ORIGINAL ARTICLE

Increase level of salivary malondialdehyde is associated with decrease mouth opening in stage-I oral submucous fibrosis

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Abstract

Objective: To compared the salivary Malondialdehyde level between Oral Submucosal fibrosis (OSMF) stage 1 patients and healthy controls.

Material and Methods: A comparative cross sectional study was conducted with eligible participants being recruited from the outpatient clinic of Department of dentistry, Ziauddin University Hospital from January 2012 till December, 2013. 80 cases and 80 controls matched for age and gender were recruited in this study. Cases were histopathologically confirmed patients of clinical stage-I Oral Submucosal fibrosis (OSMF). Socio demographic information (i.e. age, gender, ethnicity, education and occupation), oral health status, mouth opening (mm) and Malondialdehyde (mmol/L) were recorded. The study was conducted after the approval from the ethical review committee of Ziauddin University Hospital. Written informed consent was obtained from all participants prior to inclusion in the study. Data was entered and analyzed using SPSS version 21 (IBM).

Result: The overall mean (SD) of Malondialdehyde (mmol/L) for participants enrolled were 4.82 (1.65). Cases with confirmed oral submucosal fibrosis had mean (SD) Malondialdehyde (mmol/L) as 6.26 (0.60) which was significantly higher compared to controls 3.38 (0.97); the difference was significant with p-value = 0.001. Strong negative correlation was identified between mouth opening (mm) with Malondialdehyde (-0.816).

Conclusion: The salivary Malondialdehyde (mmol/L) level was significantly higher among patients with oral submucosal fibrosis and with progressively increased level of Malondialdehyde was associated with a higher risk of decreased mouth opening, among patients with OSMF. Thus, serum Malondialdehyde can be used as a biomarker for the early detection.

Keywords: free radicals, lipid peroxidation, Serum Malondialdehyde (MDA), decrease mouth opening, oral submucosal fibrosis (OSMF), salivary

Introduction:

Oral submucosal fibrosis (OSMF) being a chronic, progressive and irreversible disease.[1-2] The common features associated with OSMF are blanching (marble like appearance) due to impairment in local blood vessel, stiffening and fibrosis of any area within the oral region.[3-4] Oral submucosal fibrosis is characterized by progressive fibrosis within the mouth mucous membrane with sites involvement as soft palate, buccal and lips mucosa, and anterior pillar of fauces.[5-6] Adverse clinical outcomes are associated with it as it leads to rigidity and decreased mouth opening.[5]

Oral cancer is a significant public health concern and account for 2-4% of all malignant tumor worldwide.[7] It is a significant wide reaching health concern in Asia as well. In South-East Asia, oral cancer accounts for 40% of all cancers.[8] The epidemiological findings indicate that disease is a concern in South Asia. The inhabitants of the urban population visiting the dental practices of India, reported the prevalence of 0.2% to 1.2%.[9-10] Cases have also been reported among Pakistani population.[11] A retrospective analysis of Karachi population reported that oral cancer is the second most common cancer among all cancer in both genders.[12]

Tobacco intake induces generation of free radicals and reactive oxygen species (ROS).[13-14] Lipid peroxidation is produced by free radicals and responsible for oxidative degradation of lipids.[15-16] The end product of lipid peroxidation can be carcinogenic or mutagenic.[17] Malondialdehyde (MDA) is such end product which is highly mutagenic and carcinogenic.[18] MDA is the most widely used marker for lipid peroxidation and it leads to oxidative stress, being considered a crucial for development of premalignant lesions and cancer.[19-20]

Despite the high prevalence of OSMF in Pakistan, with a rising trend and potential to undergo malignant transformation, OSMF has not been widely investigated with respect to lipid per oxidation and antioxidants. Moreover, previous studies have estimated the level of Malondialdehyde from the blood samples. Considering, saliva sample being cost effective non-invasive and associated with low discomfort and fear the present study was conducted to compared the Malondialdehyde level between OSMF stage 1 patients and controls.

