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Original Article

Role of salivary malondialdehyde in assessment of oxidative stress among diabetics

Komal Smriti [a], *Keerthilatha Muralidhar Pai* [a], *Vineetha Ravindranath* [a], *Kalyana Chakravarthy Pentapati* [b,*]

[a] *Department of Oral Medicine and Radiology, Manipal College of Dental Sciences, Manipal University, Manipal, India*
[b] *Department of Public Health Dentistry, Manipal College of Dental Sciences, Manipal University, Manipal, India*

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ABSTRACT

Aims: To evaluate and compare the salivary content of malondialdehyde (MDA) in patients with type 2 diabetes and control subjects.
Methods: We conducted a cross-sectional study among 30 freshly diagnosed subjects of diabetes mellitus and 30 volunteers with no diabetes mellitus. Serum and salivary MDS levels were evaluated among all the subjects.
Results: The mean serum MDA in group controls and diabetics was 0.95 ± 0.13 and 3.11 ± 0.42. The mean salivary MDA in group controls and diabetics was 0.26 ± 0.05 μmol/l and 0.81 ± 0.07 μmol/l. The mean serum and salivary MDA levels were significantly higher in group diabetics than control group ($p < 0.001$ and <0.001) respectively. There was significant positive strong correlation between serum and salivary MDA levels in both controls and diabetics groups ($r = 0.857$, $p < 0.001$ and $r = 0.891$, $p < 0.001$) respectively.
Conclusion: MDA was detectable in saliva in both diabetic and control groups. There was a positive significant correlation between salivary and serum MDA in diabetic and control subjects. Hence, salivary MDA appears to be an indicator of serum MDA concentration.

1. Introduction

Type 2 diabetes or non-insulin-dependent diabetes is a multifactorial disease which develops slowly and in a stepwise order.[1–3] It starts with insulin resistance which progresses with time until the body fails to maintain glucose balance leading to glucose intolerance. These perturbations are accompanied with wide array of changes in biochemical processes (altered lipid profile and lipid peroxidation).[4] Lipid peroxidation and oxidative damage to unsaturated lipids are established general mechanisms for oxidative stress-mediated cellular injury.[5,6] Significant changes in the cell membrane could be seen due to free-radical induced lipid peroxidation[7] and are implicated in the pathogenesis of many degenerative diseases (atherosclerosis, aging, carcinogenesis, and diabetes mellitus).[8]

Excess production of reactive oxygen species (ROS) and an impaired antioxidant defense mechanism leads to increased oxidative stress in diabetes.[9,10] ROS induces membrane lipid peroxidation and the generated fatty acid peroxides cause cell malfunction.[11]

* *Corresponding author.*
E-mail address: drkalyan81@gmail.com (K.C. Pentapati).
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Malondialdehyde (MDA) assay is the most widely used lipid peroxidation technique due to its simplicity.[12] The determination of the oxidative stress requires sometimes invasive techniques such as taking blood samples. Whole saliva is an important physiologic fluid that contains a highly complex mixture of substances. Variable amounts of serum products are present in whole saliva. Exploring saliva as a diagnostic tool for assessment of oxidative stress and antioxidant markers could be of significant clinical interest.[13,14] With this background, we aimed to evaluate the salivary content of MDA (lipid peroxidation) in patients with type 2 diabetes that would accurately reflect the severity of the oxidative stress.

2. Materials and methods

We conducted a cross-sectional study in Department of Oral Medicine and Radiology and the Diabetic clinics of Kasturba Medical Hospital, Manipal. Institutional ethical committee approval was obtained before the commencement of the study. Written informed consent was obtained from all the participants.

Subjects who were newly diagnosed with diabetes and without any other co-morbidities were included in the diabetic group. A total of 30 healthy volunteers with no known comorbidities were included in control group. Subjects with decreased salivary flow, or any salivary gland disorders, oral lesions associated with bleeding, or recent antibiotic usage were excluded.

