Determination of oxidant and antioxidant parameters in the serum of children with type 1 diabetes mellitus

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The primary goal of this study was to assess the oxidant/ antioxidant balance of children and adolescents with type 1 diabetes mellitus (T1DM). It was an experimental case-control study with 38 children and adolescents diagnosed with T1DM. We found that the fasting blood glucose, haemoglobinA1c, malondialdehyde, total oxidant status, and total and native thiol values of the type-1 diabetes group were significantly higher than the control group, while total antioxidant status was significantly lower. Our results corroborate other studies showing diabetic patients are more vulnerable to oxidative stress.

Keywords: Adolescents, children, diabetes mellitus, oxidant and antioxidant parameters.

DIABETES mellitus (DM) is a common metabolic and non-communicable disease related to enhanced oxidative stress as well as psychosocial disturbances characterized by hyperglycaemia[1]. Among non-communicable diseases, DM has become a global health problem[2]. Since the prevalence of this disease is increasing exponentially all over the world, it has been accepted as the epidemic of the 21st century[3,4]. Medical attention is required because those affected by DM cannot adequately utilize carbohydrates, fats and proteins due to insulin deficiency or problems with insulin use[5]. According to the IDF Diabetes Atlas published by the International Diabetes Federation (IDF) in 2021, there are currently 643 million people living with diabetes worldwide, which is expected to rise to 783 million by 2043 (ref. 6). Type 1 diabetes mellitus (T1DM), formerly known as juvenile diabetes or insulin-dependent diabetes, is one of the most prevalent endocrine and metabolic conditions to affect children. It is characterized by elevated blood glucose levels (hyperglycaemia) because of insulin deficiency and a high risk of developing life-threatening complications[7]. T1DM has historically been considered a childhood disease, but recent epidemiological studies have shown that the incidence is similar in adults[8].

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Diabetes, a significant health issue in the 21st century, dramatically increases the risk of oxidative stress (OS) and causes many other diseases. OS affects insulin secretion during diabetes. It has a considerable role in the pathogenesis of T1DM patients with a genetic predisposition. In addition, it has been claimed that hyperglycaemia promotes OS by generating new free radicals and suppressing the antioxidant defence mechanisms[9]. OS leads to further adverse processes in patients with diabetes, such as increased cell proliferation, lipid peroxidation that results in the crosslinking of individual protein molecules, and oxidation of low-density lipoprotein (LDL) that causes early development of atherosclerotic changes[10].

An organism defends against oxidative stress by means of antioxidants and enzymes. Numerous studies have used various biochemical markers in children to examine the connection between diabetes and oxidant/antioxidant status[11].

In this study, lipid peroxidation end-product, namely malondialdehyde (MDA), total oxidant status (TOS), total antioxidant status (TAS) and thiol/disulphide homeostasis were measured in T1DM patients and compared with healthy children and adolescents.

Materials and methods

Study population

This is a case-control study that was conducted at Aydin Adnan Menderes University, Turkey, between September 2020 and June 2021. A total of 38 patients with T1DM, including 19 males and 19 females, diagnosed and followed-up in the Department of Pediatric Endocrinology outpatient clinic of the university were included in the study. DM was diagnosed according to American Diabetes Association (ADA) criteria, and patients with antibody (anti glutamic acid decarboxylase (GAD), insulin, island cells) positivity were accepted as T1DM. Patients with a family history of diabetes, phenotypical features of metabolic syndrome, secondary diabetes and microvascular or macrovascular complications, and/or coexisting conditions

such as autoimmune thyroiditis and coeliac disease were not included in the study. Thirty-four (89%) T1DM patients were treated with multiple daily insulin injections, while four (11%) patients were treated with a sensor-augmented insulin pump system (continuous subcutaneous insulin infusion). The control group for the study consisted of 38 age- and sex-matched healthy groups (19 males and 19 females) who visited the outpatient clinic for a check-up and had no known family history of T1DM or other chronic diseases.

