ORIGINAL RESEARCH

Estimation of Malondialdehyde Level in Oral Submucous Fibrosis

Poorani R,[1] Vezhavendhan N,[2] Ramesh R,[3] Vidhya Lakshmi S,[4] Sivaramakrishnan M,[5] SuganyaR[6]

ABSTRACT

Background: Oral submucous fibrosis are the common Potentially Malignant Disorders prevailing in India. The primary etiological factor include arecanut which contain numerous Reactive Oxygen Species (ROS). Malondialdehyde (MDA) is the end product of lipid peroxidation and it is mutagenic and tumorigenic. **Aims and Objectives:** To estimate the serum Malondialdehyde level in Oral submucous fibrosis. **Methodology:** The control group comprised twenty normal individuals (Group 1). The experimental group comprised twenty patients with oral submucous fibrosis (Group 2). Blood samples were obtained and evaluated for serum Malondialdehyde and antioxidants level. Serum Malondialdehyde level was estimated using TBARS assay by Spectrophotometric method. **Results:** The present study revealed a statistically significant increase in serum MDA level in OSMF patients in comparison with corresponding normal individuals. **Conclusion:** While the oxidant (Malondialdehyde) levels are increased, indicating the potential role in premalignancy status, it is important to see from a larger sample if these results are reproducable and if so can it be sensitively used in detection of these potentially malignant disorders (Oral submucous fibrosis).

Key words: Oral submucous fibrosis, Malondialdehyde

Oral submucous fibrosis is the potentially malignant disorders. Potentially malignant disorders are the ones that are described as "Not all lesions and conditions may transform to cancer rather than a morphological alteration amongst which some may have an increased potential for malignant transformation".[(1)] In Indian subcontinent, oral submucous fibrosis are the most common potentially malignant disorders caused largely by tobacco smoking and areca nut consumption. Areca nut has been declared as a known human carcinogen by IARC expert group in 2003.[(2)] Tobacco and areca nut also contains numerous free radicals and reactive oxygen species important of which includes singlet oxygen (O_2), hydroxyl radical ($OH^{\bullet}$), and nitric oxide ($NO^{\bullet}$). Free radicals are highly reactive

molecules which contain unpaired electron in the outer orbital. The unpaired electrons are unstable and they can propagate the chain reaction to produce the abundant free radicals during the chemical processes. Examples include Superoxide anion radical ($O_2^{\bullet -}$), Hydroperoxyl radical ($HOO^{\bullet}$), Hydrogen peroxide (H_2O_2), Hydroxyl radical (OH^-), Lipid peroxide radical ($ROO^{\bullet}$), Singlet oxygen (O_2), nitric oxide ($NO^{\bullet}$), and Peroxynitrate ($ONOO^{-\bullet}$), among which hydroxyl radicals are the most reactive oxidants that can produce tissue damage.[3] Free radicals can produce lipid peroxidation in membrane, Oxidative modification of proteins, and lesions in DNA.[4] Lipid peroxidation and continuous degradation produce Malondialdehyde ($CHO-CH_2-CHO$), propanediol and 4- hydroxynonenal (4-HNE).[5] Malondialdehyde is a three carbon dialdehyde that appears mostly in blood, saliva and urine and serves as an important biomarker for oxidative stress.[6]

The aim of the present study was carried out to evaluate the level of serum MDA in Oral submucous fibrosis patients.

AIMS AND OBJECTIVES:

To estimate the Malondialdehyde level in Oral Submucous Fibrosis.

MATERIAL AND METHODOLOGY:

Source of data

This study was approved by the Institutional Ethical Committee, and written consent was taken from every participant. The study group consisted of a total number of fourty subjects between the age 17 to 58 years reporting to the Department of Oral Medicine and Department of Oral Pathology, for a period of 2 years which included fourty subjects divided into two groups: Group 1: 20 healthy individuals (controls), Group 2: 20 clinically diagnosed as leukoplakia and histopathologically confirmed as epithelial dysplasia, immunocomprised patients were excluded from the study.

Sample collection

2.5ml of venous blood was collected from the antecubital vein of all the patients included in the study. For control group, blood samples were obtained from the normal individuals who underwent master health check up. The sample was then clotted at room temperature for 2 hours and centrifuged at 3000 rpm for 10 mins and the serum was separated and stored at -20^o C. Estimation of Serum Malondialdehyde level was done from the collected samples.

Serum Malondialdehyde level was estimated by using Thiobarbituric acid reagent (TBARS) assay. The principle of this method is based on serum proteins were precipitated by trichloroacetic acid. Sulphuric acid was used to hydrolyse the lipid peroxidation product from the protein to yield MDA. MDA was made to react with thiobarbituric acid (TBA) to yield MDA-TBA adduct. Upon boiling in the water bath, this yielded a pink color, which was measured at an absorbance of 530 nm, using spectrophotometry. The units of concentration of MDA in serum were calculated using the formula and expressed as µmol/L.