Material and Methods:
A comparative cross sectional study was conducted with eligible participants being recruited from the outpatient clinic of Department of Dentistry, Ziauddin University Hospital from January 2012 till December, 2013. 80 cases and 80 controls matched for age and gender were recruited in this study. Cases were histopathologically confirmed patients of clinical stage 1 Oral Submucosal fibrosis (OSMF).

Similar eligibility criteria was followed for the recruited of cases and controls. Participants with age greater than18 years, either gender and addiction of chewing habits (i.e. pan, Ghutka and betel nuts) were recruited in this study. Pregnant women and patients with inflammatory conditions i.e. arthritis and periodontal inflammation or with any systemic illness were excluded from the study. Moreover, cases having received any prior therapy for OSMF were also excluded.

The data was recorded on a pre-designed proforma. Socio demographic information (i.e. age, gender, ethnicity, education and occupation) were recorded. Moreover, oral health status (i.e. burning sensation, addiction or chewing habits, duration of addiction, habit frequency, brushing frequency) were recorded for cases and controls. Importantly, mouth opening (mm) and Malondialdehyde (mmol/L) was also recorded. Early morning saliva samples were obtained from the study participants. Mouth was rinsed with water thoroughly, and this same water was collected in the sterilized container in which PBS solution was dropped for the maintenance of PH, and then the samples were kept in storage at temperature of minimal 800C. Finally using the Malondialdehyde level Kit assay, MDA level was estimated.

The study was conducted after the approval from the ethical review committee of Ziauddin University Hospital. Written informed consent was obtained from all participants prior to inclusion in the study. The participants were completely briefed about the purpose of the research and procedures involved. The study was conducted according to the ethical guidelines of Helenski declaration and Pakistan Medical research Council (PMRC). Anonymity and confidentiality of the study participants were maintained throughout the research.

Data Analysis:
Data was entered and analyzed using SPSS version 21 (IBM). Once the data was entered in the analytical software it was weighted twice for incorrect entries. Qualitative or categorical data was presented as frequency and percentage while quantitative data was presented as mean±standard deviation. Qualitative variables were compared between cases and controls using chi square statistics. If the assumptions of chi square statistics were not satisfied Fisher exact test was used. Independent t-test was used to compare the quantitative variables between cases and controls. Correlation of addiction duration, habit frequency and mouth opening with Malondialdehyde (mmol/L) were performed and correlation co-efficient were reported. For inferential statistics p-value < 0.05 was consid-

Table 1: Comparison of socio demographic characteristics of Cases and Controls

Socio demographic Characteristics	Cases (n = 80)	Controls (n = 80)	Total (n = 160)	P-value
Age in years	22.53 ± 2.76	23.03 ± 3.67	22.78 ± 3.25	0.332
Age Categories				
< 20 years	8 (10)	8 (10)	16 (10)	0.016
20-25 years	64 (80)	50 (62.5)	114 (71.2)	
> 25 years	8 (10)	22 (27.5)	30 (18.8)	
Gender				
Male	46 (57.5)	54 (67.5)	100 (62.5)	0.253
Females	34 (42.5)	26 (32.5)	60 (37.5)	
Ethnicity				
Urdu speaking	20 (25)	26 (32.5)	46 (28.8)	0.157
Sindhi	22 (27.5)	14 (17.5)	36 (22.5)	
Punjabi	18 (22.5)	20 (25)	38 (23.8)	
Balochi	16 (20)	10 (12.5)	26 (16.2)	
Pathan	4 (5)	10 (12.5)	14 (8.8)	
Education years	9.25 ± 3.50	10.65 ± 3.78	9.95 ± 3.70	0.016
Education Categories				
≤ 5 years	24 (30)	12 (15)	36 (22.5)	0.001
6-12 years	44 (55)	32 (40)	76 (47.5)	
≥ 13 years	12 (15)	36 (45)	48 (30)	
Occupation				
Skilled	48 (60)	50 (62.5)	98 (61.2)	0.871
Unskilled	32 (40)	30 (37.5)	62 (38.8)	

Data presented as n(%) or Mean ± SD.

