

## Salivary Malondialdehyde and Uric acid in Tobacco Chewers

Sushama Dhonde<sup>1,\*</sup>, Miss Lizelle Rodrigues<sup>2</sup>, Pratap Jagtap<sup>3</sup>, G.J. Belwalkar<sup>4</sup>, Vinayak Mane<sup>5</sup>

<sup>1</sup>Professor, <sup>2</sup>MBBS Student, <sup>3</sup>Professor & Head, <sup>4</sup>Associate Professor, <sup>5</sup>Tutor, Dept. of Biochemistry, Bharati Vidyapeeth Deemed University Medical College & Hospital, Sangli

**\*Corresponding Author:**

Email: drsushamadh@gmail.com

### Abstract

**Introduction:** Tobacco in any form is responsible for the generation of free radicals which cause oxidative damage, producing lesions in the mouth like ulcers, leukoplakia, erythroplakia, sub mucus fibrosis and is responsible for the progression of oral cancer. We aimed to find out the use of salivary malondialdehyde as a marker of oxidative stress, and salivary uric acid as an antioxidant, in the tobacco chewers.

**Materials and Method:** 60 healthy tobacco chewers and 60 healthy non chewers were included in the study. Salivary MDA was measured by method of K. Satoh, and Salivary Uric acid with the help of Biochemistry analyzer.

**Results:** The mean concentration of salivary MDA of control group was  $0.0179 \pm 0.008$  and of study group was  $0.073 \pm 0.140$  n moles/L. The increase in the MDA was statistically significant ( $p < 0.004$ ). Salivary uric acid concentration of control group was  $1.97 \pm 1.38$  and of study group was  $2.11 \pm 1.41$  mg/dl. The difference was not statistically significant ( $p > 0.05$ ).

**Discussion:** Oxidative stress increased by the use of tobacco, leads in the increased lipid peroxidation followed by increased malondialdehyde. This may be the cause for the significantly increased salivary malondialdehyde concentration in the study group. Increased mean uric acid levels, though nonsignificant, may indicate initial phase of cellular injury.

**Conclusion:** Present study suggests that increased concentration of salivary Malondialdehyde and uric acid may be used as screening tool in tobacco chewers, for cellular injury.

**Keywords:** Salivary MDA, Salivary Uric acid, Tobacco Chewers.

### Introduction

The use of tobacco and tobacco containing product is very common in India. Tobacco chewing forms include pan (piper betel leaf filled sliced areca nut, lime, catechu and other spices chewed with tobacco), pan masala or gutakha (a chewable tobacco containing areca nut) and mishri (a powdered tobacco rubbed on the gums as toothpaste). The effects of these habits though not immediate are long term and can prove fatal to the user mostly resulting into oral cancer, due to oxidative damage caused by free radicals generated by tobacco chewing.<sup>(1)</sup> Oral cancer accounts for over 30% of all cancers in the country.<sup>(2)</sup> WHO predicts that tobacco deaths in India may exceed 1.5 million annually by 2020.<sup>(1)</sup> Because tobacco in any form is responsible for the generation of free radicals (F.R.), these free radicals cause oxidative damage to DNA resulting into cytotoxicity, mutations and potential for malignant changes.<sup>(3)</sup> These F.R. also damage other macromolecules, e.g. damage to lipids from membrane leads to self-perpetuating chain reaction known as lipid peroxidation. Malondialdehyde (MDA) is one of the aldehyde produced when lipid peroxidation proceeds. Damage to proteins leads to fragmentation, cross linking & aggregation. All these damages produce lesions in the mouth like ulcers, leukoplakia, erythroplakia, sub mucus fibrosis and thus are responsible for paving the way for progression of oral cancer.

Uric acid has been known to play a key role as significant antioxidants in plasma with low molecular mass in the body fluids. It is believed that it could

increase plasma antioxidant capacity. Uric acid can scavenge free radicals and it can chelate metal ions which act as pro oxidants.<sup>(4)</sup>

The first biological medium which is encountered during tobacco chewing is saliva. Saliva is a unique fluid and now a day's emerging as the diagnostic medium.<sup>(5)</sup>

In milieu of this we had undertaken the present study to find out the biochemical changes in MDA and uric acid, in the saliva of tobacco chewers. Salivary MDA as a marker of oxidative stress and salivary uric acid as an antioxidant were measured as biochemical parameters.

### Materials and Method

After ethical clearance, present study was carried out in the department of Biochemistry, BVDUMC&H Sangli from 2014 -2016. Whole saliva was collected from 60 subjects with tobacco chewing habit and 60 healthy non tobacco chewers; after written consent for voluntary participation. The subjects with tobacco chewing habit were included in 'Study group' while age and sex matched healthy subjects who were non tobacco chewers considered as controls.

**Inclusion Criteria:** Subjects consuming any form of smokeless tobacco daily, and willing to participate in the study. All these subjects were free from any dental problem as well as any other illness/disorder. Age group 15-50yrs. Duration of tobacco chewing is more than 5 years.

