Original Article

Malondialdehyde Levels in Oral Sub Mucous Fibrosis: A Clinicopathological and Biochemical Study

Shishir Ram Shetty, Subhas G Babu, Suchetha Kumari[1], Vaman Rao[2], Vijay R[3], Arvind Karikal[4]

Departments of Oral Medicine and Radiology, AB Shetty Memorial Institute of Dental Sciences, [1]Biochemistry, KS Hegde Medical Academy, [2]Director, Research and Development, Nitte University, [3]Central Research Laboratory, [4]Department of Oral Surgery, AB Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, Karnataka, India

Abstract

Background: Oral submucous fibrosis is one of the most commonly occurring potentially malignant disorders in the South-East Asia. Levels of lipid peroxidation product malondialdehyde have been recently correlated with clinical grades of oral Submucous fibrosis. **Aims:** The aims of this study were to estimate the levels of malondialdehyde in serum, saliva and tissue in patients with oral submucous fibrosis, to correlate change in levels of malodialdehyde with the histopathological grading. **Materials and Methods:** The study group comprised of 65 clinically diagnosed and histopathologically confirmed cases of oral submucous fibrosis, 21 age and sex matched controls were also enrolled into the study. The serum saliva and tissue samples in the study groups were evaluated by the thiobarbutric acid reactive substances. **Results:** There was a significant difference between the serum and salivary malondialdehyde among the histopathological grades of oral submucous fibrosis. Tissue malonaldehyde levels were significantly higher as the grading progressed but tissue levels in grade 3 oral submucous fibrosis were lower than the controls. **Conclusion:** This decrease in tissue malonaldehyde could possibly be associated to collagen cross linking occurring during the advanced stages of oral submucous fibrosis.

Keywords: Collagen, Cross-linking, Lipid peroxidation, Tissue, Saliva, Serum

Address for correspondence: Dr. Shishir Ram Shetty, Department of Oral Medicine and Radiology, AB Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, India. E-mail: drshishirshettyomr@yahoo.com

Introduction

Oral submucous fibrosis (OSMF) is a fibrotic condition of the oral cavity and is always associated with chronic epithelial inflammation and progressive deposition of collagenous extracellular matrix (ECM) proteins in the subepithelial layer of the buccal mucosa.[1] The disease is seen in those from Indian subcontinent and from many parts of South-East Asia such as Taiwan.[2] Numerous etiologies have been suggested for the pathogenesis of this condition that include consumption of chillies, nutritional deficiency, chewing of arecanut, genetic susceptibility, altered salivary constituents, autoimmunity and collagen disorders.[2] Current evidence implicates collagen-related genes in the susceptibility and pathogenesis of OSMF.[3]

Access this article online

Quick Response Code:

Website: www.najms.org

DOI: 10.4103/1947-2714.93887

The cytotoxic effects of the chewing tobacco including pan masalas are mediated through the production of the reactive oxygen species (ROS).[4] ROS induced lipid peroxidation causes a loss of cell homeostasis by modifying the structure and functions of cell membrane.[5] The most important characteristic of lipid peroxidation is to cause a considerable deoxyribose-nucleic acid malondialdehyde (DNA-MDA) adducts by interacting with cellular DNA.[6] Increased levels of MDA reported in individuals consuming large quantities of meat and fish.[7] We conducted a study to determine the levels of serum, salivary and tissue MDA in patients with OSMF. The influence of dietary pattern and habit frequency on the level of MDA has also been investigated.

Materials and Methods

Eighty-six patients between the age range of 20 and 40 years, reporting to the department of oral medicine and radiology in a dental college in south India were enrolled into the study. The study subjects included 65 histopathologically confirmed cases of OSMF and 21 age and sex matched healthy controls who required to undergo frenectomy or operculectomy. A detailed case history which included diet pattern and habit index was taken from each subject in the study. Subjects with any other long-term systemic illness and long term medication were excluded from the study. Five milliliter of unstimulated saliva was obtained by spit method after following standard precollection protocol. Five milliliter of venous blood was obtained from the antecubital vein, centifudged and stored. Tissue obtained during frenectomy, operculectomy from healthy controls and sectioned biopsy specimens from the OSMF patients were dissolved by the nitric acid method. The malonaldehyde content of the dissolved tissue, serum and saliva of the study subjects were evaluated by the TBARS procedure and sphectrophotometrically determined. The data obtained was subjected to statistical analysis using the SPSS version 17 software.

