Original Article

Comparison of salivary catalase and superoxide Dismutase levels in women with gestational diabetes mellitus and non-diabetic pregnant women

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Abstract:

Background: Pregnancy is considered a stressful event, results in higher levels of oxidative stress and considerable changes in physiology; and metabolic functions such as gestational controversies in this area, this study was undertaken to investigate the catalase and superoxide dismutase of saliva in pregnant women whit gestational diabetes in comparison to nondiabetic pregnant women.

Methods: In this cross- sectional study, unstimulated aLIC od 29 patients with salivary catalase and superoxide dismutase were assessed separately in these individuals and Mann- Whitney test.

Results: A P-value <0.05 was considered as significant in all tests. The mean value of salivary catalase level was 0.25±0.42 in women with gestational diabetes and 0.22 ± 0.14 in non-diabetic pregnant women. The mean value of superoxide dismutase level in the saliva was 0.95 ± 0.63 in women with gestational diabetes and 0.44 ± 0.83 in non-diabetic pregnant women.

Conclusion: According to the data obtained from this study, it could be stated that in women with gestational diabetes the level of catalase and superoxide dismutase are increased, compared with non-diabetic pregnant women, but none of these changes are significant.

Key words: Salivary Superoxide Dismutase, Salivary Catalase, Gestational Diabetes Mellitus, Saliva.

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Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy [1]. The definition applies whether insulin or only diet modification is used for treatment an whether or not the condition persists after pregnancy.it does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. Risk assessment for GDM should be undertaken, but there is evidence that maternal hyperglycemia is associated with increased oxidative stress [2], with may also be involved in the pathophysiology and maternal and fetal complications of GDM [3]. oxidative stress occurs when the production of reactive species , particularly reactive oxygen species (ROS), exceed the capacity of the antioxidant defense system. This process can cause damage and change the functions of biomolecules, such as lipids, proteins and DNA [4]. The most commonly produced ROS is superoxide. The depletion of antioxidant capacity may appear whether through a low abundance of non-enzymatic antioxidants (vitamins c and e, and glutathione) or enzymatic antioxidants (superoxide dismutase, glutathione peroxidases and catalase). This condition makes the cell vulnerable to oxidative attack, different from the physiologic situations where redox status in maintained through a careful balance of a low level of ROS synthesis and the pathways of cellular defense [5]. Antioxidants are present in all body fluids including saliva. Saliva may constitute a first line of defense against oxidative stress and has protective effects against microorganisms, toxins and oxidants [6,7] in the second half of the 20th century, it was suggested that saliva could be used in diagnostics, saliva has drawn the attention of researches as a readily available clinical samples has other advantages, too. In that it is easy and inexpensive to collect and store salivary samples, poses minimal risks to the can be used as a screening test for large populations as a cost-effective and noninvasive technique [8,11]. Catalase is a ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. This enzyme catalyzes the conversion of two molecules of H_2O_2 to molecular oxygen and two molecules of water (catalytic activity) [12,13].

SOD is an enzyme that removes the superoxide (O_2-) remains, repairs cell and helps to reduce the damage done to them by superoxide, which is the most common free radical in the body. This antioxidant is found in both the dermis and the epidermis, and is the key to the production of healthy fibroblasts which are the skin-building cells [14]. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide which can also be destroyed by catalase or GPx. SOD plays a critical role in the defense mechanism of cells against the toxic effects of oxygen radicals, it competes with nitric oxide (NO) for superoxide anion, which inactivates no to form Peroxynitrite.

Therefore, by scavenging superoxide anions, SOD promotes the activity of NO [15].

Method:

In the present cross- sectional study, 29 women having gestational diabetes and under diabetes treatment and 56 non-diabetic pregnant women with an age range of 20-40 years, who referred to the department of oral medicine, Ardabil Faculty of Dentistry and Alavi hospital were evaluated.

All the pregnant women underwent oral glucose tolerance test (75 g OGTT) screening between 24 and 28 weeks of pregnancy, in all cases, GDM was diagnosed according to the American diabetes Association criteria [16]. The diagnosis of GDM is made when any of the

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following plasma glucose values are exceeded: Fasting: ≥ 92 mg/ Dl (5.1 mmol/L), 1 h:≥180mg/dL (10.0mmol /L) and 2 h:≥153 mg/Dl (8.5 mmol/L)

Inclusion criteria

1. Diabetics: pregnant woman with GDM in the age group of 20-40 years
2. Controls: Non – diabetic pregnant women in the age group of 20-40 years

Exclusion criteria

1. Diabetics: Systemic diseases, patients under any medication other than oral hypoglycemic drugs and insulin, individuals with a history of any illness for the past 6 months and smoking.
2. Controls: Individuals with any chronic systemic diseases, habits, patients under any medication other than oral hypoglycemic drugs and insulin and individuals with a history of any illness for the past 6 months.

Collection of salivary samples

The salivary sample were collected by using the spitting method, the patients were asked to collect their saliva in their oral cavity and then evacuate it into sterilized Falcon tubes, the procedure was repeated every 60 seconds for 5-15 minutes, in order to collect stimulated salivary samples, the subjects were asked to refrain from eating and drinking or stimulating the oral cavity mucosa for 90 minutes before collecting the salivary samples. A total of 5 mL of saliva was collected using these methods. In order to avoid circadian changes in the morning in a fasting state, the collected salivary samples were immediately placed next to ice and transferred to the laboratory to be centrifuged at 40c at 800 g to isolate squamous cells and cellular debris, then the samples were frozen at -800c until the samples were prepared [17,18].

