Original Article

Serum and salivary myeloperoxidase in oral squamous cell carcinoma: A preliminary study

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ABSTRACT

Background: Free radical damage to biologic molecules forms a basis for cancer development. Since DNA damage and methylation states are influenced by oxidative species catalyzed by myeloperoxidase (MPO), this enzyme is postulated to have a role in the occurrence of cancer. MPO has been studied in cancers such as those of the lung, ovary, and breast. However, serum and salivary studies of MPO in oral cancer are lacking. **Aims:** (1) To determine the MPO levels in the serum and saliva of patients with primary oral squamous cell carcinoma (OSCC). (2) To compare and correlate the serum and salivary MPO levels in patients with primary OSCC and healthy controls. **Subjects and Methods:** A total of 30 subjects were involved in this study, of which study group consisted of 15 subjects and control group consisted of 15 subjects. Study group included subjects with histologically proven primary OSCC. Serum and salivary samples were collected from all the subjects. **Results:** The results showed that the serum levels of MPO were slightly higher in the study group as compared to the control group; however, the difference was not significant. In saliva, levels of MPO were slightly lower in the study group as compared to the control group. The difference was not significant. **Conclusions:** This study could not find a significant correlation between serum and salivary MPO and OSCC. However, our study consisted of a limited number of samples and as such can be considered a pilot study. Studies with larger sample size are needed to give better insight into the role of MPO in OSCC.

Key words: Myeloperoxidase, oral squamous cell carcinoma, saliva, serum

INTRODUCTION

Cancer is a complex disease with a multifactorial etiology that includes genetic and environmental factors.[1] Squamous cell carcinoma (SCC) is the most frequent malignancy in the oral cavity. It represents the sixth most frequent malignant tumor.[2]

Among the etiological factors implicated in oral SCC (OSCC), oxidative stress has been cited most often. Oxidative stress can be defined as a state when the levels of free radicals exceed antioxidant defense mechanisms.[3]

Generation of free radicals leads to damage to biologic molecules and thus forms a basis for cancer development. Since DNA damage and methylation states are influenced by oxidative species catalyzed by myeloperoxidase (MPO), this enzyme is postulated to have a role in the occurrence of cancer.[4]

Myeloperoxidase is a peroxidase enzyme most abundantly present in azurophilic granules of the neutrophils and released by a degradation process. This green enzyme is the most abundant protein in neutrophils, accounting for 5% of its dry mass.[5] MPO reacts with hydrogen peroxide converted from the extra oxygen consumed in the respiratory burst to form hypochlorous acid - a complex that can oxidize a large variety of substances.[6] This oxidative damage to biomolecules such as DNA, proteins and lipids may form the first step in carcinogenesis. MPO has also been implicated in the activation of carcinogens present in tobacco smoke, one of the major etiological factors in oral cancer.[7]

Studies have shown increased MPO production from neutrophils at the start of SCC development and

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establishment, thus implying its role in both initiation and progression.[4]

Although various studies have investigated the role of MPO in cancer,[1,3,4] studies evaluating serum and salivary MPO levels in oral cancer are rare.

We, therefore, designed a study to evaluate the salivary and serum levels of MPO in patients with primary OSCC and compare it with healthy individuals in order to determine if MPO levels can be correlated with malignancy.

SUBJECTS AND METHODS

A total of 30 subjects reporting to our institution were included in the study of which 15 formed the study group and 15 formed the control group. The study group comprised of subjects with clinical and histopathological diagnosis of primary OSCC. The control group consisted of 15 healthy subjects. All persons with any underlying systemic illness or on long term medication were excluded from the study.

Ethical clearance for the study was obtained from the Institutional Ethics Committee. Informed consent was obtained from all individuals participating in the study. Demographic parameters were recorded, and a thorough examination of the oral cavity was done to record relevant findings. Unstimulated saliva was collected by spit method. Saliva collected was transferred into sterile 2 ml glass vials. It was then centrifuged at 3000 rpm for 15 min and the supernatant was stored at − 20° till analysis. 5 ml of blood was withdrawn from the antecubital vein using aseptic method. Blood was allowed to clot, and serum was obtained through centrifugation and stored at − 20° till analysis. MPO in serum and saliva was assessed spectrophotometrically using the method by Malheston *et al*.[5]

RESULTS

The study group consisted of 11 male and 4 female subjects. All subjects were in the fifth to seventh decades of life. All cases had history of habits in the form of tobacco chewing, smoking and alcohol consumption.

Statistical analysis was performed using the Student's *t*-test [Table 1].

The serum MPO levels varied from 127 pM/L to 277 pM/L in the study group and from 158 pM/L to 218 pM/L in the control group. Comparison of the serum levels of MPO showed slightly higher levels in the study group when compared to the control group. The difference between the study and control groups was not significant [Figure 1].

The salivary levels of MPO varied from 97 pM/L to 230 pM/L in the study group and from 151 pM/L to 219 pM/L in the control group. Comparison of the salivary levels of MPO showed slightly lower levels in the study group as compared to the control group. The difference between the study and control groups was again not significant [Figure 2].

