

Estimation of Serum and Salivary MDA Levels in Tobacco Chewers with and without Lesions and Correlate the Same

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Abstract

Background and Objectives: Oral cancer is one of the major forms of cancer worldwide and is one of the most common malignancies in India. The important initiators of oral cancer in India are tobacco consumption in various forms, with prevalence of smokeless tobacco (ST) use being 20% of the population. It is also known as chewing tobacco, oral tobacco, spit or spitting tobacco. It contains many carcinogens and mutagenic chemicals which are addictive. It generates reactive oxygen species (ROS) that include oxygen derived free radicals. Most free radicals are highly reactive and short-lived. They

mediate oxidative degradation of lipid and produce various end products which results in cell damage, and one such end product is Malondialdehyde (MDA). MDA is considered to be crucial for development of potentially malignant lesions and cancer by forming adducts with DNA. Studies have shown a significantly high level of serum and salivary MDA in oral pre cancer and cancer. Correlation between serum and salivary MDA levels in tobacco chewers without lesions and with lesions are sparse. Limited research is available in tobacco chewers without lesions hence present study was done to evaluate both serum and salivary MDA levels in tobacco chewers

with and without lesions and controls and to correlate the same

Methods: 40 tobacco chewers without lesions, 40 tobacco chewers with lesions (20=Leukoplakia and 20 =OSCC) and 40 healthy controls were included in this study. Blood and saliva was collected by appropriate methods and analyzed for Malondialdehyde levels in both. Obtained serum and salivary MDA levels in cases and controls were compared and correlated by Pearson's correlation analysis XII.

Results

1. Both serum and salivary MDA levels were higher in males than in females in cases and controls.
2. Serum and salivary MDA levels were raised in tobacco chewers without lesions
3. Serum and salivary MDA levels were significantly high in tobacco chewers with lesions (OSCC and Leukoplakia) than in the tobacco chewers without lesions
4. Tobacco chewers with OSCC had increased serum and salivary MDA levels than tobacco chewers with leukoplakia
5. No correlation was found between salivary MDA levels and serum MDA levels in controls and tobacco chewers with leukoplakia, however positive correlation was found with tobacco chewers without lesions and tobacco chewers with OSCC.

Conclusion

The result indicates that, the antioxidant capacity of both blood and saliva will be reduced in tobacco chewers with and without leukoplakia or OSCC patients, which can be attributed to an increased oxidative stress. It also suggests that, as correlation between serum and salivary MDA levels showed varied results, stronger correlation studies with larger sample size are necessary to establish use of saliva as a reliable, oxidative stress marker.

Keywords: Chewing tobacco; Leukoplakia; Oral squamous cell carcinoma; Serum; Saliva; Malondialdehyde; Free radicals; Reactive oxygen species.

Introduction

Cancer is the second leading cause of death worldwide. Around 7,00,000 new cases are detected in India every year. About 50-70% of cancer-related deaths in India are due to oral cancer.¹

The habit of tobacco consumption is a known etiological factor for the development of oral cancer. Tobacco can be consumed in various forms, with prevalence of smokeless tobacco (ST) use being 20% of the population.^{2,3}

Smokeless tobacco is also known as chewing tobacco, oral tobacco, spit or spitting tobacco. It contains many carcinogens and mutagenic chemicals which are addictive. It generates reactive oxygen species (ROS) that include oxygen derived free radicals that are highly reactive and short-lived.⁴

Free radicals and ROS leads to elevated lipid peroxidation, which further damage the cellular structural blocks like lipids, proteins, and DNA. Lipid peroxidation is a chain reaction which provides continuous supply of free radicals that promotes further peroxidation in which many aldehydes are formed mainly malondialdehyde (MDA), Propanedial,4-hydroxynonenal etc.^{5,6}

MDA is one of several low molecular weight end products formed by decomposition of primary and secondary lipid peroxidation products known to serve as a reliable marker of free radical mediated tissue injury.⁷

It reacts with deoxyadenosine and deoxyguanosine in DNA and forms adducts, leading to oxidative stress, which is considered to be crucial for development of potentially malignant lesions and cancer.⁸

Previous studies have shown significantly high levels of serum and salivary MDA in oral precancer and cancer.^{9,10,11,12} However, studies estimating and correlating serum and salivary malondialdehyde levels in tobacco chewers without oral leukoplakia or oral squamous cell carcinoma are rare which might give an indication on levels of oxidative stress and the possibility of development of potentially malignant lesions and malignancy.

Thus, the present study aims to evaluate serum and salivary malondialdehyde levels in tobacco chewers with and without oral leukoplakia or oral squamous cell carcinoma and to assess if saliva can be used as an alternative to serum.

