

PAPER DETAILS

TITLE: TIP 1 DIYABET MEILLITUS'DA SERUM. LÖKÖSİT VE ERİTROSİT LİPİD

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PAGES: 47-52

ORIGINAL PDF URL: <https://dergipark.org.tr/tr/download/article-file/499887>

TİP 1 DİYABET MEİLLİTUS'DA SERUM. LÖKÖSİT VE ERİTROSİT LİPİD PEROKSİDASYONU VE ANTİOKSİDAN DURUM

Investigation of Serum, Leukocyte and Erythrocyte Lipid Peroxidation and Antioxidative Defence Systems in Type 1 Diabetes Mellitus

Ümmügülsüm CAN¹, Osman ÇAĞLAYAN², İdris MEHMETOĞLU³

ÖZET

Giriş: Tip 1 diyabet mellitus metabolik bir hastalık olup, patogeneğinde oksidatif stres rol oynamaktadır. Oksidatif stres serbest oksijen radikalleri ve lipid peroksidlerinin artışı ve antioksidan kapasitesinin azalmasına bağlıdır. Bu çalışmada tip 1 diyabet mellitus'da oksidatif stresin etkisini değerlendirmek için serum, lökosit ve eritrosit lipid peroksidasyonu, ve lökosit ve eritrosit glutatyon peroksidaz ve süperoksid dismutaz aktivitelerine ilaveten serum ve lökosit vitamin C seviyelerini sağlıklı kontroller ile karşılaştırdık.

Yöntem: Çalışmaya hasta grubu olarak 34 çocuk ve adolesan (20 kız ve 14 erkek) ve kontrol grubu 29 çocuk ve adolesan (13 kız ve 16 erkek) dahil edildi. Lipid peroksidasyonunun son ürünü malondialdehid, tiyobarbitürik asid reaktivite yöntemi, vitamin C seviyeleri dinitrofenilhidrazin metodu ile ölçüldü. Glutatyon peroksidaz ve süperoksid dismutaz düzeyleri ticari kit kullanılarak tesbit edildi. Analiz sonuçlarının istatistiksel analizleri SPSS 20.0 bilgisayar programı ile yapıldı.

Bulgular: Tip 1 diyabet mellitus çocuk ve adolesanlar kontroller ile karşılaştırıldığında serum, lökosit ve eritrosit malondialdehid seviyeleri artmasına karşın lökosit ve eritrosit glutatyon peroksidaz ve süperoksid dismutaz aktiviteleri, ve serum ve lökosit vitamin C seviyelerinde azalma tesbit edilmişti. Fakat bu değişikliklerde istatistiksel olarak anlamlı fark gözlenmedi ($p > 0.05$). Bu parametreler ile HbA1c ve glukoz seviyeleri arasında korelasyon yoktu.

Sonuç: Tip 1 diyabet mellitus çocuk ve adolesanlarda lipid peroksidasyon artışına ve antioksidan sisteminde azalmaya eğilim olmakla birlikte bu durumun düzenli kullanılan insülin tedavisi ile düzeltildiği ve diğer faktörler tarafında etkilendiği kanaatindeyiz.

Anahtar Sözcükler: *Diabetes mellitus; Lipid peroksidasyonu; Superoksid dismutaz; Glutatyon peroksidaz; C vitamini*

ABSTRACT

Background & objectives: In the present study, to evaluate oxidative stress in type 1 diabetes mellitus (IDDM), serum, leukocyte and erythrocyte lipid peroxidation and and erythrocyte and leukocyte glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, and serum and leukocyte vitamin C levels of patients and healthy controls were investigated in order to determine the effect of free radicals in these patients. Lipid peroxidation was measured in terms of malondialdehyde which is an end product of peroxidation.

Methods: Patients consisted of 34 cases (20 female, 14 male) aged 3-27 years and controls consisted of 29 subjects (13 female, 16 male) aged 3-18 years.

Results: There was at least, a tendency toward an increase in lipid peroxidation and a decrease in antioxidant systems in children and adolescents with IDDM which is corrected by insulin treatment. Additionally, there was no correlation between the above parameters and HbA1c and glucose levels.

Conclusions: These disturbances were concluded to be due to some other factors rather than diabetes itself.

