Research Article

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230-8407 [LINKING]

SALIVARY VERSUS SERUM OXIDATIVE STRESS MARKERS IN ORAL SUBMUCOUS FIBROSIS

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How to cite: Chintanippu RB, Sushma BJ, Gill N. Salivary versus serum oxidative stress markers in oral submucous fibrosis. International Research Journal of Pharmacy. 2024;15:8:17-21.

Doi:10.7897/2230-8407.110336

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ABSTRACT

Introduction: Oral cavity is considered to be mirror of the body as it reflects the health of the individual. Oral submucous fibrosis is an oral precancerous condition which is characterized by inflammation and progressive fibrosis of the submucosal tissues resulting in marked rigidity and trismus.

Aim: The present study was undertaken to determine and compare the salivary and serum levels of oxidative stress biomarkers malondialdehyde, 8-hydroxy-2-deoxyguanosine and 8-isoprostane levels and antioxidants glutathione peroxidase and superoxide dismutase in patients with different grades of OSMF.

Methodology: The observational cross-sectional study was conducted in the department of Biochemistry NIMS&R, Jaipur in association with Oral Diagnosis and Radiology at College of Dental Sciences and Research Centre, Manipur, Ahmedabad (Gujarat) after obtaining institutional ethical committee clearance. The Study population consisted of Clinically diagnosed oral submucous fibrosis patients of age 18-45 years. Unstimulated Saliva was allowed to accumulate in the floor Of mouth and collected by drooling method in a test tube. Saliva samples will be stored at -20 ^{0}C until use. Malondialdehyde was estimated by thiobarbituric acid reaction using spectrophotometer, 8-hydroxy-2-deoxyguanosine was analysed by sandwich ELISA, Glutathione peroxidase and Superoxide dismutase were analysed by spectrophotometer and 8-Isoprostane was estimated by ELISA.

Results: The present study included a total of 92 patients clinically diagnosed with oral submucosal fibrosis. It is seen that out of 92 patients, 53 were males and 39 were females accounting for 57.6% and 42.39% respectively. For all the Grades of OSMF, there were statistically highly significant (P<0.001) differences seen between the salivary and serum biomarkers. Table 6 shows very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4. Very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4.

Conclusion: When the biomarker levels were compared between serum and saliva, it is seen that there was statistically highly significant differences obtained between serum and saliva. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4. Very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4.

Key-words: oral submucous fibrosis, malondialdehyde, 8-Isoprostane, 8-Hydroxydeoxy-guanosine l, superoxide dismutase and glutathione peroxidase.

INTRODUCTION

Oral cavity is considered to be mirror of the body as it reflects the health of the individual [1]. Oral submucous fibrosis is an oral precancerous condition which is characterized by inflammation and progressive fibrosis of the submucosal tissues resulting in marked rigidity and trismus [2]. Oral submucous fibrosis is more commonly seen in south and southeast Asia-India, Bangladesh, Pakisthan, Srilanka, Taiwan etc [3] but now this condition is being reported from western countries as well due to higher rates of immigration [4]. The prevalence of oral submucous fibrosis in India has been estimated to range from 0.2 to 2.3 % in males and 1.2 to 4.6 % in females with a broad age range from 11 to 60 years [5]. Oral submucous fibrosis has multifactorial etiology [6]. Despite multifactorial etiopathogenesis, areca nut chewing in any type of formulation is considered to be major etiological factor [7]. Other possible etiological factors are capsaicin in chillies, deficiency of iron, zinc and vitamins, genetic susceptibility, autoimmunity and collagen disorders [8,9]. Supari chewing, tobacco products chewing and smoking proved to be carcinogenic and are involved in the etiology and severity of oral submucous fibrosis. Smokeless tobacco consumption in the form of gutkha, paan leads to oral submucous fibrosis [10]. The deposition of collagen in the oral submucosa was the main histopathological feature of OSMF. The activation of buccal mucosal fibroblasts has been revealed to be associated with the extent of collagen synthesis in response to the areca nut alkaloids. Transforming growth factor beta 1 was the most important factor implied in the fibrosis disease including the areca nut associated oral submucous fibrosis. Expression of collagenase and metalloproteinase inhibitors has been known to be modulated by TGF – Beta 1. Also, it has been demonstrated that the TGF-Beta pathway could be activated by areca nut and mediated fibroblast activation. It has been shown that the myofibroblast markers, alpha smooth muscle actin and gamma smooth muscle actin were both upregulated in the fibrotic tissues from OSMF patients, which suggests that myofibroblasts activation, was involved in the activation of OSMF [11]. Oral submucous fibrosis has potential for malignant transformation resulting into Oral Squamous Cell Carcinoma, which is the most common oral malignancy with high rate of mortality [12]. Auto oxidation of areca nut polyphenols in the betel quid chewer's saliva produces reactive oxygen species, which are crucial in the initiation and promotion of oral cancer [13]. Reactive oxygen species cause damage to all essential bio compounds such as DNA, Proteins and membrane lipids, causing cell death. These free radicals are counteracted by antioxidant system [14]. Oral submucous fibrosis being a premalignant condition and thought to be associated with reactive oxygen species. The antioxidant parameters in saliva and serum are altered in oral submucous fibrosis. The major advantage of using saliva in diagnosis rather than blood is easy access and non-invasive collection. This study is undertaken to determine which sample is more suitable, invasive or non-invasive to assess the oxidative stress markers and to find out levels of oxidative stress markers and antioxidant levels with severity of oral submucous fibrosis.

