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Research Paper

Salivary and serum malondialdehyde levels in type 2 diabetes mellitus with dental caries

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Abstract

The aim of the study was to evaluate the salivary and serum Malondialdehyde levels in type two diabetes mellitus patients with dental carries. The study was conducted among 80 individual, which included experimental and control group with 40 individuals each divided based on presence and absence of type two diabetes mellitus and DMFT score. The results showed significant increase in salivary and serum Malondialdehyde in type two diabetes mellitus patients with dental caries compared to the control group which comprised of individuals in absence of caries and no diabetes mellitus. Thus, confirming increased oxidative stress in type two diabetes mellitus individuals.

Keywords: *Dental Caries; Diabetes Mellitus; Malondialdehyde; Oxidative Stress; Saliva*

1. Introduction

Diabetes Mellitus consists of set of symptoms with impaired carbohydrate, protein and fat metabolism, due to abnormal insulin production, insulin action or both (MAbayon 2007). Type 2 diabetic mellitus develops in a stepwise order (Stumvoli M et al.2005) which is caused by insulin resistance and defective insulin secretion (Bergman and Lilly 1989). Diabetic individuals show impaired balance between oxidant and antioxidant levels according to various evidences. Mechanisms contributing for free radical generation as a result of oxidative stress are non-enzymatic glycosylation, auto oxidation of glycation products. Damage to proteins, lipids, carbohydrates, nucleic acids and their chemical modification is caused by free radicals in the tissue (Wolff SP 1993, Vadde R and Rama J 2007). Diabetic subjects are at an increased risk of developing oral conditions such as gingivitis, periodontal disease, and alveolar bone loss, which has been associated with persistent poor glycemic control and impaired free radical production (Taylor GW et al. 1998, Grossi SG 2001). Periodontal disease can lead to recession of the gingival margin, which can expose more tooth surfaces to caries attack (Copenhagen and Blackwell 2003). People with diabetes also experience hyposalivation and they may suffer from salivary dysfunction. The absence of copious saliva may result in minimizing buffer activity which promotes remineralization of tooth structures early in the caries process. The reduction in saliva thus decreases resistance to caries-producing bacteria(Tenovuo J et al. 1986, Selwitz RH et al. 2007).Additionally, increase in the amount of fermentable carbohydrates by oral bacteria due to high glucose levels in saliva leads to production of acidic by-products that cause teeth demineralization in dental caries(Caufield and Griffen 2000).Dental Dental caries, caries, or tooth decay, is the localized destruction of susceptible dental hard tissues by acids produced by bacterial fermentation of dietary carbohydrates.(Copenhagen and Blackwell 2003)The most widely used assay for oxidative stress evaluation is Malondialdehyde, one of the of the low molecular weight end product of lipid peroxidation (Marnett LJ 2002). Therefore, this study is designated to determine the oxidative stress effect of saliva in type two diabetes mellitus patients with dental caries. The aim of the study was to evaluate Salivary Malondialdehyde in type two diabetes mellitus patients with dental caries and systemically healthy control subjects.

2. Materials and methods

Subject population: This study was carried out between October 2014 to November 2014 in the department of Conservative dentistry and Endodontics, A.B.shetty memorial Institute of dental sciences Deralkatte Mangalore. Eighty subjects of both genders, ranged in age from 40-60 yrs were recruited in the study and were further categorized into control and experimental group as follows:

1) Experimental Group(n=40) included caries active individuals with Type 2 diabetes mellitus, was further divided into group 1 which included individuals with DMFT score 1-3, group 2 included individuals with DMFT Score 4-10, and group 3 included DMFT score more than 10.
2) Control group (n=40) included caries-free individuals with no type two diabetes Mellitus and DMFT score 0.

Subjects with any other systemic disorder other than type 2 diabetes Mellitus, subjects under long term radiotherapy or chemotherapy or under any medication that can affect the flow rate of saliva were excluded from the study. Informed consent of the participant was initially obtained. Only subjects who fulfilled all the inclusion and exclusion criteria were formally enrolled in the study. The study was conducted after obtaining the approval from the institutional ethical committee. A thorough medical and dental history was taken. All patients with Diabetes Mellitus were diagnosed as having typed two diabetes mellitus according to the criteria de-

fined by the American Diabetes association (American diabetic association 2015, p 8-16) and were treated with stable doses of oral hypoglycemic agents and/or insulin. The diabetic status was concluded based on the Fasting (no caloric intake for at least eight hours) plasma glucose of ≥126 mg/ dL (seven mmol/ L).