Keywords: *Diabetes mellitus; Lipid peroxidation; Superoxid dismutase; Glutathione peroxidase; vitamin C.*

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Geliş tarihi/Received: 08.06.2017
Kabul tarihi/Accepted: 30.03.2018
DOI: 10.16919/bozoktip.319920

Bozok Tıp Derg 2018;8(2):47-52
Bozok Med J 2018;8(2):47-52

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a metabolic disease which occurs as a result of complete or partial deficiency of insulin secretion or insulin resistance (1). Oxidative stress (OS), appears as a result of imbalance in prooxidants and antioxidants rates (1,2). Oxygen free radicals and lipid peroxides play an important role in T1DM disease and its long period complications (2). Human neutrophils go through a respiratory burst of considerably increased oxygen use during phagocytosis. The toxic action of the products in the respiratory burst kills phagocytosed bacteria, and these products including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals and oxidized halogen species such as (HOCl) (3). Free radicals cause lipid peroxidation in low density lipoprotein, which leads to atherosclerotic lesions that is the complication of diabetics (4). Biological harms effect of free radicals are prevented with vitamin E and C, glutathion and antioxidant enzymes, such as glutathione peroxidase (GPx) and superoxide dismutase (SOD). SOD converts O_2^- into more stable H_2O_2 which is then reversed to water by GPx (5,6). The transports and metabolism of Vitamin C is varied in diabetics (7). Reduced antioxidant status of serum, leukocyte and erythrocyte may cause in boosted peroxidation of cell membran lipids and therefore increased serum concentration of lipid peroxides. That's why, in the current study we investigated whether there is any change in serum, erythrocyte and leukocyte lipid peroxidation, and erythrocyte and leukocyte GPx and SOD activities, and serum and leukocyte vitamin C levels in patients with T1DM. This study's target was to further analyse the potential role of oxidative stress in patogenesis of patients with T1DM.

MATERIALS AND METHODS

The local ethics committee of Meram Medical School, Necmettin Erbakan University confirmed this study. We informed adult patients and all parents of children about the design of this study and obtained their written consents. This study population was made up of 34 patients (20 female, 14 male) with T1DM aged 3-27 years and 29 healthy controls (13 female, 16 male) aged 3-18 years. The control group consisted of the healthy children and adolescents of hospital staff. Trained

pediatricians carried out the clinical examinations of both T1DM patients and control groups. The patients who were involved in diabetes group were under the insulin treatment. Exclusion criteria were those with complications of diabetes and other chronic diseases. Any subject undergoing pharmacological treatment and vitamins were also excluded.

In total 20 ml venous blood was drawn after overnight fasting. 10 ml of the blood were passed into tubes including 20 mg solid EDTA for the separation of leukocytes by a dextran sedimentation method (8). After sedimentation, the red cells were extracted by hipotonic shock using cold distilled water and 0.6 mol/L KCl. Leukocytes in suspension were counted on a Technicon HI hemocounter. At this stage, no erythrocytes were noticed in preparation. The preparation at this stage usually involved 90% neutrophils; the other cells contained monocytes, lymphocytes and eosinophils. Through reiterated tawing and freezing process after addition of 0.2% Triton X, leukocytes were lysed. Protein level in the supernatant was determined by a commercially present kit (Protein urinairs, Biotrol, France) which can detect protein at milligram levels. Lipid peroxidation was acquired by thiobarbituric acid (TBA) reactivity (9). Malonyldialdehyde (MDA), an end product of fatty acid peroxidation, react with TBA to constitute a colored complex that has maximum absorbance at 532 nm.

For preparation process of erythrocytes, 5 ml of this sample was heparinized (9). On the Technicon HI hemocounter, the determination of erythrocytes and hemoglobin values was implemented. For erythrocytes MDA measurements, 0.2 ml packed were suspended in 0.8 ml phosphate- buffered saline (8.1 g NaCl, 2.302 g Na_2HPO_4 and 0.194 g NaH_2PO_4 /l, pH 7.4) and 0.025 ml BHT (88 mg/10 ml absolute alcohol). Subsequently, thirty percent trichloroacetic acid (0.5ml) was added. Tubes were wortexed and ensured to stand in ice for at least 2 h. Tubes were centrifuged at 2000 rev./min for 15 min. One ml of each supernatant was passed into another tube. Then, we added 0.075 ml 0.1 mol/l EDTA and 0.25 ml 1% TBA in 0.05 mol/l NaOH. Tubes were mixed and allowed to remain in a boiling water bath for 15 min. After tubes were made to cool to room temperature, absorbance was read at 532

nm. To preclude MDA formation during the assay in leukocytes and erythrocytes, butylated hydroxytoluene (BHT), which is an antioxidant, was added. Then, we calculated MDA values in nanomoles from the absorbance coefficient of MDA-TBA complex at 532, $1.56 \times 10^5 \text{ cm}^{-1} \text{ mol}^{-1}$. Serum and leukocyte vitamin C levels were measured by the dinitrophenylhydrazine (DNPH) method (10,11).

