



## Relationship of Periodontal Status with Nicotine Levels In Blood and Urine

*Dr Sabin Siddique, Department of Public Health Dentistry, MES Dental College and Hospital,  
Kerala University of health sciences, Kerala , India\**

Dr John Francis, Department of Prosthodontics, MES Dental College and Hospital,  
Kerala University of Health sciences, Kerala, India\*

Dr Justin Ninan, Department of Prosthodontics, Malabar Dental College and Research Centre,  
Kerala University of Health Sciences, Kerala , India\*\*

Dr Shanoj RP, Department of Prosthodontics, MES Dental College and Hospital,  
Kerala University of Health Sciences, Kerala, India\*

Dr Mariyam Fidha, Department of Oral Pathology , MES Dental College and Hospital,  
Kerala University of Health Sciences, Kerala ,India\*

Dr Mereena J, Department of Public Health Dentistry, MES Dental College and Hospital,  
Kerala University of Health Sciences, Kerala, India\*

Article History

Volume 6, Issue Si2, 2024

Received: 25 Feb 2024

Accepted: 16 Mar 2024

doi:  
[10.33472/AFJBS.6.Si2.2024.173-182](https://doi.org/10.33472/AFJBS.6.Si2.2024.173-182)

### ABSTRACT

**OBJECTIVES:** The study was designed to assess periodontal status between tobacco users and non tobacco users, to assess nicotine levels in blood and urine among tobacco users and to determine the relationship of periodontal status with nicotine levels in blood and urine among tobacco users.

**METHODS:** The present study was conducted on 150 male subjects, aged 35-44 years. Subjects with history of tobacco consumption were divided into 4 groups (from group 2 to group 4 based on type of tobacco use) and non-tobacco users (n=30) were in comparison group (group 1). Periodontal disease status was recorded with CPI (Community Periodontal Index) and Nicotine concentration in blood and urine samples was determined by spectrophotometric method.

**RESULTS:** There were statistically significant differences in CPI scores in subjects with and without tobacco use ( $P < 0.0005$ ). There was no statistically significant association between periodontal disease status and levels of nicotine in blood and urine.

**CONCLUSIONS:** Prevalence of periodontal disease was high among tobacco users when compared with non users. Nicotine levels in blood and urine may be measured as an excellent indicator to evaluate type of tobacco usage, but not for periodontal disease.

**KEY WORDS:** Nicotine, Periodontal, Tobacco, Smokers, Chewers, Blood, Urine

## Introduction

Tobacco usage is a menace that has grabbed millions of people all over the world, cutting across the nation and social barriers. Tobacco usage is in form of smoking, chewed and also inhaled. Dental Caries and Periodontal disease is the most commonly occurring dental diseases affecting mankind. Periodontal disease ever since the days of Hippocrates has crippled the survival of human dentition. Destruction of periodontal attachments is the effect of interaction between genetic, environmental and host factors.<sup>1</sup> For the last few decades, dentists and dental researches have become more aware about the critical role of tobacco usage to the prevalence of periodontal disease. Tobacco usage is now known as the major risk factors for periodontal disease.<sup>2</sup>

Tobacco is addictive in all its forms. Nicotine is considered the most pharmacologically active component in tobacco. Estimation of nicotine in biological fluids like saliva, blood and urine has been studied as a method for monitoring exposure to tobacco. 35-44 years is the standard monitoring age group for health condition of the adults. In this age group full effect of periodontal and other oral lesions is seen.<sup>3</sup>

Till date in India, very few studies have been reported on biochemical analysis of blood and urine among tobacco users and their association with periodontal status. Hence the research is undertaken to assess and correlate the periodontal status of 35-44 year old male tobacco users with their nicotine concentration in Blood and Urine.

## METHODOLOGY

A cross sectional institution based study was conducted to evaluate the periodontal status of different tobacco users and the nicotine concentration in their blood and urine. The study population consisted of male patients attending the Department of Community Dentistry in 35-44 year age group. The study was approved by Institutional Review Board.

Inclusion Criteria was Male subjects in the 35-44 year age group, Beedi smokers Subjects smoking more than 5 beedis per day for a period more than a year, Cigarette smokers Subjects smoking more than 5 cigarettes per day for more than a year, Tobacco chewers: Subjects consuming tobacco in any chewable method daily for more than a year. Smokers who are also tobacco chewers: Subjects using more than 5 beedis / Cigarettes per day for more than a year and also chewing tobacco, Non Smokers (Controls): Subjects who had never smoked in their life time and also Subjects who consented to be a part of the study. Subjects with systemic diseases and on long term medication or drugs which may modify the periodontal status and who have undergone periodontal therapy in the past six months were excluded from study.