**Exclusion Criteria:** Subjects who had dental diseases or any other type of illness or disorders.

**Sample Collection:** Sugar free polystyrene balls used for chewing, to stimulate saliva, and stimulated saliva (directly expectorated whole saliva) was collected in clean, dry, sterilized glass bottles and fitted with proper rubber stoppers immediately. This filtered saliva was used for analysis of salivary Malondialdehyde by the method of K. Satoh.<sup>(6)</sup> This method was based on the principle that, trichloroacetic acid precipitates the serum and this precipitate was heated with thiobarbituric acid (TBA). This causes coupling of lipid peroxide with TBA, giving pink colored chromogen. This chromogen was extracted with n-butanol and intensity of which was measured on colorimeter using filter of 530 nm. Salivary Uric acid was estimated with the help of Biochemistry analyzer – fully automated.

**Statistical analysis:** Statistical analysis was done by calculating mean and standard deviation. Paired ‘t’ test was used to compare the results of study group and control group.

## Result and Discussion

It is now well known that oral squamous cell carcinoma (OSCC) and previous lesions not only involve specially expressed genes and proteins but also changes in the concentration of endogenous metabolites.<sup>(1)</sup> For the present study MDA as a marker of oxidative stress and Uric acid as an antioxidant were considered as endogenous metabolites to measure, significantly increased MDA concentrations were observed in the saliva of study group subjects than control group.

In India tobacco is used for smoking as well as in various smokeless forms. Absorption of toxic and carcinogenic chemicals in tobacco and other ingredients added to various products are generally associated with several cancers especially oral cancers.<sup>(7)</sup> Tobacco mixture is applied between the lower labial mucosa and gingival for 4-5 minutes to 1-2 hours. This region in mouth has many capillary vessels. Therefore nicotine and other addends get quickly absorbed into circulation. This process is repeated many times during a day. Tobacco specific nitrosamines are metabolites of nicotine. Chronic inflammation may promote the carcinogenic effect of these nitrosamines through generation of reactive oxygen species (ROS).<sup>(8)</sup> ROS and reactive nitrogen species (RNS) which induce oxidative stress are principle inducers of oral squamous cell carcinoma(OSCC). It was recently demonstrated that oxidative & nitrative stress contributes to the development of oral carcinoma from leukoplakia through DNA damage.<sup>(9)</sup>

RNS in the form of nirtosamines, and ROS as super oxide radical, hydroxyl radical, H<sub>2</sub>O<sub>2</sub> play a key role in human cancer development because they can cause DNA base alterations, strand breaks, damaged tumor suppressor genes & enhanced expression of proto-oncogenes.

Nair observed HO radical in the human oral cavity during betel quid chewing.<sup>(9)</sup> Oral Lichen Planus (OLP)

is a common chronic mucosal disease with an inflammation background. It has been suggested that the occurrence of OLP could be triggered by imbalances among the antioxidants in the biological fluids and thus could play an important role in the pathogenesis of the transformation.<sup>(10)</sup>

Oral sub mucous fibrosis (OSMF) being a premalignant condition and associated with carcinogenesis is thought to be associated with reactive oxygen species. MDA levels were found significantly higher in OSMF<sup>(11)</sup> & OLP.<sup>(10)</sup>

Our results were in agreement with the results of Metin Kilinc,<sup>(12)</sup> Khanna,<sup>(13)</sup> Naciye,<sup>(7)</sup> Nair,<sup>(10)</sup> Samal<sup>(14)</sup> and Atena.<sup>(4)</sup> Thus the reactive oxygen species generated by tobacco chewing may cause damage to the membrane leading to lipid peroxidation reaction. MDA is one of the products of such lipid peroxidation reaction. As oxidative stress increased by the use of tobacco, it consequently leads in the increased lipid peroxidation followed by increased generation of MDA. This may be the cause for the significantly increased MDA concentration in the saliva of study group subjects than control group, suggesting risk of cellular damage.

**Uric Acid:** Majority of researchers showed decreased salivary uric acid levels, because of its use as an antioxidant; but in present study we found higher levels of uric acid in study group subjects than control group and the difference was not statistically significant. Our results are similar to the results of Giovanni,<sup>(15)</sup> Hyun-Sik Shin<sup>(16)</sup> while in contrast with the findings of Joanna and others.<sup>(17)</sup>

Hyun et al stated that the mechanism of raised uric acid production in the patient with terminal cancer is cellular injury and inflammatory reactions.<sup>(16)</sup> Shistated that hyperuricemia was one of the body's danger signals derived from the damaged cells.<sup>(18)</sup> Mehdi and co researchers proposed that elevated uric acid may be a true risk factor for cancer incidence.<sup>(19)</sup> Increased uric acid levels indicated as marker of progression for nasopharyngeal cancer by some researchers.<sup>(20)</sup> Considering all above scenario, increased mean uric acid levels, in our study group subjects though insignificant, may be suggestive of initial phase of cellular injury caused by tobacco chewing habit than as an antioxidant.

The finding of elevated MDA and uric acid levels in our study indicates the oxidative stress leading to cellular injury, which may proceed to lesions in the mouth like ulcers, leukoplakia, erythroplakia, submucous fibrosis and consequently may be responsible for paving the way for progression of oral cancer. Our findings suggest that increased concentration of salivary MDA and salivary uric acid can be used as a screening tool of cellular injury.

## Conclusion

The significantly elevated salivary MDA and increased uric acid may serve as screening tool in tobacco chewers, to make them aware about the future

risk of progression of cellular injury. Extended studies required with more biochemical parameters in tobacco chewers.

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