Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/medici

Original Research Article

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

*Lubov I. Kolesnikova, Marina A. Darenskaya, Natalia V. Semenova *, Lyudmila A. Grebenkina, Larisa V. Suturina, Marya I. Dolgikh, Svetlana V. Gnusina*

Department of Reproductive Pathophysiology, Scientific Centre of Family Health and Human Reproduction Problems, Siberian Branch, Russian Academy of Medical Sciences, Irkutsk, Russian Federation

ARTICLE INFO

Article history:
Received 3 March 2014
Accepted 16 January 2015
Available online 31 March 2015

Keywords:
Type 1 diabetes mellitus
Oxidative stress
Antioxidative defense
Girls

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.

* *Corresponding author at*: Timiryasev Str. 16, 664003 Irkutsk, Russian Federation.
E-mail address: natkor_84@mail.ru (N.V. Semenova).

Peer review under the responsibility of the Lithuanian University of Health Sciences.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.medici.2015.01.009

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 autoxidation 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 triacylglycerole 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 $1.500 \times g$ at 4 °C, and erythrocytes were washed three times with NaCI 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 ($K = 2.2 \times 10^5\ M^{-1}\ C^{-1}$) 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-tetraetoxypropane and

Table 1 – Baseline characteristics of diabetic and control subjects.

Clinical data	Stages					
	Prepubertal		Adolescent		Juvenile	
	T1D ($n = 13$)	Control group ($n = 13$)	T1D ($n = 30$)	Control group ($n = 24$)	T1D ($n = 17$)	Control group ($n = 19$)
Age, years	10.69 ± 2.10	11.08 ± 1.85	14.90 ± 0.80	14.63 ± 0.77	17.59 ± 1.50	17.16 ± 1.21
Duration of disease, years	3.15 ± 2.4	–	3.54 ± 1.45	–	3.21 ± 1.65	–
Hb A_{1C}, %	9.77 ± 2.32	–	9.58 ± 2.22	–	9.74 ± 2.61	–
Cholesterol (mmol/L)	4.75 ± 1.30	4.30 ± 1.32	4.44 ± 1.19	4.25 ± 1.24	4.80 ± 1.12	4.35 ± 0.91
Triacylglycerols (mmol/L)	0.82 ± 0.36	0.64 ± 0.22	0.72 ± 0.48	0.56 ± 0.21	0.74 ± 0.33	–

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 o-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 fluorometric [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 compare 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).

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

Parameters	Groups	Stage		
		Prepubertal	Adolescent	Juvenile
CDs, μmol/L	T1D	1.47 ± 0.73	1.63 ± 0.84	1.37 ± 0.68
	Control group	0.90 ± 0.36*	1.02 ± 0.52*	1.32 ± 0.76
	P	0.021	0.004	0.839
TBARs, μmol/L	T1D	1.96 ± 0.79	1.99 ± 0.73	2.39 ± 1.06
	Control group	1.38 ± 0.37*	1.86 ± 0.57	1.39 ± 0.58*
	P	0.031	0.481	0.004

Values are means ± standard deviation.
* Significant difference between control and group with T1D.

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 nonenzymated 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 3 – AOD values in patients with T1D and control group and related to stages of reproductive system development.

Parameters	Groups	Stage		
		Prepubertal	Adolescent	Juvenile
SOD, U/mg Hb	T1D	1.57 ± 0.20	1.54 ± 0.33	1.51 ± 0.26
	Control group	1.53 ± 0.20	1.51 ± 0.11	1.51 ± 0.13
	P	0.682	0.770	0.899
GSH, mmol/L	T1D	2.27 ± 0.62	2.63 ± 0.75	2.91 ± 0.75
	Control group	2.88 ± 0.73*	2.80 ± 0.56	2.90 ± 0.43
	P	0.042	0.462	0.938
GSSG, mmol/L	T1D	1.46 ± 0.35	1.57 ± 0.50	1.59 ± 0.53
	Control group	1.59 ± 0.41	1.53 ± 0.37	1.84 ± 0.47
	P	0.435	0.783	0.176
α-Tocopherol, μmol/L	T1D	8.66 ± 3.06	6.36 ± 2.89	5.21 ± 2.99
	Control group	7.90 ± 1.70	7.62 ± 2.63	7.31 ± 2.84*
	P	0.439	0.120	0.046

Values are means ± standard deviation.
* Significant difference between the control and T1D groups.

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, lipid-soluble 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].

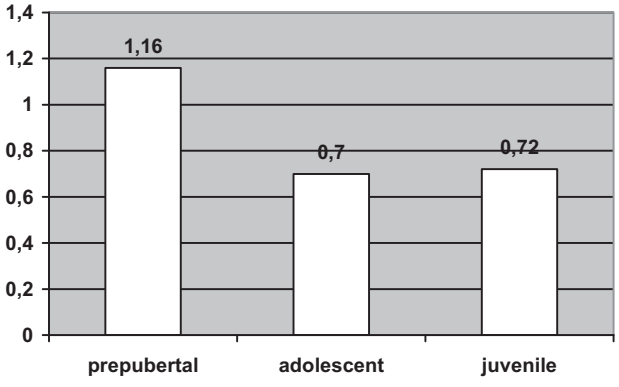

Fig. 1 – Coefficient of oxidative stress (COS).

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

This research was supported by grants from President of Russian Federation (Nos. 494.2012.7; 5646.2014.7).

