ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

*International Journal of Dental Science and Innovative Research (IJDSIR)*

*IJDSIR : Dental Publication Service*

*Available Online at:www.ijdsir.com*

*Volume – 8, Issue – 1, January – 2025, Page No. : 148 - 154*

# Comparison between Nicotine Dependence and Urine Cotinine by Fagerstrom Test for Nicotine Dependence in Smokers and Tobacco Chewers

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**Citation of this Article:** Dr. Manjiri U. Joshi, Dr. Mukta V. Vanjani, Dr. Reema Sarvesh Agrawal, Dr. Ritvi Desai, "Comparison between Nicotine Dependence and Urine Cotinine by Fagerstrom Test for Nicotine Dependence in Smokers and Tobacco Chewers", IJDSIR- January – 2025, Volume – 8, Issue – 1, P. No. 148 – 154.



**Type of Publication:** Original Research Article

**Conflicts of Interest:** Nil

## Abstract

**Context**: All tobacco products contain nicotine. The most widely used measure for assessment of nicotine dependence is Fagerstrom Test for Nicotine Dependence (FTND). Nicotine dependence is one of the most important determinants of maintenance and unsuccessful cessation of tobacco use.

**Objectives**: To assess nicotine dependence and urine cotinine in smokers and tobacco chewers, to compare these two parameters using FTND, and to test the association of urine cotinine with nicotine dependence.

**Materials and Method**: 70 patients were selected from the outpatient department and were divided into two groups [smokers = 35, tobacco chewers = 35]. The Urine Assay was carried out using COT Rapid Test Cassette kit.

**Statistical analysis used**: Statistical analysis was done using the SPSS software, version 16.0.0 (SPSS Inc. Chicago, IL, USA, 2007). Independent T test and Pearson's Chi-square test were applied.

**Results**: There was no significant statistical difference between smokers and tobacco chewers based on their

Urine Cotinine and FTND scores using the Independent T test, while a significant association was present between urine cotinine and FTND scores using Pearson's Chi-square test. ($p$=0.005)

**Conclusion**: The results of this study support the validity of using urinary cotinine levels for assessment of nicotine dependence using FTND. However, the use of a more specific alternative chemical method such as Gas Chromatography / Mass Spectrometry (GC/MS) is recommended in order to obtain a confirmed analytical result.

**Keywords**: Urine, Cotinine, Nicotine, FTND, Tobacco, Smoking

## Introduction

Tobacco consumption is the leading preventable cause of morbidity and mortality which is available in two forms - smoking and smokeless.[1] All tobacco products contain nicotine in substantial concentration. It is a drug to which virtually every member of a tobacco-smoking society is exposed whether through direct contact or second - hand inhalation. This nicotine is metabolized to form a toxic alkaloid, i.e., cotinine, which produces stimulation of the autonomic ganglia and central nervous system when in humans.[2]

Nicotine (tobacco) dependence is one of the most important determinants of maintenance and unsuccessful cessation of tobacco use.[3] The most widely used measure for assessment of nicotine dependence is the Fagerstrom Test for Nicotine Dependence (FTND), proposed by Karl-Olov Fagerström (1991).[4] This test was developed with the aim to diagnose the degree of physical dependence among tobacco users and to serve as a tool for tobacco cessation treatments. It is not just a single criterion but a complex entity that is based on various dimensions.[3]

Cotinine, a major / first stage metabolite of nicotine, has low plasma protein binding, long half-life and is the most commonly used marker for assessment of nicotine dependence in various body fluids like saliva, blood, urine. It is used to distinguish tobacco users from non-users and reflects the extent of exposure because of its greater sensitivity and specificity than other biochemical tests.[5,6,7]

There is a high correlation between blood and salivary cotinine concentrations. A widely used biomarker is urine cotinine since cotinine concentrations are four to six times higher in urine than that in blood or saliva because of its pH. The value of urine cotinine ranges from 20-550 ng/ml. A change in the cut-off value is directly proportional to sensitivity of detection.[8,9]

The literature search revealed very few studies comparing the nicotine dependence and urine cotinine in between smokers and tobacco chewers. The current study aims to compare and correlate the above-said parameters in the population of Gujarat. Hence this study was conducted to assess and compare nicotine dependence in smokers and tobacco chewers using FTND. Also it aims at comparing urine cotinine in smokers and tobacco chewers and to test its association with nicotine dependence.

## Materials and Methodology

This observational study was carried out in the Department of Oral Medicine and Radiology of a dental institute in Central Gujarat; after obtaining clearance from the institutional ethics committee [IEC/MPDC_202/OD-13/20].

With reference to the previous study, sample size selection was done in the ratio of 1:1 with a minimum of 70 subjects with 80% power.[10] In this time based hospital study which was conducted for a period of 6 months from February 2022 to July 2022, all the cases

visiting the outpatient department and consenting to be part of the study were included. They were divided into two main groups based on their pattern of tobacco consumption. Each group consisted of 35 participants.

