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How does thiol/disulfide homeostasis change in children with type 1 diabetes mellitus?

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ABSTRACT

Aims: An increase in reactive oxygen species leads to formation of covalent bonds between sulfur atoms, thus thiol/disulfide homeostasis shifts towards the disulfide direction and oxidative damage occurs. We aimed to determine thiol/disulfide homeostasis in children with T1DM.

Methods: Thiol/disulfide homeostasis was evaluated in 30 patients with T1DM and 30 age, gender matched healthy controls. Thiol/disulfide homeostasis parameters were measured using a novel automated measurement method and correlation between demographic data and parameters was measured.

Results: There weren't any significant differences in age or gender between the T1DM and control groups. T1DM group, findings were as follows: native thiol: 388.3 ± 76.7 μmol/L, total thiol: 426.2 ± 87 μmol/L, disulfide: 18.9 ± 7 μmol/L, control group findings were as follows: native thiol: 423.1 ± 45.2 μmol/L, total thiol: 455.7 ± 49.9 μmol/L, disulfide: 16.2 ± 5.6 μmol/L. The disulfide/native thiol and disulfide/total thiol ratios were significantly higher in the T1DM group ($p = 0.005$ and $p = 0.004$, respectively), whereas the native thiol level and the native thiol/total thiol ratio were significantly lower in the T1DM group than in the control group ($p = 0.036$ and $p = 0.015$, respectively). There wasn't significant correlation between demographic data and thiol/disulfide homeostasis parameters.

Discussion: This study shows that dynamic thiol/disulfide homeostasis in children with T1DM shifts towards the disulfide direction. We think that this shift is caused by oxidative damage in β-cells. Additional research on thiol/disulfide homeostasis in children with T1DM might provide techniques for early detection of oxidative damage in β-cells.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a metabolic disease caused by loss of β-cells in response to chronic autoimmune inflammation in pancreatic β-cell islets and is associated with hyperglycemia [1]. An increase in cytokine release, impaired antioxidation and damage caused by reactive oxygen species (ROS) in β-cells play a role in the pathogenesis of T1DM [2,3].

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IL-4, IL-5, and IL-10, which suppress inflammation, are secreted by CD4 + Th2 cells, whereas IL-2 and IFN-γ, which activate macrophages, are secreted by CD4 + Th1 and CD8 + T cells via induction of T cells that play a preliminary role in autoimmune injury in β-cells. Several inflammatory cytokines and ROS are secreted by active macrophages due to impairment of Th1/Th2 homeostasis [4].

Thiols are organic compounds that contain succinyl groups consisting of a sulfur atom and a hydrogen atom bound to a carbon atom [5]. The plasma thiol pool is composed of albumin thiols, protein thiols, and a small quantity of low-molecular-weight thiols. An increase in ROS leads to oxidation of sulfur atoms in the cysteine side chain of these plasma thiols and formation of covalent bonds between the sulfur atoms, which subsequently causes disulfide conversion [5–9]. Thus, dynamic thiol/disulfide homeostasis develops in the body which plays an important role in many physiological events including antioxidant defense, apoptosis, and protection of the chemical structure of proteins [8–10]. Thiol/disulfide homeostasis has been associated with many degenerative diseases. In 2014 Erel and Neselioglu developed a novel method for measuring the dynamic thiol/disulfide homseostasis, that is fully automatized and colorimetric [9]. The present study, aimed to determine thiol/disulfide homeostasis in children with T1DM using this novel method and the correlation between thiol/disulfide homeostasis parameters and demographic variables.

2. Materials and methods

The T1DM group included 30 patients aged ≤18 years that presented to Dr. Sami Ulus Obstetrics and Pediatrics Training and Research Hospital, Ankara, Turkey, and were followed-up for T1DM and had no other accompanying diagnosis. The control group included 30 healthy children without any known chronic or acute disease. T1DM was diagnosed based on the presence of ≥1 of the autoantibodies, anti-islet antibody, anti-glutamic acid decarboxylase antibody, and anti-insulin antibody, in addition to clinical findings characteristic of diabetes [11]. Pateints aged >18 years, and those with an acute infection or chronic disease accompanying T1DM were excluded from the study. Demographic data, including age and gender were recorded in the T1DM and control groups, and only in the T1DM group fasting blood glucose, hemoglobin A1c (HbA1c), age at the time of T1DM diagnosis, duration of T1DM, and presence of anti-islet antibody, anti-glutamic acid decarboxylase antibody, anti-insulin antibody were recorded.