REFERENCES

[1] Rewers M. Challenges in diagnosing type 1 diabetes in different populations. Diabetes Metab J 2012;36(2):90–7.
[2] Tuomilehto J. The emerging global epidemic of type 1 diabetes. Curr Diabetes Rep 2013;13(6):795–804.
[3] Kolesnikova LI, Darenskaya MA, Vlasov BY, Grebenkina LA, Semenova NV, Dolgikh MI, et al. Oxidative stress in mongoloids and Caucasians with type 1 diabetes. J Diabetes Metab 2014;5(376):1–4.
[4] Dedov II. Diabetes mellitus – a dangerous treat to the mankind. Vestn Russ Acad Med Sci 2012;1:7–13.

[5] Raine JE, Donaldson MD, Gregory JW, Van-Vliet G. Practical endocrinology and diabetes in children. John Wiley & Sons; 2011.
[6] Codner E. The gonadal effects of diabetes. Int J Pediatr Endocrinol 2013;2013(Suppl. 1):O9.
[7] Kolesnikova LI, Darenskaya MA, Grebenkina LA, Labygina AV, Suturina LV, Dolgikh MI, et al. Activity of lipid peroxidation in infertile women from different populations. Bull Exp Biol Med 2012;154(2):203–5.
[8] Kolesnikova LI, Madaeva IM, Semenova NV, Vlasov BY, Grebenkina LA, Darenskaya MA, et al. Antioxidant potential of the blood in men with obstructive sleep breathing disorders. Bull Exp Biol Med 2013;154(6):731–3.
[9] Takayanagi R, Inoguchi T, Ohnaka K. Clinical and experimental evidence for oxidative stress as an exacerbating factor of diabetes mellitus. J Clin Biochem Nutr 2011;48(1):72–7.
[10] Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 2011;50(5):567–75.
[11] Martin-Gallan P, Carrascosa A, Gussinye M, Dominguez C. Estimation of lipoperoxidative damage and antioxidant status in diabetic children relationship with individual antioxidant. Free Radic Res 2005;39:933–42.
[12] Hsu WT, Tsai LY, Lin SK, Hsiao JK, Chen BH. Effects of diabetes duration and glycemic control on free radicals in children with type 1 diabetes mellitus. Ann Clin Lab Sci 2006;36(2):174–8.
[13] Gleisner A, Martinez L, Pino R, Rojas IG, Martinez A, Asenjo S, et al. Oxidative stress markers in plasma and urine of prepubertal patients with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2006;19(8):995–1000.
[14] Corongiu FP, Banni S. Detection of conjugated dienes by second derivative ultraviolet spectrophotometry. Methods Enzymol 1994;233:303–10.
[15] Yagy KA. Assay for serum lipid peroxide level and its clinical significance. In: Yagi K, editor. Lipid peroxides in biology and medicine. New York: Academic Press; 1982. p. 223–42.
[16] Hisin PJ, Hilf R. Fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 1976;74:214–26.
[17] Taylor SL, Lamden MP, Tappel AL. Sensitive fluorometric method for tissue tocopherol analysis. Lipids 1976;11(7):530–8.
[18] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[19] Pan HZ, Zhang L, Guo MY, Sui H, Li H, Wu WH, et al. The oxidative stress status in diabetes mellitus and diabetic nephropathy. Acta Diabetol 2010;47(1):71–6.
[20] Kaviarasan K, Arjunan MM, Pugalendi KV. Lipid profile, oxidant–antioxidant status and glycoprotein components in hyperlipidemic patients with/without diabetes. Clin Chim Acta 2005;362:49–56.
[21] Wang X, Tao L, Hai CX. Redox-regulating role of insulin: the essence of insulin effect. Mol Cell Endocrinol 2012;349 (2):111–27.
[22] Lin CC, Huang HH, Hu CW, Chen BH, Chong IW, Chao YY, et al. Trace elements, oxidative stress and glycemic control in young people with type 1 diabetes mellitus. J Trace Elem Med Biol 2014;28(1):18–22.
[23] Repetto M, Semprine J, Boveris A. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. Lipid Peroxid 2012;1–28.
[24] de Assis AM, Rech A, Longoni A, Rotta LN, Denardin CC, Pasquali MA, et al. Ω3-Polyunsaturated fatty acids prevent lipoperoxidation, modulate antioxidant enzymes, and reduce lipid content but do not alter glycogen metabolism in the livers of diabetic rats fed on a high fat thermolyzed diet. Mol Cell Biochem 2012;361(1–2):151–60.
[25] Salmonowicz B, Krzystek-Korpacka M, Noczynska A. Trace elements, magnesium, and the efficacy of antioxidant systems in children with type 1 diabetes mellitus and in their siblings. Adv Clin Exp Med 2014;23(2):259–68.
[26] Enns GM, Moore T, Le A, Atkuri K, Shah MK, Cusmano-Ozog K, et al. Degree of glutathione deficiency and redox imbalance depend on subtype of mitochondrial disease and clinical status. PLOS ONE 2014;9(6):e100001.
[27] Stevensona D, Wokosinb D, Girkinc J, Granta MH. Measurement of the intracellular distribution of reduced glutathione in cultured rat hepatocytes using monochlorobimane and confocal laser scanning microscopy. Toxicol In Vitro 2002;15:609–19.
[28] Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J 2012;12(1):5.
[29] Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev 2013;93(1):137–88.