

# Comparative assessment of salivary cotinine level and psychological dependence among tobacco users

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## Abstract

### Background:

The aim of the study was to assess the correlation between salivary cotinine level and psychological dependence measured through Fagerstrom test for nicotine dependence (FTND) questionnaire among tobacco users.

### Materials and Methods:

This was a cross-sectional study, conducted on tobacco users. Participants with the present habit of tobacco chewing and smoking above the age of 16 years were included in the study. A standard questionnaire form of FTND revised version for smoking and smokeless form of tobacco were given to each participant. Each participant was asked to answer the questions as per their experience of tobacco consumption and calculate the total point score or FTND score. Salivary cotinine level assessment was done using commercial available NicAlert kit.

### Results:

When salivary cotinine level was correlated with different variables of both groups, it was observed that weak correlation between salivary cotinine level and FTND scoring in smokers group ( $r = 0.083$ ) and also in smokeless group ( $r = 0.081$ ). When two groups were compared for salivary cotinine level, statistically significant difference ( $P = 0.021$ ) was observed, with smokeless group showing high level of salivary cotinine level as compared to smokers group.

### Conclusion:

Salivary cotinine and psychological dependence through FTND scoring are not strongly correlating with each other. This indicates that dependence over tobacco is a separate phenomenon and cannot be assessed by salivary cotinine level. It is well accepted that salivary cotinine level is influenced by age of individual, duration of habit, and type of tobacco consumption.

**Key Words:** Cotinine, nicotine, saliva, tobacco

## INTRODUCTION

In India, since ancient times, the tobacco consumption is followed in various parts in various forms and patterns.[1] People generally know that smoking is dangerous and also know their harmful effects on the health of an individual. In spite of knowing the consequences, still people indulge in such practices. Various attempts through legal and educative approach have been made by the governmental and nongovernmental bodies to refrain the tobacco users from consuming tobacco.[2] Interestingly, the Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> Edition, Text Revision (DSMIV-TR) has included nicotine-related disorders in its list of mental disorders since 2000.[3] The standard method of assessment of psychological dependence of tobacco users is using well-known Fagerstrom test for nicotine dependence (FTND) questionnaire. Even though it is well accepted, its subjective nature warrants its validity as use alone tool for assessment. Ashley *et al.* have stated, “although nicotine is blamed as a chemical causing high level of dependence, certain byproducts and additives in tobacco are also responsible for dependence.”[4] Various studies have reflected the use of salivary cotinine assessment, for example, participants with nicotine replacement therapy, among smokeless tobacco users, chair-side evaluation in chronic periodontitis patients.[5,6,7] All these studies have supported the salivary cotinine as an important marker of tobacco consumption assessment.

The detection of exposure to tobacco in smoke or chewed form by measurement of cotinine is the preferred method. Nicotine is not considered a valid marker of smoking status due to its relatively short half-life (approximately two hours). By contrast, cotinine has an average half-life of 17 hours, and blood levels closely reflect the dose of nicotine absorbed from tobacco smoke.

[5] Thus, the cotinine assessment is preferred rather than nicotine.

## Aim

The aim of the study was to assess the correlation between salivary cotinine level and psychological dependence measured through FTND among tobacco users.

## Objectives of the study

1. To assess and compare salivary cotinine levels with psychological dependence among tobacco smokers
2. To assess and compare salivary cotinine levels with psychological dependence among tobacco chewers
3. To assess salivary cotinine level as an independent factor for influencing psychological dependence.

## MATERIALS AND METHODS

This is a cross-sectional observational study, conducted on tobacco users reporting to for dental treatment. Tobacco users and their accompanying friends and relatives were considered for this study. Ethical clearance was obtained from institutional ethical review board.

### Inclusion criteria

- Participants with the present habit of tobacco chewing and/or tobacco smoking
- Participants above age of 16 years with above-mentioned habits were included in this study.

### Exclusion criteria

- Participants who were past tobacco users and presently had quit the habit or not consumed the tobacco since last 18–20 h were excluded from the study
- Participants on tobacco cessation therapy were excluded from the study
- Participants with disturbed state of mind and not willing to voluntarily participate in the study were automatically excluded from the study
- Participants with salivary gland pathology, condition (hyposalivation and xerostomia), and on medication altering the physical property of saliva were also excluded from the study.