MATERIALS AND METHODS

The observational cross-sectional study was conducted in the department of Biochemistry NIMS&R, Jaipur in association with Oral Diagnosis and Radiology at College of Dental Sciences and Research Centre, Manipur, Ahmedabad (Gujarat) after obtaining institutional ethical committee clearance. The Study population consisted of Clinically diagnosed oral submucous fibrosis patients of age 18-45 years.

Inclusion criteria: Using purposive sampling method, a total of 92 newly clinically diagnosed Oral submucous fibrosis cases of age 18 to 45 years attending the oral diagnosis and radiology department of college of dental sciences and research centre, Manipur, Ahmedabad willing to provide voluntary informed consent were included.

Exclusion criteria: Patients who have undergone treatment or who are undergoing treatment, Patients with other oral premalignant disorders, Patients with infections and inflammatory conditions of oral cavity, Patients with cardiovascular, respiratory, hepatobiliary, gastrointestinal, CNS diseases, diabetes mellitus, epilepsy and degenerative joint diseases were excluded from the study.

Sample collection and biochemical analysis: Unstimulated Saliva was allowed to accumulate in the floor Of mouth and collected by drooling method in a test tube. Saliva samples will be stored at -20 ^{0}C until use. Venous blood samples were collected and centrifuged for the separation of serum and was used for the estimation of biomarkers. Malondialdehyde was estimated by thiobarbituric acid reaction using spectrophotometer, 8-hydroxy-2-deoxyguanosine was analysed by sandwich ELISA, Glutathione peroxidase and Superoxide dismutase were analysed by spectrophotometer and 8-Isoprostane was estimated by ELISA. The Grading of OSMF was based on based on interincisal distance, described by Lai DR (1995) [15].

Statistical analysis: After data collection, the data were coded and entered in Microsoft Excel 2019. The data were presented as mean ± standard deviation. The data's normality was checked using the Shapiro-Wilk test, which shows no significant difference with a P value > 0.05. Hence, parametric tests were applied for the comparisons of mean values. One-way Analysis of Variance (ANOVA) test was used to compare the mean difference among the groups. If the result shows a significant difference, then the pairwise comparison was done by using the post hoc Tukey test. Independent t-test was applied to compare the mean difference between two groups. Pearson's correlation coefficient (r) test: to assess the correlation between the variables. Statistical Package for Social Science version 23 (IBM SPSS Inc.) was used for statistical analysis. The level of significance was set at 5%.

RESULTS

It is seen that out of 92 patients, 53 were males and 39 were females accounting for 57.6% and 42.39% respectively. The mean age in years in males and females were 28.97±5.80 and 32.39±3.77 years respectively.

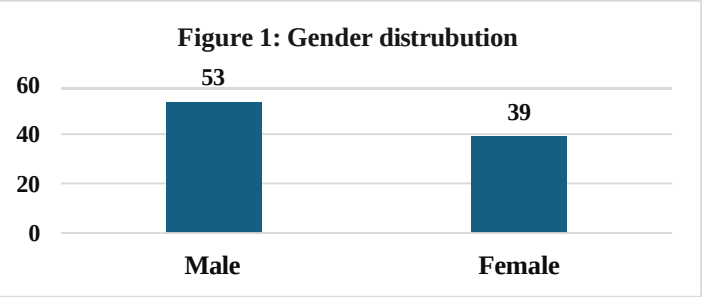

Figure 1 represents gender wise distribution of study population. It is seen that out of 92 patients, 53 were males and 39 were females accounting for 57.6% and 42.39% respectively. The mean age in years in males and females were 28.97±5.80 and 32.39±3.77 years respectively.

