Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jobcr

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*

ARTICLE INFO

Article history:
Received 23 August 2015
Accepted 4 December 2015
Available online 22 December 2015

Keywords:
Diabetes
Lipid peroxidation
Malondialdehyde
Oxidative stress
Saliva

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).
http://dx.doi.org/10.1016/j.jobcr.2015.12.004

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]

and Nakhjavani et al.[21] The various compositional changes in saliva currently indicate that salivary glands are targeted organs in diabetes mellitus. The high concentrations of MDA in saliva recorded in this study usually followed those recorded in serum which was similar to the previous studies.[13,22] However, lower MDA values in diabetic group have been reported by Hegde et al., which were probably due to the exceptionally high antioxidant activity.[23] Previous studies also reported higher salivary antioxidants in diabetics than controls.[23–25] This may clarify that the salivary MDA in diabetic patients may result from a state of systemic dyslipidemia and serum composition is reflected in the saliva composition.

Enhanced oxidative stress was indicated by increased free radicals production, lipid peroxidation, and reduced antioxidant status. Several studies have reported an increased susceptibility to lipid peroxidation in patients with diabetes mellitus. The generation of free radicals may lead to lipid peroxidation and the formation of several types of damage in diabetes mellitus. In the present study, we have observed that MDA levels, a lipid peroxidation product and a marker of oxidative stress, were elevated significantly in diabetic patients.

Salivary MDA levels are directly affected by systemic oxidative stress, since MDA levels were also elevated in the saliva of diabetic patients. The salivary MDA level was significantly increased in the diabetic group of the present study which reflects a high oxidative stress status among diabetic patients. Salivary estimation of lipid peroxide along with other lipid profiles in diabetes mellitus is therefore considered very useful as it may serve as a useful monitor to judge the oxidative stress status of the patients.

The overall salivary antioxidants increase in DM patients may result from a state of systemic oxidative stress, which induced a general increase in serum antioxidant, as to a large extent serum composition is reflected in the saliva composition. Reznick et al. have shown that oxidative stress exists in diabetic patients as evidenced by the increased total antioxidant capacity in the saliva and blood of patients.[25] Indeed, there is evidence that suggests that endogenous antioxidant capacity is reduced in diabetes, due to several factors, including the impact of non-enzymatic glycation on key enzymes, the polyol pathway, and its consumption of reducing power, as well as the constant demands of oxidative stress.

Thus, in the present study, MDA was detectable in saliva in both diabetic and nondiabetic individuals. 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. Further, studies on larger populations and in different geographic areas are needed to establish salivary MDA estimation as a monitoring tool for oxidative stress in diabetes mellitus and also to evaluate possible correlations with serum lipid profiles.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Stumvoli M, Goldestain B, Timon WH. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 2005;65: 333–346.
2. Waeber GP, Vollenweider PP. Prevention of type 2 diabetes: where do we stand? *Med France*. 2007;55.
3. Lipinski B. Pathophysiology of oxidative stress in diabetes mellitus. *J Diabetes Complications*. 2001;15:203–210.
4. Maharjan BR, Jha JC, Adhikeri D, et al. A study of oxidative stress, antioxidant status and lipid profile in diabetic patient in Western region of Nepal. *Kathmandu Univ Med J*. 2008;6: 16–21.
5. Yagi K. Lipid peroxides and altered radicals in clinical medicine. *Adv Exp Med Biol*. 1994;366:1–15.
6. Syryawansh NP, Bhutey AK, Nagdeote AN, Jadav AA, Manoorker GS. Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian J Clin Biochem*. 2006;21: 126–130.
7. Agrawal S, Banerjee S, Chattergee SN. Effects of oxygen on ferrous sulphate-induced lipid peroxidation in liposomal membrane. *Indian J Biochem Biophys*. 1985;21:331–334.
8. Nair U, Bartch H, Nair J. Lipid peroxidation-induced DNA damage in cancer prone inflammatory diseases: a review of published adduct types and levels in humans. *Free Radic Biol Med*. 2007;43:1109–1120.
9. West IC. Radicals and oxidative stress in diabetes. *Diabet Med*. 2000;17:171–180.
10. Antoine L, Karine L, Jerome G, Anne-Marie P. Value of sperm thiobarbituric acid reactive substance in fertile men. *Clin Chim Acta*. 2002;325:113–115.
11. Sanocka D, Kurpisz M. Reactive oxygen species in sperm cells. *Reprod Biol Endocrinol*. 2004;224:2–12.
12. Tappel AL. Lipid peroxidation damage to cell components. *Clin Pathol Fed Proc*. 1973;32:1870–1874.
13. Al-Rawi NH. Oxidative stress, antioxidant status and lipid profile in the saliva of type 2 diabetics. *Diab Vasc Dis Res*. 2011;8:22–28.
14. Schenkels LC, Veerman EC, Nieuw Amerongen AV. Biochemical composition of human saliva in relation to other mucosal fluids. *Crit Rev Oral Biol Med*. 1995;6: 161–175.
15. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol*. 1978;51:302–310.
16. Powers AC. Diabetes mellitus. In: Fauci A, Braunwald E, Kasper D, eds. et al. In: *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill; 2008:2275–2304.
17. Lamichhane A, Prasad S, Bhaskar N, Singh J, Pandey R. *Malondialdehyde (MDA): an oxidative stress marker in type II Diabetes mellitus with and without complications*.2012;2: 110–112.
18. Bhutia Y, Ghosh A, Sherpa ML, Pal R, Mohanta PK. Serum malondialdehyde level: Surrogate stress marker in the Sikkimese diabetics. *J Nat Sci Biol Med*. 2011;2: 107–112.
19. Mahreen R, Mohsin M, Nasreen Z, Siraj M, Ishaq M. Significantly increased levels of serum malonaldehyde in type 2 diabetics with myocardial infarction. *Int J Diabetes Dev Ctries*. 2010;30:49–51.
20. Mandal B, Bandyopadhyay R, Chakrabarti S, Bandyopadhyay TK. Assessment of oxidative stress in patients of diabetes mellitus with and without complications. *JIACM*. 2010;11: 20–25.
21. Nakhjavani M, Esteghamati A, Nowroozi S, Asgarani F, Rashidi A, Khalilzadeh O. Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels. *Singapore Med J*. 2010;51:582–585.

22. Rajeshwari SG, Choudhry AA, Gururaja A, Prabhu K. Correlation of plasma lipid profile with salivary oxidative stress markers in Type II diabetes mellitus patients. *J Clin Diagn Res*. 2014;8:CC08–CC10.
23. Hegde A, Shenoy R, D'Mello P, Smitha A, Tintu A, Manjrekar P. Alternative markers of glycemic status in diabetes mellitus. *Biomed Res*. 2010;21:252–256.
24. Arana C, Cutando A, Ferrera MJ, et al. Parameters of oxidative stress in saliva from diabetic and parenteral drug addict patients. *J Oral Pathol Med*. 2006;35:554–559.
25. Reznick AZ, Shehadeh N, Shafir Y, Naglar RM. Free radicals related effects and antioxidants in saliva and serum of adolescents with type 1 diabetes mellitus. *Arch Oral Biol*. 2006;51:640–648.