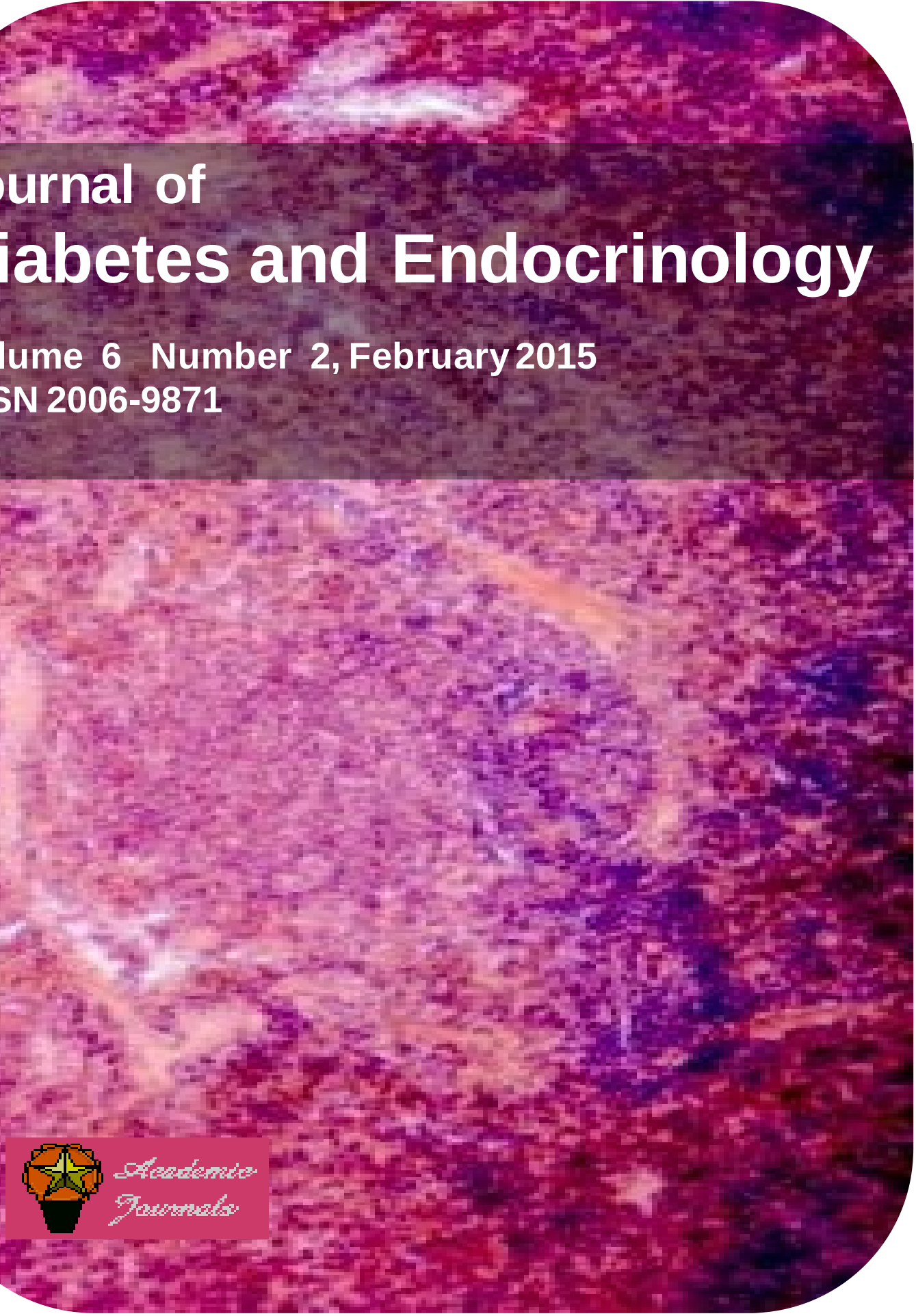
Journal of Diabetes and Endocrinology

Volume 6 Number 2, February 2015
ISSN 2006-9871



Academic Journals

ABOUT JDE

The **Journal of Diabetes and Endocrinology (JDE)** is published monthly (one volume per year) by Academic Journals.

