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Estimation of Salivary Malondialdehyde Levels in Patients with Adverse Oral Habits

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Abstract

Aims and Objectives

Aim of this study was to assess the levels of Salivary Malondialdehyde as an oxidative stress biomarker in people with adverse oral habits.

Introduction

Habit of consumption of various forms of Tobacco such as Gutka, Supari, Bidi, Cigarette a fairly common habit, especially among males. These harmful products cause imbalance between the free radicals and antioxidants , leading to oxidative stress. The levels of this oxidative stress can be measured by a specific oxidative stress biomarker, that is Malondialdehyde.

Materials and Methods

A total of 40 , medically fit males were selected for the study. Among these, 20 people had no history of any adverse oral habits.The other 20 males reported having a habit of consuming at least one or more of harmful substances such as Paan, Gutka, Supari like substances. The salivary Malondialdehyde level was calculated in these patients and subsequently, a comparison was made between those with adverse oral habits and those without any such habits.

Results: The salivary Malondialdehyde levels were found to be increased in people with adverse oral habits as compared to those without any such habits . The maximum contrast and increase was found in people of age group 40- 50 years.

Conclusion: Elevated salivary Malondialdehyde levels, as an oxidative stress biomarker is one of the reliable tools to estimate the level of oxidative stress and chance of other potential damage to the oral mucosa due to consumption of various harmful substances like Pan, Gutka, Supari, Smoking and others .

Keywords: Antioxidants, Free Radicals , Oxidative Stress , Oxidation, Malondialdehyde .

Introduction

Saliva is one of the important body fluids consisting of various elements such as proteins, immunoglobulin, antibodies, enzymes, few digestive enzymes, antioxidants, antibacterial agents, electrolytes and trace elements. Saliva as a body fluid is the first to get exposed to the ill effects of harmful substances present in Gutka ,Tobacco, Cigarette and Bidi smoke. Also the oral mucosal cells have the tendency to rapidly absorb various substances present in the oral cavity and the saliva. Thus, saliva has recently emerged, as an alternative diagnostic tool to measure levels of various substances and diagnose various pathologies. Saliva as a fluid is easy to collect, painless, readily available, and reliable as a diagnostic sample [1].

Consumption of Tobacco, Paan, Supari, Khaini ,Gutka, Bidi, Pan masala like substances cause the activation and release of free radicals and other reactive oxygen species. To counteract the effects of free radicals, salivary antioxidants also get released which leads to oxidative stress and damage. Salivary antioxidants play an important role in the oral cavity against effects of free radicals, as being highly vascular oral mucosa gets affected by free radicals and oxidative stress more than other body tissues [2]. There are certain oxidative stress biomarkers in saliva which help to assess the level of oxidative stress and damage to oral mucosa. These include , Malondialdehyde (MDA) , Vitamin C and Vitamin A. Among these MDA is one of the main biomarkers used as a reliable tool to measure the levels of oxidative stress and its damage to the oral mucosa.

Materials and Methods

A total of 40, medically fit male patients were taken up for the study who had reported to the Department of Oral and Maxillofacial Surgery, College of Dental Sciences and Hospital, Rau Indore for dental treatment. Among these total 40 patients, 20 of the patients confirmed having a habit of consuming various substances like Pan masala, Gutka , Bidi, Khaini, and Cigarette smoking. All these patients were consuming one of these various substances or in combination, more than 3-5 times a day for many months and years. The other half claimed to have never indulged in any form of tobacco product consumption or any other adverse oral habits throughout their lives, neither was their oral cavities suggestive of any such habits .

These 40 patients were divided into 4 age groups from 20 to 30, 31 to 40, 41 to 50 and 50 years and above. In each age group, 5 persons were listed who were those with the adverse oral habits, while the other 5 were in the control group who did not have any adverse oral habits.

All these patients were explained about the study and a well informed written consent was obtained. Approval of the Institutional Ethical Committee was also taken. Inclusion criteria for the study were selection of only healthy, medically fit patients for both the habits group and others the non habit group. Patients had not taken any food for the past one hour. Two ml of stimulated saliva was collected in sterile vial and sent for analysis for salivary MDA levels. The Spectrophotometric analysis was used to measure the level using various reagents.