Table 1: Results of the independent sample *t*-test

	N	Mean	Standard deviation	*t*	*P*
Serum					
Case	15	202.44	43.23	1.359	0.185
Control	15	185.70	20.17		
Saliva					
Case	15	166.41	36.94	0.569	0.574
Control	15	173.05	26.01		

N: Number of cases and controls respectively, *t*: Results of the independent sample *t*-test, *P*: Difference between the samples and controls, $P < 0.05$ is considered significant. Interpretation: Since the $P > 0.05$, there is no difference in the mean serum and saliva level between cases and controls

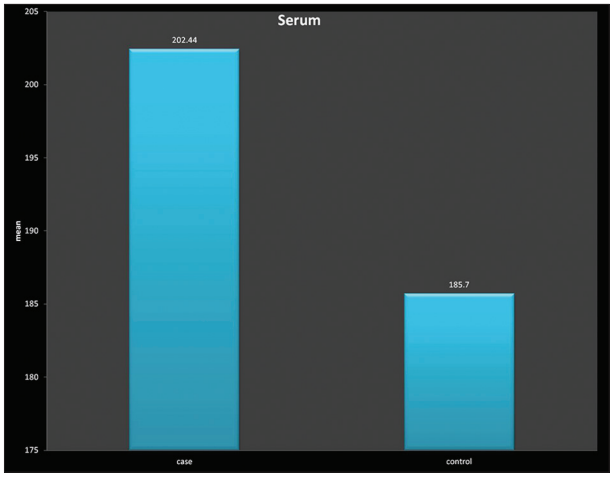

Figure 1: Comparison of serum myeloperoxidase between study group and control group

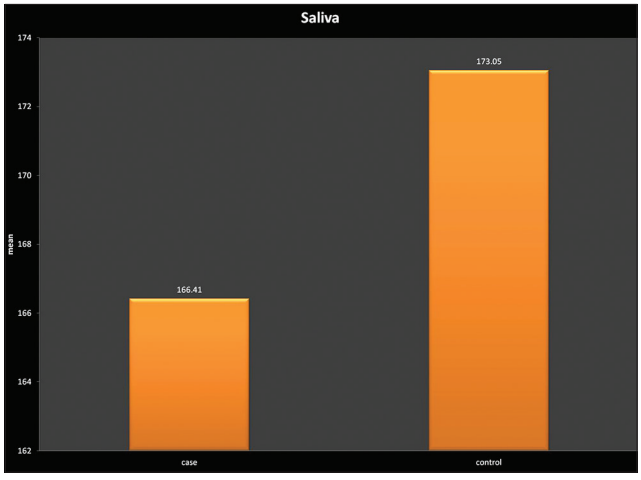

Figure 2: Comparison of salivary myeloperoxidase between study group and control group

DISCUSSION

Oral and pharyngeal cancer is the one of the most prevalent cancers and is the sixth leading cancer worldwide.[6] Despite recent advances in chemotherapy, radiotherapy and surgery, it still has a 50% mortality rate.[7] SCC is the most common form of oral and pharyngeal cancer in South and Southeast Asia.[1] The most common etiologic factors associated with OSCC include tobacco chewing and smoking and alcohol consumption.[6-8]

Serum is the traditional medium used for evaluation of various biomarkers. Salivary analysis is gaining popularity nowadays, especially in different oral diseases. Since saliva is constantly in direct contact with the oral tissues, it is reasonable to assume that the analysis of saliva would provide useful information about the local factors affecting the disease. In addition, it provides an attractive alternative to other invasive methods of analysis. Saliva is easily available, easily collected from the patient and is a noninvasive method, if proven effective.

The present study thus evaluated the role of serum and salivary MPO in OSCC. The subjects with OSCC formed the study group. All the subjects with OSCC were in the age group of 40–70 years. This is in accordance with most studies on oral cancer, which state that malignancy occurs as a result of cumulative damage at the cellular level over a long period. Thus, most cases occur in the sixth to the seventh decade of life. Mathur *et al*.[9] have stated that the mean age at diagnosis is 57.1 years in males and 52.3 years in females.

In our study, majority of OSCC was found in males (73%). This is in agreement with most studies on oral cancer which clearly state a male predilection of 2:1.[9] This is said to be due to the increased incidence of habits in males such as smoking cigarettes and bidi, chewing tobacco and paan and alcohol consumption.[9]

Of the 15 cases of OSCC included in our study, 6 (40%) cases involved the buccal mucosa, which was the most common site, followed by the tongue (26%). Here, again, our findings agree with authors who state that the buccal mucosa is the most common site, followed by the anterior two-thirds of the tongue, lower gum, hard palate, floor of mouth and upper gum.[9]

Most OSCCs in India are associated with tobacco habits. Smoking and other forms of tobacco use are associated with about 75% of oral cancer cases, caused by irritation of the mucous membranes of the mouth from smoke and heat of cigarettes, beedis, cigars or pipes.[10] Tobacco has more than 19 known carcinogens and the combustion of these carcinogens and their products lead to OSCC.[10] In the case of pan chewers, betel quid is in direct contact with the mucosa causing the carcinogens to have an enhanced effect on the mucosa at the site of contact. Some authors mention that the carcinogens from the tobacco and lime in the pan get dissolved in the saliva and thus remain in contact with the oral tissues for a long period of time leading to premalignant and malignant changes.[9] Alcohol has a synergistic effect with tobacco use, especially smoking. Alcohol is rarely the sole etiologic agent in OSCC except, maybe, in case of prolonged use of alcohol containing oral rinses.[10] In our study, all the subjects with OSCC had tobacco associated habits. The most common habit was smoking (66%) of cigarettes and beedis in males. Tobacco chewing was present in 33% (5 cases). All the females had a history of tobacco chewing. Regular alcohol use was reported by only two male subjects.