1) Group 1 :- 35 participants with habit of tobacco smoking
2) Group 2 :- 35 participants with habit of tobacco chewing / smokeless tobacco

**Inclusion Criteria**

Individuals with the present habit of tobacco smoking and/or tobacco chewing; and subjects above the age of 14 years with the above-mentioned habits were included in the study. Only those subjects who agree to enroll for TCC counseling were included in this study.

**Exclusion Criteria**

Individuals with past tobacco history and presently have quit the habit or have not consumed tobacco since the last 24–48 hours; and those individuals who are on medication for tobacco cessation were excluded from the study. Also individuals with history of any other substance abuse other than smoking and tobacco chewing, recent infection, subjects with systemic illness and poor compliance were omitted 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.

Interested subjects were registered under Tobacco Control & Cessation (TCC) Centre and a TCC questionnaire form was filled up for each of the participants. These subjects were first evaluated through counseling where FTND was assessed and then the urine cotinine test was performed in the Hematology Laboratory of the Department of Oral & Maxillofacial Pathology.

FTND is basically a six-item questionnaire scale that has a total scoring from 0-10 which is classified according to patient nicotine addiction as very low (0–2), low (3–4), medium (5), high (6–7), or very high (8–10). A higher score indicates stronger dependence.[4]

The Urine Assay was carried out using COT Rapid Test Cassette Kit [JusChek$^{+}{}_{R}$, ACRO BIOTECH, Inc., U.S.A.]. For this test, the urine specimen was collected in a clean and dry container. Urine collected at any time of the day was used. Specimens exhibiting visible precipitates were centrifuged, filtered, or allowed to settle to obtain a clear supernatant for testing.[2]

**Procedure**

Allow the urine specimens to reach room temperature (15-30°C) prior to testing.

1) Bring the COT Rapid Test Cassette pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within one hour.
2) Place the test cassette on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine (approx. 120 µL) to the specimen well (S) of the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well (S).
3) Wait for the colored line(s) to appear. The result should be read in 5 minutes. It is important that the background is clear before the result is read. Do not interpret the result after 10 minutes.

**Interpretation of the Urine Assay[2]**

**Negative*** -- Two lines appear. One colored line should be in the control line region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the cotinine concentration is below the detectable level (200 ng/mL).

*Note : The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

**Positive**: One colored line appears in the control line region (C). No line appears in the test line region (T). This positive result indicates that the cotinine concentration exceeds the detectable level (200 ng/mL).

**Invalid:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test kit.

This COT Rapid Test Device is used for the qualitative detection of cotinine. This assay provides a preliminary analytical test result only. Technical and procedural errors were omitted in this study. A positive result indicates the presence of cotinine only, and does not indicate or measure its intoxication. And a negative result does not at any time rule out the presence of cotinine in urine, as it may be present below the minimum detection level of the test.[2]

Statistical tests applied were descriptive statistics like counts, mean, percentages, standard deviation etc. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) software, version 16.0.0 (SPSS Inc. Chicago, IL, USA, 2007). Independent T test was used to compare smokers and tobacco chewers based on their Urine Cotinine and FTND scores and Pearson's Chi-square test was used to test the association of urine cotinine with nicotine dependence.

**Results**

A total of 70 study subjects participated in the study. The mean age of study participants was 34.13 ± 12.95 years. The mean age of onset of tobacco habit in smokers and tobacco chewers in this study was 25.63 and 24.77 years respectively [Table 1]. Only four females (5.71%) were part of the study. Out of 4 only one female was a smoker [Table 2]. The study participants were further grouped according to the type of tobacco smoked or consumed as depicted in [Table 3 and 4]. 15 (42.86%) smokers and 14 (40%) tobacco chewers had a positive family history for consumption of tobacco in either form.

The study subjects were evaluated for their urine cotinine test and FTND score. It was seen that the urine cotinine test was positive in 32 (91.43%) smokers and 33 (94.29%) tobacco chewers. The mean FTND score was 5.66± 2.24 in smokers and 6.4± 2.14 in tobacco chewers [Table 5 and 6].

There was no significant statistical difference between smokers and tobacco chewers based on their urine cotinine and FTND scores using the Independent T test, while a significant association was present between urine cotinine and FTND scores using Pearson's Chi-square test ($p$=0.005)

**Discussion**

An extensive variety of tobacco products are accessible for smoking as well as smokeless use. Nicotine is a highly addictive chemical compound. All tobacco products contain a substantial amount of nicotine. Tobacco consumers are inadvertently exposed to nicotine either through direct contact or passive inhalation which can pose significant health risks as nicotine is metabolized to form cotinine which is a toxic alkaloid and produces stimulation of the autonomic ganglia and central nervous system.[2]

In India, nearly 1 in 10 adolescents in the age group of 13-15 years have ever smoked cigarettes.[11] The mean age of subjects in this study was 32.60 (smokers) and 24.77 years (tobacco chewers) respectively which is in congruence with the study performed by Jhanjee *et al*. where the mean age of the subjects was found to be 37.6 years, with more than half of the subjects educated

below secondary school level and employed with low or medium monthly income.[12] Out of the 70 subjects in this study, 66 (94.29%) were males and 4 (5.71%) were females which might have been due to the social desirability bias. This can be due to the refusal of women to acknowledge tobacco use due to the associated stigma.