Results

The salivary Malondialdehyde levels in non habit groups were normal while MDA levels in habit groups

were more as compared to non habits group.[Table-1, Figure-1] Among the habit group, the maximum MDA levels were found in the age group of 40 to 50 years.In the age group of 40 -50 years, the MDA levels were .038nd . The MDA levels were the least in the non habits group aged 20- 30 years of age . It is clear in this study that Salivary MDA levels are high in people with adverse oral habits than in people without these adverse oral habits. Longer years of duration of habits, increased frequency per day, with long term contact of these harmful substances and poor oral hygiene are few factors which lead to increased levels of Malondialdehyde (MDA) in habits group. [Table-2, Figure-2]

Figure-1 and Figure 2

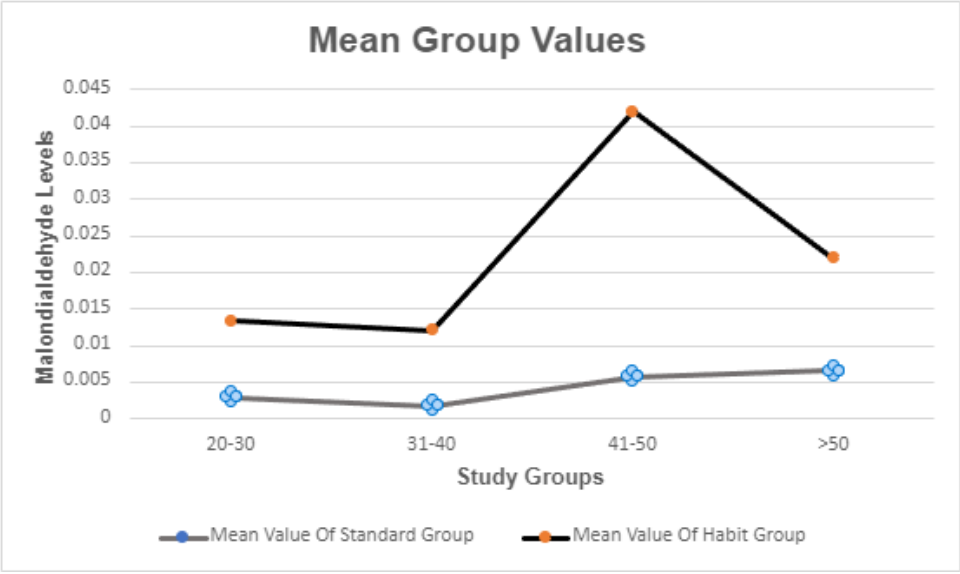

Table- 1 Comparisons of mean values in habits and non-habits group

Age	Mean Value Of Non Habits Group (In nm/dl)	Mean Value Of Habit Group (In nm/dl)
20-30	0.00288	0.01048
31-40	0.00175	0.01027
41-50	0.00569	0.0362
>50	0.00654	0.01536

Table-2 Mean group values

Age	Groups	Mean + SD	P Value
20-30	**Controls**	0.0028 ± 0.015	-
	Habits	0.0104 ± 0.011	0.1726nd
31-40	**Controls**	0.0017 ±0.0002	-
	Habits	0.0017 ± 0.0117	0.1438nd
41-50	**Controls**	0.0056 ±0.008	-
	Habits	0.036 ± 0.025	0.038nd
>51	**Controls**	0.0065 ± 0.009	-
	Habits	0.0015 ± 0.011	0.40nd

Discussion

Saliva is an important body fluid produced by three major and other numerous minor salivary glands of the oral cavity. Saliva as a fluid contains various enzymes, digestive enzymes, antioxidants, antibacterial substances, trace and micro elements, sodium chloride, bicarbonates, immunoglobulins and others. All these agents individually and collectively play an important role in oral and general body functions and health.

Antioxidants are substances produced by body cells and body fluids including saliva and play an important role in various physiologic and metabolic processes of the body. Antioxidants are substances that when present at low concentrations as compared to those of an oxidizable substrate (Example- Proteins, Lipids), help to delay or prevent oxidation of various substances [3].