Methodology

The present study was conducted in the Department of Oral Pathology and Microbiology, College of Dental Sciences, Davangere

Source of data

Patients reporting to the Out Patient Department, and to the Department Of Oral and Maxillofacial Pathology and Microbiology, College of Dental Sciences, Davangere.

Selection of subjects

Study group consisted of 120 individuals divided into three groups.

Group I: 40 healthy controls without any habits related to oral carcinoma development and systemic illnesses.

Group II: 40 individuals with the habit of tobacco chewing for a minimum of 5 years without any lesions in the oral cavity and/or any systemic illnesses.

Group III: 40 individuals with the habit of tobacco chewing for a minimum of 5 years with clinically diagnosed oral leukoplakia (n=20) (Group IIIA) or oral squamous cell carcinoma (n=20) (Group IIIB) but without any systemic illnesses.

Inclusion criteria

For cases

Group II: Subjects with the habit of tobacco chewing for a minimum of 5 years without any lesions in the oral cavity.

Group III: Subjects with the habit of tobacco chewing with clinically diagnosed and histopathologically confirmed oral leukoplakia or oral squamous cell carcinoma.

For control group

Subjects without any habit believed to cause oral squamous cell carcinoma and/or oral precancerous or cancerous lesions in the oral cavity

Exclusion Criteria

For cases and controls:

Pregnant women

- Patients under medication that could affect saliva production
- Systemic diseases
- Individuals with the habit of smoking and consumption of alcohol
- Patients undergoing radiation therapy
- Patients with recent history of hospitalization and infusion

Armamentarium

Materials used

- Sterile gloves
- Cotton swab
- Syringe
- Blood collection tubes
- Sterile container for saliva collection
- Cuvettes
- Finn Micropipettes (5- 50µl and 100-1000µl) and sterile tips
- Micro centrifuge (Denver instrument)

- Test tubes
- Test tube holder
- Distilled water
- 10%Trichloroacetic acid
- 0.67%Thiobarbituric acid
- Glass containers

Equipment used

- Spectrophotometer (US-VIS Systronics117)

Collection of Samples and Their Analysis

Collection of saliva

After obtaining the informed consent from the subjects, they were instructed not to have anything except water for 2 hours prior to the collection of saliva. Patients were made to sit upright, asked to swallow existing saliva. After a minimum of 5 minutes rest, patients were asked to spit the saliva into a sterile container for 5 minutes. Due to minimal change in chemical composition of saliva when collected from resting glands, unstimulated whole saliva was collected for analysis by spitting method.¹³ The collected samples were than centrifuged at 3000rpm for 5 minutes and the resulting supernatant of saliva was utilized for biochemical assay (MDA) on the same day using spectrophotometer [Figure 8].

Collection of blood

Under aseptic conditions 2ml of the patient’s intra venous blood was obtained.

The samples were centrifuged at 3000rpm for 2-3minutes to obtain serum. The concentration of serum Malondialdehyde (MDA) was determined on the same day of collection using spectrophotometer.

Estimation of Serum and Salivary Malondialdehyde Levels: ¹⁴

Principle

Auto oxidation of unsaturated fatty acid involves the formation of semi stable peroxides, short chain aldehydes like MDA. Malondialdehyde is an end product of fatty

acid peroxidation that can react with thiobarbituric acid to form a colored complex that has maximum absorbance at 532nm which will be measured with the help of a spectrophotometer.

Reagents used are;

- 10% Trichloroacetic acid (TCA)
10gm TCA in 100ml distilled water
- 0.67% Thiobarbituric acid (TBA)
670mg TBA in 100 ml distilled water

Procedure (Table 1):

	Distilled water	Serum in ml	Saliva in ml	TCA (10%) in ml	TBA(0.67%) in ml
Blank	0.5	-	-	3.6	1.5
Control	-	0.5	0.5	3.6	1.5
Case	-	0.5	0.5	3.6	1.5

After mixing reagents in test tube, they were kept in water bath and boiled for 10-15 minutes and allowed to cool. Later it was centrifuged for 10 to 15 minutes; optical density of supernatant was taken to measure the absorbance at 532 nm.

$$\text{Concentration of MDA} = \frac{\text{Absorbance of test (A)}}{\text{Nanomolar extinction coefficient (E)}} \times \frac{\text{Total volume (V}_s\text{)}}{\text{Sample volume (V}_T\text{)}}$$

n moles/ml

Molar extinction coefficient is 1.5×10^5 nmol/L.

$$\Rightarrow \frac{A \times 5.6}{1.5 \times 10^5 \times 0.5} \times \frac{10^3}{1000}$$

$$\Rightarrow A \times 73.33 \text{ nmol/ml}$$

The results were expressed as nmol/ml.