Journal of Diabetes and Endocrinology (JDE) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as steroid hormones, clinical chemistry and biochemistry, neuroendocrinology, hypoglycemia in diabetes etc.
The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JDE are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jde@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Diabetes and Endocrinology will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editorial Board

Journal of Diabetes and Endocrinology

Table of Content: Volume 6 Number 2 February 2015

ARTICLES

Research Articles

academicJournals

Vol. 6(2), pp. 5-11, February 2015
DOI: 10.5897/JDE2014.0083
Article Number: FC4930850423
ISSN 2141-2685

http://www.academicjournals.org/JDE

Journal of Diabetes and Endocrinology

Full Length Research Paper

Elevation of oxidative stress markers in Type 1 diabetic children

Amina Boudghene Stambouli-Guerriche[1], Nassima Mokhtari-Soulimane[1]*, Hafida Merzouk[1], Sid-Ahmed Merzouk[2] and Ahmed Salih Bendedouche[3]

[1]Laboratory of Physiology and Biochemistry of Nutrition, Department of Biology, University of Tlemcen, Algeria.
[2]Department of Technical Sciences, Faculty of Engineering, University of Tlemcen, Algeria.
[3]Pediatrics Department, University-Hospital Centre, Tlemcen, Algeria.

Received 25 November, 2014; Accepted 21 January, 2015

Children's diabetes is represented by the Type 1 diabetes mellitus (T1DM). In T1DM, the persistence of hyperglycemia has been reported to cause increased production of oxygen free radicals through glucose autooxidation and nonenzymatic glycation. The aim of this study was to evaluate markers of oxidant/antioxidant status in diabetic children of Western Algeria. This study included 40 children with T1DM with mean age of 7.5 ± 1.7 years and 40 healthy age and sex matched controls. They were subjected to assessment of indicative parameters of lipoperoxidation, protein oxidation, changes in the status of antioxidant defense systems, plasma oxygen radical absorbance capacity (ORAC), glycated hemoglobin (HbA1c), total cholesterol and triglycerides. Malondialdehyde (MDA) and carbonyl proteins levels in plasma were significantly higher (4.03 ± 0.39 versus 2.53 ± 0.4 µmol/L, 5.03 ± 0.57 versus 3 ± 0.38 nmol/mg protein, respectively; P < 0.001) and a significant reduction in plasma total antioxidant capacity and vitamin C was observed in diabetic children than the controls (1.55 ± 0.28 versus 2.5 ± 0.23 AU, 37.58 ± 5.76 versus 48.8 ± 4.47 µmol/L, respectively; P < 0.001). Erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities were significantly higher (520 ± 40.42 versus 392.7 ± 42.66 U/g hemoglobin, 71.08 ± 5.18 versus 56.6 ± 2.84 U/g hemoglobin, respectively; P < 0.001), whereas erythrocyte glutathione reductase (GSH) reduced significantly (34.98 ± 2.34 versus 42.68 ± 3.03 U/g hemoglobin, respectively; P < 0.001) in diabetic children than the control subject. The present finding suggested that young diabetic patients were susceptible to oxidative stress. Appropriate support for enhancing antioxidant supply in these patients may help prevent complications due to oxidative injury.

Key words: Children, oxidative stress, Type 1 diabetes mellitus.

INTRODUCTION

Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical

*Corresponding author. E-mail: nassima_amel@yahoo.fr. Tel: +213555879855.

toxicity (Halliwell and Whiteman, 2004; Sies, 1991). Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases (Dalle-Donne et al., 2006; Halliwell and Gutteridge, 1999).

Children's diabetes also called Type 1 diabetes mellitus (T1DM) today, was formerly represented by the insulin dependent diabetes. It is a chronic autoimmune disease caused by the specific destruction of pancreatic β cells (Maahs and Rewers, 2006). Diabetes represents one of the most common diseases in school-aged children (Linder, 2014). The increased incidence of the disease in toddlers suggests the influence of the environment of which dietary factors, enterovirus infections, but not vaccines, have been implicated. However, no single factor can be incriminated in the rapid increase of incidence observed in young children (Penno et al., 2013). Extensive studies have focused on the role of the immune system in the development of T1DM, from the initiation of disease to eventual β cells destruction (Beyan et al., 2003; Thomas and Kay, 2000). Some studies have shown that oxidative stress leads to the destruction of pancreatic islets, either by necrosis or apoptosis of β cells (Bonnefont-Rousselot, 2002). Indeed, diabetic patients are exposed to increased oxidative stress due to several mechanisms, including glucose autooxidation and non-enzymatic protein glycation (Sakurai and Tsuchiya, 1988; Wolff, 1993). Nonenzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Shiff bases and more stable Amadori products are formed (Vlassara, 1994). Advanced glycation end products (AGEs) are then formed through oxidative reactions and cause irreversible chemical modifications of proteins. Chronic hyperglycemia also leads to activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH)-dependent aldose reductase (polyol pathway), which diminishes the NADPH available for glutathione reductase (GSH); consequently, the ratio of reduced to oxidized glutathione decreases (Ou et al., 1996).

