

## ORIGINAL ARTICLE

COMPARISON OF SALIVARY ANTIOXIDANT LEVEL  
IN SMOKERS AND NON-SMOKERS

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**Background:** Saliva is the first biological medium encountered during inhalation of cigarette smoke. Uric acid in saliva possesses free-radical-scavenging properties which are produced by cigarette smoke and contributes approximately 70–85% of the total antioxidative capacity of saliva. Objective of this study was to determine and compare the salivary uric acid (SUA) level in cigarette smokers and non-smokers amongst the patients visiting Sardar Begum Dental Teaching Hospital, Peshawar. **Methods:** It was a comparative study done on 118 individuals, (59 in each group of cigarette smokers and non-smokers). Non-probability convenience sampling was used. The data was collected using structured proforma and analysed using SPSS-25. For the association of quantitative variables with smoking independent sample *t*-test was used. Chi-square test was used for qualitative variables, and  $p \leq 0.05$  was considered statistically significant. **Results:** Mean salivary uric acid level in cigarettes smokers was  $2.7 \pm 1.02$  mg/dL as compared to  $4.7 \pm 0.91$  mg/dL in non-smokers. **Conclusion:** Salivary uric acid level was found significantly lower in cigarette smokers compared with non-smokers.

**Keywords:** Antioxidant, Oxidative stress, Saliva, Uric acid

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## INTRODUCTION

Saliva is the first line of biological medium that meets outside components like food, drink, or inhaled volatile substances consumed in daily routine. Whole saliva is a bio-mixture of major and minor salivary gland secretions.<sup>1</sup> Water content of saliva is around 99% with electrolytes and proteins. These components collectively are accountable for functions such as digestion, buffering, taste, balance of re-mineralization, and anti-microorganisms. Salivary monitoring is an important factor as its composition reflects the disease severity and response to treatment.<sup>1,2</sup>

Early detection of COVID-19 is possible from saliva by gathering oral swabs and testing RNA among susceptible individuals.<sup>3</sup> Saliva plays a key role in maintenance of oral mucosa and oral health. It protects the soft and hard tissues from damage. Saliva not only lubricates the oral mucosa but also protects the oral cavity and oral mucosa from different types of harmful chemicals and microorganisms.<sup>4–7</sup> Salivary immunological defence system is predominant because of protein defence and secretory immunoglobulin A. This defence system is mainly composed of uric acid, albumin, caeruloplasmin, ascorbic acid, lactoferrin, agglutinin, glutathione, lysozyme, and transferrin.<sup>2,8</sup>

Uric acid, ascorbic acid and albumins are the essential antioxidant elements of saliva. However, uric acid proved to be the foremost antioxidant present in saliva and exhibits a concentration which is similar to

that of serum as well as has accepted clinical importance in examining oxidative stress.<sup>1,9</sup> Uric acid contributes around 70–85% of the total salivary antioxidant capacity.<sup>9</sup> Salivary uric acid (SUA) is able to chelate transition metals and to counter with biological oxidants such as reactive oxygen species, hydroxyl radicals, hypochlorous acid, and reactive nitrogen species. The SUA is achieved as an end-product in purine catabolism. Increased concentration of SUA is believed as a hallmark in oxidative stress monitoring.<sup>10</sup> Saliva represents as a first line defence against oxidative stress, and carries protective effects against oxidants, microorganisms, and toxins. Antioxidants eradicate the free radicals generated from the oxidative stress which form as a product or by-product during functional activities and cellular metabolism. Highly active free radicals due to their unpaired electrons carry a tendency to react with healthy tissues and their ingredients causing oxidative stress.<sup>11,12</sup> Oxidative stress can harm DNA, RNA, lipids, and proteins increasing risk of oral diseases and cancer, cardiovascular diseases, and autism. Antioxidants reduce the oxidative damage directly by countering with free radicals and indirectly by inhibiting free radical-generating enzymes activity.

Among defensive systems, salivary antioxidant activity may be affected by cigarette smoking.<sup>7,13</sup> Imbalance between oxidants and antioxidants makes smokers more susceptible to oxidative stress.<sup>14</sup> Oxidative stress can originate from human body itself as well as from external sources. Cigarette smoking leads to

formation of numerous hazardous compounds.<sup>15</sup> These compounds and their metabolites may initiate oxidative stress by creation of reactive species or beginning of radical chain reactions.<sup>16,17</sup>

This study aimed to evaluate the comparison of salivary uric acid level in cigarette smokers and non-smokers visiting Sardar Begum Dental Teaching Hospital, Peshawar, Pakistan.

## MATERIAL AND METHODS

It was a comparative, cross-sectional study between cigarette smokers and non-smokers for assessing association of smoking with salivary uric acid level. Study was done from 23<sup>rd</sup> Aug to 22<sup>nd</sup> Dec 2020 after approval from Ethical Committee and Board of Advance Studies and Research. Non-probability convenience sampling was used to recruit all eligible subjects. Subjects' saliva was collected from Outpatient Department, Sardar Begum Dental College and Hospital, Peshawar, and procedure was carried out in Department of Pathology, Khyber Teaching Hospital, Peshawar. Study population consisted of all male patients aged 20–60 years. Sample size was 118 using WHO Sample Size calculator. Participants were male cigarette smokers and non-smokers aged 20–60 years. Participants having any systemic diseases, drug users, or having a malignancy were excluded.

