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ENDOCRINE METHODS AND TECHNIQUES

Determination of thiol/disulphide homeostasis in type 1 diabetes mellitus and the factors associated with thiol oxidation

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Abstract In this study, we aimed to examine dynamic thiol/disulfide homeostasis in type 1 diabetes mellitus (T1DM) and identify the factors associated with thiol oxidation. Thirty-eight subjects (18 male, 20 female) diagnosed with T1DM and 38 (17 male, 21 female) healthy volunteers without any known diseases were included in the study. Thiol/disulfide homeostasis concentrations were measured by a newly developed method (Erel & Neselioglu) in this study. After native thiol, total thiol and disulfide levels were determined; measures such as disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol were calculated. In T1DM patients, compared to the control group, disulfide ($p = 0.024$), disulfide/native thiol ($p < 0.001$), and disulfide/total thiol ($p < 0.001$) were determined higher, while native thiol ($p = 0.004$) and total thiol ($p < 0.001$) levels were much lower. In the patient group, a positive correlation was determined between c-reactive protein ($r = 325$, $p = 0.007$; $r = 316$, $p = 0.010$, respectively), fasting blood glucose ($r = 279$, $p = 0.018$; $r = 251$, $p = 0.035$, respectively), and glycosylated hemoglobin ($r = 341$, $p = 0.004$; $r = 332$, $p = 0.005$, respectively) and rates of disulfide/native thiol and disulfide/total thiol. We determined that thiol oxidation increase in T1DM patients compared to the control group. We thought that hyperglycemia and chronic inflammation might be the major cause of increase in oxide thiol form. In order to determine the relationship between the status of autoimmunity and dynamic thiol/disulfide in T1DM, dynamic thiol/disulfide homeostasis in newly diagnosed-antibody positive-T1DM patients is required to be investigated.

Keywords Autoimmunity · Chronic inflammation · Hyperglycemia · Oxidative stress · Sulfide

Introduction

Oxidative stress occurs as a result of the imbalance between antioxidant molecules and reactive oxygen species (ROS) in favor of ROS [1]. ROS that increases above the physiological level causes oxidation between two electron or redox modification of radical-based cysteine residues. In this redox reaction, sulfur atom that is found in cysteine side chain gets oxidized and transforms into disulfide [2]. Thus, dynamic thiol/disulfide homeostasis moves toward disulfide form, and the first stage of the oxidative damage is attached to oxidant radicals when the cellular level begins.

Thiol/disulfide homeostasis plays a major role in the maintenance of many physiological processes that are necessary for the organism, such as antioxidant defense, apoptosis, and stabilization of protein chemical structures [3]. This shift from the balance towards disulfide form is expected to be related with some degenerative diseases (diabetes, cardiovascular diseases…) [4].

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The methods that measure dynamic thiol/disulfide homeostasis as calorimetric and duplex were not developed until 2014 [5]. For the first time, a fully automated colorimetric method was developed by Erel et al. in 2014 [4]. With this developed, simple, reliable, sensitive, new method with both high linearity and repeatability, thiol/disulfide homeostasis and native thiol, dynamic disulfide, and total thiol levels were offered the opportunity of individual assessment [4].

We have not found any study related to how thiol/disulfide homeostasis changes in type 1 diabetes mellitus (T1DM) patients with this newly developed method and factors affecting the thiol oxidation. Therefore, in this study, we aimed to examine—with this newly developed method—thiol/disulfide homeostasis in T1DM patients.

Materials and methods

Study population

This study was conducted in Ankara Numune Training and Research Hospital, Department of Internal Medicine between May, 2015 and August, 2015.

Thirty-eight cases (18 male, 20 female) diagnosed with T1DM and 38 (17 male, 21 female) healthy volunteers were included in the study. T1DM patients were selected from patients who were in clinical follow-up. Antibody levels (anti-glutamic acid decarboxylase (Anti-GAD), islet cell antibody (ICA), insulin autoantibodies (IAA)) of these patients have been measured in the last 3 months and were recorded in the patient's files.

