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Investigation of the Oxidative Stress markers in salivary, serum and erythrocyte ofEl-Oued (Algeria) Diabetic Patients

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Abstract

The aim of this study was to evaluate the state of oxidative stress during diabetes in El-Oued (Algeria) patients. The experimentation is carried out on 72 voluntary individuals were divided into three groups. The first group of healthy individuals (controls), the second group diabetic type 1and the third group diabetic type 2, on which we measured some biochemical parameters on serum, erythrocytes and saliva. The Results showed a significant decrease in serum uric acid concentration in diabetics type 1 (10.78%) and diabetics type 2 (26.83%) and a significant decrease ($p < 0.05$)in erythrocytes GSH in both types of diabetes compared to controls. On the other hand, there was a significant increase ($p < 0.05$) in serum and salivary peroxidation lipidique (MDA) in type 1 and 2 diabetics compared to non-diabetics. In addition, a significant correlation ($p < 0.05$) between serum and salivary MDA in type 1 diabetics ($R^2 = 0.750$) and type 2 diabetics ($R^2 = 0.768$) was shown in this study. In conclusion the present study reveals that diabetes induces an imbalance of oxidant / antioxidant status at serum, erythrocyte and salivary level and that salivary MDA is a good indicator of the state of oxidative stress in diabetes.

KEY WORDS: Diabetes, Oxidative stress, Uric acid, GSH, saliva MDA.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from a disorder that affects the ability of the body to synthesize or use insulin [1]. WHO predicts global growth in the prevalence of diabetes, which is expected to reach 300 million patients by 2025 [2]. Diabetes mellitus is responsible for a serious morbidity including retinopathy with risk of vision loss; Nephropathy leading to renal failure; Peripheral neuropathy with a risk of foot ulcers, amputations and increased incidence of cardiovascular disease atherosclerosis [3]. Many studies suggest that diabetes is accompanied by an oxidative stress that promotes the development of the disease by disrupting insulin secretion, favoring insulin resistance [4]. Oxidative damage due to free radicals is associated withvascular disease in people with type 1 and those with type 2 diabetes mellitus [5]. Oxidative stress defines an imbalance between the levels of reactive oxygen species produced and the ability of a biological system to detoxify the reactive intermediates, creating a perilous situation by contributing to cellular damage [6]. This imbalance appears as a common denominator in various pathological processes in which an oxidative insult causes cell death and tissue damage [7]. The regulation of redox homeostasis is fundamental for the maintenance of normal cellular functions and for the survival of cells [8]. The objective of our study is to evaluate markers of oxidative stress in serum, erythrocyte and saliva to control the state of oxidative stress in type 1 and type 2 diabetics.

Subject and methods

Ethical approval (Appendix) was sought and approved by theEthical Committee of the Department of Cellular and MolecularBiology, Faculty of natural science and life, University of ElOued.We studied patients with newly diagnosed and treatedtype 1 diabetics (24 patients)mean aged 42.08 ± 3.81 years and type 2 diabetics (24 patients), mean aged 50.67 ± 2.94 years, werejoined in this study. A total of 24 females' healthy volunteers(mean aged 35.45 ± 4.48 years) served as controls with normalserum blood glucose.All the volunteers in this study live in the El Oued area located in the southeast of Algeria.

Inclusion Criteria

Patients who had clinical diagnosis and laboratory findings ofdiabetes disease type 1 and type 2 for more than three months evidenced

Exclusion Criteria

To eliminate the factors which might affect free radicalantioxidant activity, we excluded all High blood pressure and other chronicdiseases subjects from patient groups and healthy controls.

Laboratory Investigations

Fasting blood samples were collected and transferred into EDTA tubes for hemoglobinand GSH assay and in non-heparinized tubes for serum glucose and uric acid analysis. Serum was obtained by centrifugation of the blood at 3000 × rpm for 5 min and stored at -20°C until analysis. Saliva is collected in tubes after a 12-hour fast. The samples were collected between 08:00 hours and 10:00. At least 1 hour after mouth washed. The samples were centrifuged at 4000 rpm for 5 minutes and the transparent supernatanttransferred into another tube and then stored at -20 ° C. until use.Saliva was