Statistical analysis

Statistical analysis was carried out using SPSS package (version 16). Results were expressed as mean ± SD, One way ANOVA was used for multiple group comparisons followed by post hoc Tukey’s test for group wise comparisons. Pairwise comparison was made by paired T

test for all the tests, a p-value of 0.05 or less was considered to be statistically significant.

Figure 1: Armamentarium for blood and saliva collection



Figure 2: Collection of saliva in a sterile container by spitting method



Figure 3: Salivary containers in a vaccine carrier with ice pack



Figure 4: Micro centrifuge (Denver instrument company, USA)



Figure 5: Laboratory centrifuge (REMI- RC 80)



Figure 6: Armamentarium used for MDA level estimation



Figure 7: Reagents used for serum and salivary MDA level estimation



Figure 8: Spectrophotometer



Results

The present study was conducted in the Department of Oral Pathology and Microbiology, College of Dental Sciences, Davangere. It comprised of 40 tobacco chewers without lesion, 40 tobacco chewers with lesions (20-Leukoplakia 20-OSCC) and 40 age and gender matched healthy individuals (Table 16). All the groups were assessed for MDA levels in serum and saliva

Obtained data was analyzed using One Way ANOVA test to study the comparative behavior of serum and salivary MDA levels for different test groups. Further correlation analyses were carried out between serum and saliva in the test groups. The significance level was set at $P < 0.05$. Statistical analysis was performed with the IBM® SPSS® Statistics Version 16.

Mean serum MDA levels in different test groups

The mean serum MDA levels in group I, group II, group IIIA and group IIIB were 2.68 ± 0.6 , 4.51 ± 0.8 , 5.82 ± 0.5 and 9.53 ± 0.9 nmol/ml respectively. This difference in mean serum MDA levels in cases when compared to controls was statistically significant ($p=0.000$) (Table 2, Graph 1).

Mean salivary MDA levels in different test groups

The mean salivary MDA levels in group I, group II, group IIIA and group IIIB were 0.87 ± 0.2 , 1.93 ± 0.4 , 2.84 ± 0.6 and 4.06 ± 0.6 nmol/ml respectively. This difference in mean salivary MDA levels in cases when compared to controls was statistically significant ($p=0.000$) (Table 3, Graph 2).

Comparison of mean serum and salivary MDA levels between cases and controls

The mean comparative values of serum and salivary MDA were 2.68 ± 0.6 , 4.51 ± 0.8 , 5.82 ± 0.5 , 9.53 ± 0.9 , 0.87 ± 0.2 , 1.93 ± 0.4 , 2.84 ± 0.6 , 4.06 ± 0.6 nmol/ml in group I, group II, and group IIIA and group IIIB respectively. This comparison of serum and salivary MDA levels were statistically significant with p value (0.000) less than 0.005 (Table 4, Graph 3)

Comparison of mean serum MDA levels between males and females

The mean serum MDA levels in males and females among group I, group II, group IIIA group IIIB were 2.83 ± 0.7 , 4.92 ± 0.8 , 6.02 ± 0.5 , 9.76 ± 1.1 nmol/ml and 2.54 ± 0.5 , 4.10 ± 0.7 , 5.62 ± 0.5 , 9.31 ± 0.5 nmol/ml respectively. This mean difference in serum MDA levels between males and females with P value of 0.000 was statistically significant (Table 5, Graph 4).

Comparison of mean salivary MDA levels between males and females

The mean salivary MDA levels in males and females among group I, group II, group IIIA and group IIIB were

0.89±0.2, 1.99±0.5, 2.93±0.8, 4.31±0.8 nmol/ml and 0.84±0.19, 1.87±0.3, 2.75±0.3, 3.80±0.4 nmol/ml respectively. This mean difference in salivary MDA levels between males and females with P value of 0.000 was statistically significant (Table 6, Graph 5).