Patients with known type 2 diabetes, cardiovascular and cerebrovascular disease, acute and chronic kidney or liver disease, infectious or rheumatic inflammatory disease history, malignancy diagnosis, antioxidants, lipid-lowering drugs, smoking, alcohol consumption, and vitamin supplements were excluded from the study.

The study was conducted in accordance with the Declaration of Helsinki 2013 Brazil version, and was approved by the local Ethics Research Committee. All subjects provided written informed consent prior to participation in the study.

Biochemical parameters

For the measurement of thiol/disulfide hemostasis parameters, blood sample was taken from participants into biochemical tubes after eight hours of fasting. After blood samples were quickly centrifuged at 1500 rpm for 10 min, plasma and serum samples were separated and serum samples were stored at −80° C. Then, all parameters of the entire study population were studied in the same session with same serum samples.

Fasting blood glucose (FBG) was measured with enzymatic UV Hexokinase method in Beckman Coulter in 5800 (Beckman Coulter, Inc., Brea, CA, USA) autoanalyzer. Glycosylated hemoglobin (HbA1c) was measured with cation exchange HPLC method and Arkray ADAMS A1c HA8180 A1c (Arkray Global Business, Inc., Kyoto. Japan) automatic glycohemoglobin analyzer. Albumin was measured with bromine cresol green method, c-reactive protein (CRP) was measured with immunoturbidimetric method, and creatinine, total protein, triglyceride, total cholesterol, and high-density lipoprotein cholesterol were measured with enzymatic colorimetric method in Hitachi Modular P800 (Roche Diagnostic Corp. Indianapolis, Indiana, USA) auto analyzer. Low-density lipoprotein cholesterol was calculated with the Friedewald method [6].

Thiol/disulfide hemostasis parameters

Serum thiol/disulfide homeostasis was studied with a new and fully automatic analysis method developed by Erel and Neselioglu [4]. When the result obtained by subtracting native thiol from total thiol was divided into two, dynamic disulfide level was obtained. After dynamic disulfide, native and total thiol levels were determined; disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios were calculated [4].

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL) program. Normal distribution of data was evaluated with Kolmogorov–Smirnov test. Numerical variables showing normal distribution were expressed as mean ± standard deviation and those not showing normal distribution were expressed as median (interquartile range). Categorical variables were expressed as numbers and percentage. Student's t test and Mann–Whitney U test were used to compare two groups of numerical variables. Chi-square and Fisher's Exact tests were used to compare categorical variables. The relationship between numerical variables was evaluated using Pearson and Spearman correlation analysis. A p value < 0.05 was considered statistically significant.

Results

Table 1 summarizes the characteristics and laboratory findings of the study populations. Median FBG (205 mg/dl vs. 89 mg/dl, respectively; $p < 0.001$) and mean HgA1c

Table 1 Demographic characteristics and laboratory findings of the study patients

Variables	Control *n*(38)	T1DM *n*(38)	*p*
Gender (male), *n* (%)	17 (44.7)	18 (47.3)	0.813
Age (years)	28.9 ± 8.8	29.7 ± 8.5	0.688
BMI (kg/m^2)	25.5 ± 5.3	24.8 ± 6.6	0.612
FBG (mg/dL)	89 (3.8)	205 (147)	<0.001*
HbA1c (%)	4.9 ± 0.2	10.2 ± 2.5	<0.001*
Creatinine (mg/dL)	0.7 (0.10)	0.7 (0.2)	0.822
Total protein (g/dl)	62.8 ± 8.5	64.5 ± 9.3	0.408
Albumin (g/dl)	38.4 ± 6.2	40.1 ± 5.2	0.199
Triglyceride (mg/dl)	85 (95)	91 (94)	0.865
Total cholesterol (mg/dl)	170.1 ± 34.6	178.1 ± 54.2	0.446
HDL (mg/dl)	48.2 ± 14.5	50.4 ± 18	0.560
LDL (mg/dl)	92.4 ± 30.5	98.5 ± 38.8	0.449
CRP (mg/L)	1.2 ± 0.6	3.4 ± 0.8	<0.001*
Native thiol (μmol/L)	368.5 ± 40.2	335.8 ± 54.6	0.004*
Total thiol (μmol/L)	411.0 ± 45.5	365.5 ± 64.8	<0.001*
Disulfide (μmol/L)	18.1 ± 5.3	21.3 ± 6.7	0.024*
Disulfide/native thiol (%)	4.9 ± 1.4	6.4 ± 2.0	<0.001*
Disulfide/total thiol (%)	4.4 ± 1.3	6.0 ± 2.2	<0.001*
Native thiol/total thiol (%)	89.7 ± 2.8	92.5 ± 10.1	<0.001*

