Original Research Article

Lipid peroxidation and antioxidant protection in girls with type 1 diabetes mellitus during reproductive system development

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Abstract

Background and objective: Type 1 diabetes mellitus (T1D) is found worldwide and is regarded as one of the main risks to human health. The objective of this study was to determine the state of lipid peroxidation (LPO) and antioxidant protection in girls with T1D type considering the stages of reproductive system development.

Materials and methods: This study enrolled 56 young girls with T1D and 60 healthy girls (control) matched by age. The study population was divided into 3 age groups: prepubertal, adolescent, and juvenile. The state of LPO and antioxidant system was assessed using the coefficient of oxidative stress that represented the ratio of LPO products to general antioxidative blood activity. Spectrophotometric and fluorometric methods were applied.

Results: The results of our study showed increased conjugated diene (CD) and thiobarbituric acid reactant (TBAR) concentrations as well as a decreased reduced glutathione level in prepubertal girls with T1D. Adolescent girls with T1D had a significantly greater CD level and juvenile girls with T1D had a significantly greater TBAR level and lower α-tocopherol concentration than girls in the control group. The greatest coefficient of oxidative stress (1.16) was observed in the prepubertal period.

Conclusions: The prepubertal period is characterized by the most severe state of lipid peroxidation process—antioxidant protection.

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1. Introduction

Type 1 diabetes mellitus (T1D) is found worldwide and is regarded as one of the main risks to human health [1]. T1D is characterized by varying levels of morbidity in different populations and its prevalence increase in the majority of developed countries during the last 30 years [2,3]. The manifestation and development of T1D in girls often occurs during formation of reproductive system (in Russia from 20% to 50% of patients under 15 years of age) [4]. This pathology may affect the tempo and course of pubertal growth and development, onset of menarche and the violation of menstrual function in girls [5]. Pubertal changes induce glucose metabolism or specific insulin resistance. Recent data has shown that puberty greatly increases the risk of diabetes complications and the management of this transitional age is critically important [6]. It is well known that oxidative stress (OS) is known as a component of molecular and cellular tissue that damage mechanisms in different human diseases [7,8]. The results of many clinical and experimental studies have suggested that lipid peroxidation processes are activated during different stages in different types of T1D, even in their subclinical forms [9]. The existence of hyperglycemia produces increases OS via nonenzymatic glycation, glucose autooxidation and alterations in polyol pathway activity with subsequent influence at the whole organism [10]. Many authors have observed increase OS at the early and late stages of T1D in girls [11]. It is known that certain indices of OS due to the duration of diabetes and the efficacy of glycemic control [12]. Some studies have showed that puberty in girls modulates antioxidant mechanisms of childhood diabetes that may have implications for the treatment and interventions [13]. The aim of the present study was to evaluate the state of lipid peroxidation (LPO) and antioxidant defense (AOD) in patients – girls with T1D considering stages of reproductive system development.

2. Materials and methods

2.1. Study subjects

Parameters of LPO and AOD system were measured in blood samples of 56 young girls with T1D with no clinical diabetic angiopathy and 60 healthy girls (control) matched by age. All of the patients lived in Irkutsk city (East-Siberia). The study population was divided into 3 age groups: prepubertal (7–13 years), adolescent (14–15 years), and juvenile (16–18 years) girls. The criteria for compensation of T1D at young girls were used recommendations International Society for Pediatric and Adolescent Diabetes (ISPAD) Consensus Guidelines, 2000. All groups of patients with T1D were matched for diabetes duration, glycemic control, cholesterol and triacylglycerol levels. There were no significant differences in age and number between the control group and patients with T1D. The baseline characteristics are presented in Table 1. There were no significant differences in diet habits and physical activity between the patients of all groups. All patients with T1D got insulin replacement therapy. Patients with severe somatic pathology and severe diabetic complications (chronic renal failure, macroangiopathy) were excluded out of this study. This study was approved by the Ethic Committee of Scientific Centre of family health and human reproduction problems (Siberian Branch of Russian Academy of Medical Sciences), and all participants provided patients signed written informed consent.

