Original Research Article

Evaluation of salivary and serum lipid peroxidation in oral leukoplakia and oral squamous cell carcinoma

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Abstract

Introduction: Reactive oxygen species induced damage of the cells produces lipid peroxidation end products such as malondialdehyde (MDA), a well-established biomarker of oxidative stress. Malondialdehyde is a highly toxic aldehyde molecule which is considered to be an ideal marker of lipid peroxidation.
Aim and objectives: To determine and compare serum and salivary MDA level so as to assess the degree of oxidative damage caused by oral leukoplakia and OSCC patients.
Materials and methods: A cross-sectional study was conducted on 45 subjects in the IDST College, Modinagar and the study group comprised of 15 cases of oral leukoplakia, 15 cases of oral squamous cell carcinoma (OSCC) and 15 cases of normal healthy individuals (control). Saliva and blood samples were obtained from each patient and evaluated for MDA level.
Results: In the present study, salivary and serum levels of MDA increased significantly from healthy controls to oral leukoplakia subjects and a highly significant increase was observed in oral squamous cell carcinoma.
Conclusion: In the present study, increased levels of MDA in oral leukoplakia and OSCC patients reflect interactions with several carcinogenic agents that confirm greater lipid peroxidation and oxidative stress in oral leukoplakia and OSCC patients.

Key words

Malondialdehyde, Lipid peroxidation, Reactive oxygen species, Tobacco.

Introduction

Oral cancer is one of the major forms of cancer worldwide that accounts for 30-40% of all cancers [1]. The important promoters/initiators of oral cancer in India are tobacco, chewing with betel quid or tobacco smoking and alcohol consumption. Use of tobacco leads to oral potentially malignant disorders and leukoplakia is among the important oral potentially malignant disorders with 0.13%-10% malignant transformation in India [2]. Two-thirds of oral cancer patients are diagnosed at advanced tumor stages, where survival drops to a little more than 30% and its prognosis is unpredictable [3, 4].

Tobacco is an exogenous source of reactive oxygen species (ROS) that subsequently leads to oxidative stress (OS). Tobacco products of polynuclear aromatic hydrocarbons (PAH) and nitrosamine cause increase in free radicals and ROS production, which have a pathognomonic role in multistep carcinogenesis. They initiate mutagenic events by causing DNA damage that ultimately leads to degeneration of cellular components. Thus, free radicals and ROS stimulate malignant transformation and progression [5, 6].

Reactive oxygen species induced damage of the cells produces lipid peroxidation end products such as malondialdehyde (MDA), a well-established biomarker of oxidative stress. Malondialdehyde is a highly toxic aldehyde molecule which is considered to be an ideal marker of lipid peroxidation [5, 7].

In normal cellular processes, cells are capable of neutralizing the deleterious effects of ROS and free radicals by several intracellular and extracellular antioxidative systems. Any change in one of these systems, breaks this equilibrium leading to OS and hence, resulting in an overall increase in cellular levels of ROS that can initiate lipid peroxidation and induce oxidative DNA damage [9]. The study was carried out to determine and compare serum and salivary MDA level so as to assess the degree of oxidative damage caused by oral leukoplakia and OSCC patients.

Materials and methods

A cross-sectional study was conducted on 45 subjects in the IDST College, Modinagar in the year 2016-17 and the study was approved by the ethical committee. The study group comprised of 15 cases of oral leukoplakia, 15 cases of oral squamous cell carcinoma (OSCC) and 15 cases of normal healthy individuals (control). Patients with underlying systemic diseases, cancers other than oral cancer and previously treated cases for oral cancer were excluded from the study.

Saliva and blood samples were obtained from each subject after overnight fasting. Five milliliters of venous blood was collected from the selected patients using a sterile disposable syringe. The blood was shifted to sterile test tube and left for clotting. The clotted blood sample was centrifuged at 3000rpm for 5min and obtained serum sample was used. At the same time, whole unstimulated saliva samples were collected. Subjects were asked to rinse the mouth thoroughly and then directed to spit into a sterile plastic container. The sample was then centrifuged at 3000rpm at 4˚C for 5min and saliva free of large particle debris was used for estimation of lipid peroxide in saliva.

MDA, the marker of lipid peroxidation was estimated as thiobarbituric acid-reactive substances (TBARS). To 1 ml of sample, 1.5 ml of 0.8% thiobarbituric acid (TBA) was added and then, 1.5 mL of acetic acid and 0.4 ml of 8.1% sodium dodecyl sulfate were added. Distilled water was added to make the mixture up to 5 ml, and it was then placed in a hot water bath at 95°C for 1 h. The mixture was then allowed to cool and 5ml of pyridine and n-butanol (15:1, v/v)

along with 1.0 mL of distilled water were added. The mixture was vortexed and centrifuged at 4,000 rpm for 10 min. With a spectrophotometer, absorbance of the upper layer was measured at 532 nm against distilled water. When allowed to react with TBA, MDA formed a colored complex that was measured using the spectrophotometer.