Quantitative variables are shown as mean ± standard deviation or median (IQR). Categorical variables are shown in number (%)

T1DM type 1 diabetes mellitus, *IQR* interquartile range, *BMI* body mass index, *FBG* fasting blood glucose, *HbA1C* hemoglobin A1C, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *CRP* c-reactive protein

* $p < 0.05$ statistical significance

(10.2 ± 2.5 vs. 4.9 ± 0.2 %, respectively; $p < 0.001$) levels were significantly higher in T1DM group compared to the control group.

If we look at intergroup thiol/disulfide hemostasis parameters, mean native thiol ($p = 0.004$) and total thiol levels ($p < 0.001$) were determined lower in T1DM patients compared to the control group. Mean disulfide level ($p = 0.024$) with mean disulfide/native thiol ($p < 0.001$), mean disulfide/total thiol ($p < 0.001$), and mean native thiol/total thiol ratios ($p < 0.001$) was determined to be higher in T1DM group compared to the control group.

When we examine thiol/disulfide hemostasis parameters according to the positivity of antibodies such as anti-GAD, ICA, and IAA in T1DM patients, significant differences were not determined between groups ($p > 0.05$).

When we examine the correlation analyses between hemostasis parameters and clinical and biochemical parameters, a positive correlation was determined between FBG and disulfide, disulfide/native thiol, and disulfide/total thiol. A negative correlation between HgA1c and native thiol and total thiol, and a positive correlation between disulfide, disulfide/native thiol, and disulfide/total thiol was determined. A positive correlation between CRP and native thiol and total thiol was determined, while between disulfide, disulfide/native thiol, disulfide/total thiol, and FBG, a positive correlation was determined (Table 2).

Discussion

To our knowledge, this is the first study showing the shift of dynamic thiol/disulfide homeostasis toward disulfide form in T1DM patients. Also for the first time in this study, a positive correlation was determined between FBG, HbA1c, CRP, and disulfide/native thiol, disulfide/total thiol levels.

Type 1 DM is a degenerative disease accompanied by chronic inflammation, autoimmunity, and hyperglycemia. Therefore, we consider that there is a significant relationship between T1DM and oxidative stress. However, degenerative diseases (chronic kidney disease, diabetes mellitus, cardiovascular disease) [7–9], chronic inflammation [10], autoimmunity [11], and hyperglycemia [12–14] have been shown to be associated with oxidative stress in previous studies.

In the literature review, we could not find any other studies that examine dynamic thiol/disulfide homeostasis in T1DM patients using this new method (Erel and Neselioglu). However, in a study conducted by a method developed by Ellman, serum thiol levels in T1DM patients were found to be lower compared to the control group [15]. But since disulfide form could not be determined with this method, we could not reach any data about thiol/disulfide balance. In another study conducted with adolescent patients diagnosed with T1DM, glutathione level, that shows a major part of the intracellular thiol level, was determined lower [16]. In these studies, it is thought that thiol levels were determined low due to reduction in synthesis or based on usage. In our study, we consider the major cause of the shift of dynamic thiol/disulfide homeostasis to be hyperglycemia and chronic inflammation and more toward disulfide form compared to the control group in T1DM patients. However, in our study, in T1DM group, disulfide, disulfide/native thiol, FBG, HbA1c, and CRP levels were determined higher than the control group. Besides this, in the correlation analysis, disulfide/native thiol and FBG, HbA1c and CRP were observed to be positively correlated. These results show that in the shift of thiol/disulfide homeostasis toward disulfide form, both hyperglycemia and inflammation might have a role. As mentioned above, the relation between hyperglycemia and oxidative stress is related to ROS, which are released from glycated proteins caused by hyperglycemia, increasing the oxidative stress level via advanced glycation end products specific receptors [17].