used for MDAassay.The determination of blood glucose is according to the method of Kaplan et al 1984[9],The serum uric acid assay according to the method of Schultz 1984[10].Hemoglobin is assayed automatically by the NFS auto-analyzer. The dosage of salivary and serum MDA according to the method of Nourooz-Zadeh et al., 1996[11]; MDA is the most widely used marker in lipid peroxidation. In brief After hot acid treatment, the aldehydes react with thiobarbituric acid (TBA) to form a chromogenic condensation product consisting of 2 molecules of TBA and one molecule of MDA. The intense absorption of this chromogen takes place at a 532 nm.The GSH assay according to the method of Sedlakand Lindsay 1968[12], which is based on a colorimetric method based on the oxidation reaction of GSH by 5,5'-dithiobis 2-nitrobenzoic acid (DTNB), releasing Thus thionitrobenzoic acid (TNB), the reading of the absorbance is carried out at a 412 nm.

Statistical analysis

The statistical evaluation is carried out by the student T testusingMinitab software(version 13.31)
The reported data are the means of measurements and theirstandard error of mean (SEM) values.

Results

The results obtained in this study clearly show that diabetes is confirmed by the hyperglycemia observed in type 1 and 2 diabetic patients compared to control, the results also show no significant difference in the concentration of l Hemoglobin in diabetic groups when compared to the control(Table 1).

Table 1. Description and concentrations of glucose and hemoglobin in controls and diabetics

Group description	Control (n=24)	Diabetic type 1 (n=24)	Diabetic type 2 (n=24)
Mean age (years)	35.45±4.48	42.08±3.81	50.67±2.94
Sex ratio (men:women)	12:12	10:12	14:8
Blood glucose (g/l)	0.97 ±0.037	2.03±0.12***	1.70±0.09***
Hemoglobin (g/dl)	13.13±0.39	13.65 ±0.88	13.45 ±0.39

*** p <0.001: significant difference compared to the control group. Values are mean ±SE

Our results also show that the level of salivary and serum MDA are significantly elevated ($p<0.05$)in type 1 and type 2 diabetic patients compared to the control(fig 2 and fig 3), concerning antioxidants statue, the results shows a significant decrease ($p <0.05$ and $p <0.01$)in the concentration of serum uric acid in Type 1diabetics and type 2diabetics respectively in comparison with the control(fig 1). Moreover, the erythrocyte GSH level is significantly decreased ($p <0.05$ and $p <0.01$)in both type 1 and type 2 diabetic respectively compared to controls.(fig 4)

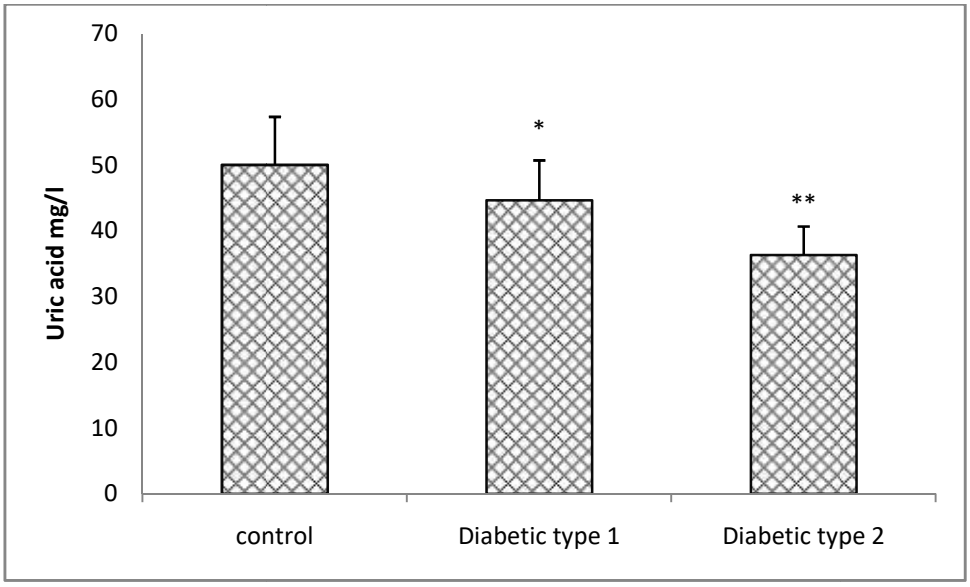

Fig. 1. Serum uric acid concentration in controls and type 1 and type 2 diabetic patients. The data are presented as Mean ± ES for (n = 24). * P <0.05, **P <0.01: significant difference compared to the control group.

