Correlation of Oral Health Status and Salivary Antioxidant Capacity in Type 2 Diabetes Mellitus

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Abstract

Objective: Dental caries is considered as one of the major complication of diabetes mellitus. Patients with type 2 diabetes mellitus (T2DM) demonstrate evidence of oxidant–antioxidant imbalances in their biological fluids such as saliva. The aim of this study was to evaluate the correlation of oral health status and salivary total antioxidant capacity (TAC) in T2DM.

Materials and Methods: Fifty T2DM patients and 50 healthy subjects were selected as study sample. Oral examination to assess the dental caries experience was carried out according decayed, missing and filled teeth (DMFT) index. Unstimulated whole saliva specimens were collected in the morning. TAC of saliva was evaluated by spectrophotometric assay. Statistical analysis was performed using an independent two-sample t test and Pearson's correlation test, by SPSS 18.

Results: The mean level of TAC in T2DM was lower than healthy people (*P*-value: 0.0001). The mean of DMFT scores in T2DM were significantly higher than non-diabetics (*P*-value: 0.0001). Moreover, there was a significant negative correlation between TAC level and DMFT scores in both groups (*P*-value: 0.006).

Conclusion: TAC of saliva could be a marker of dental caries activity among T2DM and healthy adults. Therefore, in order to decrease the risk of oxidative damage, it is recommended that T2DM patients take more natural antioxidant food.

Keywords: Antioxidants, Dental caries, Diabetes, Saliva

Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease determined by persistent hyperglycemia and disorder in carbohydrate, fat and protein metabolism. Type 2 DM (T2DM) is most common in adults, whereas type 1 DM (T1DM) affects children and adolescents (1). It is a critical global health problem affecting millions of people in the world, and its outbreak is increasing in many countries, including Iran (2).

Many studies have indicated that hyperglycemia condition is detected as one of the major factors contributing to oxidative

stress by various mechanisms (3-5). T2DM patients demonstrate evidences of oxidant–antioxidant imbalance in their biological fluids such as saliva (6).

Saliva, which serves as the first line of defense against oxidative stress is described as a heterogeneous fluid contained different biochemical compounds including proteins, glycoproteins, electrolytes, and small organic molecules, as well as antioxidants (7). Antioxidants are the several enzymatic, and non-enzymatic compounds exist in the tissues and biological fluids of our body and mediate the potential complications of free radicals in the body manufactured by oxidation reaction (8). If the antioxidant systems do not work properly, dental caries may occur (9). The aim of this study was to evaluate the correlation of oral health status and salivary total antioxidant capacity (TAC) in T2DM.

Materials and Methods

The study was performed at the Department of Biochemistry, College of Medicine and University of Mazandaran. After getting permission from the ethical committee and informed consent from the participants.

The study sample were 100 age matched adults (40 female and 60 male) divided into 2 groups; group I were 50 known T2DM patients and group II were 50 healthy subjects as control. All of 50 T2DM patients were from Sari, coming to Mostafavian clinic in Imam Khomeini Hospital -sari. They were between 21‑69 years old. The Inclusion criteria was, Fasting blood glucose (FBG) >126 mg/dl and glycated hemoglobin levels (HbA1c)>7%. The exclusion criteria were: patients diagnosed with T1DM, systemic diseases requiring long term medications, severe diabetic complications, regular alcohol and tobacco abuse, salivary gland disorders, supplementation with vitamin E, C, A and folic acid.

Method of collection of saliva: Patients were asked to sit and hold their head slightly down. During the period of collection, patients did not swallow or move their tongue or lips. Unstimulated saliva was collected in the mornings before the consumption of food. The saliva was accumulated in their mouth within 2 min followed by asking them to spit the accumulated saliva into a disposable sterile laboratory container with wide opening and lid. Two milliliters of saliva was clarified by centrifugation at 4000 rpm for 10 minutes at a temperature of 4 ℃ and supernatant was isolated and then transferred to Eppendorf micro tubes through volume samplers. After marking, they were stored at a temperature of –20 ℃ until the implementation of the experiment.

TAC level of saliva was clarified by evaluating their ability to reduce Fe^{3+} to Fe^{2+} by the FRAP method as already set up by Benzie and Strain (10). Briefly, in this assay, the medium is exposed to Fe^{3+} and the antioxidants present in medium start to reduce Fe^{3+} and produce Fe^{2+} at low pH as an antioxidant activity. The reagent included 300 mmol/L acetate buffer, pH 3.6 and 16 mL $C_2H_4O_2$ per liter of buffer solution, ten mmol/L TPTZ in 40 mmol/L HCl, 20 mmol/L $FeCl_3$. $6H_2O$. Working FRAP reagent was made by blending 2.5 mL TPTZ solution, 25 mL acetate buffer, and 2.5 mL $FeCl_3$. $6H_2O$ solution. Ten µL of H_2O-diluted sample was then added to 300 µL freshly prepared reagent warmed In water bath at 37 C. The complex between Fe^{2+} and TPTZ forms a blue color with absorbance at 593 nm. A standard linear curve was drawn according to the absorbance of different concentrations of ferrous sulfate as the standard solution, and TAC levels of the samples were calculated by using a standard curve by FRAP index. The results were expressed as µmol/L.