The mean age of onset of tobacco habit in smokers and tobacco chewers in this study was 25.63 and 24.77 years respectively, which is higher than that of a study conducted by Guan NC *et al*. where the mean age of smoking initiation was 19.61 ± 11.47 years.[13] In the present study; among the smokers group, 85.71 % of the subjects smoked cigarettes and 14.29 % of the subjects smoked bidi which is in contrast to the study performed by Jhanjee *et al*. where three fourth of the subjects reported beedi use.[12] Beedi could be the preferred mode of tobacco use due to economic reasons as it is cheaper than cigarettes. Whereas 77.14 % of the subjects in the tobacco chewers group had the habit of chewing tobacco in the form of padiki and 22.86 % of the subjects chewed khaini. Out of the 70 subjects, 29 (41.43%) had a positive family history of tobacco consumption whereas 41 (58.57%) of the subjects were lacking that family history.

Cotinine is a metabolite of nicotine and is the most commonly used marker for assessment of nicotine dependence. Concentration of cotinine is four to six times higher in urine than that in blood or saliva. In the present study, there was no significant statistical difference between smokers and tobacco chewers based on their urine cotinine concentration and FTND scores. Contrarily, in the study by Oberoi SS *et al*. mean cotinine concentrations were significantly higher among smokeless tobacco users than in smokers.[14] No differentiation between the cigarette and bidi smokers was made in the present study, whereas in the study by Behera *et al*. cigarette smokers had a higher value of urinary cotinine concentration than bidi smokers.[15]

The mean FTND score for smokers in this study was 5.66 which refers to the moderate nicotine dependence, approximately similar to a study conducted by Jhanjee *et al*. where FTND was found to be high.[12] However, in the study conducted by Priyonugroho G *et al.* the median FTND score was 4, and more than half of the participants had mild nicotine dependence.[16]

In this study, it was observed that there was a significant association between urinary cotinine levels and FTND, which is in accordance with a study by Jung *et al*. who stated that the level of urinary cotinine is independently associated with the degree of nicotine dependence and that cotinine is a valid biomarker for prediction of high nicotine dependence.[17] However, in a study by Priyonugroho G *et al*. there was no correlation between urinary cotinine level and FTND score.[16] The different findings might be due to the variability in cotinine levels per cigarette smoke/tobacco consumption and also due to the time elapsed since the last cigarette smoked/tobacco consumed and the urine collection, as revealed by Muscat et al.[18]

**Conclusion**

The results of this study support the validity of using urinary cotinine levels for assessment of nicotine dependence in smokers and tobacco chewers using Fagerstrom Test for Nicotine Dependence (FTND). However, the use of a more specific alternative chemical method such as Gas Chromatography / Mass Spectrometry (GC/MS) is recommended in order to obtain a confirmed analytical result.

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**Legend Tables**

Table 1: Mean age of onset in smokers and tobacco chewers

| Groups | N | Mean Age (In Years) | Standard deviation |
|---|---|---|---|
| Smokers | 35 | 25.63 | 9.04 |
| Non-Smokers | 35 | 24.77 | 9.17 |
| Total | 70 | 25.20 | 9.05 |

Table 2: Distribution of study subjects according to gender

| Gender | Number | Percentage |
|---|---|---|
| Male | 66 | 94.29% |
| Female | 4 | 5.71% |
| Total | 70 | 100% |

Table 3: Distribution of smokers according to the type of tobacco they use

| Sr.No. | Type of tobacco | Number | Percentage |
|---|---|---|---|
| 1 | Cigarette | 30 | 85.71 % |
| 2 | Bidi | 5 | 14.29 % |
| Total | | 35 | 100% |

Table 4: Distribution of tobacco chewers according to the type of tobacco they use

| Sr.No. | Type of tobacco | Number | Percentage |
|---|---|---|---|
| 1 | Padiki | 27 | 77.14 % |
| 2 | Khaini | 8 | 22.86 % |
| Total | | 35 | 100% |

Table 5: Comparison of study subjects according to Urine Cotinine test results

| Type of tobacco consumption | Urine Cotinine [n (%)] | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Smoker | 32 (91.43%) | 3 (8.57%) | 35 (100%) |
| Nonsmoker | 33 (94.29%) | 2 (5.71%) | 35 (100%) |
| Total | 65 (92.86%) | 5 (7.14%) | 70 (100%) |

Table 6: Distribution of study subjects according to FTND score

| Groups | Mean | Standard deviation |
|---|---|---|
| Smokers | 5.66 | 2.24 |
| Non-Smokers | 6.4 | 2.14 |