Table 2: Comparison of Oral health status of Cases and Controls

Oral health status	Cases (n = 80)	Controls (n = 80)	Total (n = 160)	P-value
Burning Sensation				
Yes	54 (67.5)	2 (2.5)	56 (35)	0.001
No	26 (32.5)	78 (97.5)	104 (65)	
Addiction (Chewing habits)				
Pan	10 (12.5)	8 (10)	18 (11.2)	0.001
Ghutka	50 (62.5)	26 (32.5)	76 (47.5)	
Betel nuts	20 (25)	46 (57.5)	66 (41.2)	
Addiction duration (years)	6.55 ± 4.20	3.80 ± 1.17	5.18 ± 3.37	0.001
Habits frequency (packets/ day)	5.43 ± 2.26	3.38 ± 1.17	4.40 ± 2.16	0.001
Brushing frequency	1.75 ± 0.44	1.98 ± 0.27	1.86 ± 0.38	0.001
Brushing frequency Categories				
Once a Day	20 (25)	4 (5.1)	24 (15.2)	0.001
Twice a Day	60 (75)	74 (94.9)	134 (84.8)	
Mouth opening (mm)	31.10 ± 2.48	42.23 ± 3.48	36.66 ± 6.34	0.001
Mouth opening (mm) Categories				
26-35 mm	76 (95)	2 (2.5)	78 (48.8)	0.001
36-45 mm	4 (5)	64 (80)	68 (42.5)	
> 45 mm	0 (0)	14 (17.5)	14 (8.8)	

Data presented as n(%) or Mean ± SD.

ered significant.

Results:

The table 1 gives details of the comparison of socio demographic characteristics of cases and controls enrolled in this study. There was no significant difference in mean age in years, gender, ethnicity, and occupation between cases and controls. However, significant difference was found in mean education years, with controls having higher mean years of education (10.65 years) as compared to cases (9.25). Similarly, significant difference was also found in education year categories with greater proportion of controls (45%) attained thirteen or more years of education as compared to cases (15%).

The table 2 gives details of the comparison of burning sensation, addiction (chewing habits), duration of addiction in years, habits frequency, brushing frequency and mouth opening (mm) between cases and controls. Significant difference was found in burning sensation, addiction, duration of addiction in years, habits frequency, brushing frequency and mouth opening between cases and controls. Greater proportion cases (67.5%) had burning sensation compared to controls (2.5%) with p-value = 0.001. Moreover, among eighty cases, around sixty three percent were addicted to Ghutka compared to only around thirty three percent among controls. The mean addiction duration in cases and controls were (6.55 Vs. 3.80; p-value = 0.001) with mean frequency habit significantly higher among cases (5.43) compared to controls (3.38). The brushing frequency was significantly lower among cases compared to controls (1.75 Vs. 1.98; p-value = 0.001). Importantly, the overall mean mouth opening among participants enrolled was 36.66 mm, however cases had significantly lower mouth opening (31.10) compared to controls (42.23) with p-value = 0.001. No cases had mouth opening greater than 45 mm compared to around 80 percent controls in the similar category.

The Figure 1 showed comparison of mean Malondialdehyde (mmol/L) between cases and controls. The overall mean (SD) of Malondialde-

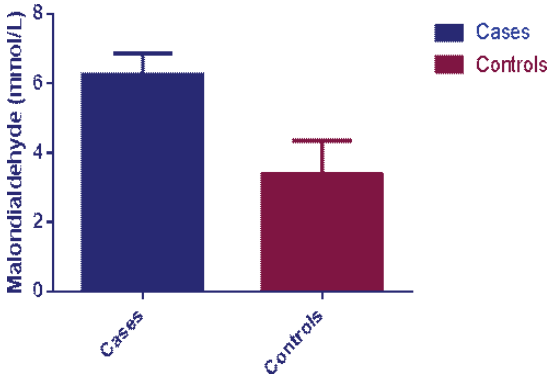

Figure 1: Comparison of Malondialdehyde (mmol/L) between Cases and Controls

Table 3: Correlation of Addiction duration (years) and Mouth Opening (mm) with Malondialdehyde (mmol/L) among Cases and Controls