After collecting saliva, teeth clinical examinations were performed by an expert dentist under standard conditions. DMFT (D: decayed; missing; F: filled; T: teeth of permanent dentition) index was recorded according to the table of the World Health Organization (WHO).

Statistical analysis

Statistical analysis was performed by a computer using SPSS 18 software (Chicago, Ill., USA). The normal distribution of the variables was determined by Kolmogorov-Smirnov test, before analyzing of an independent two-sample t test and Pearson's correlation test. Data are represented as a mean ± standard error. The difference and correlation between variables were considered significant when *P*-value <0.05.

Results

Demographic and medical features between diabetic and non-diabetic groups were shown in Table 1 and Table 2, respectively.

In both groups, possible correlation between TAC level and DMFT score were also studied. The correlation coefficient between TAC level and DMFT score levels in diabetic and non-diabetic groups were (r = -0.398, *P*= 0.006) and (r = -0.253, *P*= 0.009), respectively. There was a significant negative correlation between TAC level and DMFT scores in both groups of participants.

Discussion

In this study, we evaluated the TAC of saliva. The reactive oxygen species (ROS) and antioxidant defense system appear to activate in combination rather than separately and measurement of each antioxidant may be less illustration of the whole antioxidant capacity (11). Finally, we have found that there was a significant difference in the mean level of TAC in unstimulated saliva and DMFT scores between diabetic and non-diabetic groups. Also there was a significant decrease in the TAC level with increasing the DMFT scores. We attribute these results to three major reasons.

Past studies showed that oxidative stress is involved in T2DM incidence. Oxidative stress occurs when the amount of oxidant production oversteps the amount of oxidant scavenging (12). The lack of intake of glucose by adipose tissue and muscle due to insulin resistance leads to increasing glucose concentrations in blood. Therefore, glucose uptake by insulin-independent tissues augments. This augmentation disrupts the balance of oxidants and antioxidants defenses by generating various radicals such as superoxide anion ($O2^{-}$), hydroxyl radical (OH^{-}), and hydrogen peroxide (H2O2), nitric oxide (NO^{-}) through different interacting pathways (13). The anti-oxidants present in the saliva as the first line of defense protect against oxidative stress. Hence, reduction TAC of saliva leads to initiate and spread of many diseases of oral cavity like tooth decay because of oxidative stress destructive factors including free radicals or non-radical oxygen derivatives (14).

Second, uric acid (UA) as the major antioxidant existent in human saliva: UA is

Table 1. Demographic and medical features of diabetic and non-diabetic

Variables	Diabetic (n=50) Mean ± SE	Non diabetic (n=50) Mean ± SE	*P*-Value
Age	49.19±1.8	50.65±4	0.27
Sex, n (%)			
Male	20(40)	21(42)	0.70
Female	30(60)	29(58)	
Fasting blood glucose (mg/dL)	173.0±12.3	74.75±4.3	0.001
HbA1c (%)	7.9±0.1	5.1±0.2.7	0.001

Abbreviations: SE: Standard error

Table 2. Comparison of saliva TAC and DMFT between diabetic and non-diabetic groups

Variables	Diabetic (n=50) Mean ± SE	Nondiabetic (n=50) Mean ± SE	*P*-Value
TAC (µmol/L)	1023.8±87.0	1227.5±91.0	0.0001
DMFT	32.4±1.5	25.0±1.5	0.0001

Abbreviations: TAC, Total Antioxidant Capacity ; DMFT, D: decayed; missing; F: filled; T: teeth of permanent dentition; SE: Standard error

substantially gained from the diet containing fructose, sucrose and glucose. It is produced by Streptococcus mutans and lactobacilli that can metabolize mentioned sugars (15). Serum UA can raise rapidly after ingestion of fructose, and it was determined in researches in which fructose (or sucrose) diets was prescribed that even fasting UA levels will increase after several days. Thereby increase of salivary UA concentration, as to a large scale of serum components is reflected in saliva components, due to the increase in the TAC of saliva (16). Thus, in diabetic patients due to reduced consumption of sugar might be responsible for the increase in UA level and TAC in the diet, UA level and TAC in saliva comes down and subsequently increases dental caries.

The third factor is enhanced activity of neutrophils and monocytes in the oral cavity. Neutrophils and monocytes are essential part of our body defense. Several reports have demonstrated an increase in superoxide anion production by these invading leucocytes in diabetic patients. In other words, over production of ROS leads to reduce TAC of saliva (17,18).

There are various studies that show conflicting results with our findings (16,19,20). The probable reasons for this contradiction could be because dental caries is a multi-factorial infectious disease which can be influenced by oral health habits, differences in dietary habits, socioeconomic and cultural status, lifestyle, bacterial load, age of study groups, tooth structure, function and morphology of the teeth in various communities (21). Further studies regarding the role of these factors, the contribution in TAC of saliva, and the association with dental caries are required to better comprehend this phenomenon.

Conclusions

From our result, it can be concluded that the TAC of saliva has an inverse linear relationship with dental caries as the severity of caries increases, the TAC level decreases. Thus oxidative stress play a major role in the onset and development of dental decay in diabetes, the TAC of the diabetic patient can also be moderate by antioxidant-rich foods to meliorate the antioxidant defense system. Also more serious oral health training and regular periodic oral and dental examinations should be considered in the schedule care of diabetic patients.

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