Sample collection

Height was measured using a Harpenden stadiometer with a sensitivity of 0.1 cm and weight was measured using a scale with a sensitivity of 0.1 kg (SECA, Hamburg, Germany). Each subject's weight was measured with all clothing removed except undergarments.

Following a 12 h fast, venous blood samples were taken from the participants into tubes containing EDTA and centrifuged for 10 min at 4000 rpm. The obtained plasma was transferred to 2 ml eppendorfs and stored at the Department of Nutrition and Dietetics laboratory of Aydin Adnan Menderes University until analysis at –20°C.

Biochemical measurement

Fasting blood glucose (FBG) and haemoglobinA1c (HbA1c) values of the participants were measured in the Department of Biochemistry laboratory of the university. FBG was measured employing the Abbott Architect c800 brand device and kit using the hexokinase method. HbA1c was measured by the chromatographic/photometric method.

Thiol/disulphide homeostasis was measured with the help of the Rel Assay Diagnostic branded kit (Gaziantep, Turkey).

MDA level

The determination of MDA level was based on the reaction of thiobarbituric acid (TBA) and MDA to give a coloured compound that can be measured at 532 nm wavelength. MDA levels in the cell were determined according to the technique outlined by Ohkawa *et al.*[12]. MDA in the sample reacts with TBA and trichloroacetic acid (TCA) at 95°C to give a pink colour by forming the TBA–MDA adduct. Running Elisa reader at 532 nm against air.

Thiol/disulphide homeostasis

Chemicals and instruments used for thiol/disulphide homeostasis determination were as follows: GSG, GSSG, 2-mercaptoethanol, EDTA, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), chloramine-T, TRIS, $NaBH_4$, NaOH, H_2O_2, formaldehyde, methanol, Sigma-Aldrich and Merck commercial kits, pure water and pure reagents. Shimadzu UV-1800 spectrophotometer and Cobas c501 automatic analyzer were also used in the study. The principle of this method is the conversion of disulphide bonds in the sample to functional thiol groups by $NaBH_4$. Unused $NaBH_4$ residues were eliminated by formaldehyde. In this way, extra-reduced DTNB and future disulphide bonds are prevented. The content of total thiol in the sample was estimated with Ellman's reagent. The following formula was used to determine the quantity of serum disulphide[13] (serum total thiol–serum native thiol)/2.

TOS and TAS levels

The measurement of TAS and TOS was done according to Erel[14,15].

Ethics

The Ethics Committee approval for the study was obtained by Aydin Adnan Menderes University, Health Sciences Institute (2020/005-1). All participants and their families gave their signed, informed consent.

Statistical analysis

The statistical package for social sciences (SPSS), version 22.0 (IBM), was used to perform statistical analysis on the data. In the analysis of descriptive statistics (body weight, age, height, gender, body mass index (BMI)), standard deviation (SD), arithmetic mean ($\bar{X}$), and minimum and maximum value statistics were evaluated. Whether the data showed normal distribution was evaluated with the Kolmogorov–Smirnov test. Mean ± SD analysis was used for normally distributed data. Independent samples *t*-test was performed to compare the numerical values between the two groups. The relationships between the variables were analysed with the Pearson and Spearman correlation coefficients. A *P*-value <0.005 was considered to be statistically significant.

Results

Mean age of the patients with T1DM and healthy subjects was 13.2 ± 2.8 and 12.9 ± 2.5 years respectively ($P > 0.05$). Similarly, the results were insignificant in the height comparison ($P > 0.05$). The difference observed as a result of the comparison made for body weight was not statistically significant ($P > 0.05$), while the results obtained from BMI comparison were also not statistically significant ($P > 0.05$) (Table 1).

The participants in the group who were diagnosed with T1DM had substantially higher FBG and HbA1c levels than the healthy participants ($P < 0.01$) (Table 2).

