Evaluation of the Salivary Total Antioxidant Capacity and Lipid Peroxidation Status in Type 2 Diabetes Mellitus Patients

Mehryar Zargari[1], Hourolein Arab[2], Zahra Ghafouri[3*]

1. Department of Biochemistry Biophysics and Genetics, Faculty of Medicine, Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Biochemistry Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
3. Department of Biochemistry Biophysics and Genetics Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

***Correspondence:**
Zahra Ghafouri, Department of Biochemistry Biophysics and Genetics Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
Tel: (98) 939 969 6804
Email: ghafourizara@gmail.com

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Abstract

Objective: Type 2 diabetes mellitus (T2DM) is a multifactorial disorder with various disturbances in biochemical processes such as antioxidant status and lipid peroxidation. The aim of present study was evaluation of the oxidative stress markers in saliva of T2DM patients.

Materials and Methods: In this study, 80 (40 T2DM and 40 control) individuals were assessed. The salivary total antioxidants capacity and lipid peroxidation were determined by the ferric reducing antioxidant power (FRAP) method and Thiobarbituric acid reactant substances (TBARS) assay, respectively.

Results: Total salivary antioxidants value was significantly lower in T2DM patients compared with healthy controls (*P* -value: 0.01). A significant increase in lipid peroxidation was seen in T2DM (*P*-value: 0.0001).

Conclusion: The findings of the present study suggest that diabetic patients confront a disturbed state of antioxidant defense, especially the reduction in total antioxidant capacity of body.

Keywords: Lipid peroxidation, Saliva, Total antioxidant capacity, Type 2 diabetes mellitus

Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by impairment in carbohydrate, fat, and protein metabolism that affects millions of people around the world (1). Type 2 diabetes mellitus (T2DM) is also a multifactorial disorder. It starts with insulin resistance, the glucose homeostasis failure and glucose intolerance (2). Moreover, these disturbances are accompanied with changes in a variety of biochemical processes, especially lipid peroxidation (3).

Peroxidation of the lipid membrane is related to the pathogenesis of many degenerative diseases, such as atherosclerosis, aging, rheumatoid arthritis, carcinogenesis, heart disease and DM. Reactive oxygen species (ROS) and lipid peroxides are responsible for the pathogenesis of mentioned disorders (4,5). Overproduction of ROS and decreased efficiency of antioxidant defenses of body, referred to as oxidative stress, contribute to the extent of chronic diabetes complications (6). For instance, ROS induce membrane lipid

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peroxidation and this production of fatty acids peroxides toxically causes cell malfunction. Malondialdehyde (MDA), the most popular at the same time facile element which is usually measured as Thiobarbituric acid reactive substances (TBARS), has been widely used to assess lipid peroxidation profile in diabetics (7). On the other hand, antioxidants such as vitamin C (Ascorbic acid), vitamin E (α-tocopherol), thiols, Bilirubin, Albumin, Ceruloplasmin, uric acid (UA), catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) in a relatively high concentration are believed to prevent macromolecular oxidation. Antioxidants convert reactive free radicals into inactive substances by a set of electron-donating processes and this way cease toxicity of oxidized products (5,7,8).

Invasive techniques such as taking blood samples in order to achieve serum and plasma are useful to determine the oxidative stress and antioxidant concentrations. Recently, great clinical interest has been allocated to investigating saliva for oxidative stress and antioxidant markers, an indication of the redox status of the body (9). Whole saliva is a principal physiological fluid that contains diverse amounts of antioxidants including uric acid, vitamin C (ascorbic acid), reduced glutathione (GSH), oxidized glutathione (GSSG), and others which exist in whole blood, serum and plasma (10,11). According to various researches on the issue of salivary antioxidants and oxidants, there is contradictory data. Diabetic patients sustained intensified oxidative stress, due to increased free radical production, lipid peroxidation, and reduced antioxidant defenses (12). The aim of our study was to measure the saliva antioxidant and oxidant status in T2DM patients in comparison with the normal controls.