A variety of natural antioxidants exist to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is enzymatic (intracellular), which include superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT). In addition to enzymatic antioxidants, the major natural antioxidants, most of them derived from natural sources by dietary intake are vitamin A, vitamin C, vitamin E and carotenoids. Also, numerous small molecules are synthesized or produced within the body that has antioxidant capacity (Azen et al., 1996; Halliwell and Gutteridge, 1999; Heistad, 2006; Maritim et al., 2003). These non-enzymatic antioxidants act as terminators of free radicals' chain reactions caused by lipid peroxidation (Halliwell and Gutteridge, 1999).

As both free-radical production and antioxidant defenses may be disturbed in diabetes (Lyons, 1991), it has been suggested that oxidative stress may be partly responsible for the development of diabetic complications (Baynes, 1991). Consistent with this, oxidative stress has been implicated in the pathogenesis of T1DM in several studies (Jain, 1989; Sato et al., 1979). Increased levels of lipid peroxidation products and altered antioxidative enzyme activity were also reported in type 2 diabetes mellitus (T2 DM) (Kaji et al., 1985).

To our knowledge, there are a few data regarding the relationship between the diabetes in children and oxidant/antioxidant status. It was, therefore, thought worthwhile to undertake a study in order to evaluate markers of oxidant/antioxidant status in diabetic children in Western Algeria.

METHODOLOGY

Patients

This cross-sectional study was conducted at the Department of Pediatrics of the Hospital of Tlemcen, Algeria, from April 2011 to February 2012. It included 40 patients with T1DM diagnosed in accordance with the criteria for classification and diagnosis of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997). They were 22 males and 18 females. Their ages ranged from 5 to 10 years with a mean age of 7.5 ± 1.77 years. Forty age and sex matched healthy individuals were included as the control group. They were 21 males and 19 females. Their age ranged from 6 to 10 years with mean age of 7.7 ± 1.33 years. Most diabetic children in this study present with severe symptoms, very high blood glucose levels, marked glycosuria, and ketonuria. The diagnosis is confirmed without delay by blood glucose measurements, and treatment is initiated immediately, often as a life-saving measure.

Care was taken to ensure the parity of the subjects. The characteristics of both groups are as shown in Table 1. An informed consent has been completed and signed by the parents of all subjects and the study was approved by the Scientific Committee on Research Involving Human Subjects of Tlemcen Hospital.

Blood samples

Fasting venous blood samples were collected in heparinized tubes, centrifuged and plasma was separated for glucose, vitamin C, the oxygen radical absorbance capacity (ORAC), malondialdehyde (MDA) and carbonyl proteins determinations. The remaining erythrocytes were washed three times in isotonic saline, hemolysed by the addition of cold distilled water (1/4) and the cell debris was removed by centrifugation (2000 g, 15 min). The hemolysates were assayed for antioxidant enzyme activities.

Biochemical determination

Plasma glucose was determined by glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA, USA). Markers of diabetes control were evaluated on regular medical check-ups of diabetic children; they were represented by the determination of glycated hemoglobin (HbA1c) using isolab column chromatography (Kaplan et al., 1982).

Lipid determination

Plasma triglyceride and total cholesterol contents were determined by enzymatic methods (Kits from Sigma).

Scavenging capacity of plasma

Plasma ORAC was measured by a fluorimetric method (Cao et al., 1993). A fluorescent protein, allophycocyanin (APC) was used in this assay (Courderot-Masuyer et al., 2000). ORAC employs the oxidative loss of the intrinsic fluorescence of APC. APC fluorescence decay shows a lag or retardation in the presence of antioxidants, related to the antioxidant capacity of the sample. The reaction mixture (2 ml) contained a final concentration of 37.5 nmol/L APC in 75mmol/L phosphate buffer (pH 7.0) at 37°C in the absence (blank) or presence of 20 µl of trolox (1 µmol/L) or plasma, respectively. The reaction was initiated by the introduction of 9 µmol/L of $CuSO_4$ and 0.3% H_2O_2 as redox catalysts. This reaction was followed spectrophotometrically by the decrease in fluorescence at 651 nm emission and 598 nm excitation, using a spectrofluorometer SFM25 Kontron. Trolox was used as a reference antioxidant for calculating the ORAC values, with one ORAC unit defined as the net protection area provided by 1 µmol/L final concentration of trolox. ORAC value of the samples was calculated as:

ORAC ((A sample – A blank)/(A trolox – A blank)),

where A refers to the area under the quenching curve of APC.