Correlation between serum and salivary MDA levels in different test groups

Statistically significant negative correlation with r value - 0.455 was obtained between serum and salivary MDA levels in group I. Significant negative correlation with - 0.01 was obtained in group IIIA. A positive correlation between serum and salivary MDA levels with r value of 0.406 and 0.586 was obtained in group II and group IIIB and this was found to be statistically significant. (Table 7, Graph 6, 7, 8, 9)

Table 2: Sample size

Sample size	Controls (Group I)	Tobacco chewers without lesions (Group II)	Tobacco chewers with Leukoplakia (Group IIIA)	Tobacco chewers with OSCC (Group IIIB)
Male	20	20	10	10
Female	20	20	10	10
Total	40	40	20	20

Table 3: Mean serum MDA levels in different test groups

Serum MDA levels in nmol/ml Mean±SD	Groups			
	Group I	Group II	Group III A	Group III B
	2.68±0.6	4.51±0.8	5.82±0.5	9.53±0.9
P value		0.000(HS)	0.000(HS)	0.000(HS)

SD-Standard deviation; HS-Highly significant; p value < 0.05 was considered significant.

Table 4: Mean salivary MDA levels in different test groups

Salivary MDA levels in nmol/ml Mean±SD	Groups			
	Group I	Group II	Group III A	Group III B
	0.87±0.2	1.93±0.4	2.84±0.6	4.06±0.6
P value		0.000(HS)	0.000(HS)	0.000(HS)

SD-Standard deviation; HS-Highly significant; p value < 0.05 was considered significant

Table 5: Comparison of mean serum and salivary MDA levels between cases and controls

Subjects	Mean serum MDA levels ± SD	Serum F	Mean Salivary MDA levels ± SD	Saliva F	Serum and Saliva Sig.
Group I	2.68±0.6	361.03	0.87±0.2	211.45	0.000(HS)
Group II	4.51±0.8		1.93±0.4		
Group IIIA	5.82±0.5		2.84±0.6		
Group IIIB	9.53±0.9		4.06±0.6		

SD-Standard deviation; HS-Highly significant; S-significant; NS-Non significant; p value < 0.05 was considered significant.

Table 6: Comparison of mean serum MDA levels between males and females

Serum MDA levels in nmol/ml	Group I	Group II	Group III A	Group III B
Males Mean±SD	2.83±0.7	4.92±0.8	6.02±0.5	9.76±1.1
Females Mean±SD	2.54±0.5	4.10±0.7	5.62±0.5	9.31±0.5
P value		0.000(HS)	0.000(HS)	0.000(HS)

SD-Standard deviation; HS-Highly significant; p value < 0.05 was considered significant.

Table 7: Comparison of mean salivary MDA levels between males and females

Salivary MDA levels in nmol/ml	Group I	Group II	Group III A	Group III B
Males Mean±SD	0.89±0.1	1.99±0.5	2.93±0.8	4.31±0.8
Females Mean±SD	0.84±0.2	1.87±0.3	2.75±0.3	3.80±0.4
P value		0.000(HS)	0.000(HS)	0.000(HS)

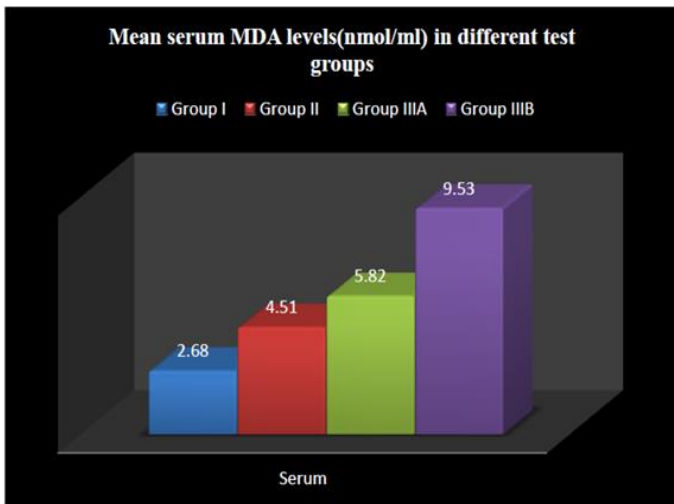
S.D-Standard deviation; HS-Highly significant; p value < 0.05 was considered significant.

Table 8: Correlation between serum and salivary MDA levels among test groups

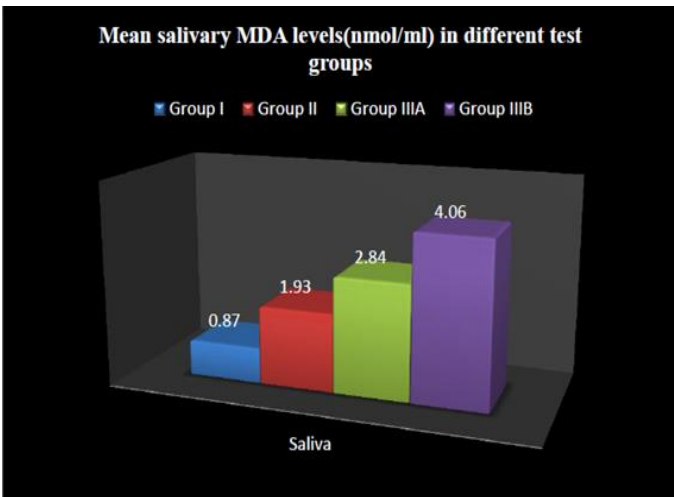
Groups	Correlation r value	P value
Group I	-0.455	0.003*(HS)
Group II	0.406	0.009*(HS)
Group IIIA	-0.010	0.966(NS)
Group IIIB	0.586	0.007*(S)

HS-Highly significant; S-Significant; NS-Non significant; p value < 0.05 was considered significant.