The data were analysed with Students unpaired‘t’ test is employed to compare the serum oxidative stress level (MDA) in control group and in patients with OSMF. All statistical analysis was performed with the program Statistical Package for the Social Science Program (SPSS Version 21) software and p value of ≤ 0.05 was accepted as statistically significant.

RESULTS AND OBSERVATIONS:

Table 1 shows comparison of serum MDA level between control and oral submucous fibrosis: There was a statistically significant increased serum MDA level in OSMF (Group 2) patients when compared to normal individuals (Group 1) [Graph 1].

DISCUSSION:

Oral sub m ucous fibrosis (OSMF) which were previously categorised as premalignant condition respectively by WHO in 1978[7] are now categorised as potentially malignant disorders (PMD). PMD has the prevalence rate of 0.2 to 0.5 % with malignant transformation potential of 0.13 to 17.5 %. [8] Oral submucous fibrosis is defined as "An insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by fibro-elastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat".[9]

Areca nut is the known etiological factors for oral submucous fibrosis. Areca nut contains arecoline, arecaidine, guvacoline and guacine which can directly stimulate the carcinogenic effect in cells. Numerous Reactive oxygen species (ROS) and reactive nitrogen species present in areca nut can indirectly stimulate the carcinogenic effect in the cell. Deleterious effect of ROS are neutralised by intracellular and extracellular antioxidant system. Estimation of the level of ROS in Oral submucous fibrosis patient may serve as important biomarkers to analyse the progression of the disease status and its malignant transformation.

ROS is a collective term that includes free radicals and certain non-radicals that are either oxidizing agents and/or easily converted into radicals. Free radicals can be defined as a

Variables	Group 1 (N=20)		Group 2 (N=20)		t value	P value
	Mean	SD	Mean	SD	3.459	0.001**
	2.70	1.34	4.26	1.50		

**Highly Significance in comparison to controls

Table 1: Comparison of serum malondialdehyde between control and OSMF

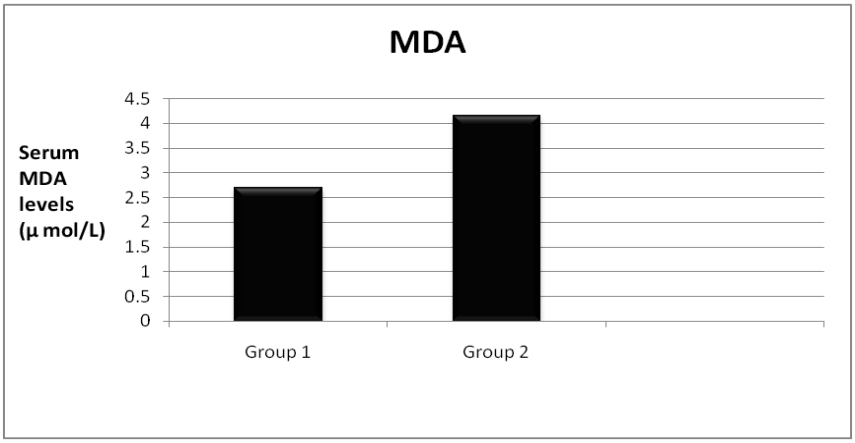

Fig 1: Serum MDA levels(µ mol/L)

molecule or molecular fragments that contain one or more unpaired electrons, in its outer orbital.[3] The free radicals are formed by partial reduction of oxygen. Oxygen is a stable gas that is vital for aerobic life. In aerobic life, the oxygen is completely reduced to water by oxidative reaction. [11] The partial reduction of oxygen produces highly reactive free radicals like Hydrogen peroxide (H_2O_2), Hydroxyl radical ($OH^{\bullet}$) and Superoxide anion (O_2^-). The free radical especially Hydroxyl radical is an extremely aggressive oxidants that can damage most of the biological molecule of the cell by lipid peroxidation, oxidative modification of proteins, DNA base alteration, damage the tumor suppressor gene and enhance expression of proto-oncogene.[10] The cellular membranes are vulnerable to the oxidation by ROS due to the presence of high concentration of unsaturated fatty acids in the lipid component. ROS react with the membrane lipids and causes lipid peroxidation resulting in the formation of lipid hydroperoxyl (LOOH) which can further decompose to an aldehyde such as Malondialdehyde, 4 – Hydroxylnonenal and Propandiol. Among these MDA is the frequently used biomarker that provides overall information regarding lipid peroxidation.[11]

MDA is the naturally occurring endogenous product of lipid peroxidation and it is mutagenic and tumorigenic.[12] The MDA reacts with both the Deoxyadenosine and Deoxyguanosine in the DNA and produce DNA-MDA adduct.

The present study was carried out to estimate the level of serum MDA in oral submucous fibrosis patients. The study group consisted of one control group, Group 1 (consisting of 20 normal individuals) Group 2 (consisting of 20 patients with OSMF). MDA level was evaluated by TBARS assay using spectrophotometry.