Determinations of plasmatic levels of vitamin C

Vitamin C levels were determined in plasma using a biochemical method (Roe and Kuether, 1943). After protein precipitation with 10% trichloroacetic acid and centrifugation, the supernatant (500 µl) was mixed with 100 µl sulfuric acid (9 N) containing 30 mg/ml dinitrophenylhydrazine, 4 mg/ml thiourea and 0.5 mg/ml copper sulfate and incubated at 37°C for 3 h. Following the addition of 750 µl of 65% (v/v) sulfuric acid, the absorbency was recorded at 520 nm.

Determination of plasma MDA

The MDA level, a marker of lipid peroxidation, was determined in plasma by the reaction of MDA with thiobarbituric acid at 95°C (Ohkawa et al., 1979).

Determination of plasma carbonyl proteins

Plasma carbonyl proteins (marker of protein oxidation) were evaluated following the 2,4-Dinitrophe- nylhydrazine assay (Levine et al., 1990).

Determinations of erythrocyte antioxidant enzyme activities

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by the NADPH oxidation procedure (Elstner et al., 1983), and expressed as units of SOD per g hemoglobin. CAT (EC 1.11.1.6) activity was measured by spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm (Aebi et al., 1974). Enzyme activity was expressed as U/g hemoglobin. Glutathione reductase (GSSG-Red, EC 1.6.4.2) activity was determined by measuring the rate of NADPH oxidation in the presence of oxidized glutathione (Goldberg and Spooner, 1992). The unit of enzyme activity was defined as the amount of enzyme which oxidized 1 mmol of NADPH/min.

Statistical analysis

Values are presented as means ± standard deviation (SD). Statistical analysis of the data was carried out using STATISTICA (version 4.1, Statsoft, Tulsa, OK). The significance of the differences between two groups was determined by Student's t-test. Correlations between parameters were performed by Pearson coefficient. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Clinical and biochemical parameters

The results showed no significant difference in the mean age, body mass index (BMI) between diabetic children with their controls (Table 1).

As expected, fasting glucose and HbA1c were significantly higher in diabetic children compared with controls (Table 1). Diabetic patients demonstrated significantly higher plasma levels of total cholesterol, and triglycerides compared with their controls (Table 1).

Markers of oxidative stress

Plasma ORAC and vitamin C were statistically lower in diabetic children when compared with controls; while MDA and carbonyl proteins were significantly higher in diabetic children when compared with controls (Table 2). The erythrocyte antioxidant enzyme activity of CAT and SOD were significantly higher in diabetic children when compared with controls; however, GSH was significantly lower in diabetic children (Table 3).

Correlations between SOD and oxidative stress parameters

Table 4 showed correlation coefficients between SOD and oxidative stress biomarkers in the diabetic children. In the diabetic children, SOD activity was positively correlated with carbonyl proteins ($p < 0.001$), ORAC ($p < 0.01$), vitamin C ($p < 0.001$), and GSH ($p < 0.05$). In the control group, correlations between SOD and these parameters were not significant (results not shown).

DISCUSSION

The present study examined the changes in oxidant/antioxidant status in a children suffering from T1DM. Several studies have shown that chronic hyperglycemia induces an increase in oxidative stress in

Table 1. Clinical and biochemical characteristics of diabetic and control groups.

Characteristic	Control	Diabetic	P
Number	40	40	-
Age (years)	7.7 ± 1.33	7.5 ± 1.77	NS
BMI (kg/m^2)	18.61 ± 1	18.81 ± 1.11	NS
Diabetes duration (years)	-	2.84 ± 0.76	-
Fasting glucose (mmol/L)	4.78 ± 0.56	9.83 ± 2.22	<0.001
Glycated hemoglobin (%)	5.7 ± 0.67	10.14 ± 1.6	<0.001
Total cholesterol (mmol/L)	3.66 ± 0.41	4.07 ± 0.59	NS
Triglycerides (mmol/L)	1.18 ± 0.10	1.36 ± 0.17	<0.01

Values are presented as means ± standard deviations (SD). BMI: Body mass index (weight/height^2). NS: Not significant.

Table 2. Serum oxidative stress markers in diabetic and control group.