The principally common method for measuring catalase activity is the UV spectrophotometric method, which depends on monitoring the change of 240 nm absorbance at high levels of hydrogen peroxide solution (≥30 Mm). High levels of hydrogen peroxide (H_2O_2) immediately lead to inhibition of the catalase enzyme by altering its active site structure, although there is variation in the extent to which this occurs.

Catalase catalyzes the following reaction:

2 H_2O_2-Catalase 2 H_2O+2 O

Catalase activity was assessed by incubating the enzyme sample in 1.0 ml substrate (10 Umol/ml hydrogen peroxide in 50 mmol / l sodium – potassium phosphate buffer , Ph7) at 370 c for 2 minutes , the reaction was stopped with ammonium molybdate .absorbance of the tallow complex of molybdate and hydrogen peroxide is measured at 240 nm against to bland [19].

Superoxide dismutase assay

Superoxide dismutase activity was analyzed by the reduction of nitroblue terrazolium (NBT) by superoxide, which formed formazan and detected spectrometrically at 560 nm using genesis 10 UV and expressed in terms of U/ml [10]. I11umination of riboflavin in the presence of O_2 and electron donors, such as methionine generates superoxide radicals, which have been used as the basis for this assay and the reduction of NBT by O_2 was followed at 560 nm [20].

Statistical analysis

Statistical analysis was done with SPSS 20 statistical analysis software, the Kolmogorov-Smirnov test was used for assessing the distribution of the variables. variables with normal distribution were compared by t-test and the results represented as mean ±standard deviation (SD). The Mann–Whitney test was used for variables with no- normal distribution and the results expressed as median (interquartile range) in this study P-value < 0.05 was considered statistically significant.

Results:

From 85 cases , 29 cases were women with gestational diabetes and 56 others were nondiabetic pregnant women average catalase levels in the saliva of women with gestational

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diabetes was 0.42 ± 0.25 and in non – diabetic pregnant women was 0.22 ± 0.14. According to the data average, level of salivary catalase has increased in women with gestational diabetes. However, comparison of salivary catalase levels with Mann- Whitney non parametric test showed that this difference was not significant (P-value = 0.084). Also, the mean level of superoxide dismutase in the saliva of women with gestational diabetes was 0.63 ± 0.95 and non–diabetic pregnant women was 0.44 ± 0.83. According to the data average, the level of salivary superoxide dismutase has increased in women with gestational diabetes, however, comparing these two levels with t-test parametric test showed that this difference was not significant (P-value = 0.308).

Discussion:

Pregnancy is a physiological period with increased susceptibility to insulin resistance and increased oxidative stress, because the placenta acts in the production of diabetogenic hormones and contributes to the generation of ROS, creating an environment rich in mitochondria with high oxygen pressure [21,22] in normal pregnancy, the ROS production rate is compensated by an increased synthesis of antioxidants [23]. However, when the pregnancy is complicated by diabetes, excessive production of ROS overpowers antioxidant defenses, leading to increased oxidative stress [24,25]. Different studies with various results have been conducted on salivary compounds of people with diabetes based on the type of diabetes, their different metabolic control and other factors [18,26,27,28,29] the present study compares the unstimulated salivary sanoke in 29 women with GDM and 56 non-diabetic pregnant women in terms of catalase enzyme and SOD level. The results showed that the level of salivary SOD level in women with GMD was higher than non - diabetic pregnant women. studies done by Al-Rawi [30] on saliva and serum and Padalkar *et al* [31] on serum also showed an increase in the levels of SOD in diabetic group when compared with that of the control group. They proposed that this increase in the antioxidant SOD may be due to the body's mechanism to enhance the antioxidant defense so as to counterbalance the increasing oxidative stress Also, Reznick *et al.* (2006) conducted a study on 20 patients affected by type 1 diabetes in 13-19 age range. They came to the conclusion that the level of salivary antioxidant (peroxidase, superoxide dismutase and total antioxidant capacity) increased they reported that increment of HbA1C level leads to the increased level of antioxidant [32]. Xontrary results were observed by Taheri *et al.* explained that, since SOD forms the first line of defense against free radicals, the activities of this enzyme may be affected first by oxidative stress before any other antioxidant enzymes. There is evidence to show that hyperglycemia is accompanied by the loss of copper, which forms an essential cofactor in SOD activity, and SOD is inactivated by glycosylation in erythrocytes [33].

Ibuki *et al* as well as Leite *et al.* implemented separate studies and found that the level of salivary catalase in experimental rats under induced diabetic condition will increase [34,35]. In another study, the salivary catalase in diabetic patients was estimated. The level of saliva catalase in people affected by type 1 diabetes was higher than the non –diabetic people. It should be declared that the catalase deficiency is associated with oxidative effects of diabetes [11]. In contrast, some studies showed that catalase antioxidant levels in patients with type II diabetes mellitus wire significantly lower than those of healthy subjects [28].

Conclusion:

The use of saliva as a "diagnostic tool" is an upcoming area of research, it offers the advantage over serum as the collection process

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of saliva is noninvasive. It can be performed easily and cost effectively in a number of clinically challenging situations such as obtaining samples from children, disabled or anxious patients, etc. in whom blood sampling could be a difficult act to perform.

The results of our study helped us to arrive at the conclusion that saliva can indeed be used as an excellent medium for biochemical analysis. further extensive studies are required to be conducted with larger samples along with antioxidant therapy. This will help in establishing the reliability of superoxide dismutase and catalase in saliva as a potential biomarker of oxidative stress in Gestational diabetes mellitus. Further, it may also help in establishing the role of oxidative stress in the pathogenesis of GDM and is complications.

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