Original Article

Comparison of total antioxidant capacity (TAC) of saliva in patients with type 1 diabetes compared to non-diabetics patients

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Abstract:

Background: Oxidative stress plays a crucial role in the onset and also the progression of vascular and neurological complications of diabetes. The present study was aimed to determine and compare the antioxidant capacity of saliva in patients with type 1 diabetes compared with those do not have diabetic.

Methods: In this case-control study, the patients aged 20-40 years referred to Imam Khomeini Hospital in Ardabil were categorized into two groups including 30 patients with type 1 diabetes as a case group and 30 non-diabetics as a control group. These two groups were compared in terms of TAC levels in saliva. The TAC of saliva the patients measured by 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. The analysis of data was carried out through t-test statistic using "SPSS Statistics Version 24" at a significant level of $P < 0.05$.

Results: Based on the data achieved from the present study, the mean and standard deviation of TAC of saliva in patients with type 1 diabetes was 0.380 ± 0.120 (µmol / l) and in non-diabetics was 0.306 ± 0.122 (µmol / l). The increment of TAC of saliva in the case group compared to the control group was significant based on T-test ($p = 0.003$).

Conclusion: Since there is a significant increment in the level of TAC of saliva in patients with type 1 diabetes compared to non-diabetics, the determination of the level of TAC of saliva in patients with type 1 diabetic can be used as an alternative method for early diagnosis of diabetes.

Keywords: Diabetes, Antioxidants, Oxidative stress, Saliva, Total antioxidant capacity (TAC) of saliva

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Introduction:

Diabetes mellitus is a metabolic disorder in which insulin secretion happens incompletely or do not happen at all, and causes physiological changes in most parts of the body (1). Diabetes is the most common endocrine and metabolic diseases that can reduce life expectancy by a third. Chronic microvascular complications induced from diabetes are responsible for a high incidence rate of deaths in diabetes (3,2). Based on the estimation of World Health Organization (WHO) diabetes was the direct cause of death for 1.5 million people worldwide in 2012. However, hyperglycemia and its side effects were the cause of 2.2 million deaths worldwide in 2012 (4). It has been estimated that by 2030 there will be about 9.2 million people with diabetes in Iran (6). Previous studies have showed that the production of reactive oxygen species and impaired antioxidant capacity are both common in patients with diabetes (7). Antioxidants can react by multiple mechanisms, including oxygen uptake, uptake of catalytic metal ions, and uptake of reactive oxygen species such as superoxide and hydrogen peroxide, and interruption of chain reactions (8).

Oxidative stress (OS) is a situation in which an imbalance between oxidants and antioxidants happens in the body and causes the accumulation of oxidants in the body (9). Oxidative stress is involved in pathological conditions such as cancer, cardiovascular disease, neurological disorders, rheumatoid arthritis, diabetes and getting old (10). After the reaction of free radicals with unsaturated fatty acids or lipoproteins, lipid peroxidation increases. Uncontrollable products of this peroxidation cause oxidative stress and damage the cells. Since lipid peroxidation is caused because of oxidative stress, a variety of markers can be examined in this process (11). Due to the great variety of antioxidants in biological fluids and the their synergistic effects on each other, measuring all the antioxidant components is very difficult and time consuming, and the measurement of one of the antioxidants cannot reflect the activity of all of them. Consequently, the measurement of antioxidants capacity in biological fluids can be a good parameter to assess the body's antioxidant conditions under oxidative stress (10). On the other hand, saliva acts as the first defense barrier against oxidative stress with different mechanisms such as enzymatic and non-enzymatic systems of the body against external damaging factors entering the mouth. Hormonal, infectious, immunological and toxicological markers of diseases can be measured through saliva (11).

Saliva is a biological fluid which is easy to collect and storage, it is available, safe, inexpensive and non-invasive compared to other diagnostic techniques of diseases (12). Due to non-invasive property and easy accessibility of saliva samples compare to other samples such as blood and biopsy, it provide the possibility of studying a broad range of molecules and biomarkers in comparison with blood (13). Since saliva has antioxidant capacity and oxidative stress impairs antioxidant capacity (15, 14), TAC is used as an indicator of total antioxidants due to its role in representing the TAC of saliva (16). Any damages and imbalances in the levels of free radicals and those oxygen species which react with antioxidants may play an important role in the onset and development of several oral inflammatory diseases (17). Dr. Zamani et al. (18) revealed that determining the total level of TAC of saliva during pregnancy can be an alternative method for early diagnosis of diabetes. Another study also revealed that the amount of alpha-amylase (α-amylase) and salivary catalase enzyme increases in people with type 2 diabetes compared to non-diabetics (19). In their study Abdolsamadi et al. (20) reported that using saliva samples could be effective, simple, and safe factor for predicting

possible complications, responding to treatments and controlling the disease. Another study compared plasma lipids and oxidative markers in pregnant patients with diabetes and without diabetes. Based on their data, oxidative stress decreased significantly in the diabetic patients compared to healthy non-diabetic pregnancies as control group (21). Due to limited and contradictory researches carried out in this field and the importance of antioxidants in the prevention and treatment of many diseases, our study compares the level of TAC of saliva in patients with type 1 diabetes compared to non-diabetics in Ardabil in 2018.