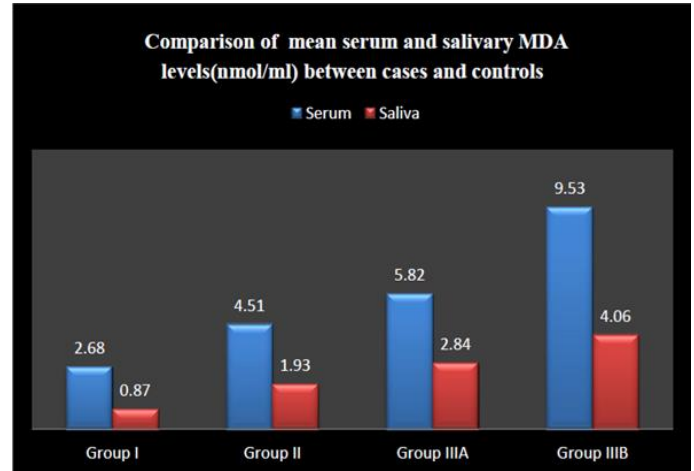
Graph 1: Mean serum MDA levels in different test groups



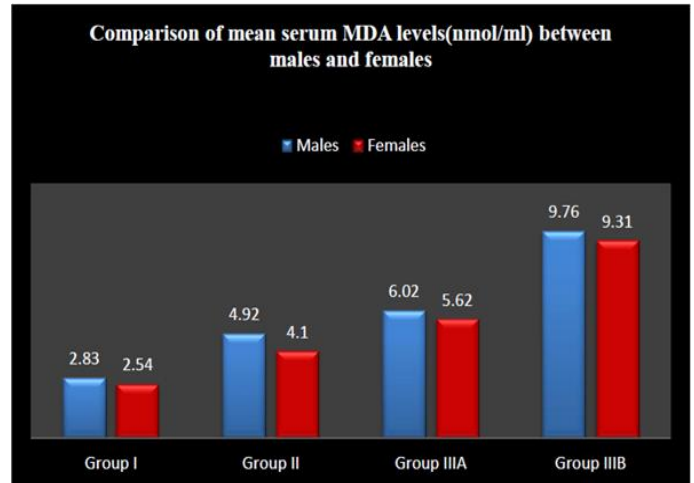
Graph 2: Mean salivary MDA levels in different test groups



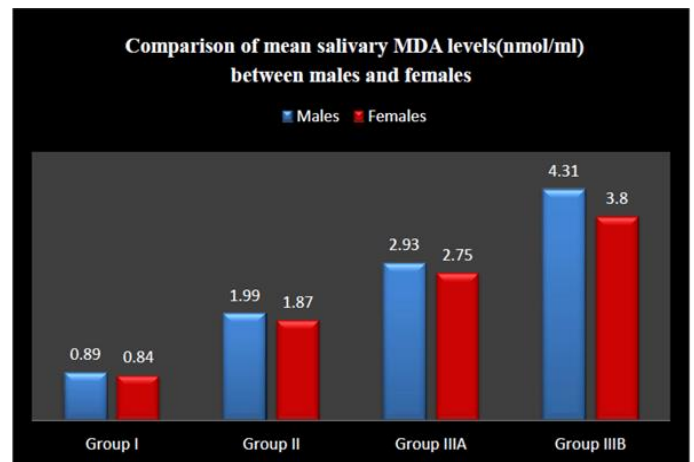
Graph 3: Comparison of mean serum and salivary MDA levels between cases and controls