Table 1: Comparison of mean values of different salivary and serum biomarkers for Grade 1 OSMF

Biomarkers	Salivary (n=23)	Serum (n=23)	95% CI	P value
Superoxide Dismutase (U/ml)	0.82 ± 0.04	150.60 ± 3.39	-151.20, -148.35	<0.001**
Malondialdehyde (nM/ml)	0.23 ± 0.03	21.94 ± 0.96	-22.11, -21.30	<0.001**

Mean ± SD were compared by independent t test; CI=Confidence Interval; **P<0.001 highly significant

Table 2: Comparison of mean values of different salivary and serum biomarkers for Grade 2 OSMF

Biomarkers	Salivary (n=23)	Serum (n=23)	95% CI	P value
Superoxide Dismutase (U/ml)	0.63 ± 0.10	125.59 ± 2.24	-125.90, -124.02	<0.001**
Malondialdehyde (nM/ml)	0.28 ± 0.03	25.0 ± 8.62	-28.35, -21.10	<0.001**

Mean ± SD were compared by independent t test; CI=Confidence Interval; **P<0.001 highly significant

Table 3: Comparison of mean values of different salivary and serum biomarkers for Grade 3 OSMF

Biomarkers	Salivary (n=23)	Serum (n=23)	95% CI	P value
Superoxide Dismutase (U/ml)	0.56 ± 0.06	90.64 ± 3.01	-91.35, -88.81	<0.001**
Malondialdehyde (nM/ml)	0.35 ± 0.05	28.03 ± 7.13	-30.68, -24.69	<0.001**

Mean ± SD were compared by independent t test; CI=Confidence Interval; **P<0.001 highly significant

Table 4: Comparison of mean values of different salivary and serum biomarkers for Grade 4 OSMF

Biomarkers	Salivary (n=23)	Serum (n=23)	95% CI	P value
Superoxide Dismutase (U/ml)	0.42 ± 0.09	70.24 ± 1.72	-70.45, -68.78	<0.001**
Malondialdehyde (nM/ml)	0.42 ± 0.04	31.71 ±10.55	-35.85, -27.15	<0.001**

Mean ± SD were compared by independent t test; CI=Confidence Interval; **P<0.001 highly significant

Table 5: Correlation coefficients of salivary and serum biomarkers for different Grades of OSMF

Grade	Superoxide Dismutase	Malondialdehyde
1	0.10 (0.65)	0.25 (0.24)
2	-0.31 (0.15)	-0.23 (0.30)
3	0.03 (0.90)	0.03 (0.87)
4	0.49 (0.02)*	-0.01 (0.98)

Correlation coefficients (r), figure in parenthesis shows P Value, *P<0.05 significant.

DISCUSSION

OSMF is a persistent, sneaky illness that affects the mouth. Even with tobacco use under control, there is still a comparatively significant risk of malignant transformation. OSCC is the most prevalent head and neck cancer, making up between 40% and 50% of all cancer cases in India. ROS play a major influence on a number of cancer hallmarks. Lipid peroxidation byproducts called isoprostanes are a sensitive and particular indicator of oxidative stress. They also operate as pathophysiologic mediators of oxidative stress because of their wide range of physiological effects. When 8-isoprostane levels in OSMF and OSCC were compared to those in the normal population, differences were seen in the isoprostane plasma levels within the control group. There are a number of reasons for this diversity, including age, gender, dietary pattern, physical exercise, body mass index, psychological stress, and smoking and alcohol consumption. The present study included a total of 92 patients clinically diagnosed with oral submucosal fibrosis. It is seen that out of 92 patients, 53 were males and 39 were females accounting for 57.6% and 42.39% respectively. The mean age in years in males and females were 28.97±5.80 and 32.39±3.77 years respectively. OSMF was classified into 4 grades, the comparison of salivaery and serum oxidative stress biomarkers Malondialdehyde (nM/ml), 8-Isoprostane (ng/ml), 8-Hydroxydeoxy-guanosine (ng/dl) and antioxidant biomarkers Superoxide Dismutase (U/ml) and Glutathione Peroxidase (U/L) was done between different grades of OSMF. Table 2 to Table 5 shows the comparison of mean values of different salivary and serum biomarkers for different Grades of OSMF. For all the Grades, there was a statistically highly significant (P<0.001) difference between the salivary and serum biomarkers. Table 6 shows very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4. Very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4.

CONCLUSION

The results of our study showed that the concentration of MDA, isoprostane, 8-Hydroxydeoxy-guanosine levels were increased significantly as the clinical stage and histopathological grade of OSMF advances suggesting these biomarkers can be used as a reliable biochemical marker and also a prognostic marker to assess the extent of oxidative damage in OSMF. The concentration of antioxidant enzymes showed progressive decline as OSMF advances; indicating the potential role of supply of antioxidants to reduce the progress of disease. For all the Grades of OSMF, there were statistically highly significant (P<0.001) differences seen between the salivary and serum biomarkers. Table 6 shows very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4. Very weak correlations were observed between the salivary and serum biomarkers for all the Grades of OSMF. A significant (P=0.02) moderate correlation (r=0.49) was observed for salivary and serum Superoxide Dismutase for Grade 4.

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