Makers	Control	Diabetic	P
ORAC (Arbitrary units)	2.50 ± 0.23	1.55 ± 0.28	<0.001
Vitamin C (µmol/L)	48.8 ± 4.47	37.58 ± 5.76	<0.001
MDA (µmol/l)	2.53 ± 0.40	4.03 ± 0.39	<0.001
Carbonyl proteins (nmol/mg protein)	3.00 ± 0.38	5.03 ± 0.57	<0.001

Values are presented as means ± standard deviations (SD). ORAC: Plasma oxygen radical absorbance capacity; MDA: malondialdehyde

Table 3. Erythrocyte antioxidant enzyme activities in diabetic and control group.

Enzyme activity	Group 1	Group 2	P
SOD (U/g hemoglobin)	392.7 ± 42.66	520 ± 40.42	<0.001
CAT (U/g hemoglobin)	56.60 ± 2.84	71.08 ± 5.18	<0.001
GSH (U/g hemoglobin)	42.68 ± 3.03	34.98 ± 2.34	<0.001

Values are presented as means ± standard deviations (SD). SOD: Superoxide dismutase; CAT: catalase; GSH: reduced glutathione.

Table 4. Correlation coefficients (Pearson's) between SOD and oxidative stress parameters in diabetic children.

Parameter	r
MDA (µmol/L)	-0.16
Carbonyl proteins (nmol/mg protein)	0.42***
ORAC (Arbitrary units)	0.40**
Vitamin C (µmol/L)	0.47***
GSH (U/g hemoglobin)	0.34*
CAT (U/g hemoglobin)	0.22

Values represent correlation coefficients (r). MDA: Malondialdehyde; ORAC: plasma oxygen radical absorbance capacity; GSH: reduced glutathione; CAT: catalase. Statistically significant: *P < 0.05; **P < 0.01; ***P < 0.001.

diabetic children (Dominguez et al., 1998; Lin et al., 2014; Mishra and Singh, 2013). Our results are in accordance with those of previous findings which show that increased glucose level induces overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation.

Indeed, the plasma concentration of MDA which is a final product of the peroxidation of polyunsaturated fatty acids was increased in patients compared with controls. These results are in agreement with previous studies (Lin et al., 2014; Mishra and Singh, 2013; Varvarovská et al., 2003). Another study demonstrated the elevated concentrations of plasma MDA, 8 days after clinical onset of diabetes when metabolic control had returned to normal; this suggests that oxygen free radicals may already have exerted their cytotoxic effects in the early clinical stage of the disease (Dominguez et al., 1998). Furthermore, in children and adolescents with T1DM, MDA levels continued to rise over the course of the disease, indicating overproduction of free radicals and leading to lipid peroxidation and cell oxidative injury, which is considered by some authors to be related to the development of diabetic complications (Velazquez et al., 1991; Wolff, 1994).

It is important to note that glycemic control plays an important role in peroxidation of fatty acids (Wierusz-Wysocka et al., 1995) and the well-controlled diabetic patients (HbA1c<6.5%) demonstrate a lower level of lipid peroxidation markers (Griesmacher et al., 1995; Jain and McVie, 1999; Vantyghem et al., 2000). Since the mean HbA1c in the diabetic children was >8%, therefore this can explain the increase in MDA in this study. However, we did not find a statistically significant correlation between any parameter of oxidative stress and diabetes control; similar findings were presented by Varvarokská et al. (2003).

The identification of oxidation proteins was made by assay of carbonyl proteins, whose concentration increased in plasma of diabetic children when compared with control; these results are in agreement with previous studies (Dominguez et al., 1998; Hernández-Marco et al., 2009). Carbonyl group formation is considered an early and stable marker for protein oxidation. Oxidized proteins constitute a substantial fraction of the catalytically inactive or less active forms of enzymes, which may have direct metabolic consequences (Jain and McVie, 1999; Velazquez et al., 1991).

Elevation in pro-oxidant species and peroxidation of lipids and proteins observed in diabetic patients was associated with increase of antioxidant erythrocyte of SOD and CAT activity; we can presume that this increase could be interpreted as a positive feedback mechanism that reflects a favorable response of the organism to oxidative stress. These results are in agreement with previous studies (Dominguez et al., 1998; Zivić, et al., 2008).

The increase in erythrocyte SOD activity found in patients could protect against the elevation of the superoxide anion. In fact, SOD, catalyzing the decomposition of the superoxide anion to hydrogen peroxide (H_2O_2), prevents against eventual generation of free radicals (Yu, 1994). Superoxide radicals are converted by SOD in H_2O_2 which are destroyed by CAT. This process may result in lipid peroxidation if H_2O_2 is not decomposed immediately (Gumsulu et al., 2002).