Table 2 The correlation analysis of findings related to dynamic thiol/disulfide homeostasis in type 1 diabetes mellitus group

Variables	Native thiol		Total thiol		Disulfide		Disulfide/native thiol		Disulfide/total thiol		Native thiol/total thiol	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	−0.040	0.743	−0.083	0.494	−0.135	0.261	−0.121	0.315	−0.050	0.676	0.115	0.339
BMI	0.146	0.226	0.154	0.201	0.050	0.678	−0.007	0.953	−0.010	0.935	−0.033	0.786
FBG	−0.120	0.318	−0.141	0.240	0.280	0.031*	0.279	0.018*	0.251	0.035*	0.085	0.479
HbA1c	−0.302	0.011*	−0.330	0.005*	0.253	0.033*	0.341	0.004*	0.332	0.005*	0.130	0.280
Creatinine	−0.244	0.040*	−0.241	0.043*	−0.021	0.859	0.088	0.464	0.082	0.498	0.028	0.818
CRP	−0.298	0.017*	−0.305	0.009*	0.262	0.030*	0.325	0.007*	0.316	0.010*	0.140	0.380
TG	−0.096	0.583	−0.005	0.978	−0.201	0.248	−0.157	0.369	−0.191	0.271	−0.180	0.300
TC	0.066	0.706	0.115	0.512	−0.234	0.175	−0.253	0.143	−0.249	0.150	−0.125	0.473
HDL	0.015	0.934	0.059	0.738	−0.340	0.046*	−0.324	0.058	−0.303	0.077	−0.112	0.521
LDL	0.177	0.310	0.139	0.424	−0.060	0.730	−0.129	0.462	−0.089	0.610	0.042	0.811

BMI body mass index, *FBG* fasting blood glucose, *HgA1C* hemoglobin A1c, *CRP* c-reactive protein, *TG*:triglyceride, *TC* total cholesterol, *HDL* High-density lipoprotein, *LDL* Low-density lipoprotein

* $p < 0.05$ statistical significance

In our study, another reason for the increase of oxidized thiol levels in T1DM patients is chronic inflammation. Although CRP was determined higher compared to the control group, a positive correlation was found in the patient group between CRP and disulfide, disulfide/native thiol levels. Between native and total thiol levels, a negative correlation was determined. In previous studies, pro-inflammatory cytokines were shown to cause the increase of ROS via oxidase enzyme in the case of inflammation [10, 18]. In addition, we think that in our study, the main reason for CRP level to be higher compared to the control group is hyperglycemia. Because in a correlation analysis we made, a positive correlation was determined between CRP and FBG. We attribute hyperglycemia-related increase in the chronic inflammation, to the increasing blood glucose and the fact that nuclear factor-κB (NF-κB) is activated, because it has been shown in previous studies that both the release of pro-inflammatory cytokines and oxidative stress level increase due to the activation of NF-κB [19, 20].

The main limitation of our study is cross-sectional design. We determined that thiol oxidation increases in T1DM patients compared to the control group. We considered that hyperglycemia and chronic inflammation might be the major causes of increase in oxide thiol form.

Compliance with ethical standards

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

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Statement of Human and Animal Rights All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institution and with the Declaration of Helsinki 2013 Brazil version)

Statement of Informed Consent Informed consent was obtained from all patients for being included in the study.