Antioxidants can be natural or synthetic. These antioxidants function to either delay the repair or even prevent cell damage mainly due to the action of various free radicals and reactive oxygen species. Various dietary sources of antioxidants include vitamin-C, vitamin A containing fruits, vitamin E , carotenoids,

selenium, lycopene and various fruits and vegetables [4]. Human Saliva is rich in antioxidants with the major source being the parotid gland.

Salivary antioxidants can be classified as enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase, catalases, and peroxidases. Non-Enzymatic Antioxidants include,Ascorbic Acid,Albumin, Glutathione, Uric acid and Lactoferrin [5]. Among these, Uric acid accounts for more than 85% of the total antioxidant capacity of saliva.

Salivary antioxidants can be classified as per their function,

1) Group 1- Preventive Antioxidants - These inhibit the production of free radicals.
   Salivary Superoxide dismutases, Carotenoids ,Peroxidases ,and Glutathione.
2) Group 2 - Sweeping Antioxidants- These include Vitamin A, Vitamin E, Uric acid, Bilirubin, Albumin.
   - These help to eliminate free radicals in order to reduce starting and spreading of cell damage
3) Group 3 - These are repairing antioxidants- These have a function of repairing cell damage. These include Proteases, Transferases and Lipases[6].

Saliva has lots of antioxidants. These function both individually and also collectively. All of these salivary antioxidants together form the total antioxidant capacity of saliva. Total antioxidant capacity of saliva is defined as moles of oxidants neutralized by one liter of solution as a biomarker measuring the antioxidant potential of salivary fluid [7].

In daily functions of life, various physiological and biochemical reactions and processes keep taking place, which lead to formation of substances termed as free radicals and reactive oxygen species. Metabolic processes by the immune defense body system during stress in the body, dietary intake and few environmental factors also release free radicals [8].

Free radicals are atoms or molecules containing one or more unpaired electrons in the outermost atomic or molecular orbital. These free radicals are also defined as molecules or molecular fragments with an unpaired electron which imparts certain characteristics to the free radicals such as reactivity [9].

Some of these free radicals have good physiologic body functions like killing of pathogenic organisms, to help decrease inflammation, control smooth muscle functioning and in proper working of some other body organs. Free radicals are also said to have a role in the aging process also. Few other free radicals are said to have a role in the pathogenesis of various diseases and ailments such as Alzheimer's disease and Parkinsonism. In the oral cavity Leukoplakia, Erythroplakia, Oral Fibrosis like lesions can be seen as effects of actions of free radicals.

Formation of free radicals is a continuous and unavoidable process. In excess, free radicals damage oral mucosal cells by disturbing DNA function and cell growth. Lipid peroxidation in the cells directly leads to increase in the MDA levels, which are indicative of the levels of oxidative stress.

Side effects of excess free radicals can be visualized in the oral mucosa and can lead to common pathologies such as dental caries, periodontitis, mucositis, certain white and red lesions, partly due to the

exposure to harmful substances as nicotine[10].

Saliva in the oral cavity is the first line of defense and is also the first to get exposed to ill effects of harmful substances such as Gutka, tobacco, supari, khaini alone or together in combination with any two or more substances . Various salivary antioxidants now get activated as a response to these free radicals. This fight between free radicals and antioxidants, damages the oral mucosa and leads to oxidative stress. This oxidative stress leads to cell damage, DNA defects, lipid peroxidation which predisposes the oral mucosa to various oral pathologies[11].

There are certain oxidative stress biomarkers which can be used to estimate the degree of oxidative stress and damage to the oral mucosa. These include vitamin A, vitamin C, Carotene, Malondialdehyde, Lipids, Peroxidases, Protein and Carbonyl. Among these MDA is one of the main oxidative stress biomarkers present in the saliva. Salivary MDA originates from systemic sources and is also produced in saliva. MDA is also found in a few food products such as yogurt, ice cream, fish meat, nuts, and dry fruits. MDA levels get altered with dietary habits, oral hygiene conditions , consumption of certain food products and even drugs. Gutka, tobacco like products directly increase oxidative stress levels and lead to increase in level of salivary MDA[12].