Methods:

Participants

In the present study, as a case-control study consisted of the patients aged between 20-40 years who referred to Imam Khomeini Hospital in Ardabil. The participants were categorized into two groups of type 1 diabetes (30 patients) and non-diabetic patients (30 patients). Pregnant women, menopauses, systemic disease except for type 1diabetes, those taking drugs upsetting the balance of the antioxidant defense system, and those with periodontal disease were excluded from the study. To take a non-stimulated saliva sample, all patients were asked to keep away from eating, drinking, brushing, and chewing gum for 90 minutes before sampling. All samples were collected between 8 and 9 am. Non-stimulated saliva sample was collected from all patients by spitting. Patients continued to spitted every 60 seconds for 5-15 minutes. Through this method about 5 ml of saliva was collected. The samples were collected in special plastic tubes and immediately kept near the ice and transferred to the laboratory. The collected samples were centrifuged at 800 rpm speed for 10 minutes at 4 ° C to remove debris and squamous cells. The supernatant was transferred to the microtubes, then were coded and kept at -80 ° C until all samples were prepared. When all the samples were collected, the required tests were performed using a randox TAC Assay Kit.

Estimation of TAC

Aimed to estimate TAC, ABTS assay was performed on saliva samples. ABTS assay is incubated with peroxidase and hydrogen peroxide to achieve ABTS cation. This solution has a relatively stable blue-green color which is measured at a wavelength of 600 nm. The antioxidant in the sample reduces the formation of this dye, which is associated with the concentration of antioxidants.

Statistical Analysis

The data were analyzed using statistical software "SPSS Statistics Version 24". The index of dispersion (mean and standard deviation) was evaluated in the case group and the control group and then statistically judged using the t-test. The level of significance was considered equal to 0.05 in our study.

Results:

For determining the level of TAC of saliva in non-diabetic patients, the average data related to those patients was used. Based on the data achieved from the present study, this value was 0.306 ± 0.122. On the other hand, in order to determine the level of TAC of saliva in patients with type 1 diabetes, the average data about the total level of TAC of saliva was used based on which the obtained value was 0.380 ± 0.120. Based on the data presented in figure (1), the difference between the mean of the two studied groups of diabetic and non-diabetic patients in terms of TAC was significant. In this regard, the level of TAC in type 1 diabetic patients was higher compared to that in non-diabetic patients (t=288.3, p=0.003).

Discussion:

Type 1 diabetes is associated with myocardial infarction, heart failure, and ischemic attacks (22). Diabetes mellitus accounts for 44% of end-stage renal disease (ESRD) cases in the United States (23). OS plays the major role in the onset and the progression of vascular and neurological complications of diabetes disease

(24). The source of OS is active oxygen species from mitochondrial proton leak (25). In our study, in which 30 patients with type 1diabetes and 30 non-diabetics were examined for evaluating the level of TAC of saliva, it was observed that the mean level of TAC of saliva in patients with type 1 diabetes was 0.380 + 0.120 and in non-diabetic participants was 0.306 + 0.122. Different studies have examined these salivary biomarkers, which we will discuss and compare below. Various studies have examined these salivary biomarkers, which we will discuss and compare below.

Zamani et al. (26) compared TAC of saliva and reported a significant increase in OS and consequently an increase in salivary total antioxidant levels of TAC of saliva in women with gestational diabetes compared to non-diabetic women. Maleki et al. (27) showed that the level of salivary alpha-amylase (sAA) and catalase were higher in people with type 2 diabetes compared to the non-diabetic patients. Regarding the total antioxidant capacity of saliva in patients with type 1 diabetes, Cutando et al. (28) observed an increase in the antioxidant capacity of saliva. Lin et al (29) conducted a study entitled "prolonged oxidative stress with altered periodontal disease in patients with type 2 diabetes" and Blaszczak (30) conducted in a study entitled " Total antioxidant capacity and low concentration of molecular antioxidants in the plasma of patients with type 2 diabetes with different metabolic attenuation levels and diabetic nephropathy" claimed that the level of TAC in type 2 diabetic patients was increased compared to healthy individuals, which was also mentioned in response to oxidant-induced damage. In contrast to the abovementioned studies, which are in line with the findings of the present study, a number of studies have reported conflicting results. Arif et al. (31) revealed that the levels of superoxide dismutas (SOD) and TAC in type 2 diabetic patients were reduced compared to healthy individuals. Honarmand et al (32) reported that the average TAC of saliva in type 2 diabetics was lower in comparison with the healthy individuals. Studies by Lodovici (33) and Emmanuel (34) have shown that serum antioxidant capacity is reduced in diabetics compared to healthy individuals. Kuppusamy (35) and Seghrouchni (36) showed that the TAC of saliva in type 2 diabetic patients was reduced compared to healthy individuals. The results of these studies contradict the findings of the current study that can be due to differences in diet and high consumption of natural and medicinal antioxidants, differences in participants' age, differences in sample size and duration of the disease. In this regard, it is recommended future studies should be done with identical samples.