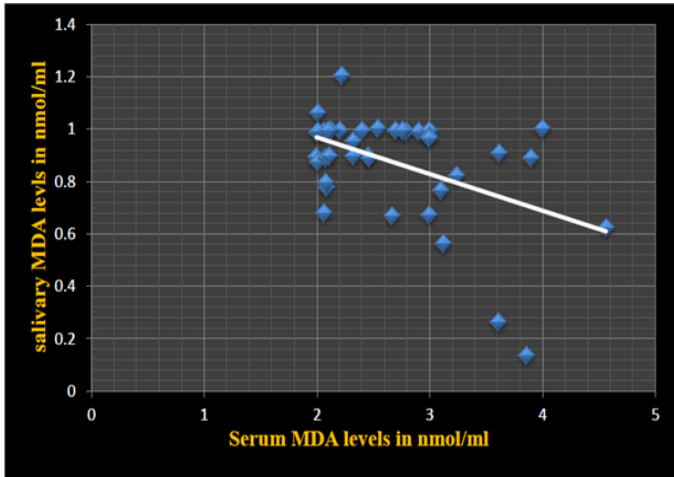
Graph 4: Comparison of serum MDA levels between males and females



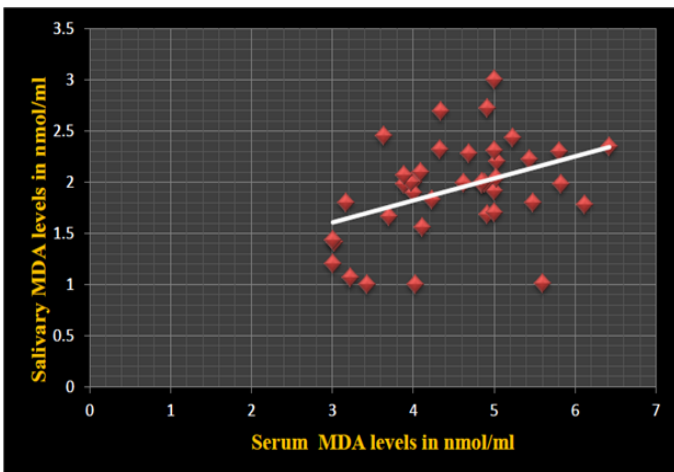
Graph 5: Comparison of salivary MDA levels in males and females



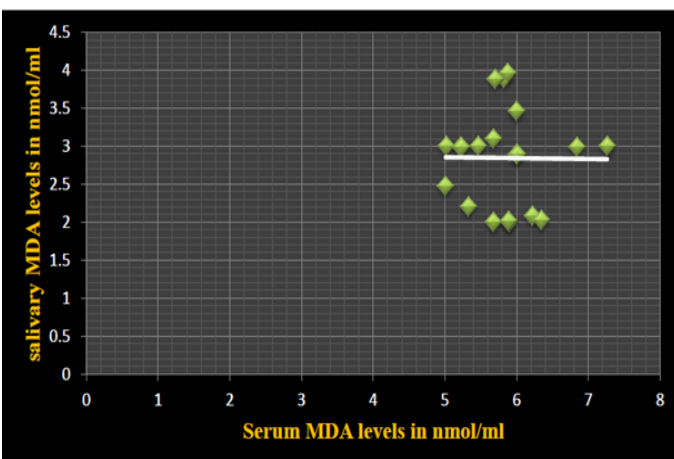
Graph 6: Correlation between serum and salivary MDA levels in group I



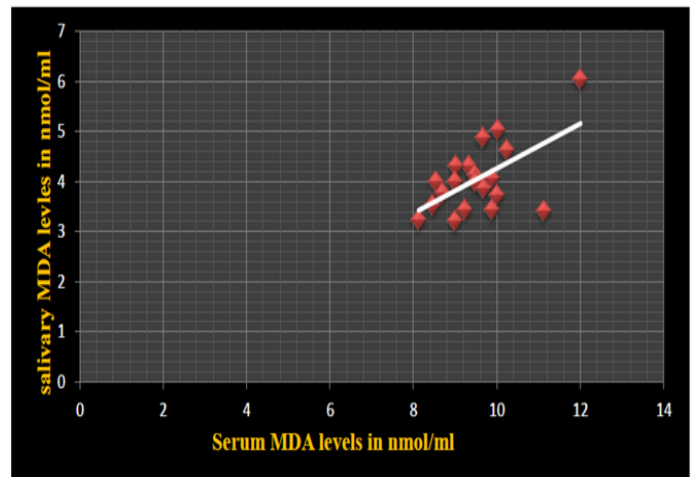
Graph 7: Correlation between serum and salivary MDA levels in group II



Graph 8: Correlation between serum and salivary MDA levels in group III A



Graph 9: Correlation between serum and salivary MDA levels in group IIIB



Discussion

Oral cancer is one of the 10 most frequent cancer and accounts for 2-4% of all global cancer incidences worldwide.¹⁵ Five year mean survival rate remains very low, despite improvements in diagnostic and treatment modalities.^{16,17} Two-thirds of oral cancer patients are diagnosed at advanced tumor stages, where survival drops to little more than 30% and its prognosis is unpredictable.^{18,19} It is also the leading cancer site in India, and its high incidence reflects tobacco as a major etiological cause.²⁰