Thus, participants selected with all inclusion and exclusion criteria were given an information sheet in local language regarding the methodology and purpose of study, and written informed consent in local language was obtained from each participant.

All the participants were divided into two main groups based on pattern of tobacco consumption.

- Group A: Participants with habit of tobacco smoking
- Group B: Participants with habit of tobacco chewing/smokeless tobacco.

First, the demographic details of participating participants were recorded. This included the participant's age, gender, address, type of tobacco consumption, duration, and frequency of tobacco consumption. Participants were also asked for information related to attempts to quit the habit, last consumption of tobacco, and any tobacco cessation therapy. Those participants following strict criteria of inclusion and exclusion were further subjected to through oral examination to rule out any oral pathological lesion and/or salivary gland lesion, the condition of saliva, etc. Participants passing all the norms of inclusion in the study were further subjected to psychological dependence assessment through FTND questionnaires and salivary cotinine level assessment.

A standard questionnaire form of FTND revised version for smoking given by Heatherton *et al.*[8] and smokeless form of

tobacco given by Ebbert *et al.*[9] were given to each participant. These questionnaires were converted in local language for better understanding and effective answering. Each participant was asked to answer the questions as per their experience of tobacco consumption and calculate the total point score or FTND score. Based on total point score, the dependence was assessed as follows:

- 7–10: Person is highly dependent on nicotine
- 4–6: Person has low to moderate dependence on nicotine
- Below 4: Person has low addiction.

Salivary cotinine level assessment was done following the guidelines of the User's instruction manual of NicAlert™ (NYMOX Sales Corporation, New Jersey, USA).

## Saliva collection

Saliva collection was avoided within 12 h after consuming alcohol. Since acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. All the participants were asked to rinse mouth thoroughly with water for 10 min before collection of sample. Unstimulated whole saliva was then collected by asking the patient to drool the saliva through a small funnel into the collection tube (both included in the NicAlert kit) until the tube is half full. The funnel was then discarded and the tube was capped. These tubes were stored in deep freezer (−20°C) until further analysis.

## Assessment of salivary cotinine

Cotinine levels were measured by NicAlert strip which works on principle of enzyme-linked immunosorbent assay. The test strip displays seven zones with each zone representing a range of level of cotinine (e.g., zone “0” [0–10 ng/ml, a nonsmoker] to zone “6” [>1000 ng/ml, a heavy smoker]). The results were recorded as values from 0 to 6 [Figure 1].



Figure 1

NicAlert test strip is pasted on NicAlert test card. The test strip displays seven zones (0–6) with each zone representing a range of level of cotinine.

Before assessment, the stored tubes with preserved saliva were kept at room temperature for 1 h. The test strip was pasted on test indicator card. Few drops of saliva were squeezed out on sample zone at the bottom of test strip. The care was taken not to touch the tube to strip and also excess droops were avoided. After the complete disappearance of blue band on test strip, the level of cotinine was observed. The lowest colored band was considered as level of salivary cotinine [Figure 2]. The results were recorded as values from 0 to 6.



Figure 2

The test strip indicates the disappearance of blue band (blue arrow) and the lowest colored band (black arrow) is considered as level of salivary cotinine.

0 1–10 ng/ml

1 10–30 ng/ml

2 30–100 ng/ml

3 100–200 ng/ml

4 200–500 ng/ml

5 500–2000 ng/ml

6 2000+ ng/ml.

## Statistical analysis

Thus, collected data were statistically analyzed using statistical test. Significance was assessed at 5% confidence interval. Karl Pearson's correlation test was applied for correlation analysis. Chi-square test and Mann–Whitney U-test were applied for comparison.

## RESULTS

During the study period, a total of 65 participants were screened and assessed for tobacco consumption habit. Out of 65 participants, 31 had habit of tobacco smoking and 34 had habit of consumption of smokeless tobacco. Out of 31 participants in smokers group, 3 participants had intraoral precancerous lesions and 2 participants did not volunteer to give the consent, so they were excluded from the study. Out of 34 participants in smokeless group, 1 participant was below the age of 16, three participants had not consumed tobacco for the past 24 h, and two participants did not volunteer to give the consent, so they were excluded from the study. Participants remaining after all inclusion and exclusion criteria were as follows:

- Group I: Smokers group consists of 26 participants
- Group II: Smokeless group consists of 28 participants.