Data were statistically analyzed by statistical software (SPSS version 21.0) using unpaired *t*-test for significance of differences between each group. Mean and standard deviation were calculated. P values of less than 0.05 were considered to be statistically significant.

Results

In this study, the average age of the patients in this study was 25-62 years and majority of the subjects were males. The mean and standard deviation values of MDA in saliva and serum of healthy controls were 18.75±0.72 nmol/dl and 3.21±0.28 nmol/dl with a statistical significant P-value (<0.005). In leukoplakia subjects, the mean and standard deviation of MDA in saliva and serum were 22.45±1.04 nmol/dl and 4.27±0.71 nmol/dl respectively with a statistical significant P-value (<0.001). The mean and standard deviation values of MDA in saliva and serum of OSCC subjects were 35.26±3.34 nmol/dl and 6.92±0.32 nmol/dl respectively with a statistical significant P-value (<0.001) (**Table - 1**). In the study, MDA level of saliva and serum were found to greater in OSCC followed by leukoplakia and healthy controls.

Discussion

In aerobic life cycle, oxygen free radicals are formed in normal cell metabolism from molecular O_2, even though body has antioxidant defenses and oxygen free radicals that can cause constant damage to oxidizable molecules which are repaired or replaced in a dynamic equilibrium. However, there is an oxidative stress either in the form of increase production of oxygen free radicals or from the deficiency of antioxidant defense or repair mechanism and hence, there can be irreversible tissue injury [10, 11].

Table - 1: Mean ± SD values for salivary and serum MDA in healthy controls, oral leukoplakia and OSCC patients.

MDA			
Groups	**Saliva (nmol/dl) (Mean ±SD)**	**Serum (nmol/ml) (Mean ±SD)**	**P-value**
Healthy controls	18.75±0.72	3.21±0.28	<0.005
Leukoplakia	22.45±1.04	4.27±0.71	<0.001
OSCC	35.26±3.34	6.92±0.32	<0.001

MDA- Malondialdehyde, SD- Standard deviation

Normal cells can be transformed into malignant cells as a result of oxidative modification. These transformed tumor cells produce high levels of ROS, which in turn increases lipid peroxidation levels. Owing to high cytotoxic properties, lipid peroxidation products like MDA modulate cell growth by activating signal transduction pathways, therefore acting as tumor promoters and co-carcinogenic agents. MDA is a major genotoxic carbonyl compound generated by lipid peroxidation and during arachidonic acid metabolism for the synthesis of prostaglandins. Hence, MDA levels are used to indicate oxidative and cellular damage to tissues due to ROS and free radicals [6, 8].

The important exogenous cause of oxidative stress associated with oral potentially malignant disorder and cancer is the use of tobacco. Continuous exposure to carcinogenic benzopyrene and nitrosamine present in tobacco and areca nut predisposes the mucosal surface to malignant transformation. Nicotine which is present in tobacco causes pH alterations during

chewing that causes partial decrease of oxygen and produces highly reactive free radicals such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and superoxide anion (O_2^-) in the body fluid and enhance lipid peroxidation levels of biological molecules [11, 12]. The present study was done to determine and compare serum and salivary MDA level so as to assess the degree of oxidative damage caused by oral leukoplakia and OSCC patients.

In the present study, salivary and serum levels of MDA increased significantly from healthy controls to oral leukoplakia subjects and a highly significant increase was observed in oral squamous cell carcinoma. These results were in accordance with the study done by Metgud R, et al. and Ganesan A, et al. [9, 13].

Tobacco is consumed in various different forms like smoking and chewing. They generate free radicals like ROS which lead to oxidative DNA damage to the adjacent tissues. Consumption of tobacco gives rise to increase in nicotine exposure as well as causes heat production and pH changes during smoking and chewing respectively affecting bodily fluids such as blood and saliva, thus resulting in the generation and stabilization of free radicals [14, 15]. Thus, consumption of tobacco causes overproduction of ROS and free radicals that enhance lipid peroxidation levels and serum as well as salivary MDA levels are thus increased as seen in oral leukoplakia and OSCC patients [16, 17].

Increases in MDA levels in both saliva and serum are not only owing to consumption of tobacco, however occur as a result of the magnitude of oxidative stress, supporting the hypothesis that cancer cells have noticeably altered ROS metabolism which lead to production of large amounts of ROS than that of non-neoplastic cells and the suppression of the antioxidant system that mediate body's defense mechanisms [17, 18].

Conclusion

In the present study, salivary and serum levels of MDA was greater in OSCC than that of oral leukoplakia and healthy controls. Thus, increased levels of MDA in oral leukoplakia and OSCC patients reflect interactions with several carcinogenic agents that confirm greater lipid peroxidation and oxidative stress in oral leukoplakia and OSCC patients.

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