2.2. Study methods

Patients’ blood samples were collected after 12-h overnight fasting, centrifuged for 5 min at 1,500 × g at 4 °C, and erythrocytes were washed three times with NaCl 0.9% (wt/ vol). Aliquots of ethylenediaminetetraacetic acid plasma and washed erythrocytes were used immediately or kept frozen in −40 °C, but not more than one month. Lipid peroxidation, estimated in terms of the concentration of conjugated dienes (CDs) and thiobarbituric acid-reactive substances (TBARS) in plasma of blood. The CDs concentrations were detected on absorbance of plasma heptanes extracts at 232 nm [14]. We used the coefficient of molar absorption (K = 2.2 × 10^5 M^-1 cm^-1) for conversion of absorption units to μmol/L. Thiobarbituric acid reactants (TBARS) levels were detected by fluorometry [15]. The supernatant, prepared as given for determination of enzymes, was mixed with 29 mM 2-thiobarbituric acid (TBA) in 8.75 M acetic acid and heated at 95 °C for 1 h. After cooling, TBARS were extracted to n-butanol and the fluorescence of the organic layer was measured at 515 nm (excitation) and 554 nm (emission). The concentration of TBARS was estimated by referring to the standard 1,1,3,3-tetraethoxypropane and

<table>
<thead>
<tr>
<th>Table 1 – Baseline characteristics of diabetic and control subjects.</th>
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<tbody>
<tr>
<td>Clinical data</td>
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<td></td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Duration of disease, years</td>
</tr>
<tr>
<td>HbA1C, %</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
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</table>

Values are means ± standard deviation.
expressed in μmol/L. Reduced (GSH) and oxidized (GSSG) glutathione levels were detected in hemolysate by fluorometric [16]. The essence of the method lies in the ability to specifically react with GSH α-phthalaldehyde (OPT) at pH 8.0 and the formation of fluorescent product, which can be activated at 350 nm with a peak emission at 420 nm. Determination of GSSG was performed similarly with OPT fluorimetric method, but in a more alkaline environment (pH 12). Also, to prevent oxidation of GSH in a sample GSSG added N-ethylmalienit. Terms of fluorescence detection were identical. The measurements were performed on spectrofluorophotometer Shimadzu RF-1501 (Japan) at 350 nm (excitation) and 420 nm (emission). GSH and GSSG. The concentration expressed in mmol/L. Superoxide dismutase (SOD) activity was measured in erythrocytes with using a commercially available kit (Ransel; Randox Lab, Crumlin, U.K.). α-Tocopherol levels were detected in plasma by fluorimetric [17]. After saponification of the sample with KOH in the presence of ascorbic acid, α-tocopherol was extracted to n-hexane. The fluorescence of the organic layer was measured at 294 nm (excitation) and 330 nm (emission) on Spectrofluorophotometer Shimadzu RF-1501 (Japan). Content α-tocopherol an expressed in μmol/L. The hemoglobin concentration in milligrams per milliliters was determined by the cyanmethemoglobin method [18]. Cholesterol and triacylglycerols levels were measured enzymatically using commercial kits “Bio Systems” (Spain).

Also we calculated the coefficient of oxidative stress (COS). This formula is the ratio of primary (CDs), end products (TBARs) of lipid peroxidation levels to the activity system of AOD (SOD activity, GSH, GSSG, α-tocopherol levels). The value of the corresponding control group was taken as 1.

2.3. Statistical analysis

Visual graphical method and the Kolmogorov–Smirnov test with the Lilliefors and Shapiro–Wilk corrections were used to test the quantitative traits for normal distribution. The Mann–Whitney test (U test) was used for analysis of between group differences for independent samples. The Fisher exact test was used to compare for qualitative values and frequencies the different groups of patients. The data are shown as the means and standard deviation of means. The critical statistical significance level was 5% (0.05). Statistical analysis was performed by STATISTICA 6.1 software (Stat-Soft Inc., USA).

3. Results

CD and TBAR levels were significantly elevated in prepubertal girls in comparison to the control group (P < 0.05) (Table 2). We observed that adolescent girls had an increased CDs level compared with the control group (P < 0.001). The plasma TBARs level was significantly increased in juvenile girls compared to the control group (P < 0.01). SOD activity in red blood cells was similar to the corresponding control group (Table 3). The GSH concentration in erythrocytes was significantly lower in the prepubertal girls than the control group (P < 0.05). There were no significantly differences in the GSH level in the other groups. GSSG concentrations in erythrocytes were also similar in all groups with T1D compared to the control group. There were no significantly differences in plasma α-tocopherol concentrations between prepubertal and adolescent girls (Table 3). However, the α-tocopherol level was significantly lower in juvenile girls with T1D than in the control group (P < 0.05). The greatest coefficient of oxidative stress was recorded in prepubertal girls as compared with adolescent and juvenile girls (1.16 vs. 0.70 and 0.72, respectively, P < 0.05) (Fig. 1).