Tobacco is an exogenous source of ROS, the chemical species that either possess free radicals containing at least one unpaired electron or composed of reactive non-radical compounds.²¹ Among tobacco consumers, high endogenous levels of prooxidants and deficiencies in antioxidants levels lead to Oxidative stress, which cause accumulation of ROS, inducing cellular injury, oxidizing cellular macromolecules such as DNA and playing a crucial role in carcinogenesis.²²

Normal cells can transform into malignant cells due to oxidative modification. These transformed cells produce high levels of ROS, which in turn increases lipid peroxidation levels. Owing to high cytotoxic properties,

lipid peroxidation products such as MDA modulate cell growth by activating signal transduction pathways, thereby acting as tumor promoters and co-carcinogenic agents.^{23,24} Estimating the levels of these free radicals can help us to understand their role in malignant transformation of cells and in treatment and prognosis

Due to the very short half-life of free radicals, their direct measurement is impractical. Indirect methods of Oxidative stress evaluation include estimation of secondary lipid peroxidation products, such as MDA. The extent of lipid peroxidation and free radical mediated damage can be assayed by serum and salivary MDA levels.^{12,25}

MDA is an end product of lipid peroxidation of poly unsaturated fatty acids. Individuals with elevated serum MDA levels have shown to be at higher risk for the development of premalignancy and malignancy.^{8,26}

The present study was specifically meant to estimate and compare and correlate the serum and salivary MDA levels in tobacco chewers without lesions (Group II) and tobacco chewers with lesions (Leukoplakia and Oral squamous cell carcinoma) (Group III A and B), along with age and gender matched controls (Group I).

In the present study, the range of serum MDA levels was 3.004 – 12.004 nmol/ml in cases and 1.996 – 4.5626 nmol/ml in controls and that of salivary MDA levels was between 1.005 – 6.062 nmol/ml in cases and 0.134-1.206 nmol/ml in controls.

Serum MDA levels in Group II and Group III (A and B) compared to Group I

The mean serum MDA levels were 2.68±0.6, 4.51±0.8, 5.82±0.5 and 9.53±0.9 nmol/ml in group I, group II, group IIIA and group IIIB respectively. The serum MDA levels were high in group II and group III(A and B) compared to the levels in group I. Our findings were in

accordance with similar studies estimating MDA levels in premalignancy and malignancy.^{2, 10,11,12}

This increase in the level of serum MDA levels in group II group IIIA and group IIIB can be attributed to the free radicals and ROS generation by tobacco chewed, which are responsible for the high rate of lipid peroxidation leading to elevated MDA levels.¹¹

Increased serum MDA levels have also been reported in patients with other forms of potentially malignant disorders such as oral submucous fibrosis (OSF)^{27,28} and Oral Lichen Planus (OLP).²⁹ The increase in lipid peroxidation product in OSF and OLP as compared to control group has been attributed to the poor antioxidant system, excessive free radical formation due to various tissue abuse habits and decomposition of polyunsaturated fatty acids present in cell membranes.^{28,29}

Studies on oral cancer and various human cancers such as lung cancer gastrointestinal cancer and cervical carcinoma have reported and highlighted similar findings as noticed in our study, validating the fact that there exists a relationship between free radical activity, lipid peroxidation and malignancy.^{30,31.}

Other than in cancer, literature reports, increased MDA levels in periodontitis, chronic obstructive pulmonary disease and H. pylori-infected gastric mucosa.^{33,34}

Salivary MDA levels in Group II and Group III (A and B) compared to Group I

The mean salivary MDA level was 0.87±0.2, 1.93±0.4, 2.84±0.6 and 4.06±0.6 nmol/ml in group I, group II, group IIIA and group IIIB respectively. The salivary MDA levels were high in cases compared to the levels in group I. Our finding mimicked the results of studies estimating the salivary MDA levels in pre malignancy and malignancy.^{9,10,12,27,35} Increased salivary MDA levels in group II and group III (A and B) compared to group I is probably a result of tobacco consumed in

different ways (smoking and chewing). Increase in nicotine levels and pH changes during tobacco chewing, affects saliva, resulting in the formation and stabilization of free radicals, generated free radicals including ROS leading to oxidative DNA damage of surrounding tissue. Oxidative damage of the salivary gland, possibly continuous local irritation caused by tobacco chewed can lead to injury related chronic inflammation and oxidative stress. Increase in salivary oxidative stress may be related to the alteration of salivary MDA levels.^{10,27}