According to the mentioned studies, it can be concluded that because diabetes is a multifactorial disease and various factors such as race, nutrition and environment are involved in it, as well as the total antioxidant capacity of saliva can be affected by various factors. Such as the level and potential of antioxidants, the production of free radicals, the basis of individual genetics, food intake, smoking, physical activity, hormones and stress. And periodontal disease is involved in the amount of antioxidants, so it is better to select patients with the same local and systemic conditions in future research. On the one hand, diabetes is a multifactorial disorder and various factors such as race, nutrition and environment are involved in its development, and on the other hand total antioxidant capacity of saliva could be affected by various factors such as the level and potential of antioxidants, personal genome sequencing, food intake, smoking, physical activity, hormones and stress. Moreover, different studies on saliva have shown that environmental conditions of the mouth, including caries and periodontal disease, are involved in the level of TAC, in this regard it is better to conduct future studies with the

patients with the same local and systemic conditions.

Conclusion:

Based on the data achieved from this study, it can be stated that in patients with type 1 diabetes compared to non-diabetics, the level of TAC of saliva is significantly increased. It should be noted that the biomarkers of saliva can be used as an indicator tool for evaluating general health and early diagnosis of diseases. Besides, saliva is also easy to collect and store and can be collected in sufficient quantities for analysis. Moreover, collecting saliva for the patient as a non-invasive sampling technique greatly reduces anxiety and discomfort. In this regard, determining the level of TAC in type 1 diabetics could be an alternative method for early diagnosis of diabetes.

Study limitations:

In this study, there were limitations such as Inability to equalize physiological, hormonal, nutritional and environmental conditions, as well as Oral health status, prevention status reports (PSR), drug therapy and blood sugar levels that need further investigation in the future. It is recommended to conduct more studies in this field by selecting larger sample sizes and considering the intervening factors and at the same time comparing different types of diabetes

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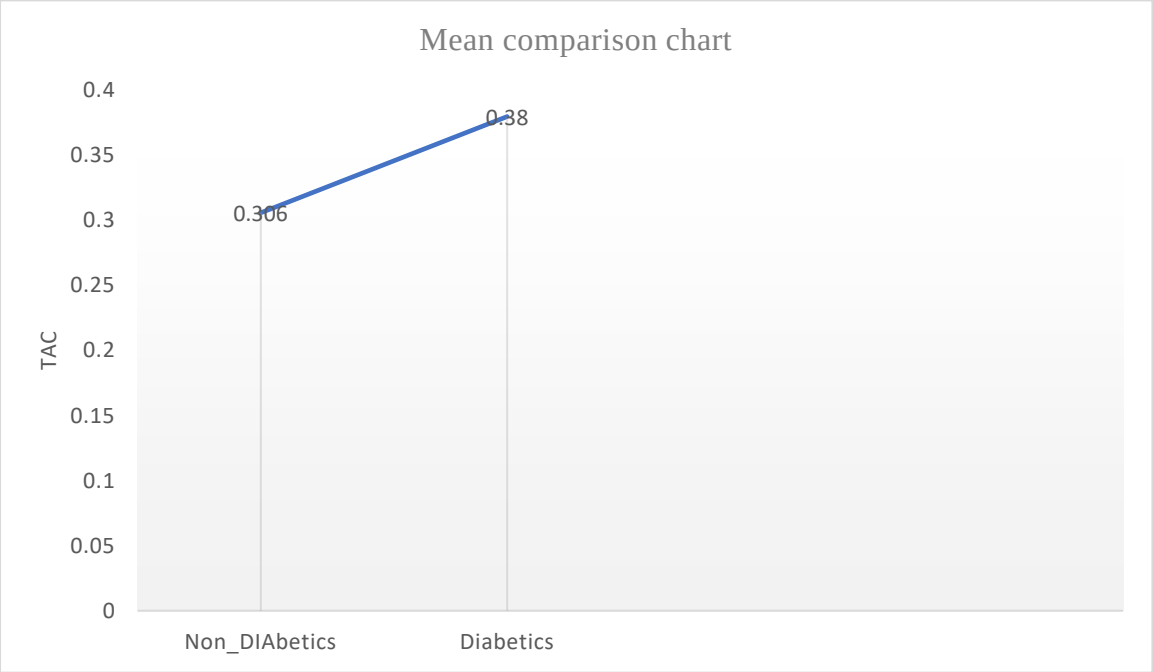

Figure 1. The difference between the mean of diabetic and non-diabetic patient groups in terms of TAC