Oral health status	Cases (n = 80)	Controls (n = 80)	Total (n = 160)
Addiction duration (years)	-0.129	-0.122	0.316**
Habits frequency (packets/ day)	-0.090	-0.089	0.383**
Mouth opening (mm)	-0.068	-0.264**	-0.816**

*Data presented as r (correlation coefficient); * represent p-value < 0.05 and ** represent p-value < 0.01*

hyde (mmol/L) for participants enrolled were 4.82 (1.65). Cases with confirmed oral submucosal fibrosis had mean (SD) Malondialdehyde (mmol/L) as 6.26 (0.60) which was significantly higher compared to controls 3.38 (0.97); the difference was significant with p-value = 0.001.

The table 3 give details of correlation of addiction duration (years) and habit frequency with Malondialdehyde (mmol/L). For the participants enrolled in this study moderate positive correlation existed between addiction duration in years with Malondialdehyde (0.316); moderate positive correlation existed between habits frequency with Malondialdehyde (0.383); and strong negative correlation between mouth opening (mm) with Malondialdehyde (-0.816). Significant weak negative correlation also existed between mouth opening (mm) with Malondialdehyde (-0.264) among controls.

Discussion:

The present study findings highlighted that cases with confirmed oral submucosal fibrosis (OSMF) had higher mean Malondialdehyde (mmol/L) levels as compared to controls. Moreover, strong negative correlation between mouth opening (mm) with Malondialdehyde was found, thereby indicating increase in Malondialdehyde levels were associated with decrease in mouth opening.

In the present study conducted it was identified that cases with OSMF had mean (SD) Malondialdehyde (mmol/L) as 6.26 which was significantly higher compared to controls 3.38 with the difference being highly significant. The results are consistent with the evidence in the literature. A case control study that recruited 48 male patients with Squamous cell carcinoma and 16 healthy subjects reported a significantly higher and elevated level of serum Malondialdehyde among cases compared to controls.[21] Another study was conducted on 25 patients with leukoplakia, 47 with oral sub mucous fibrosis and 62 with oral cancer and 50 healthy subjects reported the significantly elevated levels of MDA observed in Periodontitis, leukoplakia, oral submucous fibrosis and cancer as compared to controls thus indicating the role of MDA in its pathogenesis.[22] A study that recruited 30 normal individuals and 30 patients each with histopathologically diagnosed oral precancer, and oral cancer reported the lower mean serum Malondialdehyde level in the control group as 5.107 mol/ml, whereas it was 9.33 mol/ml and 14.34 mol/ml in oral precancer and oral cancer, respectively. The study highlighted a statistically significant increase in serum Malondialdehyde levels in the oral precancer and oral cancer patients compared with the control group.[23] A comparative study conducted among 65 cases of OSMF also reported a significant increase in serum and salivary MDA levels.[24]

Elevated MDA level have been reported in oral cell proliferation and lipid peroxidation in transformed leukoplakia, cancer and Periodontitis.[25] The body containing a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants as they form, or to repair damage caused by reactive oxygen species.[26]

The study had certain limitations. Firstly, cases i.e. patients with stage one oral submucosal fibrosis were recruited. Secondly, the sample size was limited and participants were recruited from

only clinical site. Therefore, in future a larger comparative cross sectional study with cases and controls being recruited from multiple clinical sites is desirable to be conducted.

Conclusion:

From the present study it was evident that Malondialdehyde (mmol/L) levels were significantly higher among patients with oral submucosal fibrosis. Moreover, progressively increased level of Malondialdehyde was associated with a higher risk of decreased mouth opening, among patients with OSMF. Thus, serum Malondialdehyde can be used as a biomarker for the early detection as well as successful management of OSMF, thereby arresting it at an early stage and reducing the possible consequences of malignancy.

Conflict of interest: None

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Role and contribution of authors:

Dr Humera Akhlaq, Assistant Professor, Oral Pathology, collected the data, references and wrote the initial writeup.

Dr Nosheen Mehmood, Lecturer Pathology, collected the data, references and helped in introduction writing.

Dr Maryam Azfar, Assistant Professor, Community Dentistry, critically review the article and made the final changes in result, discussion, and conclusion.

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