The study instrument consisted of a questionnaire with three parts. The first part was for recording information on tobacco usage. The second half was for recording clinical data. The periodontal disease was recorded using CPI. The third part was to record the nicotine concentration in blood and urine. 3-5 subjects were examined in a day. The examinations

were carried out by making the subjects sit on the dental chair. A trained recorder was part of the study.

Blood samples are withdrawn by standard venipuncture technique and collected in tubes containing EDTA. The precipitate obtained is separated at 4 °C by centrifugation (2000 \* g) for 10 minutes and then analyzed. Urine sample was collected in a sterile glass bottle. Blood and Urine samples was placed in a ice box with temperature maintained at -4 °C and transferred for estimation of nicotine concentration in the samples. The biochemical analysis was started within 45 minutes of collection of samples. The method of Asthana (2004) for assessing nicotine concentration in blood and urine samples by spectrophotometric method.<sup>4</sup>

The data obtained was coded and entered in to the Microsoft Excel sheets. The data was then fed into the SPSS (Statistical Package for Social Studies) software-22 for analysis. In the software the data was renamed into different variables, based on the questionnaire used in the study and analyzed. Karl Pearson test and Chi-square test was used in analysis as the data were in frequencies of more than one categories and also it was able to find the significance (p value) if any in the same data. Karl Pearson Correlation tests were also performed to see if there was any significant correlation between the CPI and LOA (Loss of Attachment) scores and nicotine levels in blood and urine samples in various groups.

## RESULTS

Periodontal status was assessed using Community Periodontal Index (CPI). Comparison of different forms of tobacco and non tobacco users with the related CPI scores based on highest of all the 6 scores in an individual showed non tobacco users with the highest percentage of healthy sextants (13.3%) and sextants with bleeding on probing (46.7%). Calculus was found to be elevated among tobacco chewers (50%) with other users. Periodontal pockets with 4 to 5 mm found to be higher among beedi chewers (36.7%). Periodontal pockets with 6 mm or more was higher among users of both forms of tobacco (30%). When the community periodontal index scores of various tobacco users and non tobacco users were compared it was found to be statistically significant ( $p < 0.0005$ ) (Chart-1). When attachment scores were compared with different forms of tobacco users, cigarette smokers had highest attachment loss with 0-3 mm (62.9%). Attachment loss with 4-5 mm (29.9%) was higher among beedi smokers. When loss of attachment score of various forms of tobacco and non tobacco users were compared it was found to be statistically significant ( $P < 0.0005$ ).

Nicotine levels in blood were associated with various forms of tobacco users, the levels detected were highest in beedi smokers (797.6 ng/ml) and least in cigarette smokers (261.4 ng/ml). Among non tobacco users maximum blood nicotine levels observed was (43.5 ng/ml) and minimum (0.0 ng/ml). The difference in nicotine levels with blood among the various tobacco and non tobacco users was found to be significant ( $P < 0.0005$ ). ( Table -1 Chart- 1).

When nicotine levels in urine with various forms of tobacco users was compared it was found to be highest in beedi smokers (3461.8 ng/ml) and minimum in cigarette smokers (261.4 ng/ml). Among non tobacco users maximum urine nicotine levels observed was (100.8 ng/ml) and minimum (0.0 ng/ml) . The difference in nicotine levels with urine among

various tobacco and non tobacco users was found to be significant ( $P < 0.0005$ ) ( Table – 2, Chart-2).

When community periodontal index scores were associated with nicotine levels of non tobacco users it decreased with increase in CPI scores and was not significant ( $R = -0.086$ ,  $P > 0.05$ ). Nicotine levels with blood was found to increase with increase in CPI and not significant ( $R = 0.136$ ,  $P > 0.05$ ). Correlation of loss of attachment scores with levels of nicotine in blood and urine of non tobacco users showed a negative correlation, not significant ( $R = -0.369$ ,  $P > 0.05$  and  $R = -0.225$ ,  $P > 0.05$ ).

When community periodontal index scores were associated with levels of nicotine in blood and urine of cigarette smokers, urine nicotine levels was found to decrease with increase in CPI scores, not statistically significant ( $R = -0.031$ ,  $P > 0.05$ ). Nicotine levels with blood showed an increase in levels with increase in CPI scores ( $R = 0.023$ ) not statistically significant ( $P > 0.05$ ). Loss of attachment scores with nicotine levels in blood and urine of cigarette smokers showed a positive correlation, which was not statistically significant ( $r = 0.031$ ,  $p > 0.05$  and  $r = 0.131$ ,  $p > 0.05$ ).