Materials and Methods

A total of 40 T2DM patients, ranged between 21 to 55 years old, stricken for at least 2 years were included in this case-control study. The patients were selected from diabetic patients of dentistry center of Mostafavian health care unit, Sari, Iran. The control subjects included 40 healthy individuals (age and sex matched) recruited at the Mazandaran University of Medical Sciences (MAZUMS) of Sari, Iran. Both case and control subjects were selected from non-smoker individuals with no systemic disease such as hypothyroidism, no radiotherapy background at head and neck area, no alcohol use, no anti-histamine and anti-cholinergic drug use. The study was conducted in full accordance with MAZUMS ethical principles, with ethical code of IR.MAZUMS.REC.94.1446 and proposal registration code of 37 and after being informed of the aim of the study, a written consent was received from each individual before his/her enrollment in the study.

Saliva samples were obtained in the morning (between 9 and 11 AM) after an over-night fast, in order to reduce circadian rhythms, during which subjects were requested not to drink (except water) or chew gum especially 1 hour before sampling. In addition, they washed their mouth 1 minute prior to sampling. Then seated in the coachman's position, head slightly down and did not swallow or stimulate saliva production (13). The collection time was five minutes. The saliva samples were collected in sterile graded containers, and then centrifuged immediately to remove cell debris (× 1000 g for 10 min at 4°C). The supernatant was extracted and stored in small aliquots at - 80°C until further biochemical analyses.

Total antioxidant power of saliva was determined by measuring the ability to reduce Fe^{3+} to Fe^{2+}. Basically, in this test the sample is exposed to Fe^{3+}, and Fe^{2+} is produced by the reduction ability of antioxidants present in the sample (14). The reagent contained 300 mmol/L acetate buffer, pH 3.6 and 16 mL Glacial acetic acid ($C_2H_4O_2$) per liter of buffer solution, 10 mmol/L TPTZ in 40 mmol/L HCL, 20 mmol/L $FeCl_3\,6H_2O$. Working Ferric Reducing Ability of Plasma (FRAP) solution was prepared as required by mixing 50 mL

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acetate buffer, 5 mL TPTZ solution, and 5 mL $FeCl_3$ $6H_2O$ solution. Then 50 µL of saliva sample added to 1.5 mL freshly prepared reagent warmed at 37°C. The complex between Fe^{2+} and TPTZ gave a blue color, which the absorption was read at 593nm by spectrophotometer.

Thiobarbituric acid reactive substances (TBARS) assay

In this test, the reaction of MDA, the end product of the oxidation of poly unsaturated fatty acids, with Thiobarbituric acid (TBA) provides a complex that is investigated spectrophotometrically, and the sample absorbance explains the degree of lipid peroxidation (15). Practically, 500 µL of saliva sample was added into a tube, then 300 µL TBA 0.375 %, 300 µL of TCA 15%, 300 µL HCl 0.25 N were added in sequence to the sample tube. After incubating at 95°C in water bath for 30 minutes and cooling the tubes, they were centrifuged at 1000g for 10 min. Then, the absorption of the supernatant was recorded at 532 nm by spectrophotometer.

Statistical analysis

All data were analyzed with SPSS-21. The significance of differences in the mean and standard deviation of groups were calculated using Mann-Whitney U test. A *P*-value < 0.05 was considered statistically significant.

Results

The results of the present study were presented in Table 1. The mean ages of the control and case groups were 38.1±9.83 and 42.40 ±8.13 years, respectively (Table 1). The mean TBARS (MDA index) values of saliva for diabetic and control individuals were 0.955 ± 0.655 µmol/L and 0.709 ± 1.16 µmol/L ,respectively. The saliva MDA level, a marker of lipid peroxidation, increased in diabetic patients in comparison with control group (*P*=0.0001). The mean salivary total antioxidant capacity (FRAP) level of patients was 940.2±623.6 µmol/L and the mean total antioxidant capacity of saliva in control group was 1333.2±629.4 µmol/L. The mean salivary antioxidant of diabetic group was statistically lower than control group (*P*=0.01) (Table 1).

Discussion

The mortality of T2DM patients has increased and chances of developing cardiovascular, cerebrovascular and peripheral vascular disease are high. The association of oxidative stress and pathogenesis of the complications of NIDDM, particularly vascular disease, has been widely proposed(16). Diabetes-associated oxidative stress is resulted from the over production of free radicals and a reduced anti oxidative defense capacity (17).

