Risk Factors Associated With Decreased Mouth Opening Among Patients With Oral Submucous Fibrosis (OSMF)

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Abstract

Obective: To determine the risk factors associated with decreased mouth opening among patients with OSMF.
Methods: A cross-sectional study was performed in out-patient Dental Clinics,Tertiary care hospital and cases (diagnosed with OSMF), and the controls (who did not have OSMF) were recruited. The study questionnaire to collect information on demographics and clinical features were distributed among cases and controls and saliva samples were obtained for malondialdehyde and superoxide dismutase. The variable "decreased mouth opening" was determined as a dependent/outcome variable. A descriptive comparative analysis was performed. The characteristics of cases and controls were compared. Co-relations were studied between various continuous variables. Cross-tabulations were performed between two categorical variables. A multivariate regression analysis was performed to determine the associations between various independent variables, and the dependent variable - decreased mouth opening.
Results: Our study demonstrated that the mean age of our patients was cases 22.5 ± 2.8 vs. controls 23.0 ± 3.7 year, and they were more male cases as compared to controls. The cases were predominantly from lower socio-economic background. A multivariate regression analysis demonstrated that male gender, educational status (lesser education), higher levels of malondialdehyde, and lower levels of superoxide dismutase were associated with a greater risk of decreased mouth opening among patients with OSMF.
Conclusion: Our study concluded that male gender, educational status (lesser education), higher levels of malondialdehyde, and lower levels of superoxide dismutase were associated with a greater risk of decreased mouth opening among patients with OSMF.
Keywords: Oral submucous fibrosis, Socio-economic Status, Malondialdehyde, Superoxide Dismutase, Decreased mouth opening (ASH & KMDC 19(1):3;2014).

Introduction

Oral Submucous Fibrosis (OSMF) is a pre-malignant and crippling condition reported mainly in the Indian-Pakistan sub-continent, and accounts for 40% of all cancer in this region[1]. According to a study from Pakistan, oral cancer is one of the ten most common malignancies[2]. Another study from Pakistan by Alamgir et al rank oral cancer as the second most common cancer[3]. The aetiology of OSMF believed to be multi-factorial includes areca nut chewing, ingestion of chillies, genetic and immunologic processes, nutritional deficiencies and other factors.

The diagnosis and prognosis of OSMF can be established by means of biopsy, which is an invasive, time-consuming procedure and causes psychological trauma to some patients.

Thus the need of the hour is that the test ought to be simple, less invasive, less time consuming, easy for interpretation, economical and yet quite confirmatory for its diagnosis and prognosis. Apart from routine histopathology other diagnostic and prognostic methods such as biochemical inves-

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tigations are of great importance too. It may also useful to monitor the response of therapy. Moreover, biochemical alteration reflects tissue changes at cellular level.

The epidemiological and experimental studies have implicated reactive oxygen species (ROS) induced lipid peroxidation i.e.,malondialdehyde (MDA) in the development of pre-cancer and cancer4. The extent of oxidative damage caused by ROS can be exacerbated by a decreased efficiency of antioxidant defense mechanisms of the body. Enzymatic antioxidant superoxide dismutase (SOD) plays a key role in detoxification of superoxide anion radical, and hence diminishes the toxic effects of this radical and other free radicals such as hydrogen peroxide and hydroxyl radical from secondary reactions[4]. Thus measurement of lipid peroxidation product and antioxidants is valuable in OSMF to assess tissue burden because they reflect the bioavailability of antioxidants as well as their increased utilization to scavenge lipid peroxidation products.

In spite of its high prevalence in the India-Pakistan sub-continent and the potential to undergo malignant transformation, OSMF has not been widely investigated with respect to lipid peroxidation and antioxidants. With this concern in mind, the present study was undertaken to estimate the salivary levels of lipid peroxidation product MDA, and antioxidants SOD, and to study its association with decreased mouth opening a hall mark of OSMF. The objective of our study is to determine the risk factor associated with decreased mouth opening among patients with oral submucous fibrosis. To our knowledge this is the first study on this subject from Pakistan. The local risk factors associated with the development of OSMF in Pakistan will help in identifying the patients at high risk of developing this disease, and then to formulate recommendations for its prevention.