References

1. I. Ates, N. Ozkayar, C. Topcuoglu, F. Dede, Relationship between oxidative stress parameters and asymptomatic organ damage in hypertensive patients without diabetes mellitus. Scand. cardiovasc. J **49**(5), 249–256 (2015). doi:10.3109/14017431.2015.1060355
2. C.M. Cremers, U. Jakob, Oxidant sensing by reversible disulfide bond formation. J. Biol. Chem. **288**(37), 26489–26496 (2013). doi:10.1074/jbc.R113.462929
3. F.Q. Schafer, G.R. Buettner, Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. **30**(11), 1191–1212 (2001)
4. O. Erel, S. Neselioglu, A novel and automated assay for thiol/disulphide homeostasis. Clin. Biochem. **47**(18), 326–332 (2014). doi:10.1016/j.clinbiochem.2014.09.026
5. J.R. Winther, C. Thorpe, Quantification of thiols and disulfides. Biochim. Biophys. Acta **1840**(2), 838–846 (2014). doi:10.1016/j.bbagen.2013.03.031
6. W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. **18**(6), 499–502 (1972)
7. S.D. Rodrigues, G.B. Batista, M. Ingberman, R. Pecoits-Filho, L.S. Nakao, Plasma cysteine/cystine reduction potential correlates with plasma creatinine levels in chronic kidney disease. Blood Purif. **34**(3–4), 231–237 (2012). doi:10.1159/000342627
8. E. Matteucci, O. Giampietro, Thiol signalling network with an eye to diabetes. Molecules **15**(12), 8890–8903 (2010). doi:10.3390/molecules15128890
9. Y.M. Go, D.P. Jones, Cysteine/cystine redox signaling in cardiovascular disease. Free Radic. Biol. Med. **50**(4), 495–509 (2011). doi:10.1016/j.freeradbiomed.2010.11.029
10. Q.N. Dinh, G.R. Drummond, C.G. Sobey, S. Chrissobolis, Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed. Res. Int. **2014**, 406960 (2014). doi:10.1155/2014/406960

11. B.T. Kurien, R.H. Scofield, Autoimmunity and oxidatively modified autoantigens. Autoimmun. Rev. **7**(7), 567–573 (2008). doi:10.1016/j.autrev.2008.04.019
12. S. Tangvarasittichai, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes **6**(3), 456–480 (2015). doi:10.4239/wjd.v6.i3.456
13. E.L. Feldman, Oxidative stress and diabetic neuropathy: a new understanding of an old problem. J. Clin. Invest. **111**(4), 431–433 (2003). doi:10.1172/JCI17862
14. I. Ates, M. Kaplan, B. Inan, M. Alısık, O. Erel, N. Yilmaz, S. Guler, How does thiol/disulfide homeostasis change in prediabetic patients? Diabetes Res Clin. Pract. (2015). doi:10.1016/j.diabres.2015.09.011
15. E. Matteucci, G. Malvaldi, F. Fagnani, I. Evangelista, O. Giampietro, Redox status and immune function in type I diabetes families. Clin. Exp. Immunol. **136**(3), 549–554 (2004). doi:10.1111/j.1365-2249.2004.02470.x
16. D. Darmaun, S.D. Smith, S. Sweeten, B.K. Sager, S. Welch, N. Mauras, Evidence for accelerated rates of glutathione utilization and glutathione depletion in adolescents with poorly controlled type 1 diabetes. Diabetes **54**(1), 190–196 (2005)
17. P.M. Abuja, R. Albertini, Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin. Chim. Acta **306**(1–2), 1–17 (2001)
18. N.D. Vaziri, Causal link between oxidative stress, inflammation, and hypertension. Iran. J. Kidney Dis. **2**(1), 1–10 (2008)
19. D. Cai, T. Liu, Inflammatory cause of metabolic syndrome via brain stress and NF-κB. Aging **4**(2), 98–115 (2012)
20. H.L. Pahl, Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene **18**(49), 6853–6866 (1999). doi:10.1038/sj.onc.1203239