Results

Of the 65 subjects in the control group, 22, 20, and 23 were categorized under grade 1, 2, and 3, respectively [Figures 1a–c] after histopathological examination. The mean serum, salivary, and tissue malondialdehyde level of control group was significantly lower (P<0.001) than the cases [Table 1]. Increased levels of serum and salivary malondiadehyde was observed in all three study groups as the grading progressed but tissue malondialdehyde levels were lower in grade 3 OSMF (0.0244±0.01043 nmol/mg) compared to controls (0.0255±0.00593 nmol/mg). There was positive correlation between serum and salivary MDA levels, in case and control groups but negative correlation between serum MDA when compared to tissue levels. Salivary MDA had no significant correlation with tissue levels in case and control groups [Table 2]. There was no significant difference between the frequency of habits (gutka chewing) and mean serum, salivary, and tissue levels of MDA. No significant difference was observed in diet pattern (predominantly vegetarian/nonvegetarian) between cases and controls. When intercomparison of the diet status was done among the groups, no significant difference was observed [Table 3].

Table 1: Comparion of malonaldehyde levels between cases and controls (Student's unpaired *t*-test)

Parameter	*N*	Mean tissue (nmol/mg) serum/ saliva (nmol/mg)	Standard deviation	*t*-test
Serum MDA				
Cases	65	1.1434	0.53934	6.42900
Controls	21	0.3597	0.24463	*P*<0.001 vhs
Salivary MDA				
Cases	65	0.4344	23486	3.24100
Controls	21	0.2242	32212	*P*<0.001 vhs
Tissue MDA				
Cases	65	0.0470	02432	3.98300
Controls	21	0.0255	00593	*P*<0.001 vhs

MDA: Malondialdehyde; *N*: Number; vhs: Very highly significant

Table 2: Correlation of serum, salivary, and tissue levels in cases and control

Parameters	Sa MDA	Tis MDA
Cases Se MDA *r*	0.461	−0.435
P	<0.001	<0.001
N	65	65
Cases Sa MDA *r*		−0.115
P		0.360
N		65
Controls Se MDA *r*	0.514	0.164
P	0.017	0.478
N	21	21
Controls Sa MDA *r*		0.233
P		0.310
N		21

P: P value; N: Number; MDA: Malondialdehyde; Se: Serum; Sa: Salivary; Tis: Tissue; r: Correlation Coefficient

Discussion

So far studies have been conducted on serum and salivary levels of lipid peroxidation end product malondialdehyde in cancer and precancerous conditions.[5,8,9] Most of the studies report of increase in the levels of MDA in oral cancer and OSMF.[8-10] Similar observations were reported in our study. A study were subjects were graded according to the classification by Bhat and Dholakia revealed significant elevation in serum MDA levels as grading progressed.[10,11] We have also analyzed the tissue levels of MDA in different histopathological grades of OSMF (based on grading proposed by Kiran *et al.*).[2] Tissue levels of MDA were consistently higher in Grade 1 and Grade 2 OSMF when compared to controls. Elevated tissue MDA levels have been observed in experimental animals when exposed to cigarette smoke and ischemic conditions.[12,13] Significantly higher levels of tissue MDA were detected in tumor tissues of breast cancer patients.[14] Tissue levels of MDA were reduced in Grade 3 OSMF when compared to controls. Possible reason could be

Table 3: Intergroup comparison of diet pattern

Diet pattern	Grade 1	Grade 2	Grade 3	Controls	Total
Non-veg count	10	8	10	8	36
%	45.5	40	43.5	38.1	41.9
Veg count	12	12	13	13	50
%	54.5	60	56.5	61.9	58.1
Total count	22	20	23	21	86
%	100	100	100	100	100