Subjects and Methods

A cross-sectional study was carried out during the period from January, 2012 to January, 2014 in the Department of Pathology Ziauddin Hospital, (tertiary care hospital).

Patients were recruited from OPD of the Department of Pathology, Ziauddin Hospital. The relevant history of each patient was recorded. Diagnosis was established on the basis of a strong history of consuming pan/gutka/betelnut for a long duration, and clinical signs of OSMF, especially decreased mouth opening.

For selection of controls an equal number of age and gender- matched healthy subjects without any abuse of drugs/addiction habits and without any clinically obvious oral lesions or systemic diseases were selected as the control group.

The salivary samples were obtained from early morning samples of saliva. Water was collected in a sterilized container after rinsing the oral cavity thoroughly with it. Phosphate Buffered Saline (PBS) solution was put in each container to maintain the pH. The sample was stored at a temperature of minus eighty degrees centigrade. MDA and SOD levels were estimated.

In the present study, all patients of Group 1 Functional Classification according to Haider et al5 (Group 1 subjects with mouth opening > 20mm, Group 2 subjects with mouth opening between 11-19mm) were recruited. However, to differentiate between our patients from a point of view of the severity of their disease, we divided them into: Group 1A (mild mouth opening defect) - 46mm to 51mm; Group 1B (moderate mouth opening defect) - 36mm to 45mm; and Group 1C (severe mouth opening defect) - 26mm to 35m.

The characteristic of all the patients in the descriptive analysis was studied. The categorical variables like gender was presented as proportions (percentage) and the continuous variables like age were presented as mean ± SD. The characteristics of the cases and controls were compared. The continuous variables were compared using an independent t-test, while the categorical variables were compared using a chi-square test. p-values less than 0.05 were considered as statistically signifi-

cant (see Table 1). All data was analyzed using SPSS version 16, statistical analysis software.

Correlation of Mouth opening with Malondialdehyde and Superoxide Dismutase was done by correlation coefficient of mouth opening (mm) with malonaldehyde (mmol/L) and superoxide dismutase (mU/L) comparison. (see Fig1 and Fig 2)

A multivariate regression analysis was performed to determine the risk factors associated with decreased mouth opening among patients with OSMF. The dependent/outcome variable was decreased mouth opening (in mm). The independent variables were age, gender, duration of habits (pan chewing, gutka eating and betel nut eating), malonaldehyde and superoxide dismutase (see table 2). Relative risk was determined for each independent variable in the equation, 95% confidence levels and p-values were also estimated. A p-value of < 0.05 was considered as statistically significant.

Results

Of the routine out-patient clinic patients who reported to the Department of Pathology, Tertiary care hospital (Clifton Campus) Ziauddin Hospital, 80 cases were selected who had clinically stage 1 OSF. Similar number of age and gender matched controls (patients who did not have oral sub-mucous fibrosis) were selected.

Comparison of characteristics of cases and controls showed similar mean age of both group (22.5 ± 2.8 Vs. 23.0 ± 3.7, p-value = 0.31). There were more male patients among cases compared to controls (69% vs. 31%, p-value = 0.0001). The majority of OSMF patients were Urdu speaking (55%), followed by Baluchi (17.5%), Sindhis (12.5%), Punjabi (10%), and Pathans (5%). As far as the educational status was concerned, 47.5% were illiterate, 50% had high-school education, while only 2.5% were graduates. The majority of the cases (72%) were earning less than Rs 8000 per month, 25.5% were earning between Rs 8000-20,000, and only 2.5% cases were earning more than Rs 20,000 per month. The majority of the addictions wasdue to gutka (62.5%), 25% were due tobetel-nut, and 12.5% were addicted to pan. All the cases belonged to Group 1 mouth opening criteria given by Haider et al.5.However, for our convenience we categorized our patients into 3 categories; stage 1A mouth opening between 46-51mm, 1B mouth opening between 36-45mm, 1C mouth opening between 26-35mm. The majority of our patients (85%) were 1C category or severe mouth opening defect, 10% had 1B or moderate mouth opening defect, while 5% had IA mild mouth opening defect. The mean salivary malondialdehyde level was 6.25 ± 0.60, while the mean superoxide dismutase level was 3.02 ± 0.44, and there was a statistically significant difference between the salivary malondialdehyde levels, and the salivary superoxide dismutase levels among the cases and controls, Table 1.