Diagnosis of Dental Caries: The diagnosis of dental caries is done through a comprehensive assessment of all patient information by a visual examination of tooth surfaces. A dental probe or explorer is sometimes used to provide tactile information. Caries in between adjacent teeth are visualized using bitewing radiographs or using light sources via transillumination. Caries diagnosis is based principally on clinical examination and review ofradiographs and DMFT index was calculated. After calculating DMFT they were further divided into different groups depending on initiation and progression into

1) Control: DMFT = 0
2) Case: Group 1: DMFT 1-3score, Group DMFT -10 score, Group 3: DMFT more than 10 score.

Saliva sampling and processing:

The saliva samples were collected from all the participants, before to the collection procedure, the subjects began by rinsing their mouth thoroughly with water and then resting quietly for three min. Unstimulated whole saliva (~3 ml) was collected by means of spitting method(Navazesh M 1993). Subsequently, saliva was allowed to accumulate in the floor of the mouth. The participant then spits the accumulated saliva into an sterile container, prior to analysis collected saliva is centrifuged at 3000 g for 15 min at 4 ºC. The supernatant fraction was then aliquot into storage vials and kept at -70º C until required for analysis.

Biochemical assay:

The salivary samples were estimated for Malondialdehyde levels byThiobarbituric Acid Reactive Substances (TBARS) assay. Thiobarbituric acid reactive substances are naturally present in the biological systems and include lipid hydroperoxides and aldehydes, which increase in concentration as a response to oxidative stress. TBARS assay uses a fluorometric microplate assay method, and the values are usually reported in malondialdehyde (MDA) equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides. The TBARS assay is a well-recognized, established method for quantifying MDA in biological samples. This assay is based in the reaction of a chromogenic reagent, 2-thiobarbituric acid (TBA) with MDA at 25ºC. Here, one molecule of MDA reacts with two molecules of 2-thiobarbituric acid to yield a coloured complex with an absorbance maximum at 532 nm (Armstrong et al. 1994, Botsoglou N.A et al. 1994, Yagi K 1998].The The Results obtained was expressed in µM/L.

Statistical Analysis: The association between the MDA levels and the clinical parameters were calculated using pearson's correlation (two tailed) and expressed by Pearson's correlation coefficient. A P-value of <0.05 was considered has been statistically significant. The data packages were statistically analyzed using SPSS statistical package.

3. Results

The characteristics of the clinical parameters in the preliminary study are shown in Table 1 and Table 2. The comparison of clinical parameters among the Control and Test groups is shown in Table 3.

Table 1: Represents Serum and Salivary Malondialdehyde Levels in Serum and Saliva in Control Group

MDA LEVELS (µMOL/L)	CONTROL
SALIVA	1.5±0.05
SERUM	2.0±0.18

Table 2: Represents Serum and Salivary Malondialdehyde Levels in Serum and Saliva in Experimental Group

MDA levels (µMOL/L)	EXPERIMENTAL		
SALIVA	Group 1 1.6±0.16	Group 2 1.8±0.05	Group 3 2.0±0.10
SERUM	Group 1 2.13±0.18	Group 2 2.29±0.05	Group 3 3.35±0.25

Table 3: Comparison of Serum and Salivary Malondialdehyde in Control and Experimental Group

M DA LEVELS(µMOL/L)	CONTROL	Experimental	
SALIVA	1.5±0.05	GROUP I	1.6±O.16
		GROUP II	1.8± 0.05*
		GROUP III	2.0±0.10*
SERUM	2.0±0.18	GROUP I	2.13± 0.18
		GROUP II	2.13±0.18*
		GROUP III	3.35±0.25*

*Significant differences among group I and II in comparison with control group in saliva and serum seen.

The observed value for MDA in saliva and serum of control group is 1.5±0.05 µMOL/L and 2.0±0.18µMOL/L respectively. The comparison of MDA level in test and control group showed increased Malondialdehyde levels in the test group compared control group. MDA levels in saliva of test group I, II and III are 1.6±0.16, 1.8±0.05, 2.0±0.10 µMOL/L respectively, and serum is 2.13±0.18, 2.29±0.05 and 3.35±0.25 µMOL/L respectively.