In Group I, 34% ( $n = 9$ ) of individuals were below 40 years of age and 64% ( $n = 17$ ) were above 40 years of age. In Group II, 35% ( $n = 10$ ) of individuals were below 40 years of age and 65% ( $n = 18$ ) were above 40 years of age. With this distribution, it appears to be almost equal number of participants in both groups. There was nonsignificant gender variability in both the groups.

In Group I, 42% ( $n = 11$ ) of individuals had habit of tobacco smoking for about 11–20 years, whereas in Group II, 46% ( $n = 13$ ) had habit of tobacco chewing for about 11–20 years. When two groups were observed for frequency of tobacco consumption per day, it was observed that in Group I, majority of individuals, 34% ( $n = 9$ ), had habit of smoking for 8 times/day, whereas in Group II, majority of individuals, 35% ( $n = 10$ ), had habit of chewing tobacco for 8 times/day.

In relation to FTND total scoring in Group I, majority of individuals, 46% ( $n = 12$ ), had total scoring of 7 (highly dependent on nicotine), whereas in Group II, majority, i.e., 46% ( $n = 13$ ) had total scoring of 6 (moderately dependent on nicotine) and 42% ( $n = 12$ ) had total scoring of 7 (highly dependent on nicotine). When salivary cotinine levels were observed for both groups, in Group I, majority of individuals, 42% ( $n = 11$ ), had level 2 (30–100 ng/ml), whereas in Group II, majority of individuals, 46% ( $n = 12$ ), had significantly higher level that is level 3 (100–200 ng/ml).

When salivary cotinine level was correlated with different variables of both groups, it was observed that weak correlation was observed between salivary cotinine level and FTND scoring in smokers group ( $r = 0.083$ ) and also in smokeless group ( $r = 0.081$ ).

In smokers group, when salivary cotinine level was correlated with age and duration of habit, positive correlation ( $r = 0.3$  for age and  $r = 0.2$  for duration of habit) was observed, whereas with frequency of habit of tobacco smoking, very weak correlation ( $r = 0.06$ ) was observed. This indicates that, as age and duration of habit increases, level of salivary cotinine level also increases and also true for vice versa. When same things were observed with smokeless group, exactly opposite results were observed. This means that in smokeless group, when salivary cotinine level was correlated with age, negative correlation ( $r = -0.07$ ) was observed and with duration of habit, weak correlation ( $r = 0.05$ ) was observed. Whereas with frequency of habit of tobacco chewing, positive correlation ( $r = 0.2$ ) was observed.

When two groups were compared for salivary cotinine level, statistically significant difference ( $P = 0.021$ ) was observed [Table 1], with smokeless group showing high level of salivary cotinine level as compared to smokers group. Similarly, when two groups were compared for FTND scoring, statistically nonsignificant difference ( $P = 0.941$ ) was observed.

Table 1  
Comparison of salivary cotinine level grades among the two study groups

Groups	Salivary cotinine grades		
	0	1	2
Tobacco consumers group	0	7	11
Smokeless tobacco consumer group	1	1	10

Chi square statistic is 11.467, P=0.021788. The result is significant at

Table 1

Comparison of salivary cotinine level grades among the two study groups

## DISCUSSION

Cotinine, the major proximate metabolite of nicotine, has been widely used as a biomarker of tobacco exposure. Cotinine concentrations in plasma, urine, and saliva of nonsmokers have been used in assessing population exposure to environmental tobacco exposure.[5,6] Plasma levels of cotinine are considered as good indicator of tobacco exposure. However, the routes of nicotine intake are different in many people and so nicotine is metabolized differently which leads to different levels of nicotine[10] Oral intake of nicotine first undergoes first-pass metabolism in intestine and then goes to liver and systemic circulation and urine. Hence, it takes at least 24 h for cotinine to appear in systemic circulation after oral intake.[11] Hence, especially for nonsmokers, saliva would be preferred medium for assessment of cotinine as compared to plasma.