Comparison of Socio-Demographic Characteristics across Mouth Opening Categories showed that male patients had a much higher proportion of patients in 1C (more severe form of mouth opening defect), compared to females (59% Vs. 36%). Patients with a greater duration of habits like eating pan, gutka and betel-nut had more severe form of mouth opening defect in a higher proportion of patients i.e. those with duration of 10-15 years on pan/gutka/betel-nut, 71% of patients had a severe grade of mouth opening defect. Illiterate patients (60%) had a much higher proportion of severe grade of mouth opening defect (grade 1C), compared to 35% among high school educated patients and 25% among graduates. Patients in the lowest income group (Rs 8000 per month) had a much higher proportion of severe grade of mouth opening defect (50% had grade 1C among lowest income group patients compared to 32% among patients in the income Rs 8000-20000 group, and 18% among patients in the income group Rs 20000 or more).

Comparison of Malondialdehyde and Superoxide Dismutase across Mouth Opening Categories showed that the patients with 1C grade of mouth opening (severe mouth opening defect) had the highest levels of serum malondialdehyde. The patients with higher levels of superoxide dismutase

had mild mouth opening defect, while those with lower levels of superoxide dismutase had more severe mouth opening defect.

Correlation of Mouth opening with Superoxide Dismutase and Malondialdehyde showed mouth opening (in mm) and superoxide dismutase (mU/L) were positively correlated, (r = 0.84). The two variables were directly proportional to each other, with increasing levels of superoxide dismutase there was an increase in mouth opening Fig.1. Moreover, the correlation mouth opening (in mm) and malondialdehyde (mmol/L) were negatively correlated (r = -0.816). The two variables were inversely proportional to each other, with increasing levels of malondialdehyde there was a decrease in the mouth opening Fig. 2.

A multivariate linear regression analysis was performed to determine the risk factors associated with decreased mouth opening. The independent variables gender, educational status, malondialdehyde (per unit increase), and superoxide dismutase (per unit increase) were found to have an independent association with decreased mouth opening, Table 2.

Discussion

Our study results show that the cases (those who had OSMF) were more likely to be male and Urdu speaking. The majority (47.5%) were illiterate with earnings less than Rs. 8,000 (72%). The majority of the addictions were due to gutka (62.5%), 25% were using betel-nut, and 12.5% were addicted to pan. The majority of our patients (85%) were 1C category or severe mouth opening defect, 10% had 1B or moderate mouth opening defect, while 5% had IA mild mouth opening defect. The mean serum malondialdehyde level was 6.25 ± 0.60, while the mean superoxide dismutase level was 3.02 ± 0.44, and there was a statistically significant difference between the serum malondialdehyde levels, and the serum superoxide dismutase levels among the cases and controls.

OSMF is highly prevalent in the Indian-Pakistan population. According to one of the study, the prevalence of OSMF has been 5 million people (0.5% of the Indian population)[4]. Unfortunately, there is no prevalence reported from Pakistan. However, considering the popularity of pan/gutka addiction in young males, un-educated, low-income wage-earners the prevalence should be somewhat similar to India.

Oral submucous fibrosis is known to affect younger patients as reported by Seedat et al.[6]. The mean age reported in the above mentioned study was 43 years. In our study the mean age of the cases were 22 ± 2.8 years only. Other studies have reported that the mean time to develop clinical features of OSMF is 3.5 years[7].For this reason our youth has to be educated to the serious outcomes like cancer developing as a consequence of this bad habit. Proper education might help them realize the serious consequences of this addiction and make them stay away from these bad habits of pan/gutka and betel-nut use.

Studies have reported a dose-dependent association between the use of betel-nut (areca-nut) and the causation of OSMF[5,9,11]. Also in our study, patients with a greater duration of using pan/gutka/betel-nut were more likely to have OSMF. This has also been emphasized by other researchers, as well[7,9,10,11].