The SOD level correlated positively with the carbonyl proteins which suggest that this enzyme is susceptible to glycation and may have its activity inhibited through blockage of the active site itself or by structural alteration which in turn affects the active site. The SOD from the plasma has been shown to be glycated *in vivo*; the proportion of glycated SOD being considerably higher in diabetic patients (Adachi et al., 1991).

GSH is a ubiquitous tripeptide that presents in red cells and participates in glutathione peroxidase (GPx) reaction. When H_2O_2 is detoxified by GPx; the GSH is simultaneously converted to oxidized glutathione (GSSG).

In some studies (Likidlilid et al., 2007; Mishra and Singh, 2013; Varvarovská et al., 2003), the authors found that GSH levels in T1DM patients were significantly lower than that in the same age-matched control subjects. These results are in good agreement with results found in this study.

As already mentioned, GSH serves as an essential cofactor for the enzyme GPx and GSSG during the enzyme processes. Thus, increases in GPx activities imply higher consumption of GSH. Other mechanisms that may explain the GSH reductions in diabetes are that the GSH is regenerated by the enzyme GSH, using reducing equivalents from NADPH. The NADPH is generated in red blood cells through the pentose phosphate pathway, which is stimulated by insulin (Wierusz-Wysocka et al., 1995), and in T1DM, NADPH production may be sluggish, probably resulting in lowered GSH activity and reduced GSH recycle.

It was found out that there was a positive correlation between carbonyl proteins and GSH, suggesting that enhanced oxidative stress in diabetes may result in increased protein glutathionylation, having an adverse effect on cellular glutathione levels (Livingstone and Davis, 2007).

The present study has also demonstrated a significantly lower plasma ascorbate in diabetic children compared with their controls; these results are in agreement with previous studies (Ramakrishna and Jailkhani, 2007).

Because of the relative difficulty in measuring each antioxidant separately, some assays have been designed to measure the plasma ORAC (Cao et al., 1993). ORAC has been found to be a good index of oxidative stress in diabetes mellitus (Merzouk et al., 2004). Our data revealed that the total antioxidant activity (ORAC) decreased in the plasma of children with diabetes in favor of an oxidative stress in such patients. These results are in agreement with previous studies (El Samahy et al., 2013; Varvarovská et al., 2003).

There is a positive correlation between SOD and ORAC. This suggests that the increase in enzymatic activity of SOD in return can induce increase in ORAC. Indeed several studies have considered that improvement

in SOD activity may explain the higher plasma ORAC in diabetic patients. Our results corroborate this hypothesis (Ginty and Conklin, 2012; Lluís et al., 2013).

Conclusively, our results showed an imbalance in the oxidant/antioxidant status in diabetic children. It may be appropriate to evaluate markers of oxidative stress in addition to routine laboratory assessments in evaluation of T1DM pediatric patients. Antioxidant supplementation may be required to reduce oxidative stress and prevent complications of diabetes.

Conflict of interest

The authors have no relevant conflict of interest to enclose.

ACKNOWLEDGEMENTS

The authors appreciate the participation of the children and their parents in the study.

REFERENCES

Adachi T, Ohta H, Hirano K, Hayashi K, Marklund SL (1991). Non-enzymic glycation of human extracellular superoxide dismutase. Biochem. J. 279:263-267

Aebi H (1974). Catalase. In: Methods of Enzymatic Analysis. Bergmeyer HU (ed.), Academic Press, Inc., New York. 2:673-684.

Azen SP, Qian D, Mack WJ, Sevanian A, Selzer RH, Liu CR, Liu CH, Hodis HN (1996). Effect of supplementary antioxidant vitamin intake on carotid arterial wall intima-media thickness in a controlled clinical trial of cholesterol lowering. Circulation 94(10):2369-2372.

Baynes JW (1991). Role of oxidative stress in development of complications in diabetes. Diabetes 40(4):405-412.

Beyan H, Buckley LR, Yousaf N, Londei M, Leslie RD (2003). A role for innate immunity in Type 1 diabetes. Diabetes Metab. Res. Rev. 19(2):89-100.

Bonnefont-Rousselot D (2002). Glucose and reactive oxygen species. Curr. Opin. Clin. Nutr. Metab. Care 5(5):561-568.

Cao G, Alessio HM, Cutler RG (1993). Oxygen-radical absorbance capacity assay for antioxidants. Free Radic. Biol. Med. 14(3):303-311.

Courderot-Masuyer C, Lahet JJ, Verges B, Brun JM, Rochette L (2000). Ascorbyl free radical release in diabetic patients. Cell. Mol. Biol. 46(8):1397-1401.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A (2006). Biomarkers of Oxidative Damage in Human Disease. Clin. Chem. 52(4):601-623.