4. Discussion

It is well known that OS is characterized by redox imbalance with isolated or combined changes in concentration of pro- or antioxidative substances. The results of our research demonstrate significantly increase CDs levels in prepubertal and adolescent girls with T1D compare to control group. Several studies have reported significant increase CDs concentrations in patients with T1D [19]. CDs are a group of peroxide and radical compounds that are developed by the separation of hydrogen from molecules of polyunsaturated fatty acid during the first stage of LPO. In doing so, olefinic links of natural fatty acids migrate to conjugated ones with formation of π-systems extended in 5 carbons. As a result, CDs become highly reactive in regards to cell substructures. For instance, increase CDs generation induces abnormal permeability of cell membranes for ions, electrolytes and macromolecules. This effect underlies many of the pathogenic mechanisms including diabetic vascular disorders are induced by LPO during continuous hyperglycemia [20]. Moreover, high concentration of lipid hydroperoxides in T1D, which also contain conjugated links, decreases as a result of intensive insulin treatment [21]. TBARs are lipid peroxidation products appearing at the late stage of LPO. In our study its level was higher at prepubertal and juvenile girls with T1D than in corresponding control groups. Some studies have reported significant increase LPO by estimation of levels TBAR in diabetic children and adolescents [22]. TBARs compounds can develop nonenzymed modification of cells components in reactions with amino groups of intracellular proteins, nitrogenous bases of deoxyribonucleic acid and the others [23]. It is suggested that high TBARs

### Table 2 – LPO values in patients with T1D and control group and related to stages of reproductive system development.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Stage</th>
<th>Prepubertal</th>
<th>Adolescent</th>
<th>Juvenile</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDs, μmol/L</td>
<td>T1D</td>
<td>Control group</td>
<td>1.47 ± 0.73</td>
<td>1.63 ± 0.84</td>
<td>1.37 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>P</td>
<td>0.90 ± 0.36</td>
<td>1.02 ± 0.52</td>
<td>1.32 ± 0.76</td>
</tr>
<tr>
<td>TBARs, μmol/L</td>
<td>T1D</td>
<td>Control group</td>
<td>1.96 ± 0.79</td>
<td>1.99 ± 0.73</td>
<td>2.39 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>P</td>
<td>1.38 ± 0.37</td>
<td>1.86 ± 0.57</td>
<td>1.39 ± 0.58</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation.
' Significant difference between control and group with T1D.
concentration can cause carbonyl modification of hemoglobin. Moreover, TBARs modify sulfhydryl groups in some redox-sensitive molecules leading to their impairment [24]. Thus, elevation of CDs and TBARs levels at girls with diabetes compared to corresponding control group confirms activation of prooxidative processes that occurs in T1D.

We did not observe increased SOD activity in diabetic girls that has been reported by several authors [25]. The highest SOD activity in red blood cells of diabetes was interpreted by the authors as a compensatory activation mechanism due to increase superoxide radical generation. Our results showed that erythrocyte GSH was decreased at prepubertal girls with T1D in compare to the control group. This is consistent with the findings of other investigators in T1D adolescents suggesting that GSH metabolism is altered in T1D. It is well known that the GSH level is a sensitive indicator of redox trend of metabolism [26] especially in a view of the fact that tissue glutathione concentration is hundreds times higher than other antioxidants [27]. Therefore, changes in GSH redox status could be considered as particularly sensitive indicator of oxidative stress. Thus in this study, a decrease in the GSH level was documented in prepubertal girls with diabetes may disturb antioxidant defenses. Increase oxygen free radical activity may be acceleration of the oxidative damage already present in this stage. Plasma α-tocopherol levels at juvenile girls were significantly lower by 29% than in the control group.

It is known that extracellular fluids contain nonenzymatic antioxidants that may delay or inhibit the oxidative processes [28]. Tocopherol is the primary in vivo chain-breaking, liposoluble antioxidant in human serum and particularly effective in lipid peroxidation inhibition [10]. Therefore relative decrease in plasma – tocopherol may be attributed to its consumption while scavenging free radicals in biomembranes or lipoproteins. For a more accurate diagnosis of oxidative stress in the study groups we used the coefficient that represented ratio of lipid peroxidation products to antioxidant defense components. This coefficient was significantly higher at prepubertal girls than in other groups. This fact may be explained initial intensive hormonal changes in this age. Moreover, this group had the manifestation of diabetes at early age than adolescent and juvenile girls. It is known that the early onset of diabetes leads to its severe course [29].

5. Conclusions

In summary, our results showed that an imbalance in the oxidant/antioxidant ratio is already present at the prepubertal girls with T1D. Diabetes induced oxidative damage increases from juvenile period. These results suggest that puberty modulates antioxidant mechanisms in T1D which may have implications for therapy and intervention.

Conflict of interest

The authors state no conflict of interest.

Acknowledgment

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References