According to Ranganathan et al.[8] and Ariyawardana etal.[12] smoking and alcohol consumption alone, habits common to areca nut chewers, have been found to have no effect in the development of OSF[12].

During the last 5-6 years it has been observed in one of the major hospitals in Karachi that the disease is appearing more in younger individuals, youngest being 12 years old[13,14].

A higher frequency of OSMF among males in our study is probably related to greater access to pan/gutka and betel-nut. Men in our country are bread-earners hence have the money and can buy these addiction giving products easily, whereas females are mostly confined to homes and do not have time and money to buy these products. Other

studies have also shown a male predominance similar to our study[15,16].

The majority of the cases in our study were less educated (totally illiterate or high-school educated only). The cases also were less affluent (the majority were earning less than Rs 8000 per month).Other studies coming mainly from India[8] have reported OSMF in people with low socio-economic status. This also shows that the less educated and low income group are more vulnerable.

The majority of our patients (85%) were 1C category or severe mouth opening defect, 10% had 1B or moderate mouth opening defect, while 5% had IA or mild mouth opening defect. So we have shown that our cases although all belonged to Group of 1(of Haider et al)[5], the majority had a significant mouth opening defect, and our main predictors malondialdehyde and superoxide dismutase had a significant association with decrease in mouth opening (our outcome).

On a multivariate regression analysis there was a significant association between the independent variables gender, education status and our predictors of interest, malondialdehyde and superoxide dismutase. Our study has again highlighted the fact that males and less educated are more likely to get into the bad habits of pan/gutka/betelnut, and hence develop the clinical features of OSMF. Surprisingly, the independent variable ethnicity was not found to be statistically significant.

Recently, there has been growing interest in studies that concern with reactive oxygen in various disease[4]. Several studies have shown the role of oxidant-antioxidants in the causation and progression of various types of cancer including oral cancer. However, considering a high prevalence in India-Pakistan and the potential to undergo malignant transformation OSMF has not been widely investigated with respect to lipid peroxidation and antioxidants. This definitely has developed a responsibility over the oral pathologists to find out the exact role of lipid peroxidation and antioxidants in OSMF. In a multivariate analysis looking at the factors associated with decreased mouth opening among patients with OSMF, our study showed a significant association of malondialdehyde (a lipid peroxidation product) and superoxide dismutase (an anti-oxidant). This is a result similar to other studies done on this subject.

The present study was undertaken and an attempt was made to correlate the serum levels of lipid peroxidation product malondialdyhyde (MDA), and antioxidant superoxide dismutase (SOD) in relation to clinical and histopathological grading of OSMF. The progressively increased MDA and progressively decreased SOD levels has positive correlation with clinical grades of OSMF. Despite the fact that we only included Group 1 OSMF patients (patients with mild disease), yet we could see a very significant association between the disease in our study and our predictors of interest, malondialdehyde and superoxide dismutase.

We recommend that the people at a higher risk of developing OSMF, the younger persons, the people belonging to a lower socio-economic status should be targeted for education programs. The ministry of health and other health planners should prioritize to strictly limit the sale of these addiction products, as they are not only making people addicts but also result in poor health outcomes like oral cancer. The people consuming pan/gutka/betelnut are very much vulnerable to develop worst outcomes, like oral cancer.

Conclusion

OSMF is associated with younger age, male gender and more commonly among patients with low socio-economic status. Progressively increased levels of malondialdehyde, and decreased levels of superoxide dismutase are associated with a higher risk of decreased mouth opening, among patients with OSMF.