The comparison in values of group I and group II with test group showed statistical significant values both in saliva and serum ($P < 0.05$), whereas no significant difference in values of MDA among group I and control group in saliva and serum was observed ($P > 0.05$).Bar diagram represents the comparison of MDA levels in Saliva and Serum in test and control group.

4. Discussion

Saliva is body fluid with complex composition and specific roles. Biochemical constituent analysis in saliva is of great help in diagnostics of diseases in oral cavity as well as in monitoring general health of organism (Todorovic et al. 2006and Mithra NH et al. 2014).Based Based on numerous studies, it has been proved that in diabetic patients, there is a modification of organic and inorganic constituents of saliva (Mata AD et al .2004]. There is an abundance of literature supporting relationship between type two diabetes mellitus and oral health status. This study evaluates parameters of oxidative stress in saliva and serum of type 2 diabetic mellitus with dental caries. However, the exact mechanism by which Diabetes Mellitus exerts its deleterious effects on the initiation and progression of dental caries is still unclear. The study showed increased Malondialdehyde levels in saliva and serum of patients with type two diabetes mellitus patients at different levels of initiation and progression of caries, which attributes to the increased oxidative stress, compromised host defence, chronic systemic inflammation, vasculopathy, and impaired salivary secretion (Surdacka A et al. 2011).

Diabetes mellitus has been associated with oxidative stress. In the development and progression of diabetes increased oxidative stress plays an important role (Cerielo A 2000). The imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to be ready detoxify the reactive intermediates is oxidative stress. Decreased production of antioxidants; or excessive production of free radicals may cause imbalance. Free radicals are formed disproportionately by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins in diabetes. Decline of antioxidant defense mechanisms and abnormal high levels of free radicals simultaneously leads to damage of cellular organelles and enzymes, increased lipid peroxidation thus development of insulin resistance. The development of complications of diabetes mellitus are mainly due to consequences increased oxidative stress (AC Martim and JB Watkins 2003).In In the study Ayaz K. Mallick et

al showed significant levels in increased erythrocyte membrane lipid peroxidation as increased Malondialdehyde (MDA) levels. There is a significant positive correlation between the erythrocyte Malondialdehyde (MDA) levels and glycated haemoglobin. This is due to auto oxidation of glucose, which causes persistent generation of ROS or Malondialdehyde (MDA) pointing towards the fact that prolonged hyperglycemia appears to be a cause for increased oxidative stress which in turn leads to life-threatening complications (Ayaz K et al. 2011, Meenakshi and Dinesh 2014).Many Many studies have been conducted on the oral health status of diabetic individuals in recent years. Caries, periodontal disease, xerostomia and tooth loss have been studied by different authors. The frequency of dental caries in diabetic patients is a controversial issue. Various studies relate diabetic patients to high caries frequency due to poorly controlled metabolism, poor oral hygiene, systemic complications (nephropathy), age, sex, presence of microorganisms and location of caries on the tooth [Carda C et al. 2006]. Some studies have evaluated the association between diabetes mellitus and the location of caries, variables on the root surfaces and coronal caries, stimulation of salivary function, oral hygiene, gingival recession, periodontal condition. Conversely, an association between diabetes and the increase in caries has been reported, explained by increase in salivary glucose, lack of metabolic control, recticular fluid and decrease in saliva flow.

This study showed an increase in MDA levels in DM patients with increase in caries prevalence, which is in keeping with the results of previous studies. The association between diabetes and the increase in caries has been, explained by lack of metabolic control, increase in salivary glucose, crevicular fluid and decrease in saliva flow. MDA marker is found to be increased in saliva of patients with type two diabetes, indicating that the increase of MDA may be a high-risk marker for complications in patients with poorly controlled. Diabetes (Al-Rawi 2011).

5. Conclusion

This study gives convincing support in favor of the etiologic role of free radical injury in type 2 diabetic mellitus with dental caries. The increased Malondialdehyde levels' attributes to oxidative stress as a result of excessive production of free radicals generation attributable to Diabetes Mellitus. Thus, importance should give oral health care among diabetes mellitus individuals to prevent worsening of their oral health status.

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