Total of 118 subjects fulfilling the inclusion criteria, were enrolled in this study after informed consent. Data was recorded on a structured Performa. The enrolled subjects were divided into cigarette smokers and non-smoker groups. Each group included 59 subjects. The record included demographics, smoking status, and salivary uric acid level.

About 2.5 mL clear saliva was collected from each subject in a saliva collection tube by spitting method<sup>18</sup>. Supernatants of saliva was achieved by centrifuging the collected saliva at 4,000 rpm for 10 minutes.<sup>19</sup> Salivary uric acid was determined with colorimetric method<sup>20</sup> on Cobas Mira Autoanalyzer (HITACHI (Roche Pakistan Limited) C 501.

Quantitative variables were calculated as Mean±SD, and qualitative variable as frequencies and percentage. For the association of quantitative variables with smoking, independent sample *t*-test was used and for qualitative variables Chi-Square test was used. The data was analysed on SPSS-25, and  $p \leq 0.05$  was considered statistically significant.

## RESULTS

There were 59 smokers and 59 non-smokers in a total of 118 patients. All subjects were male with mean age 38.19±11.30 years (Range: 20–60). Mean age of cigarette smokers was 40.37±11.0 years, and mean age of non-smokers was 36.02±11.23 years. Independent *t*-test showed that there was a statistically significant

difference in age between the two groups ( $p < 0.05$ ). (Table-1).

Mean salivary uric acid level in cigarettes smokers was 2.73±1.02 mg/dL compared to 4.73±0.91 mg/dL in non-smokers. There was a statistically significant difference in salivary uric acid levels between the two groups ( $p < 0.001$ ). (Table-2).

**Table-1: Ages of the study subjects (Mean±SD)**

Group	N	Age (Years)	<i>p</i>
Smokers	59	40.37±11.0	<0.05
Non-smokers	59	36.02±11.23	
Total	118	38.19±11.30	

**Table-2: Salivary uric acid level in study subjects**

Group	N	Salivary Uric Acid (mg/dL)	<i>p</i>
Smokers	59	2.73±1.02	<0.001
Non-smoker	59	4.73±0.91	

## DISCUSSION

Smoking is a risk factor for oral diseases such as periodontal disease, oral cancer, alveolar bone loss and black hairy tongue. Pregnant women's smoking is also a risk factor for cleft lip and palate in the baby. Cigarette smoking has a direct correlation with DNA damage.<sup>21</sup>

The current study corroborates findings of Hanna *et al*<sup>21</sup> which assessed serum uric acid levels in smokers and non-smokers. They concluded that although serum uric acid level in smokers did not reach the lower reference range, it is significantly lower than the non-smoker group ( $p < 0.001$ ).<sup>21</sup>

Our findings are in agreement with Greabu *et al*<sup>22</sup> that indicated significant decrease in important salivary antioxidant levels and the loss of activity of salivary enzymes with antioxidant actions can be considered as one of the mechanisms by which the toxic effects of cigarette smoke initiate oral inflammatory diseases, promote precancerous transformations and destroy the oral cavity homeostasis.<sup>22</sup>

The findings of present study contrasted with a study by Zappacosta *et al*<sup>23</sup> which showed that there was no statistical difference in SUA level in cigarette smokers and non-smokers. The results of present study were also in contrast with Fawaz Pullishery *et al*<sup>7</sup> who reported that the SUA levels with number of cigarettes smoked in tobacco users and non-tobacco users had no significant differences.

The results of present study were in agreement with Miricescu *et al*<sup>24</sup> who observed that SUA level was significantly less in smokers compared to controls. They observed that decrease SUA levels were found in periodontitis and oral lichen planus, and smoking may initiate oxidative stress which causes toxic effects of free radicals, and can in turn initiate precancerous transformations, oral diseases and destroy the oral cavity homeostasis.<sup>24</sup>

The results of present study are in agreement with Fatima *et al*<sup>1</sup> who evaluated that the SUA level in smokers with periodontitis and periodontally healthy individuals and concluded with significantly decrease SUA level in smokers. According to them the reduced level of salivary antioxidant could be a direct underlying factor in periodontal and other oral disease.<sup>1</sup>

This study also corroborates with A. Abdolahinia *et al*<sup>25</sup> who showed an association of smoking with the age. Their analysis presented a significant correlation between age and number of daily cigarette smoking. The current study also agrees with Abdolsamadi *et al*<sup>26</sup> that there is a parallel relationship between smoking and age as the smoking increase with age.<sup>26</sup> Same result was observed by Dube *et al*<sup>27</sup> that there was a significant association between age and smoking as cigarette smoking increases with age and puts adverse effects on physical health.

## LIMITATIONS

Number of cigarettes, chronicity of smoking and effect of smoking cessation was not considered in this study.

## CONCLUSION

Cigarette smokers had significantly low uric acid level in their saliva as compared to non-smokers.

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## Contribution of Authors:

HMABS: Performed study

MAW: Review of manuscript

AM: Interpret results

ZS: Write Methodology

AK: Write Introduction

MS: Write discussion

SMAS: Study supervision

EM: Proofreading and review

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