In subjects who smoked beedi when community periodontal index scores were associated with levels of nicotine in blood and urine of beedi smokers, it was found to decrease with increase in CPI scores in blood, not significant ( $R = -0.025$ ,  $P > 0.05$ ). Nicotine levels in urine was found to increase with increase in CPI scores in beedi smokers, which was not significant ( $R = 0.100$ ,  $P > 0.05$ ). Correlation of attachment loss with levels of nicotine in blood and urine, of beedi smokers showed positive correlation, not significant ( $R = 0.146$ ,  $p > 0.05$ ,  $R = 0.256$ ,  $P > 0.05$ ). Loss of attachment scores with nicotine levels in urine of tobacco chewers was found to increase with loss of attachment scores, not significant ( $R = 0.030$ ,  $P > 0.05$ ). Nicotine levels with blood was found to increase with attachment score, not significant ( $R = -0.087$ ,  $P > 0.05$ ). Mean urine concentration of nicotine was found to be the highest in beedi smokers, ( $2030.88 \pm 645.51$ ) followed by users of both forms of tobacco ( $2018.28 \pm 634.46$ ). Non smokers also showed mean concentration of  $58.24 \pm 26.95$ .

Correlation of community periodontal Index scores with nicotine levels in urine of users of both forms of tobacco showed a positive correlation ( $R = 0.106$ ,  $r = 0.126$ ) not significant ( $P > 0.05$ ). Nicotine levels in blood showed a negative correlation ( $R = -0.024$ ) which was not statistically significant ( $P > 0.05$ ). In the present study when attachment loss was correlated with nicotine levels in blood and urine was found to increase with increase in attachment scores ( $R = 0.147$ ,  $r = 0.251$ ) which was not statistically significant ( $P > 0.05$ ).

## DISCUSSION

Tobacco consumption is the single largest cause of death in the developed world.<sup>5</sup> Tobacco use is directly related to a variety of medical problems including low birth weight, pulmonary and cardiovascular diseases.<sup>15</sup> Results of the study showed significant differences in the periodontal status with respect to CPI scores and LOA scores in tobacco and non tobacco users. This is in agreement with most of the previous studies Haber et al 1993<sup>7</sup>;

Linden and Mullaly 1994<sup>8</sup>; Martinez Canut et al 1995<sup>9</sup>; Machuca et al 2000<sup>10</sup>; Hashim et al 2001<sup>11</sup> and Alwahadni and Linden 2003.<sup>12</sup>

Sites with calculus detected was observed more in tobacco users than non tobacco smokers. This is similar in lines with research conducted by Muller et al 2002.

Sites with periodontal pockets 4 -5 mm was found to be higher in tobacco smokers in comparison with non tobacco users in whom it was considerably low. This is in agreement with the studies conducted by Linden and Mullaly 1994<sup>8</sup>; Martinez Canut et al 1995<sup>9</sup>; Axelsson et al 1998<sup>14</sup>; Machuca et al 2000<sup>10</sup>; Haffajee et al 2001<sup>17</sup>; Calcina et al 2002<sup>16</sup> and Tanner et al 2005.<sup>17</sup>

Tobacco chewers had high prevalence of calculus when compared to tobacco smokers and users of both forms of tobacco. This is due to the cumulative affect of placement of tobacco for longer duration in the mouth and also more irritants seen in smokeless tobacco products.<sup>18</sup>

Nicotine is metabolized in the body in to two major pharmacologically inactive metabolites: cotinine and nicotine-N-oxide. Half life of nicotine following inhalation or parenteral administration is approximately 2 hours and the half-life of its metabolite, cotinine, is about 19 hours. Nicotine and/or cotinine have been isolated in plasma, urine, saliva and GCF of cigarette smokers.<sup>19</sup>

The results demonstrated the presence of higher amount of nicotine in blood and urine of tobacco users compared to non tobacco users.

Mean urine concentration of nicotine was found to be the highest in beedi smokers followed by users of both forms of tobacco and non smokers. Oral intake of tobacco in chewable form also increase the excretion as nicotine can be absorbed from oral mucosa. This was in tune with the studies conducted by Behera et al 2003<sup>20</sup>, Heinrich et al 2005<sup>21</sup> and Asthana et al 2004.<sup>4</sup>

Mean nicotine levels in blood in various tobacco users showed highest concentration in users of both forms of tobacco  $505.58 \pm 205.79$  followed by beedi smokers  $504.38 \pm 208.25$ . Non smokers also showed nicotine levels in blood. This was in agreement with Asthana et al 2004<sup>4</sup> and Hengen and Hengen 1978.<sup>22</sup>

In our study nicotine levels in blood and urine showed no positive correlation with periodontal status. There are no previous studies in this regard and hence no comparison could be made.