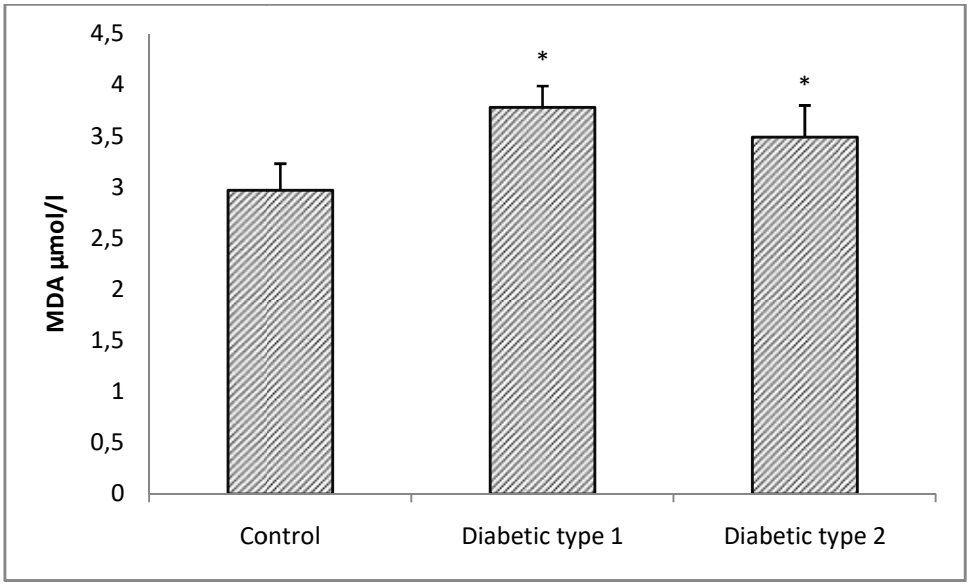

Fig. 2. Serum MDA concentration in controls and type 1 and type 2 diabetic patients. The data are presented as Mean ± ES for (n = 24). * P <0.05: significant difference compared to the control group.

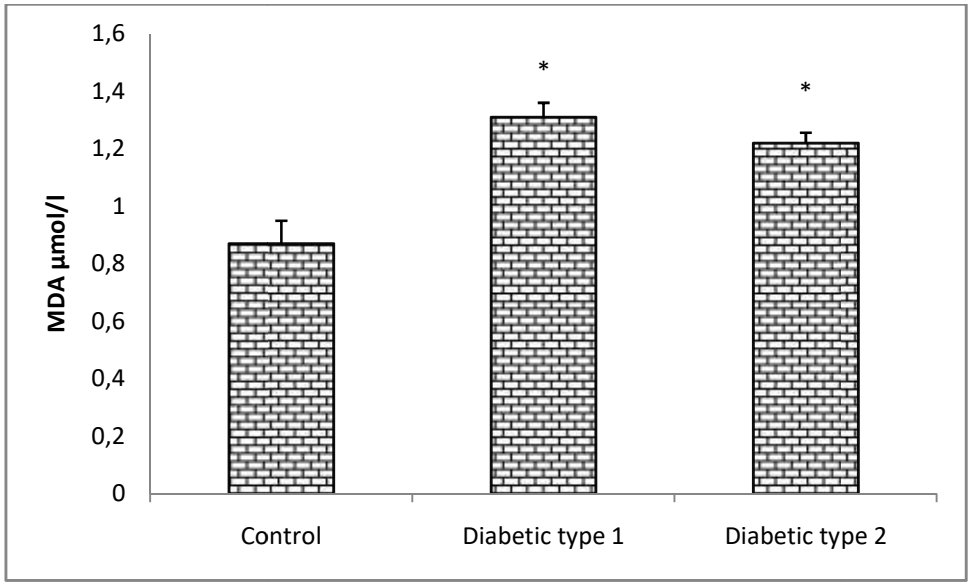

Fig. 3.Saliva MDA level in controls and type 1 and type 2 diabetic patients. The data are presented as Mean ± ES for (n = 24). * P <0.05: significant difference compared to the control.