$\chi^2 = 0.292$, $P = 0.961$ not significant

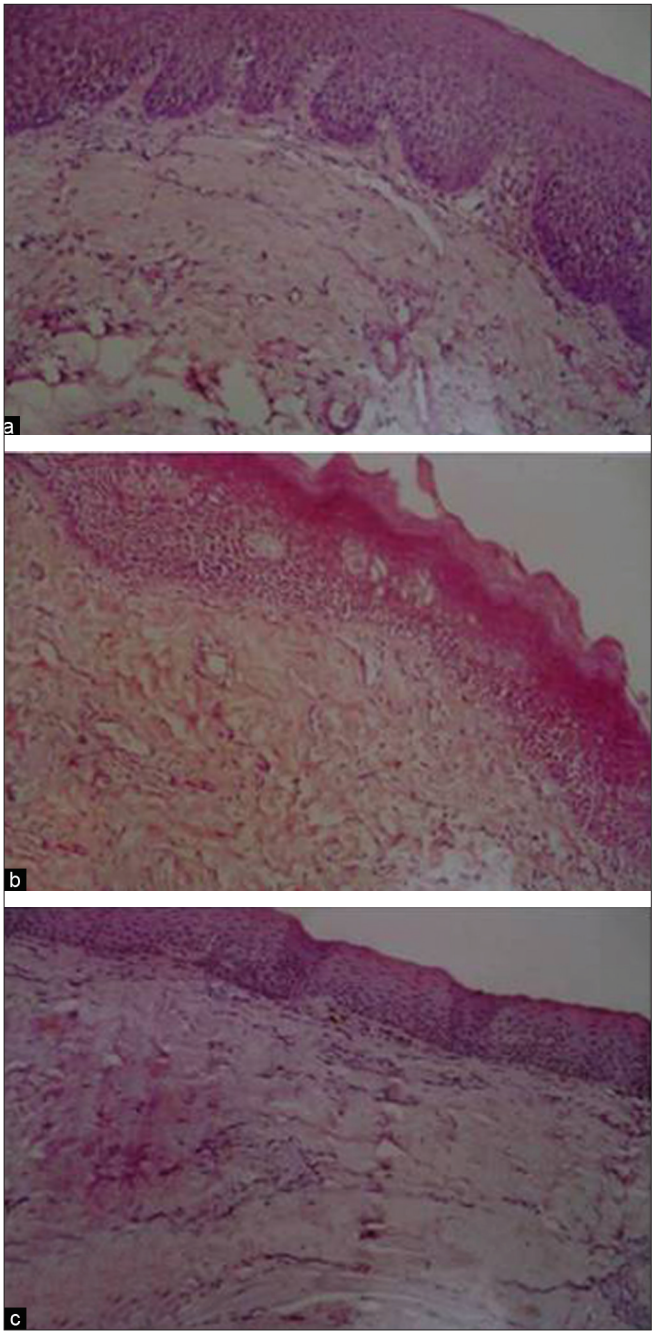

Figure 1: (a) Photomicrograph (×40) showing loose thin and thick fibers; (b) photomicrograph (×40) showing loose thin or thick fibers with partial hyalinization; (c) photomicrograph (×40) showing complete hyalinization

due to utilization of malondialdehyde in crosslinking of collagen.[15,16] It has been shown by an amino acid analysis that malonaldehyde reacts in a significant way on lysine and tyrosine residues. [16] Previous studies have revealed fibroblast stimulating properties of malondialdehyde in culture.[17] Addition of MDA to cultured fibroblasts increased collagen production by 2–3 times.[18] We used nitric acid dissolution method for tissue dissolution and thiobarbutric acid method (TBARS) for evaluation of malondialdehyde. Similar nitric acid dissolution method was used for determination of tissue malondialdehyde in zinc deficient rats.[19] We also investigated the dietary pattern and habit frequency of the subjects; however, no relevant information was obtained.

Conclusion

The decreased levels of tissue MDA in the advanced histopathological grades of OSMF could be probably due its utilization in pro-fibroblastic and collagen cross linking activities. However this finding requires further research which will enhance current knowledge about pathogenesis and progression of OSMF.