Table 1. Comparison of Characteristics of Cases (those who had Oral Sub-Mucous Fibrosis) Vs. Controls (those who did not have Oral Sub-Mucous Fibrosis)

Characteristics (Variables)	Patients with Oral Sub Mucous Fibrosis (Cases)	Patients without Oral Sub Mucous Fibrosis (Controls)	p - values
Age	22.5 ± 2.8	23.0 ± 3.7	0.31
Gender			0.0001
Male	69%	31%	
Female	36%	64%	
Ethnicity			0.0001
Urdu speaking	55%	60%	
Sindhi	12.5%	0%	
Punjabi	10%	20%	
Baluchi	17.5%	5%	
Pathans (reference)	5%	15%	
Educational status			0.0001
Illiterate	47.5%	0%	
High school	50%	65%	
Graduate	2.5%	35%	
Income status			0.0001
Rs 8000	72%	58%	
Rs 8000-20,000	25.5%	15%	
Rs 20,000	2.5%	27%	
Addictions*			0.0001
Pan	12.5%	6%	
Gutka	62.5%	30%	
Betel-nut	25%	10%	
No addiction	0%	54%	
Dose of addicted stuff (mean packets/day)	5.3 ± 2.9	0.81 ± 1.0	0.0001
Duration of addiction (years)	**6**.2 ± 4.1	0.73 ± 0.90	0.0001
Mouth Opening Categories			0.0001
26-35 mm	85%	2.5%	
36-45 mm	10%	80%	
46-51 mm	5%	17.5%	
Malondialdehyde (MDA) (mmol/L)	6.25 ± 0.60	3.37 ± 0.97	0.0001
Superoxide dismutase (m U/ L)	3.02 ± 0.44	6.9 ± 1.2	0.0001

*Controls only occasionally took pan(6%), Gutka (30%) and betal-nut (10%) i.e once every month or so. While remaining 54% of the controls had no addiction.

Table 2. Multivariate Linear Regression Analysis to determine risk factors associated with mouth opening among patients with Oral Sub mucous Fibrosis

Variable	Beta	95% CI	p-value
Gender (Ref = Female)	-1.75	-2.78. -0.72	0.001
Education Level (Ref = Graduates)	0.85	-0.01, 1.71	0.053
Ethnicity (Ref = Pathans)	-0.11	-0.44, 0.22	0.51
Malondialdehyde (per unit increase)	-1.27	-1.80, -0.74	0.0001
Superoxide dismutase (per unit increase)	1.68	1.29, 2.07	0.0001

Figure 1. Correlation of Mouth opening (in mm) with Superoxide Dismutase (mU/ L) showed mouth opening (in mm) and superoxide dismutase (mU/L) were positively correlated, (r = 0.84). With increasing levels of superoxide dismutase there was an increase in mouth opening.

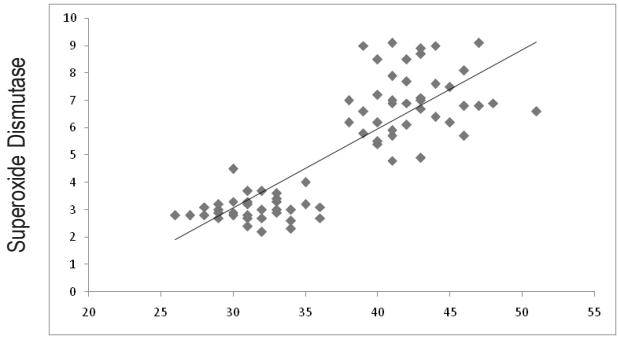
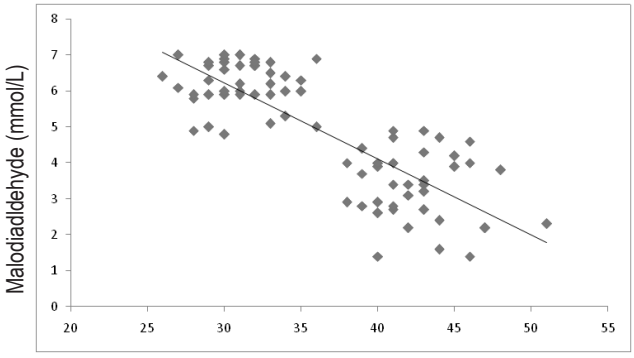


Figure 2. Correlation of mouth opening (in mm) and malondialdehyde (mmol/L) were negatively correlated (r = -0.816). The two variables were inversely proportional to each other, with increasing levels of malondialdehyde there was a decrease in the mouth opening.



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