Cotinine is formed by cytochrome P450-mediated C-oxidation of nicotine and is more stable.[12] Nicotine is not considered a valid marker of smoking status due to its relatively short half-life (approximately 2 h). By contrast, cotinine has an average half-life of 17 h, and blood levels closely reflect the dose of nicotine absorbed from tobacco smoke. Saliva samples are easier to obtain, however, and saliva levels are highly correlated and can be used interchangeably with blood levels.[13] Hence, in the present study, salivary cotinine level was assessed as preferred method.

In most investigations, psychological dependence was examined exclusively through a self-administered questionnaire, the validity of which is often questioned because of underestimation.[14] On the other hand, self-reported measures are likely to be imprecise indicators of intake of tobacco smoke.[15] A quantitative assessment of tobacco smoke exposure through evaluation of its metabolites would help overcome such drawbacks. Hence, in our study, semi quantitative assessment of salivary cotinine levels was done.

Descriptive statistics of the present study shows that both groups had an equal proportion of participants in respect to age of individuals, duration of habit, and frequency of tobacco consumption. This blind proportion has helped the study results to be more precise and valid.

Weak correlation ( $r = 0.081$ ) of salivary cotinine with FTND total scoring in both groups indicates that salivary cotinine level is weakly influenced by psychological dependence in both tobacco smokers and tobacco chewers. These results are in accordance with results of study conducted by Asha and Dhanya ( $r = 0.2$ )[16] and Abram *et al.* ( $r = 0.3$ )[17] carried out to assess the correlation between salivary cotinine level and psychological dependence. However, Etter *et al.* have shown strong correlation between salivary cotinine level and FTND scoring ( $r = 0.67$ ).[18] Contrasting results in our study as compared to study conducted by Etter *et al.* may be due to difference in population, lesser female population in our study, and also may be due to subjective nature of FTND questionnaire.

Salivary cotinine level in the present study is weakly correlating with age ( $r = 0.3$ ) and duration of habit (0.2) of tobacco smoking. This indicates that as age and duration of habit increases, the level of salivary cotinine level also increases. These facts can be well explained by the altered physiology of saliva as age advances; saliva secretion decreases and dilution factor saliva changes lead to more concentrated of saliva and hence more value of salivary cotinine. Study conducted by Figueiredo *et al.* has shown that there is a weak correlation of salivary cotinine and age of individual.[19] Study conducted by Etter *et al.* showed that age was slightly associated with the cotinine concentration, but this association weakened after adjustment for the number of cigarettes per day.[18] However, smokeless group shows contrasting features as compared to smokers group when we consider age and duration of habit. This may be because chewing stimulates more salivary secretion and so dilution factor decreases which leads to low salivary cotinine concentration. Weak correlation ( $r = 0.2$ ) between frequency of smoking and salivary cotinine level is in accordance with study results published by Etter *et al.*[18]

The present study has shown that participants who consume smokeless tobacco show high level of salivary cotinine as compared to participants who consume tobacco in smoking form and this difference is statistically significant ( $P = 0.0217$ ) [Table 1]. This results in accordance with study conducted by Etter *et al.*[18] and Asha and Dhanya.[16] The reason for high level of salivary

cotinine in smokeless group can be attributed as the cotinine requires more time to get metabolized through the local routes of administration rather than the systemic route.

When FTND scoring was compared between two groups, statistically nonsignificant difference was observed among both groups. This indicates that individuals are psychologically dependent on tobacco irrespective type of habit of tobacco consumption. These findings are in accordance with study conducted by Jadhav and Singh.[14]

Weak or negative correlation between salivary cotinine level and tobacco consumption within 5 min after waking up indicates again salivary cotinine level is independent of dependence. These results are in contrast to the results of study conducted by Figueiredo *et al.*[19] The variations in the results may be due to the neurological influence of an individual while providing the information for the questionnaire and as on other aspect there is no influence to be seen regarding the salivary cotinine levels.

## CONCLUSION

Here, in the study, we tried to find the relation between the salivary cotinine levels and psychological dependence among the tobacco users. The study showed that the salivary cotinine is not influenced by the level of psychological dependence among the tobacco users. Rather, it is noticed that the levels of cotinine are strongly associated with the pattern of tobacco consumption (smoking/smokeless) followed by the path of metabolism accordingly. Furthermore, multicentric studies with large sample size are required for setting a significant relation between the salivary cotinine levels and tobacco dependence.

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## Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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