Blood glucose was identified by routine methods by means of glucose oxidase on a Technicon RA-XT autoanalyser. HbA1c was measured by a commercially available kit based on column chromatography (Helena Laboratories, Beaumont, TX).

The activities of erythrocyte and leukocyte GPx and SOD were estimated by commercially present kit (Randox Laboratories, UK). Activities of these enzymes

were reported as U/mg protein. We examined the data in terms of statistical values by the help of the x2 test, Student's t- test and Spearman correlation test.

RESULTS

Table 1 indicates the laboratory findings of the patients and control subjects. As far as the table is concerned, there was significantly ($p < 0.05$) increase in the serum glucose and HbA1c.

There was observed a mildly increase in lipid peroxidation (serum, erythrocyte and leukocyte MDA) and a mildly decrease in antioxidant systems (erythrocyte and leukocyte GPx and SOD activities, and serum and leukocyte vitamin C levels in patients that had T1DM, but there was no difference between all groups and controls ($p > 0.05$). Also, no correlation was observed between both the above parameters, and HbA1c and glucose levels.

Table 1. Laboratory findings of patient and control groups

	Diabetic patients (n=34)	Controls (n=29)	p
Glucose (mg/dL)	247.97 ± 119.82	78.07 ± 11.29	<0.05
HbA1c (%)	11.43 ± 3.28	4.51 ± 1.66	<0.05
Serum Vitamin C (mg/dL)	0.88 ± 0.35	0.93 ± 0.30	>0.05
Vitamin C (µg/108 leuk)	30.37 ± 11.05	30.76 ± 14.36	>0.05
Leukocyte GPx (U/ mg protein)	4.81 ± 4.57	4.86 ± 4.59	>0.05
Erythrocyte GPx (U/gHb)	26.47 ± 10.52	27.33 ± 0.04	>0.05
Leukocyte SOD (U/ mg protein)	1.23 ± 0.70	1.44 ± 0.87	>0.05
Erythrocyte SOD (U/gHb)	1406.4 ± 280.4	1487.7 ± 355.9	>0.05
Serum MDA (nmol/mL)	2.82 ± 1.41	2.62 ± 0.98	>0.05
Leukocyte MDA (nmol/mg protein)	12.28 ± 13.06	12.10 ± 5.82	>0.05
Erythrocyte MDA (nmol/ gHb)	6.30 ± 4.93	6.23 ± 4.11	>0.05
All values are mean ± standart deviations.			
GPx;glutathione peroxidase, MDA;malonyldialdehyde SOD;superoxide dismutase			

DISCUSSION

The existence of hyperglycemia leads to boosted production of oxygen free radicals by means of glucose autooxidation and nonenzymatic glycation in diabetes. Reduced activity of the antioxidant defence systems or risen oxidative stress (OS) are pathological processes in T1DM (12). In prior researches, the erythrocyte SOD (12,13) and GPx (12), and serum SOD (13) levels in young patients with T1DM looked quite similar to those in controls. No significant difference were indicated between plasma MDA levels of T1DM and healthy subjects (15,16). Correspondingly, no significant correlations between the levels of MDA and degree of glycemic control (HbA1c) were observed (15). Conversely, there appeared significant increases in the values of soluble form of a receptor for advanced glycation end products (sRAGE). They considered that increased plasma levels of sRAGE in T1DM were to provide protection against cell damage and to be adequate to wipe out surplus circulating MDA during first years after disease was diagnosed (16).

In other studies, no difference was observed between the ascorbic acid (AA) in polymorphonuclear cells (17) and plasma AA levels (18) of T1DM subjects and controls. In accordance with the findings detected in prior researches, we didn't found a statistically significant difference the serum erythrocyte and leukocyte MDA levels, and erythrocyte and leukocyte GPx and SOD activities, and serum and leukocyte vitamin C levels between patients with T1DM and control groups in our study. We regard that there was an slightly inclination toward an increase in lipid peroxidation and a slightly drop in antioxidant status in patients with T1DM, which case insulin treatment can correct.