The vasoactive properties of nicotine may possibly affect the pathogenesis of periodontal disease. Further studies needed to accurately quantitate nicotine in blood and urine, perhaps with more specific and sensitive technique such as radioimmunoassay and High performance liquid chromatography. Also further research should be aimed at examining the effect of nicotine on periodontium by means of longitudinal studies with extended follow up of the subjects in order to better understand its possible role in the periodontal disease process.

## CONCLUSION

The study was conducted to evaluate and correlate the periodontal status of 35-44 years male tobacco and non tobacco users with their nicotine concentration in blood and urine. The findings suggested a marked association between tobacco use and periodontitis. The study showed statistically significant difference in the periodontal status with respect to CPI scores among tobacco and non tobacco users.

In the study when the loss of attachment scores of tobacco and non tobacco users were compared the difference was found to be statistically significant. The study showed non tobacco users had significantly better periodontal status compared to various tobacco users. Increased attachment loss was observed in tobacco users with non tobacco users.

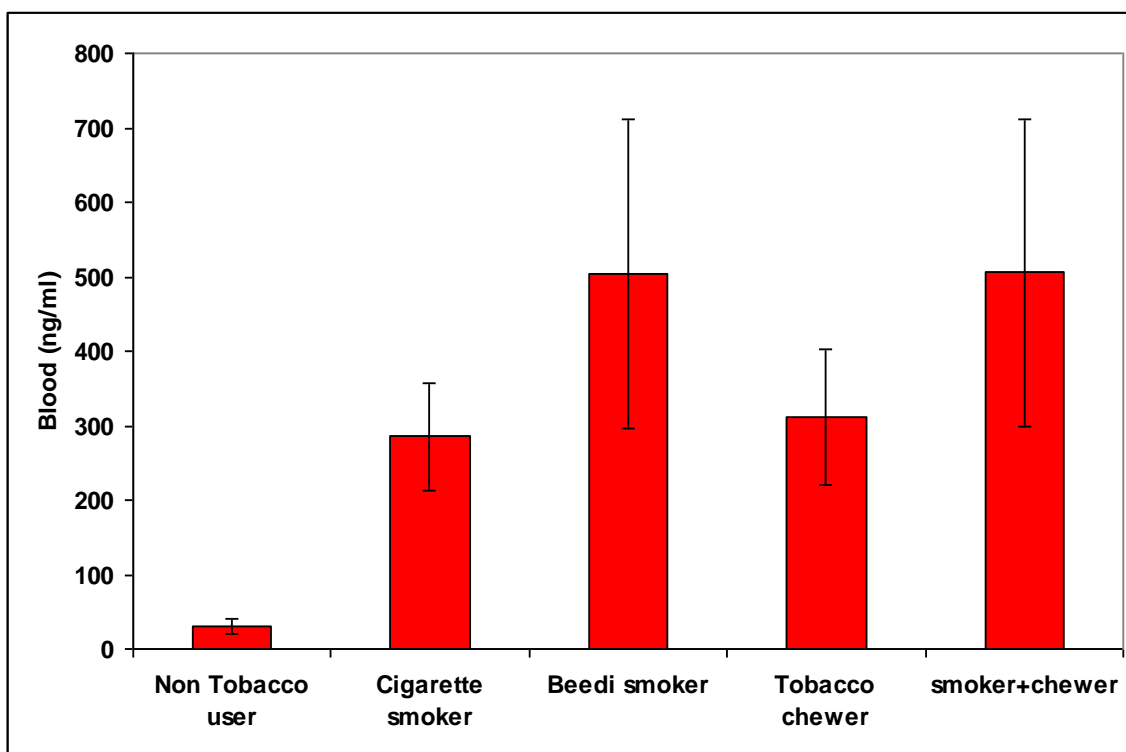
The study showed that nicotine levels in blood and urine nicotine is higher in various tobacco users compared to non tobacco users. Our results indicate that nicotine levels in blood and urine may be considered as excellent indicator to assess the exposure to tobacco.