Blood samples from all the participants were collected into biochemical tubes and centrifuged at 3600 rpm for 10 min, and then the separated serums were stored at −80 °C, all samples were dissolved at the same time and the serum thiol/disulfide parameters were measured at Yildirim Beyazit University Atatürk Training and Research Hospital Biochemistry Laboratory, Ankara, Turkey, using Erel&Neselioglu's novel technique and Roche Hitachi Cobas c501 automatic analyzer. The study protocol was approved by the Ankara Keçiören Training and Research Hospital Ethics Committee and written informed consent was obtained from all parents and children aged >8 years.

2.1. Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows v.20.0 (IBM, Corp., Armonk, NY). The normality of the distribution of numerical variables was determined via the Kolmogorov-Smirnov test. Numerical variables with normal distribution are shown as mean ± SD and those not normally distributed are shown as median. G*Power v.3.0.10 (G*Power Franz Faul, Universitat, Kiel, Germany) was used to determine the sample size required for the study [12]. Taking a sample of ≥ 60 cases (30 cases per group) was calculated to achieve 85% power with d = 0.80 effect width, α = 0.05 type 1 error, β = 0.15 type 2 error. Differences between groups were assessed using the *t* test in independent groups when parametric test assumptions were provided. Correlations between numerical variables were determined using Pearson's correlation coefficient. The level of statistical significance was set at $P < 0.05$ was considered statisticaly significant [13].

3. Results

The T1DM group included 17 female and 13 male T1DM patients, and the control group included 12 female and 18 male children. Mean age in the T1DM group was 11.75 ± 2.71 years, versus 11.54 ± 2.55 years in the control group. Age and gender did not differ significantly between the 2 groups ($p = 0.196$, $p = 0.751$, respectively). Thiol/disulfide homeostasis parameters in the control group were as follows; native thiol: 423.1 ± 45.2 μmol/l, total thiol: 455.7 ± 49.9 μmol/l, disulfide: 16.2 ± 5.6 μmol/l, disulfide/native thiol: 3.8 ± 1.3, disulfide/total thiol: 3.5 ± 1.1, native thiol/total thiol: 92.9

Table 1 – Demographic characteristics and laboratory findings in study groups.

Variables	T1DM *n* (30)	Control *n* (30)	*p*
Age (years) (±SD)	11.75 ± 2.71	11.54 ± 2.55	0.196
Gender (male), *n* (%)	18 (60)	13 (43)	0.751
Native thiol (μmol/L)	388.3 ± 76.7	423.1 ± 45.2	0.036*
Total thiol (μmol/L)	426.2 ± 87	455.7 ± 49.9	0.113
Disulfide (μmol/L)	18.9 ± 7	16.2 ± 5.6	0.111
Disulfide/Nativethiol (%)	4.8 ± 1.3	3.8 ± 1.3	0.005*
Disulfide/Total thiol (%)	4.3 ± 1	3.5 ± 1.1	0.004*
Native thiol/Total thiol (%)	91.3 ± 2.4	92.9 ± 2.2	0.015*

* $p < 0.05$ statistical significance.

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	0.24	0.06	0.22	0.08	0.27	0.83	−0.11	0.39	−0.11	0.37	0.66	0.61
Gender	0.16	0.19	0.16	0.19	0.09	0.47	0.01	0.89	0.01	0.9	−0.56	0.67
FBG	0.27	0.13	0.27	0.14	0.17	0.34	0.06	0.97	0.11	0.95	−0.07	0.67
HbA1c	0.24	0.19	0.24	0.19	0.17	0.34	0.01	0.96	0.02	0.89	−0.06	0.75
Antibody	−0.1	0.75	−0.1	0.76	−0	0.91	0.01	0.92	0.01	0.96	0.11	0.53
Age at the time of T1DM diagnosis	0.23	0.2	0.25	0.18	0.25	0.18	0.14	0.44	0.14	0.45	−0.15	0.41
Duration of T1DM	−0.01	0.93	−0.03	0.86	−0.1	0.52	−0.16	0.39	−0.16	0.39	0.09	0.63

FBG: Fasting Blood Glucose HbA1c: glycolysed hemoglobin. T1DM: Type 1 Diabetes Mellitus.
$p < 0.05$ statistical significance.