Similarly, MDA ($P < 0.01$) and TOS ($P < 0.01$) values were found to be significantly higher in the T1DM group compared to the healthy group. For TAS, it was found to be significantly lower in the T1DM group than in the healthy group ($P < 0.01$). In the case of total thiol, it was found that the statistics of the patient group was significantly higher than that of the control group ($P < 0.05$), and a significant result was obtained on comparison of the native thiol values of the participants ($P < 0.01$). Although it was observed that the disulphide values of the participants were higher in the T1DM group than in the control group, this difference was not found to be statistically significant ($P > 0.05$). Similarly, the native/total thiol ratio revealed no significant change ($P > 0.05$). Comparison of the disulphide/total thiol ratio of the participants showed no statistically significant difference ($P > 0.05$). In the case of disulphide/native thiol ratio of the participants, the value for the T1DM group was found to be significantly lower than the healthy group ($P < 0.05$).

Correlation analysis revealed a moderate and positive relationship between age and BMI, which was statistically significant ($r = 0.586$; $P < 0.01$). Additionally, there was a high positive correlation between the native thiol and disulphide variables ($r = 0.845$; $P < 0.01$). Although the correlation coefficient was calculated between all the other variables in this study, these values were not statistically significant (Table 3).

Discussion and conclusion

Hyperglycaemia or DM, is a recurring metabolic condition. Along with the usual clinical signs and symptoms, DM also causes significant biochemical alterations, such as the generation of non-enzymatic advanced glycation products, protracted oxidative stress and modifications in the activity of the polyol pathway[16]. Age has a major effect on the epidemiology, risk and progression of T1DM. Young people are more affected than adults. Thus, field studies should also consider the effect of age on the disease, and different possibilities should be evaluated. The first is that adult and pediatric pathophysiology are actually distinct. The second is that the immune systems in children are less strong than in adults[17]. T1DM is a common chronic autoimmune disease that is more frequent in children than adults. Significant progress has been made in glucose monitoring and insulin therapy, with advancements resulting from the introduction of continuous glucose monitoring. The present study aimed to determine how children with T1DM change their oxidant/antioxidant status. Several studies have demonstrated that diabetic children experience an increase in OS due to chronic hyperglycaemia[18]. Our findings are consistent with those of earlier studies demonstrating that elevated glucose levels cause excessive oxygen free-radical generation as well as protein and lipid oxidation.

Table 1. Comparison of demographic data of children with type 1 diabetes mellitus (T1DM) and control group

Variables	T1DM ($n = 38$)	Control group ($n = 38$)	P value
Age (years)	13.2 ± 2.8	12.9 ± 2.5	0.705
Height (cm)	155.7 ± 13.6	157.8 ± 14.9	0.508
Weight (kg)	52.2 ± 14.5	57.6 ± 19.3	0.175
BMI (kg/m^2)	21.1 ± 3.3	22.4 ± 4.8	0.168

Data presented as mean ± SD.

In a study by Yesilkaya *et al.*[19] in Turkey, children diagnosed with T1DM were mostly found in the age group of 10–14 years (39.8%). In the present study, the mean age of children included who were diagnosed with T1DM (patient group) was about 13 years. The mean age of the healthy children who participated in this study (control group) was about 12 years. Following these findings were those for the 15–18 age group with 35.7%, the 5–9 age group with 19.1%, and the 0–4 age group with 5.4%. It has been observed that T1DM peaks between the age of 10 and 14 years[19].

In previous studies, diabetic groups were found to have higher MDA levels in serum than healthy groups, which is consistent with our findings[20–26]. In the present study, as in previous studies, it was found that the lipid profile of the patient group, T1DM was found to be lower in comparison to the healthy group. MDA levels, a biomarker of lipid peroxidation in OS, were found to be significantly higher in the T1DM group than in the healthy group. Studies have shown that hyperglycaemia increases lipid peroxidation and decreases antioxidant levels[27]. It was found that high FBS increased lipid peroxidation in the T1DM group. However, no correlation was detected between FBG and MDA levels in the T1DM group. Unlike in the present study, a correlation was observed between MDA and FBG in other studies[28,29].