Subjects were instructed to fast overnight (minimum 8 h) and come for fasting blood sugar and MDA levels estimation in the morning. A total of 2 ml blood sample for plasma MDA and glucose estimation was collected in plain vacutainers. MDA was assessed by using thiobarbituric acid as a substrate in serum. The collected samples were immediately transported to the biochemistry laboratory and were analyzed on the same day or within two hours (μmol/l). Samples were first centrifuged at 4000 rpm for 15 min and clear supernatants were processed immediately for estimation of glucose and MDA.

Subjects were instructed to rinse the mouth thoroughly with tap water 2–3 times and spit to clean any food debris. Unstimulated whole saliva was collected by spitting method into a sterile sample container over the next 10 min. MDA was measured using the method outlined by Buege and Aust where MDA reacts with thiobarbituric acid (TBA) to yield a pink-colored product. The absorbance of 3 ml colored layer was measured at 335 nm spectrophotometrically.[15]

All the analysis was done using SPSS version 18 (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered statistically significant. Comparison of mean values between the groups was done using independent sample t test. Pearson correlation coefficient was done to evaluate correlation between serum and salivary MDA levels.

3. Results

A total of 30 patients with recently diagnosed diabetes mellitus and 30 age- and gender-matched nondiabetic individuals were included in the study. Among controls, there were 50% male while in diabetics, there were 56.7% males. However, there was no significant difference in the distribution of gender between controls and diabetics ($p = 0.605$) (Table 1). The mean age in controls and diabetics was 57.73 ± 7.92 and 54.77 ± 8.99. There was no significant difference in the mean age between controls and diabetics ($p = 0.18$).

Table 1 – Distribution of gender between controls and diabetic groups.

	Controls N (%)	Diabetics N (%)	p-value
Gender			
Female	15 (50.0%)	13 (43.3%)	0.605
Male	15 (50.0%)	17 (56.7%)	

Chi-square test.

Table 2 – Comparison of serum and salivary MDA levels between controls and diabetic groups.

	Controls Mean ± SD	Diabetics Mean ± SD	p-value
Serum MDA (μmol/l)	0.95 ± 0.13	3.11 ± 0.42	<0.001
Salivary MDA (μmol/l)	0.26 ± 0.05	0.81 ± 0.07	<0.001

Independent sample t test.

The mean serum MDA in controls and diabetics was 0.95 ± 0.13 μmol/l and 3.11 ± 0.42 μmol/l. The mean salivary MDA in controls and diabetics was 0.26 ± 0.05 μmol/l and 0.81 ± 0.07 μmol/l. The mean serum and salivary MDA levels were significantly higher in diabetics than control group ($p < 0.001$ and <0.001) respectively (Table 2). There was significant positive strong correlation between serum and salivary MDA levels in both controls and diabetics ($r = 0.857$, $p < 0.001$ and $r = 0.891$, $p < 0.001$) respectively.

4. Discussion

Diabetes mellitus is a group of chronic metabolic changes (insulin deficiency, cellular resistance to insulin action, or both, resulting in hyperglycemia) and associated with serious complications of various organ systems that might impair quality of life and shorten the lifespan.[16]

In diabetes mellitus, abnormally increased levels of lipids, lipoproteins, and lipid peroxides in plasma may be due to the abnormal lipid metabolism.[8] Patients with type 2 diabetes have an abnormal blood lipid profile consisting of moderately elevated LDL-C, moderately decreased HDL-C, and high TC and triglycerides.[13] Thus, inadequate levels of HDL-C, in conjunction with more atherogenic forms of LDL-C may contribute to atherogenesis.[13] One of several byproducts of lipid peroxidation processes is MDA which can be used as indicator for oxidative stress.

The results of our study showed a three-fold increase in serum levels of MDA for diabetic group when compared with control group. This was similar to that reported by Lamichanne et al.,[17] Bhutia et al.,[18] Mahreen et al.,[19] Mandal et al.,[20]