± 2.2. The thiol/disulfide homeostasis parameters in the T1DM group were as follows: native thiol: 388.3 ± 76.7 μmol/l, total thiol: 426.2 ± 87 μmol/l, disulfide: 18.9 ± 7 μmol/l, disulfide/native thiol: 4.8 ± 1.3, disulfide/total thiol: 4.3 ± 1, native thiol/total thiol: 91.3 ± 2.4. There wasn't a significant difference in the mean total thiol or disulfide levels between the 2 groups ($p = 0.113$, $p = 0.111$, respectively). The disulfide/native thiol and disulfide/total thiol ratios were significantly higher in T1DM group than in the control group ($p = 0.005$ and $p = 0.004$, respectively), but the native thiol level and the mean native thiol/total thiol ratio were significantly lower in the control group ($p = 0.036$ and $p = 0015$, respectively). Table 1 summarizes the characteristics and laboratory findings in the T1DM group.

Mean age at the time of T1DM diagnosis was 8.9 ± 3.5 years, mean duration of T1DM was 2.7 ± 2.5 years, the mean fasting blood glucose level was 183 ± 83 mg/dl, and mean HbA1c was 7.9% ± 1.5%. There wasn't significant correlation between thiol/disulfide homeostasis parameters, and age at the time of T1DM diagnosis, duration of T1DM, the fasting blood glucose level, HbA1c, or diabetes autoantibodies. Correlation analysis results and figures are shown in Table 2 and Fig. 1 respectively.

4. Discussion

In the present study, disulfide/native thiol, and disulfide/total thiol ratios were significantly higher whereas the native thiol level and native thiol/total thiol ratio were significantly lower in the T1DM patients than in the controls. To the best our knowledge the present study is the first to report that dynamic thiol/disulfide homeostasis in children with T1DM shifts towards the disulfide direction. Type 1 diabetes mellitus is a chronic inflammatory disease, characterized by hyperglycemia and is caused by destruction of pancreatic insulin-releasing β-cells [1,14]. Studies on β-cell pathology have shown that inflammatory cells, such as T cells, B cells, macrophages, and natural killer cells destroy the β-cells via various inflammatory cytokines and ROS. As pancreatic β-cells have a low level of antioxidant enzymes, they are highly susceptible to the toxic effects of inflammatory cytokines. Reactive oxygen species directly damage β-cells and exacerbate inflammation in β-cells [4,14,15]. Experimental animal studies reported that specific inhibition of oxidative stress in β-cells prevents the development of T1DM [14,15], indicating that oxidative stress plays an important role in β-cell degradation, which is supported by a growing body of evidence [16]. Accordingly we think that there is a significant relationship between oxidative stress and T1DM, and that thiol/disulfide homeostasis plays a major role in antioxidant defense.

Thiols are organic compounds composed of a sulfur atom and a hydrogen atom bound to a carbon atom. Thiols occur in all cells of the body and via oxidation in the presence of oxidants, form reversible disulfide bonds, thusly establishing dynamic thiol/disulfide homeostasis. This homeostasis is important for antioxidant protection, detoxification, signal transduction, intracellular signal transduction mechanisms, and regulation of the activity of apoptosis enzymes and transcription factors. Impaired thiol/disulfide homeostasis is