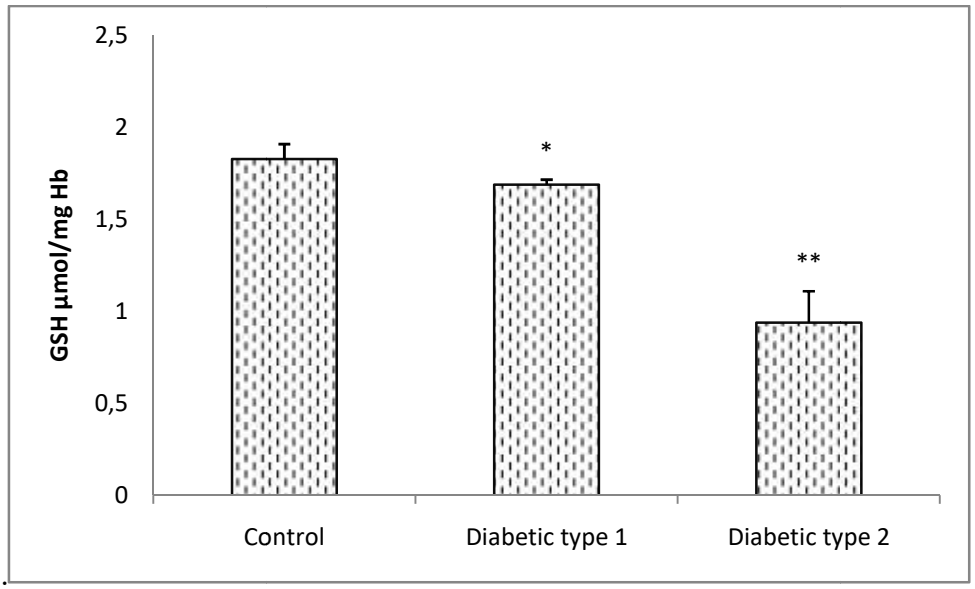

Fig. 4.Erythrocyte GSH concentration in controls and type 1 and type 2 diabetic patients. The data are presented as Mean ± ES for (n = 24). * P <0.05, **P <0.01: significant difference compared to the control group.

In addition, the statistical evaluation(fig 5 and 6)showed there was a good correlation (p <0.05)between serum and salivary MDA concentration in both types1Diabetes and type 2 diabetes with correlation coefficient ($R^2 = 0.750$) and ($R^2 = 0.768$) respectively.

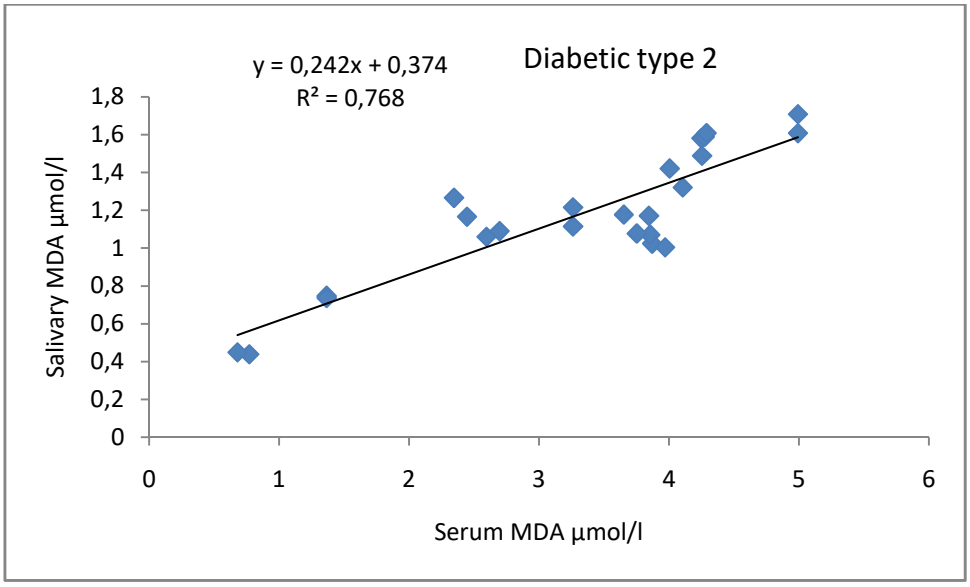

Fig. 5. Showing the correlation between serum MDA and Salivary MDA in type2 diabetes (The number of samples used in this correlation was 24).