Thiol and disulphide values of the present study were insignificant compared to other studies[30–32]. Compared to the T1DM group, the levels of native and total thiol in the control group were found to be lower. Only disulphide values were higher in the T1DM group than in the healthy group, but no significance was observed.

This study demonstrates that children with T1DM have considerably higher TOS values than those without the disease, similar to previous studies[33–36]. Based on these results, the oxidant types of children in the T1DM group were found to be high. Thus diabetes is a chronic condition that elevates oxidant levels in the body.

TAS gives the sum of both exogenous and endogenous antioxidants. Thus, a complete picture of antioxidant status is obtained. This method is more important than measuring individual antioxidants because some antioxidants work synergistically to combat oxidative damage caused by free radicals[37]. In the present study, the TAS levels for the T1DM group were considerably lower than the healthy group. Studies have revealed that the total antioxidant status of

Table 2. Comparison of biochemical results of children with T1DM and control group

Variables	T1DM (n = 38)	Control group (n = 38)	P value
FBG (mg/dl)	183.8 ± 98.7	87.1 ± 8.0	0.000**
HbA1c (%)	7.8 ± 1.6	4.7 ± 0.3	0.000**
MDA (nmol/ml)	2.1 ± 0.1	1.0 ± 0.2	0.000**
TOS (μmol H_2O_2 equivalent/l)	13.8 ± 0.7	10.4 ± 0.2	0.000**
TAS (mmol Trolox equivalent/l)	2.0 ± 0.2	2.7 ± 0.1	0.000**
Total thiol (μmol/l)	479.8 ± 133.1	398.2 ± 125.0	0.007*
Native thiol (μmol/l)	215.5 ± 73.3	145.7 ± 47.3	0.000**
Disulphide (μmol/l)	132.1 ± 65.2	126.2 ± 59.7	0.681
Native thiol/total thiol (%)	48.4 ± 18.6	40.2 ± 17.2	0.053
Disulphide/total thiol (%)	25.7 ± 9.3	29.8 ± 8.6	0.053
Disulphide/native thiol (%)	69.3 ± 42.3	91.7 ± 45.7	0.030*

Data presented as mean ± SD. **P < 0.01; *P < 0.05.
FBG, Fasting blood glucose; HbA1c, HaemoglobinA1c; MDA, Malondialdehyde; TOS, Total oxidant status and TAS, Total antioxdiant status.

Table 3. Correlation matrix between variables in children with T1DM

	Age	BMI	FBG	HbA1c	MDA	TOS	TAS	Total thiol	Native thiol	Disulphide
P-value	–	0.5**	0.1	–0.04	–0.2	–0.1	–0.03	–0.1	0.01	–0.1
		–	0.2	0.2	–0.09	–0.002	–0.1	0.07	0.07	0.03
			–	0.2	–0.02	0.05	–0.2	–0.1	–0.2	–0.05
				–	–0.02	0.2	–0.2	0.2	0.3	0.1
					–	–0.2	0.02	–0.06	–0.1	0.002
						–	–0.1	0.3	0.2	0.1
							–	0.02	–0.02	0.04
								–	0.3	0.8**
									–	–0.2
										–

BMI, Body mass index.

the patient group is lower than the healthy group regardless of the type of diabetes[38–40]. The antioxidants involved in the elimination of free radicals in diabetic patients were lower than those in the healthy group.

Contrary to many studies, in this study, no correlation was observed between OS parameters in terms of FBG and HbA1c values in T1DM. This may be because the duration of diabetes, diet and lifestyle of children included in the study differ from those of other studies. However, FBG, HbA1c, MDA and TOS values were higher in the T1DM group than in the healthy group, while TAS values were found to be significantly lower.

In conclusion, our results support the hypothesis that hyperglycemia activates cells and tissue damaged by OS. This study may offer new perspectives on the treatment of T1DM. Although this study determined oxidative damage with various oxidant–antioxidant parameters in children with T1DM, the biochemical mechanisms underlying the disease can be elucidated by conducting more extensive studies in the field of free radicals and oxidants–antioxidants.

Conflict of interest: The authors declare no conflicts of interest.

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