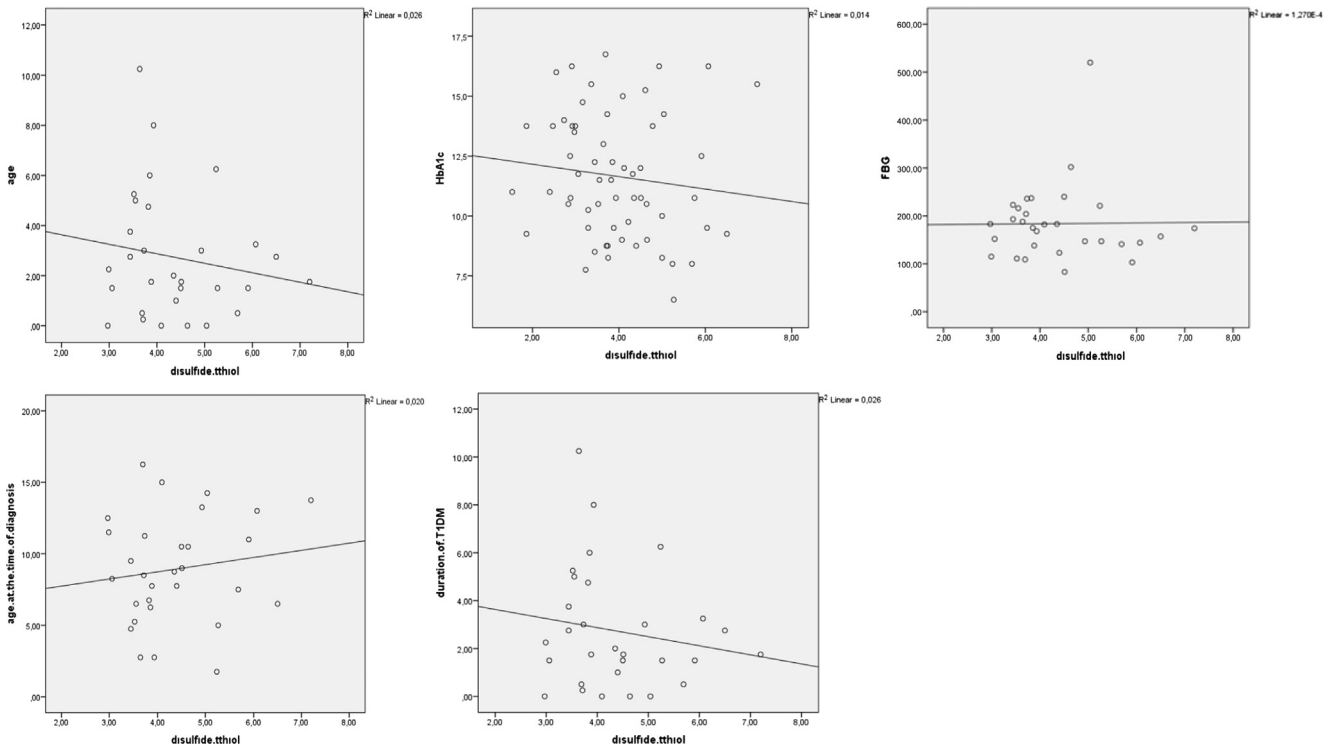

Fig. 1 – Scatter plot graphs for correlation results. Abbreviations: FBG = Fasting blood glucose, HbA1c = glycolysed hemoglobin, T1DM = Type 1 Diabetes Mellitus.

thought to play a role in the pathogenesis of many diseases [9,17].

The literature includes studies on such low-molecular-weight thiol compounds as cysteine and glutathione, which represent a small fraction of the total thiol pool in the organism [18]. One study that included adolescent T1DM patients and examined glutathione level, which is important for protecting against oxidative stresses, reported that the glutathione level was low in the T1DM patients, because of an increase in glutathione consumption associated with a high blood glucose level [19] however, based on a review of the literature dynamic thiol/disulfide homeostasis in pediatric T1DM patients has not been studied using this novel Erel&Neselioğlu's method. A study that included 38 adult T1DM patients and 38 healthy adult controls observed that, dynamic thiol/disulfide homeostasis shifted towards the disulfide direction in the patient group [8]. In the present study, the disulfide/native thiol, disulfide/total thiol ratios were significantly higher in children with T1DM than in the controls. Unlike adults, the native thiol level and native thiol/-total thiol ratio were lower in present study's pediatric T1DM patients than in healthy controls, which indicates that the thiol/disulfide homeostasis shifts towards disulfide in children with T1DM. Research has shown that there is a positive correlation between the disulfide/native thiol ratio, and fasting blood glucose, HbA1c, and C-reactive protein in adult T1DM patients [8]. In the present study there wasn't a significant correlation between any of the thiol/disulfide homeostasis parameters, and age at the time of T1DM diagnosis, duration of T1DM, fasting blood glucose, HbA1c or diabetes autoantibodies, which we think is due to the fact that HbA1c and fasting blood glucose in children with T1DM (7.5% and 185 mg/dl respectively) are lower than in the adults with T1DM (10.2% and 205 mg/dl respectively) included in the study of Ateş et al.'s [8].

The present study's 2 limitations are its small study population and lack of comparison of thiol/disulfide homeostasis parameters with other enzymatic and non-enzymatic measures of oxidative stress.

5. Conclusion

The present study shows that thiol/disulfide homeostasis, which is an important marker of oxidative stress, shifts towards the disulfide direction in pediatric T1DM patients. We think that this shift is caused by the oxidative damage in pancreatic β-cells that occurs during the pathogenesis of T1DM. It is our hope that the present findings lay the foundation for new prospective multicenter studies on the association between thiol/disulfide homeostasis and early detection of oxidative damage in β-cells for the prevention of T1DM.

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Conflict of interest statement

The authors declare there are no conflicts of interest—financial or otherwise—related to the material presented herein.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.01.027.

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