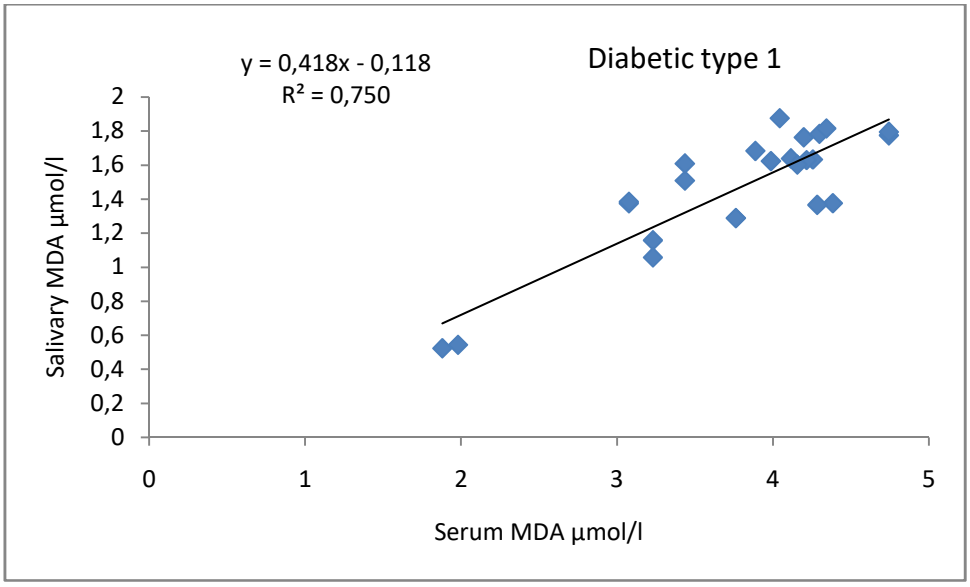

Fig. 6. Showing the correlation between serum MDA and Salivary MDA in type1diabetes (The number of samples used in this correlation was 24).

4. Discussion

The aim of our study is to evaluate markers of oxidative stress in serum, erythrocyte and saliva to control the state of oxidative stress in diabetes. In our study diabetes is confirmed by the hyperglycemia observed in type 1 and 2 diabetic patients, resulting from a disorder that affects the body's ability to manufacture or use insulin [13]. In our study, we also found that the level of serum uric acid is decreased in type 1 and 2diabetics. The decrease in uric acid can be explained by the increased the formation of free radicals showing that uric acid as an antioxidant is used in the fight against oxidative stress [14]. Oxidative stress is theconsequence of both increased production of free

radicals andreduced capacity of the anti-oxidative defense such as uric acid [15].In addition, serum uric acid level is low due to increased urate clearance [16], a positive relationship has been described between glycosuria and uricosuria, therefore there would be interference between reabsorption Tubular glucose and tubular capacity to reabsorb urate. Hypouricemia may be a marker for the onset of diabetic kidney disease [17]. Our results also show that the level of salivary and serum MDA is significantly elevated in diabetic patients. Saliva may be the first line of defense against oxidative stress [18]. The various changes in saliva compositions indicate that the salivary glands are targeted organs in diabetes mellitus, and then the increase in lipid peroxidation can be discussed by a high concentration of lipid fractions in the saliva and blood as Shown in the Natheer study, 2011 generally follow those seen in the serum which is significantly different from that of the control group. This may indicate that the salivary lipid profile in diabetic patients may result from a systemic composition of dyslipidemia and the serum is reflected in saliva composition [19]. Proportionately increased vulnerability of the diabetic patient to cardiovascular complications. In this study, the results revealed a significant decrease in reduced glutathione (GSH) in diabetics compared to controls.This decrease in GSH levels may be due to its consumption in the scavenging free radicals [20] generated by Diabetes.The latter may cause oxidative stress by numerous routes, such as glucose autoxidation, polyol pathway [21] and glycation of proteins [22], this last glycation resulted from the formation of a covalent bond between the aldehyde function of glucose and the free amino groups of the proteins. This bond gives products known as Amadori which have the particularity of possessing a ketol group which can, in the presence of transition metals, yield an electron to molecular oxygen, leading to the formation of superoxide anions [23]. In addition, elevated glucose concentration may lead to increased GSH oxidation and decreased regeneration [24].The results showed there was a correlation between serum and salivary MDA concentration in both types of Diabetes.This correlation between saliva and oxidative serum biomarker enhances the utility of saliva as a valid diagnostic fluid [25].

Conclusion

In conclusion the present study reveals that diabetes induces significant oxidative stressshown very clearly by serum, salivary and erythrocyte markers, and that salivary MDA agood way of monitoring oxidative stress states associated with type 1 or type 2diabetes.

Conflict of interest

There is no conflict of interest.

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