On the contrary a study done on biopsy tissue of precancer showed decreased levels of salivary MDA levels as compared to controls which was statistically insignificant reasoning that, lipid peroxidation was altered in tumour cells (having dysplastic features).³⁶ This peroxidation of lipids was measured by MDA levels, But in their study, they didn't find any dysplastic features in biopsy tissue, so cells were not altered and peroxidation of lipids in the membrane was not affected significantly according to MDA levels. Compared to controls MDA level in leukoplakia was decreased in this study which they attributed to the difference in sample size (Leukoplakia-9 and controls -11).³⁶

Other than leukoplakia, literature reported increased salivary MDA levels in Oral sub mucous fibrosis and OLP.^{27,29} Patients with oral premalignant lesions (oral leukoplakia, oral lichen planus and erythroplakia) were found to have two times higher levels of salivary TBARS (including MDA) in comparison to age-matched healthy controls.^{37,38}

MDA Levels and Gender

A higher value of serum and salivary MDA levels was found in males as compared to females, both in cases and controls. Our finding was in accordance with Knight et al³⁹ and contrary to Chole RH et al² where males showed less MDA level than females, while Richard et al showed

that there did not exist any gender-related difference for MDA levels. Increased serum and salivary levels in males could be due to the increased consumption of tobacco and increased oxidative stress in males compared to females and gland size and flow rate in females will be significantly lesser than those in males in controls and cases.^{40,41,42}

Correlation of serum and salivary MDA levels in various test groups.

Only few studies correlating serum and salivary MDA levels in leukoplakia and OSCC patients has been reported in the past¹⁰ and the studies related to MDA levels in tobacco chewers without lesions are sparse. The results of our study showed negative correlation between serum and salivary MDA levels in group I and group IIIA and positive correlation in group II and group IIIB. Similar to the positive correlation demonstrated in group II and group IIIB, the MDA levels variation in serum and saliva is expected to show a positive correlation simulating any other serum component. But numerous factors may lead to variations in salivary MDA levels in individuals like variations in salivary basement membrane permeability with age, salivary flow rate variation with age and gender, deterioration of salivary gland function with age, gland size variations among genders.^{40,41,42,43} The above mentioned factors possibly contribute to the variation in the serum and salivary MDA levels in our study group I which were not considered.

A significant positive correlation between serum and salivary MDA levels in group II and group IIIB indicate increased oxidative stress leading to increased MDA levels in serum and saliva. Studies done in oral pre cancer and cancer showed increased tissue MDA levels and localized pouring of MDA contributing to high MDA

levels in saliva. Thus salivary MDA levels can be either dependent or independent of serum MDA levels.^{9,10}

Our study demonstrated negative correlation in group IIIA, possible reasons being small sample size and non-categorizing of cases depending on histological grading of dysplasia.

As ROS and free radicals have predominant deleterious role in inducing and promoting carcinogenesis, the present study suggests a role of MDA levels as a diagnostic biomarker and innovative tool to monitor oxidative stress and their impact on prognosis of oral cancer.

As correlation between serum and salivary MDA levels showed varied results, studies with larger sample size are necessary before establishing use of saliva as a predictable marker of oxidative stress.

Conclusion

From the results obtained from 120 subjects of our study, following conclusions can be arrived at:

- Both serum and salivary MDA levels will be higher in males than in females in cases and controls.
- Serum and salivary MDA levels will be raised in tobacco chewers without lesions
- Serum and salivary MDA levels will be significantly high in tobacco chewers with lesions (OSCC and Leukoplakia) than in the tobacco chewers without lesions
- Tobacco chewers with OSCC will have increased serum and salivary MDA levels than tobacco chewers with leukoplakia
- No correlation was found between salivary MDA levels and serum MDA levels in controls and tobacco chewers with leukoplakia, however positive correlation was found in tobacco chewers without lesions and tobacco chewers with OSCC.

Thus, the findings of the present study suggest that the antioxidant capacity of both blood and saliva will be reduced in tobacco chewers with and without leukoplakia or OSCC which can be attributed to increased oxidative stress.

It also suggest that, as correlation between serum and salivary MDA levels showed mixed results, stronger correlation studies with larger sample size are necessary to establish saliva as a reliable alternative to serum and a oxidative stress marker.

Limitations of the Study

- Small sample size
- Age wise grouping of sample was not done
- Categorization of cases according to frequency and duration of the habit was not considered
- Histopathological grading for leukoplakia and oral squamous cell carcinoma was not considered

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