Dominguez C, Ruiz E, Gussinye M, Carrascosa A (1998). Oxidative stress at onset and in early stages of type I diabetes in children and adolescents. Diabetes care 21(10):1736-1742.

El Samahy MH, Matter RM , Youssef OI, Shams El Din El Telbany MA, Kamal NA (2013). Relation between carotid intima media thickness and oxidative stress markers in Type 1 diabetic children and adolescents. J. Diabetes Metab. Disord. 12(1):50.

Elstner EF, Youngman R, Obwad W (1983). Methods of Enzymatic Analysis vol. III. In: Bergmeyer HU, Grabl BM (eds.), Enzymes oxidoreductases 3rd ed. Weinheim: Verlag-Chemie, 3:293-302.

Ginty AT, Conklin SM (2012). Preliminary evidence that acute long-chain omega-3 supplementation reduces cardiovascular reactivity to mental stress: a randomized and placebo controlled trial. Biol. Psychol. 89(1):269-272.

Goldberg DM, Spooner RJ (1992). Glutathione reductase. In: Bergmeyer HB (Ed.), Methods of Enzymatic Analysis. 3rd ed. 3:258-265.

Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, Pietschmann P, Prager R, Schnack C, Schernthaner G (1995). Enhanced serum levels of thiobarbituric acid-reactive substances in diabetes mellitus. Am. J. Med. 98(5):469-475.

Gumsulu S, Sarikcioglu SA, Sahin E, Yargicoglu P, Agar A (2002). Influence of different stress models on the antioxidant status and lipid peroxidation in rats erythrocytes. Free Radic. Res. 36(12):1277-1282.

Halliwell B, Gutteridge JMC (1999). Free Radicals in Biology and Medicine, 3rd ed. Oxford University Press, New York. pp. 617-783.

Halliwell B, Whiteman M (2004). Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142(2):231-255.

Heistad DD (2006). Oxidative stress and vascular disease: 2005 Duff lecture. Arterioscler. Thromb. Vasc. Biol. 26(4):689-695.

Hernández-Marco R, Codoñer-Franch P, Pons Morales S, Del Castillo Villaescusa C, Boix García L, Valls Bellés V (2009). Oxidant/antioxidant status and hyperfiltration in young patients with Type 1 diabetes mellitus. Pediatr. Nephrol. 24(1):121-127.

Jain SK (1989). Hyperglycemia can cause membrane lipid peroxidation and osmotic fragility in human red blood cells. J. Biol. Chem. 264(35):21340-21345.

Jain SK, McVie R (1999). Hyperketonemia can increase lipid peroxidation and lower glutathione levels in human erythrocytes in vitro and in Type 1 diabetic patients. Diabetes 48(9):1850-1855.

Kaji H, Kurasaki M, Ito K, Saito T, Saito K, Niioka T, Kojima Y, Ohsaki Y, Ide H, Tsuji M, Kondo T, Kawakami Y (1985). Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulin-dependent) diabetic women. Klin. Wochenschr. 63(16):765-768.

Kaplan LA, Cline D, Gartside P, Burnstein S, Sperling M, Stein EA (1982). Hemoglobin A1 in hemolysates from healthy and insulin-dependent diabetic children, as determined with a temperature-controlled mini column assay. Clin. Chem. 28(1):13-18.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, Ahn B-W, Shaltiel S, Stadtman ER (1990). Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186:464-478.

Likidlilid A, Patchanans N, Poldee S, Peerapatdit T (2007). Glutathione and Glutathione Peroxidase in Type 1 Diabetic Patients. J. Med. Assoc. Thai. 90(9):1759-1767.

Lin CC, Huang HH, Hu CW, Chen BH, Chong IW, Chao YY, Huang YL (2014). Trace elements, oxidative stress and glycemic control in young people with Type 1 diabetes mellitus. J. Trace. Elem. Med. Biol. 28(1):18-22.

Linder B (2014). Overview of Diabetes in Children and Adolescents, From the National Diabetes Education Program (NDEP). Available at: http://ndep.nih.gov/media/Overview-of-Diabetes-Children-508_2014.pdf

Livingstone C, Davis J (2007). Targeting therapeutics against glutathione depletion in diabetes and its complications. Br. J. Diabetes Vasc. Dis. 7(6):258-265.