Nicotine in cigarette, gutka like similar substances tends to get very rapidly absorbed via oral capillaries in the oral mucosa and further into systemic circulation. This leads to increase in the salivary MDA levels indicating a high oxidative stress[13].

This lipid peroxidation chain reaction leads to increase in the levels of salivary MDA . There is cell damage, DNA mutations which predispose the oral mucosal cell to various oral pathologies such as oral leukoplakia, Oral sub mucous fibrosis, squamous cell carcinoma like malignancies which can be life threatening . In this study salivary MDA levels were reported to be high in people with various adverse oral habits as compared to those people without any such habits.

The result of this study clearly indicates that increased MDA levels in oxidative stress are seen due to harmful products in gutka, supari like substance. These patients are more prone to suffer from various oral pathologies. This study is in accordance with a study by S Dhonde Et al, wherein it is clearly reported that people with various adverse oral habits are more likely to develop oral pathologies as compared to those without these habits [14].

One other study also reports similar findings regarding the increase in the level of salivary MDA in people with adverse oral habits. Lipid peroxidation due to free radicals leads to increase in the levels of salivary MDA. This increased level of salivary MDA is used as a good tool for diagnosing the susceptibility of , getting affected by various oral morbidities such as leukoplakia and oral submucous fibrosis. When diagnosed in early stages, these various oral pathologies can be treated well with a good prognosis [15]. One other such similar study also has been done by Guven Y , Unuy K , E Uslu where in , the stress on the importance of detecting increased levels of salivary MDA for diagnosis of various oral pathologies has been emphasized [16].

Role of MDA in other oral diseases have also been studied and reported. People with periodontitis have been reported to have an increase in the level of salivary MDA as compared to people who do not suffer from periodontitis. Periodontitis causes increased oxidative stress in the oral cavity thus increasing the salivary MDA levels [17]. Salivary MDA levels are also reported to be high in smokers as compared to nonsmokers. Nicotine in cigarettes causes excess free radical release and oxidative stress in oral mucosa and oral cavity. Increased salivary MDA levels in cigarette smokers has also been reported by Tongue MO, Ozturk O, Sutcu R , Et al [18].

Relation between Dental Caries and increased salivary MDA levels have also been reported. A study has been done on salivary and serum antioxidant and oxidative stress markers in dental caries. This study reports Dental Caries causes an increase in salivary MDA levels which are indicative of the extent of oxidative stress[19].

While few other studies differ and do not agree that dental caries cause oxidative stress and increase in salivary MDA levels. A study on salivary antioxidant and oxidative damage among smokers and non smokers with dental caries reports that dental caries do not influence salivary MDA levels . They claim that there is no association between dental caries and salivary lipid damage measured by MDA [20].

Consumption of tobacco in both forms as chewing tobacco and smoking together increases the salivary MDA levels more than in people who consume it as a single form .The MDA level was found to be high in people who had the habit of both forms of tobacco consumption [21].
In this study people with adverse oral habits had increased salivary MDA levels as compared to the controlled group, the people without these habits. The increased salivary MDA levels were due to oxidative stress and lipid peroxidation in oral mucosa cells.

Salivary MDA levels are often used nowadays as a diagnostic marker to detect the extent of cell damage, oxidative stress, the risk of DNA mutation and probability of developing various oral pathologies in the oral mucosa.

Thus estimating levels of salivary MDA can help us to treat and even prevent the development of various premalignant lesions and conditions , whenever possible especially in early stages .
People with adverse oral habits should be counseled regarding ill effects of such substances. They should be motivated to stop these adverse oral habits immediately and completely .

Conclusion

Imbalance between free radicals and the antioxidants, with the balance is shifted more towards the free radicals, leads to oxidative stress and can predispose to development of oral pathologies, premalignant lesions and conditions.These premalignant lesions and conditions are often difficult to treat especially when in advanced stages. Estimation of salivary MDA levels, extent of oxidative damage and stress can be taken as one of the important steps in diagnosis and management of various oral pathologies in their initial stages itself, thus having a good treatment outcome and prognosis.

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