In our study, salivary levels of Thiobarbituric acid reactive substances increased in all diabetic patients when compared to those in controls. According to our data, type II diabetics are vulnerable to severe oxidative stress, compared to non-diabetic individuals, due to a depletion of the total antioxidant capacity after a lapse of years. In case of total antioxidant capacity of saliva, diabetic patients showed a significantly lower value of FRAP test in comparison with normal individuals, which expresses the role of diabetes in reducing total antioxidant capacity of body. In fact, a significant lowered antioxidant level of the saliva of diabetic patients compared to controls is a proof for validating the idea that declares oxidative stress contributes to the extent of chronic diabetes complications.

Most studies have shown a decrease in the salivary antioxidants and increase in lipid

Table 1. Study group data

Parameters	Case (T2DM)	Control (healthy)	*P*- value*
Age(Mean ± *SD*)	42.40±8.13	38.00±9.83	
Male	18(45%)	22(55%)	
FRAP (µmol/l)	940.2±623.6	1333.2±629.4	*P*=0.01 (S)
TBARS (µmol/l)	0.955±0.655	0.709±1.16	*P*=0.0001 (S)

*Man-Whitney U test: *P* value<0.05 considered as statistically significant (S) in non-parametric assay

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peroxides in diabetic patients compared to normal individuals, (15,18-21). However, few authors reported the contrary result, declaring elevated total antioxidant capacity and catalase activity in diabetic patients, as well as a high oxidative stress (7,22), which is in contradiction with our results. According to data from another study, no correlation between diabetes duration and severity of oxidative stress was reported (21).

Among the studies that measured total antioxidant capacity of diabetic patients in the blood and saliva, there were conclusions declaring that total antioxidant capacity of diabetics was higher than normal individuals (7). However, another study proved that the level of oxidative stress and free radical production in type II diabetic patients was more than control group (23). According to Pisoschi et al. after the diabetics' antioxidant defense weakens, oxidative stress occurs in diabetics (24). Other approving studies (25,26) confirmed that thiol groups of proteins and uric acid of diabetics were significantly lower than control group.

In a comprehensive study Aouacheri et al. different parameters of oxidative stress including MDA, GSH, GPx, GR, G6PDH, SOD in diabetic patients were measured, concluding an increase in SOD and a decrease in other parameters in diabetics compared to normal ones (21). According to a study that assessed the salivary content of lipid peroxides and antioxidants in type II diabetics, the rate of oxidative stress was significantly higher than controls (27), which approves our results. As oxidative stress plays important role in progression and development of diabetes and its complications, consumable or behavioral therapies can reduce the impact of oxidative stress (28). Ramakrishna et al. assessed IDDM patients during a study. They found a statistically significant higher values of MDA and lipid peroxides and a decrease in the antioxidant levels compared to normal healthy controls (29). All in all, assessment of lipid peroxidation and total antioxidant capacity of physiological fluids of the body especially saliva in diabetic patients is a beneficial way of better understanding of how oxidative stress contributes to some extent of diabetes complications(30,31).

According to the most recent data since 2012 until now, saliva has been a diagnostic tool aiding in the detection of systemic diseases, too (32,33). In case of cardiovascular disease (CVD), an important complication of type 2 diabetes mellitus, oxidative stress plays a major role in the development of CVD, with the increase in salivary TBARS and UA levels and the decrease in the TAC levels (34,35). In another study, the salivary alpha amylase and catalase in diabetic patients were estimated (36). The level of salivary alpha-amylase and catalase in people affected by type 1 diabetes was higher than the non-diabetic people (36). It should be declared that the catalase deficiency is associated with oxidative effects of diabetes (36). Many of studies exploring the role of salivary malondialdeyde in assessment of oxidative stress among diabetics have concluded that salivary MDA appears to be an indicator of serum MDA concentration (37). Thus exploring saliva for antioxidant markers that accurately reflect the redox status of the body is worthwhile (38).

The findings of the present study suggest that diabetic patients confront with a disturbed state of antioxidant defense, in particular, the reduction in total antioxidant capacity of body.

Conclusions

In our present work, we clearly proved the deficiency of total salivary antioxidants and a higher lipid peroxidation in type II diabetic patients in comparison with normal individuals. This study expresses the importance of antioxidant capacity and oxidant status as an effective assessment in diabetic patients. Besides, salivary measurement of redox status that has been a wide area of interest can be an effective tool in diabetic parameters assessment.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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