Lluís L, Taltavull N, Muñoz-Cortés M, Sánchez-Martos V, Romeu M, Giralt M, Molinar-Toribio E, Torres JL, Pérez-Jiménez J, Pazos M, Méndez L, Gallardo JM, Medina I, Nogués MR (2013). Protective effect of the omega-3 polyunsaturated fatty acids: Eicosapentaenoic acid/Docosahexaenoic acid 1:1 ratio on cardiovascular disease risk markers in rats. Lipids Health Dis. 12(1):140.

Lyons TJ (1991). Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes. Diabet. Med. 8(5):411-419.

Maahs DM, Rewers M (2006). Mortality and renal disease in Type 1 diabetes mellitus progress made, more to be done. J. Clin. Endocrinol. Metab. 91(10):3757-3759.

Maritim AC, Sanders RA, Watkins JB 3rd (2003). Diabetes, oxidative stress and antioxidants: a review. J. Biochem. Mol. Toxicol. 17(1):24-38.

Merzouk S, Hichami A, Sari A, Madani S, Merzouk H, Yahia Berrouiguet A, Lenoir-Rousseaux JJ, Chabane-Sari N , and Khan NA (2004). Impaired oxidant/antioxidant status and LDL-fatty acid composition are associated with increased susceptibility to peroxidation of LDL in diabetic patients. Gen. Physiol. Biophys. 23(4):387–399.

Mishra N, Singh N (2013). Blood viscosity, lipid profile, and lipid

peroxidation in type-1 diabetic patients with good and poor glycemic control. N. Am. J. Med. Sci. 5(9):562-566.
Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95(2):351-358.
Ou P, Nourooz-Zadeh J, Tritschler HJ, Wolff S (1996). Activation of aldose reductase in rat lens and metal-ion chelation by aldose reductase inhibitors and lipoic acid. Free Radic. Res. 25(4):337-346.
Penno MA, Couper JJ, Craig ME, Colman PG, Rawlinson WD, Cotterill AM, Jones TW, Harrison LC (2013). Environmental determinants of islet autoimmunity (ENDIA): a pregnancy to early life cohort study in children at-risk of Type 1 diabetes. BMC Pediatr. 13(1):124.
Ramakrishna V, Jailkhani R (2007). Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients. Diagn. Pathol. 2:22.
Roe JH, Kuether CA (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivatives of dehydroascorbic acid. J. Biol. Chem. 147:399-407.
Sakurai T, Tsuchiya S (1988). Superoxide production from nonenzymatically glycated protein. FEBS. Lett. 236(2):406-410.
Sato Y, Hotta N, Sakamoto N (1979). Lipid peroxide level in plasma of diabetic patients. Biochem. Med. 21(1):104-107.
Sies H (1991). Oxidative stress: Oxidants and antioxidants. New York, Academic Press.
Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997). Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 20:1183-1197
Thomas HE, Kay TWH (2000). Beta cell destruction in the development of autoimmune diabetes in the non-obese diabetic (NOD) mouse. Diabetes Metab. Res. Rev. 16(4):251-261.
Vantyghem MC, Balduyck M, Zerimech F, Martin A, Douillard C, Bans S, Degand PM, Lefebvre J (2000). Oxidative markers in diabetic ketoacidosis. J. Endocrinol. Investig. 23(11):732-736.
Varvarovská J, Racek J, Stozický F, Soucek J, Trefil L, Pomahacová R (2003). Parameters of oxidative stress in children with Type 1 diabetes mellitus and their relatives. J. Diabetes Complications 17(1):7-10.
Velazquez E, Winocour PH, Kesteven P, Alberts KG, Laker MF (1991). Relation of lipid peroxides to macrovascular disease in type 2 diabetes. Diabet. Med. 8(8):752-758.
Vlassara H (1994). Recent progress on the biologic and clinical significance of advanced glycosylation end products. J. Lab. Clin. Med. 124(1):19-30.
Wierusz-Wysocka B, Wysocki H, Byks H, Zozulinska D, Wykretowicz A, Kamzmierczak M (1995). Metabolic control quality and free radical activity in diabetic patients. Diabetes Res. Clin. Pract. 27(3):193-197.
Wolff SP (1993). Diabetes mellitus and free radicals. Br. Med. Bull. 49(3):642-652.
Yu BP (1994). Cellular defences against damage from reactive oxygen species. Physiol. Rev. 74(1):139-162.
Zivić S, Vlaski J, Kocić G, Pesić M, Cirić V, Durić Z (2008). The importance of oxidative stress in pathogenesis of Type 1 diabetes--determination of catalase activity in lymphocytes of diabetic patients. Med. Pregl. 61(9):458-463.

Journal of Diabetes and Endocrinology