very recently, Gulpamuk et al. analyzed the patients with type 2 DM and different stages of diabetes induced retinopathy and they found that thiol/disulphide parameters increased with the presence of diabetes and the significance continued to increased with the progression of retinopathy [18]. Similar to above study, we found that total and native thiols were decreased and disulphide levels were increased in patients with diabetes and the decrease in native thiol levels was associated with severity of nephropathy. In study of Ates et al. with prediabetes, the authors indicated that low native thiol level may be related with the high proteinuria in group with prediabetes [16]. Our results also support their hypothesis. On the other hand, correlation of thiol/disulfide homeostasis parameters with glucose levels demonstrated the close relationship between hyperglycemia and oxidative stress as explained previously. Impaired thiol/disulfide homeostasis indicates pathogenesis of diabetic nephropathy unlike to the proteinuria demonstrating a reason of nephropathy. Thus, understanding this situation may help to introduce new treatment modalities to prevent diabetic nephropathy.

Our study has some limitations; primarily it was cross-sectional design. For this reason, the generalization of our result may not be a realistic. Secondly, we do not speculate that patients with diabetes who have altered thiol/disulfide homeostasis tend to developed nephropathy due to design of our study. Thus, more comprehensive and longitudinal studies can answer this question.

In conclusion, thiol/disulfide homeostasis was deteriorated with the progression of nephropathy in type 2 DM. This deterioration may be due to hyperglycemia induced oxidative stress or progression of nephropathy. More studies are needed to validate the results of the present study.

Declarations of interest

None.

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