References

1. Murthi PR, Bhonsle RB, Gupta PC, Daftafray DK, Pindborg JJ, Mehta FS. Etiology of oral submucous fibrosis with special references to the role of arecanut chewing. J Oral Pathol Med 1995;24:145-52.
2. Kiran KK, Saraswathi TR, Ranganathan K, Uma Devi M, Joshua E. Oral Submucous Fibrosis: A clinico histopathological study in Chennai. Indian J Dent Res 2007;18:106-11.
3. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2005;42:561-8.
4. Bagchia M, Balmooria J, Bagchia D, Stohsa SJ, Chakrabartib J, Das DK. Role of reactive oxygen species in the development of cytotoxicity with various forms of chewing tobacco and pan masala. Toxicology 2002;179:247-55.
5. Manoharan S, Kolanjiappan K, Suresh K, Panjamurthy K. Lipid peroxidation and antioxidants status in patients with oral squamous cell carcinoma. Indian J Med Res 2005;122:529-34.
6. Abidi S, Ali A. Role of oxygen free radicals in the pathogenesis and etiology of cancer. Cancer Lett 1999;142:1-9.
7. Brown ED, Morris VC, Rhodes DG, Sinha R, Levander OA. Urinary Malondialdehyde-Equivalents during ingestion of meat cooked at high or low temperatures. Lipids 1995;30:1053-6.

8. Guven Y, Unur M, Bektas K, Uslu E, Belce A, Demirez E, *et al*. Salivary malondialdehyde levels in patients with oral leukoplakia. Turk J Med Sci 2005;35:329-32.
9. Rai B, Kharb S, Jain R, Anand SC. Salivary lipid peroxidation product malonaldehyde in various dental diseases. World J Med Sci 2006;1:100-1.
10. Gupta S, Reddy MV, Harinath BC. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Indian J Clin Biochem 2004;19:138-44.
11. Bhatt AP, Dholakia HM. Mast cell density in oral submucous fibrosis. J Indian Dent Assoc 1977;49:187-91.
12. Kim DH, Suh YS, Mun KC. Tissue levels of malondialdehyde after passive smoke exposure of rats for a 24-week period. Nicotine Tob Res 2004;6:1039-42.
13. Ilhan N, Halifeoglu I, Ozercan HI, Ilhan N. Tissue malondialdehyde and adenosine triphosphatase level after experimental liver ischaemia-reperfusion damage. Cell Biochem Funct 2001;19:207-12.
14. Portakal O, Ozkaya O, Inal ME, Bozan B, Kosan M, Sayek I. Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. Clin Biochem 2000;33:279-84.
15. Svadlenka I, Davídková E, Rosmus J. Interaction of malonaldehyde with collagen. II. Reaction of collagen with certain malondialdehyde derivatives. Z Lebensm Unters Forsch 1973;153:263-8.
16. Svadlenka I, Davídková E, Rosmus J. Interaction of malonaldehyde with collagen. III. Binding site characteristic of malonaldehyde with respect to collagen. Z Lebensm Unters Forsch 1975;157:312-5.
17. Maher JJ, Tzagarakis C, Gimenez A. Malondialdehyde stimulates collagen production by hepatic lipocytes only upon activation in primary culture. Alcohol Alcohol 1994;29:605-10.
18. Chojkier M, Houglum K, Solis-Herruzog J, Brenner DA. Stimulation of collagen gene expression by ascorbic acid in cultured human fibrcoblasts. J Biol Chem 1989;266:16957-62.
19. Bor NM, Unver Y, Kilinc K, Dereagzi H. Tissue malondialdehyde levels in zinc deficient rats. J Islam Acad Sci 1994;7:74-7.

How to cite this article: Shetty SR, Babu SG, Kumari S, Rao V, Vijay R, Karikal A. Malondialdehyde Levels in Oral Sub Mucous Fibrosis: A Clinicopathological and Biochemical Study. North Am J Med Sci 2012;4:125-8.

Source of Support: Nil. **Conflict of Interest:** None declared.