Myeloperoxidase is a heme enzyme present in the azurophilic granules of the neutrophils. It has been associated with the microbicidal property of these cells. Along with NADPH oxidase it is associated in the formation of reactive oxygen species (ROS).[11] Increased plasma levels have been found in cases of neutrophil proliferation and degranulation.[11] The hypochlorous acid produced by MPO can cause damage to nucleic acids, proteins and unsaturated lipids as well as cause the release of ROS.[11] This damage to bystander cells and host DNA could lead to malignancy at sites of inflammation.[1] Because of these mechanisms, MPO association has been extensively studied in various cancers such as lung, breast, prostate, colorectal and ovarian cancers.[1,11-15] Other conditions where MPO has been implicated include sepsis, chronic obstructive pulmonary disease and cardiovascular conditions.[11,16]

The association of OSCC and MPO has been described well by Lai *et al*.[6] Betel quid chewing, cigarette and beedi smoking and alcohol consumption can cause trauma and irritation to the oral mucosa leading to chronic inflammation. This chronic inflammation can lead to the release of MPO catalyzed hypochlorous acid as well as other ROS. This, in turn, can lead to damage to biological molecules causing the inflamed epithelium to convert to malignancy through the release of nitryl chloride or chloramines.[6] Thus, increased MPO expression can be associated with increased risk of malignancy.[6] Also, MPO-derived oxidants are involved in the bioactivation of carcinogens such as polycyclic aromatic hydrocarbons, which bind to DNA forming DNA adducts. This may lead to mutations in oncogenes and tumor suppressor genes. Hypochlorous acid is also reported to be a potent inhibitor of DNA repair. Another procarcinogenic mechanism of MPO is by inhibition of apoptotic activity leading to the proliferation of mutated cells and increased tumor growth.[6]

Myeloperoxidase polymorphisms have been extensively studied in a variety of malignancies. Lung cancer, ovarian cancer, prostate cancer, colorectal cancer etc., are some of the malignancies associated with MPO expression. In the oral cavity, MPO was found to be associated with an increased risk for the occurrence of second primary tumors.[1] A meta-analysis using 22 case control studies dealing with the MPO 2463 G A polymorphism in lung cancer did not find any significant association between MPO polymorphism and risk of lung cancer after adjusting for ethnicity and smoking status. This study, therefore, concluded that MPO polymorphism was not associated with lung cancer risk.[17]

In our study, the serum and salivary levels of MPO were evaluated in OSCC subjects. The mean serum MPO levels in our study subjects was 202.44 (±43.23 standard deviation [SD]) and in our controls was 185.70 (±20.17). These results show that there is a wide variation in the serum levels of MPO both in the cases and in the controls. The serum levels of MPO were seen to be slightly higher in the subjects with OSCC as compared to the controls. However, the difference is not found to be statistically significant. The serum levels of MPO thus did not show a correlation with oral cancer risk.

In the present study, salivary analysis of MPO was done. The study group had an average MPO of 166.41 (±36.94 SD) while the control group showed an average MPO level of 173.05 (±26.01 SD). In our study, there was wide variation in the MPO levels in saliva of both study and control groups. The study group showed slightly lower MPO level as compared to the control group, although the difference is slight and nonsignificant. Thus, based on our study results no significant correlation was obtained between the serum and salivary levels of MPO and OSCC.

Previous studies showing serum and salivary MPO in OSCC are lacking. A study on MPO gene polymorphism in ovarian cancer found that the GG genotype was found to be overrepresented in patients with early stage ovarian cancer as compared to healthy controls.[18]

Another study demonstrated differences in the MPO levels in leucocytes from cancer patients as compared to healthy controls. They suggested that the reduced MPO levels might indicate a cellular defect in leucocytes from cancer patients.[19]

A study evaluated the risk of breast cancer, MPO polymorphism and antioxidant status. They found that dietary intake and plasma antioxidant levels may modify the association between the MPO polymorphism and breast cancer risk. They, however, observed no significant association between a functional polymorphism in the MPO gene and risk of breast cancer.[20]

Thus, MPO and its polymorphisms have been studied in different cancers of the body with conflicting results. There are not many studies, which deal with serum and salivary MPO levels. Therefore, correlations with previous studies are not easy. However, based on our results and the available literature our study can conclude that, within the limitations of our study, we observed no correlation between serum and salivary MPO in OSCC.

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