Serum MDA level was increased in OSMF patients when compared to the normal healthy individuals and this observation is in line with the results obtained from the previous study conducted by Soma Gupta et al in 2004[13], in oral submucous fibrosis. Suryakant Metkari et al in 2012[14], analysed the MDA level in different histopathological grades of OSMF and stated that there was no statistically significant difference. Shishir Ram Shetty et al in 2012[15], analysed the malondialdehyde level in different grades of OSMF, and found that tissue levels of MDA were increased in grade I and grade II and decreased in grade III OSMF when compared to controls, the reason of which might be due to utilization of malondialdehyde in crosslinking of collagen. Comparison among different grades of PMD was not possible in this study due to inadequate sample size.

The increased level of serum MDA in OSMF may be due to increased lipid peroxidation and continuous action of free radical on the lipid membrane. The free radical initiates peroxidation of lipid membrane by attraction of hydrogen atom from the methylene group of polyunsaturated fatty acids. The molecular derangement of membrane forms peroxyl radical which themselves attract a hydrogen atom from another fatty acid and thus propagate the chain reaction.

The study revealed a statistically significant increase in MDA level in OSMF in comparison with control groups.

Evaluation of serum MDA level may be regarded as potential biochemical parameters for evaluating the disease process of Oral submucous fibrosis.

REFERENCE:

1. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med.2007; 36:575–80.
2. World Health Organization. World Health Organization Classification of Tumours. In: Barnes L, Eveson JW, Reichart P, Sidransky D. Pathology & Genetics. Head and Neck Tumours. Lyon: International Agency for Research on Cancer (IARC) IARC Press.2005;2:177–9.
3. Free radicals and antioxidants. Vasudevan, Sreekumari, Kannan. Textbook of Biochemistry. Seventh edition, Jaypee,2013.p.433-438.
4. Kumar, Abbas, Fausto, Aster. Cellular Responses to Stress & Toxic Insults: Adaptation, Injury & Death. Robbins and Cotran Pathologic Basis of Disease. Eighth edition, Elsevier,2010.p.20-22.
5. Revant H Chole, Ranjitkumar N Patil, Anjan Basak, Kamlesh Palandurkar, Rahul Bhowate . Estimation of serum Malondialdehyde in Oral Cancer and Precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. Journal of Cancer Research and Therapeutics.2010;6 (1):487-491.
6. Michael Lieberman, Allan D. Marks. Oxygen toxicity and free radical injury. Mark's Basic Medical Biochemistry. Fourth edition, Lippincott Williams and Wilkins,2013.p.437-455.
7. World Health Organization Collaborating Centre for Oral Precancerous lesions. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol.1978;46:518–39.
8. Phatak A. Fibrin producing factor in oral submucous fibrosis. Indian Journal of Otolaryngology and head and neck surgery. 1979; 31(4): 103-4.
9. Amagasa T, Yamashiro M, Ishikawa H. Oral leukoplakia related to malignant transformation. Oral Sci Int. 2006; 3: 45–55.
10. Darius K.Paissat. Oral submucous fibrosis. Int.J.Oral Surg.1981;10:307-312.
11. Gutteridge. Lipid peroxidation and Antioxidants as Biomarkers of Tissue Damage. Clin. Chem. 1995; 41: 1819-1828.
12. Niedernhofer et al. Malondialdehyde, a Product of Lipid Peroxidation, Is Mutagenic in Human cells. The Journal of biological chemistry. 2003; 278 (33): 31426 –31433.
13. Soma Gupta, M. V. R. Reddy et al. Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. Indian J Clin Biochem. 2004; 19(1): 138–141.
14. Suryakant B Metkari, Tupkari, Barpande. An estimation of serum malondialdehyde, superoxide dismutase and vitamin A in oral submucous fibrosis and its clinicopathologic correlation. JOMFP. 2007; 11 (1): 23-27.
15. D'Souza D, Subhas BG, Shetty SR, Balan P. Estimation of serum malondialdehyde in potentially malignant disorders and post- antioxidant treated patients: a biochemical study. Contemp Clin Dent. 2012; 3(4): 448-451.

Address for correspondence:
R. Poorani,
5, Pappammal Nagar, Kondur,
Cuddalore-607001.
rpoornibds@gmail.com

Authors:
[1]Post graduate student, [2]Reader, [4,5,6] Senior lecturer
Dept. of Oral Pathology & Microbiology,
Indira Gandhi Institute of Dental Sciences'
Sri Balaji Vidyapeeth, Puducherry

[3]Professor & Head,
Dept. of Biochemistry,
MGMCRI, Puducherry.

How to cite this article:
Poorani R, Vezhavendhan N, Ramesh R, Vidhya Lakshmi S, Sivaramakrishnan M, SuganyaR . Malondialdehyde Level in Oral Submucous Fibrosis. Journal of Scientific Dentistry 2014;4(2):8-13

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