According to the literature, there was a contradicting data regarding oxidant and antioxidant status in T1DM. Varvarovska et al. (19) detected that children with T1DM revealed significantly lower GPx and higher MDA than healthy children. In other studies, there wasn't a difference between the erythrocyte SOD activity of control and T1DM groups, whereas erythrocyte GPx activities reduced (18,20). Antioxidant enzymes convert ROS into less reactive forms and reduce their

deterious effect (21). O₂⁻, the initial reactive radical, is increasingly produced in the mitochondrial respiratory chain during hyperglycaemia (21). Other critical reason of ROS in diabetes are excessive-activated NADPH oxidase and doubled nitric oxide synthase (21). Rise of ROS leads to significant cellular harm since ROS are able to change the structure and function of all kinds of molecules such as proteins, membrane lipids and nucleic acids (21). Compensatory rise of erythrocyte SOD, which serve as a essential enzyme scavenging O₂⁻, would thus be useful (21). Paltry levels of erythrocyte SOD observed in T1DM patients could be the result of enzymatic antioxidant system consumption or down-regulation of enzyme expression owing to causes irrelevant to age, diabetes period or compensation (21). T1DM geneally starts in childhood with a lifelong inclination for hyperglycaemia and a high risk of appearance severe long-term complications (22). The study by Indran (22), the erythrocyte SOD and GPx activities significantly decreased while plasma MDA significantly increased in T1DM patients compared to control subjects. Serum MDA levels of T1DM patients in metabolically poorly controlled were remarkably higher than controls (23,24) and serum SOD levels considerably increased (23). In the same way, Erciyas et al. (25) indicated that serum MDA levels significantly increased in metabolically poorly controlled in proportion to metabolically well-controlled pediatric T1DM patients and were similar in metabolically well-controlled DM patients in proportion to control groups. Kocic et al. (26) performed intensive insulin cure was short-acting insulin following every meal and medium-acting insulin prior to night-sleep, and conventional insulin cure had two (morning and evening) doses in the T1DM patients. They discovered that intensive insulin treatment significantly enhances total antioxidative status and the plasma MDA in opposition to the conventional therapy. They deduced that DM complications triggered by free radicals can be impeded by an proper treatment preference. In another study (27), erythrocyte MDA levels increased more than two fold and antioxidant enzymes activities virtually remained unaltered in patients with diabetes vs. controls. Besides, the red cell MDA is not associated with patients' age, disease lasting, or presence of vascular complications. Biosynthetic insulin treatment

caused entire recovery of MDA level in patients with angiopathies aged under 35 and with disease lasting of less than 10 years. In diabetics with angiopathies aged over 35 and disease lasting of more than 10 years red cell MDA level dropped under the impact of human insulin treatment, but still continued to remain high vs. that in controls by 1.5 times. Red cell SOD activities dropped during insulin therapy in all the examined groups of diabetics. Martin- Gallan et al. (28) observed that plasma and erythrocyte MDA significantly increased, unlike decrease of erythrocyte GPx activity at beginning in T1DM patients compared with controls. Insulin therapy during first year improved oxidant-antioxidant status. In a study by Firoozrai et al. (29), MDA level raised significantly in T1DM patients. GPx activity in erythrocytes was not significantly different between the patients and control groups. They stated that an increased vulnerability to OS and decreased antioxidant defense in patients with T1DM, which may be owing to weak glycemic control (29). SOD and GPx activities of the leukocytes decreased in T1DM patient group as compared to controls (30). The plasma MDA levels were higher in diabetic children and adolescents than in controls. Whereas erythrocyte SOD activity was significantly higher, GPx was significantly lower in diabetic children and adolescents as opposed to controls (31). In contrast to no differences in erythrocyte GPx and Cu/Zn SOD, MDA were higher in T1DM (32). Plasma AA levels were considerably lower in T1DM. Increased OS in diabetic patients was indicated by the rise in plasma MDA, the reduce in antioxidant molecules (AA) (32). In a study by Eltayeb et al., (33) vitamin C level was significantly reduce in the children with T1DM. The level of plasma MDA was remarkably higher in T1DM patients than in controls (34). Furthermore, plasma AA decreases significantly in diabetic group. That the ratio plasma AA/DHA remarkably lessened in T1DM depends on increased MDA levels (34). Total antioxidant activity reduced significantly in patients with T1DM. The reason of this situation was the lower level of vitamin C (35).

The reason why lipid peroxidation (serum, erythrocyte and leukocyte MDA) and an antioxidant systems (erythrocyte and leukocyte glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities), and serum and leukocyte vitamin C levels were detected

to be quite different in previous studies might be related to the treatment of diabetics and appearance of complications and the period of disease.

Conclusion

That antioxidant defenses decreased in diabetic patients is bound to increase OS in diabetes. Further studies are required to explain the association between lipid peroxidation and antioxidative function and their 'pathophysiological significance in diabetes.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgement

We would like to thank all participants for contributing in the present survey.

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