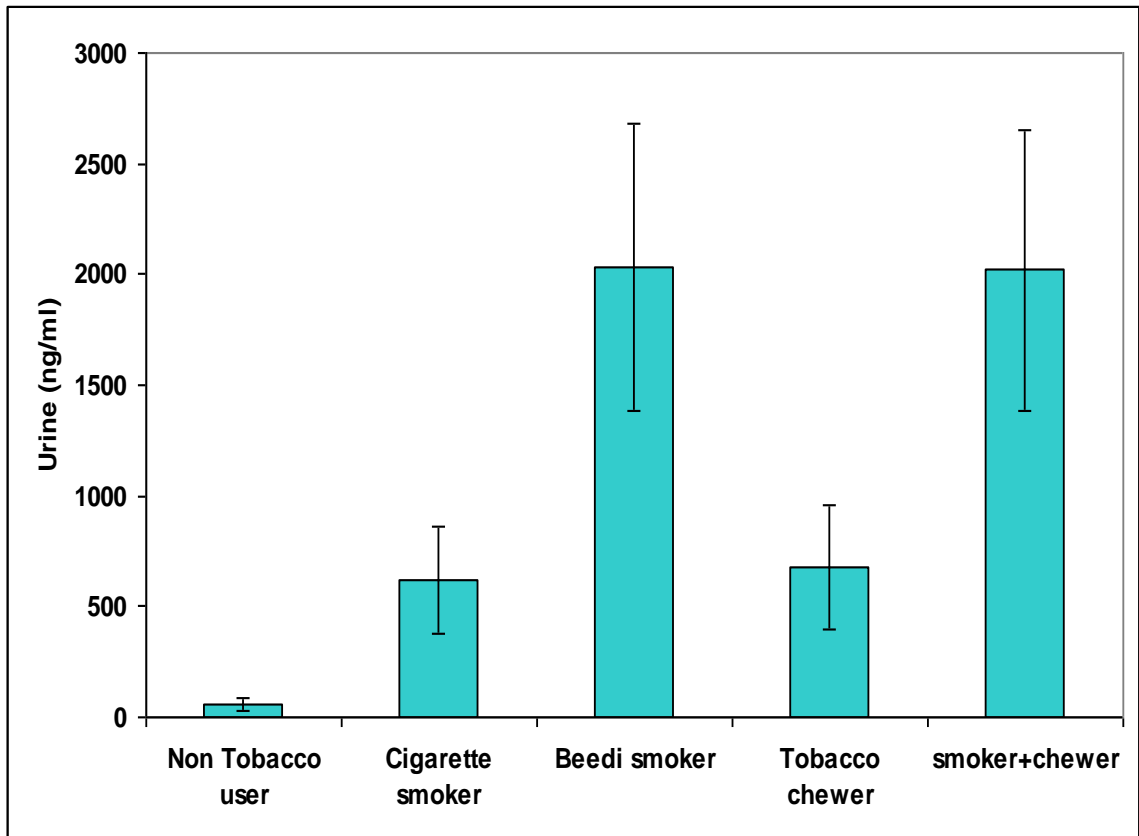
**Table 1 : Comparison Of Blood Nicotine Levels With Tobacco Users And Non Tobacco Users**

<b>Group</b>	<b>n</b>	<b>Nicotine levels (Mean )</b>	<b>Standard Deviation</b>	<b>Minimum level detected (ng/ml)</b>	<b>Maximum level detected (ng/ml)</b>
NON TOBACCO USER	30	30.91	9.84	0.0	43.5
CIGARETTE SMOKER	30	284.88	73.31	182.3	436.2
BEEDI SMOKER	30	504.38	208.25	158.4	797.6
TOBACCO CHEWER	30	311.94	91.86	151.7	487.9
SMOKER + CHEWER	30	505.58	205.79	166.8	786.1
<b>TOTAL</b>	<b>150</b>	<b>378.52</b>	<b>202.52</b>	<b>0.0</b>	<b>797.6</b>

**Table 2 : Comparison Of Urine Nicotine Levels With Tobacco And Non Tobacco Users**

<b>GROUP</b>	<b>n</b>	<b>Nicotine levels (Mean)</b>	<b>Standard Deviation</b>	<b>Minimum level detected (ng/ml)</b>	<b>Maximum level detected (ng/ml)</b>
NON TOBACCO USER	30	58.24	26.95	0.0	100.8
CIGARETTE SMOKER	30	618.52	245.05	261.4	1391.6
BEEDI SMOKER	30	2030.88	645.51	898.9	3461.8
TOBACCO CHEWER	30	678.86	278.72	266.6	1856.1
SMOKER + CHEWER	30	2018.28	634.46	898.9	3271.4
<b>TOTAL</b>	<b>150</b>	<b>1146.24</b>	<b>901.68</b>	<b>0.0</b>	<b>3461.8</b>

**Figure 1 : Comparison Of Blood Nicotine Level In Tobacco And Non Tobacco Users**

**Figure 2 : Comparison Of Urine Nicotine Levels In Tobacco And Non Tobacco Users**



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