Original Article

Assessment of Urinary Cotinine Level among Smokers in Chennai

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Abstract

Introduction: Tobacco use in India is characterized by a high prevalence of smoking and smokeless tobacco use, with dual use also contributing a noticeable proportion. Cotinine, a metabolite of nicotine, is commonly used as a biomarker for the evaluation of smoking, as well as the exposure to tobacco smoke and passive smoking. Urinary excretion of cotinine is a good marker as it is less influenced by the flow of urine and pH. Cotinine quantification thus provides an objective quantitative measure which is more reliable than the smoking history or counting the number of cigarettes smoked per day. **Aim:** The aim of the study was to assess the urinary coitinine level and to correlate with the level of tobacco dependence among smokers. **Materials and Methods:** It is a cross-sectional study conducted among company employees, Chennai. Sample size $n = 44$. Study subjects with age group between 20 and 55 years with a habit of smoking for a period of 2 years were included in the study. First section includes the collection of demographic information such as name, age, gender, occupation, and marital status. The second section comprises questionnaire to assess about duration of tobacco usage, reason for tobacco usage, and Fagestron nicotine dependence scale were used to assess the level of dependence of study participant. The third section includes a collection of urine samples. Urine samples are to be collected in empty plastic sterile containers. Subject code to be marked in the sterile container. It is frozen at 20°C. The coitinine content was assessed using COT rapid test caseette. **Results:** The mean age and smoking duration of study subjects were 28.8 years and 6.6 years. About 34 (77.3%) of study subjects belong to the age group of 20–30 years. In 20–30-year age group, 75% (33) and 2.3% (1) belong to skilled and semi-skilled worker. About 2.3% of 51–60-year age group of participant belong to skilled worker. Low nicotine dependence level was found in 40.9% (18), 13.6% (6), and 4.5% (1) of study participants. Urinary coitinine level was found to be more than 200 ng among all study participants. It shows the chronic nature of smoking habit of study subjects. **Conclusion:** Urinary coitinine level was found to be positive among smokers. Smoking habit has a negative effect on oral as well as on general health. Hence, Tobacco control policies in India should adopt a targeted, population-based approach to control and reduce tobacco consumption in the country.

Keywords: *Cigarette, coitinine, skilled worker, smokers, tobacco dependence, urine*

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Introduction

Tobacco is used in India in many forms. In India, tobacco is consumed both in smoking and nonsmoking forms. Smoking forms include cigarette, bidi, *hooka,* and

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chutta (a reverse form of smoking, in which smoking is done with the burning end inside the mouth).[1] Tobacco chewing is the main nonsmoking form of tobacco use.

Self-reported smoking rates are likely to give a substantial underestimate of the true prevalence of smoking and as many as one-sixth of smokers who claimed to be nonsmokers, actually may be positive for urinary cotinine,[2] which could lead to an underestimation of the effect of smoking on the course of disease and could prejudicially affect decisions on patient management.

Tobacco usage was more prevalent in both developing and in developed countries. The high burden of tobacco use is associated with a high mortality burden. According to the World Health Organization, nearly 6 million deaths occur every year due to tobacco use, which may escalate to 8 million deaths a year by 2030.[3]

Nicotine is highly addictive chemical found in tobacco that makes one difficult to stop smoking once initiated, and this property of nicotine is similar to those of heroin and cocaine.[3] Hence, nicotine dependence is a substance-related disorder which is an obstacle in the smoking cessation among smokers.[4] Nicotine addiction is the cause of premature death for one person in every 6 s and 80% of them are from the low- and middle-income countries.

Cotinine, a metabolite of nicotine, is commonly used as a biomarker for the evaluation of smoking, as well as the exposure to tobacco smoke and passive smoking. The level of cotinine in the blood is proportional to the degree of the exposure to tobacco, thus making it a valuable indicator of smoking, including the passive one.[5] A number of biochemical markers such as thiocynate, nicotine, cotinine, and carbon monoxide in the expired air and carboxyhemoglobin in blood have been used to validate claims of nonsmoking.[6,7] Levels of thiocyanate and carbon monoxide/carboxyhemoglobin are easier to determine but can be raised through exposures unrelated to smoking such as traffic emissions and diet. Cotinine is possibly the best marker for situations where accuracy is paramount.[8-10]

Cotinine is a major metabolite of nicotine. Urinary excretion of cotinine is a good marker as it is less influenced by the flow of urine and *p*H. For study of nicotine and cotinine levels, it is preferable to have noninvasive methods. The choice of body fluids for cotinine assay in smoking studies should depend on practical rather than pharmokinetics considerations. Cotinine, which is stable in body fluids, has a long half-life, low plasma protein binding (2.6%), and doseindependent disposition kinetics. These factors make cotinine a good marker for estimating both active and passive exposure to tobacco smoke.[11]

In urine, levels from 11 ng/mL to 30 ng/mL may be associated with light smoking or moderate passive exposure, while the levels in active smokers typically reach 500 ng/mL or more. Cotinine quantification thus provides an objective quantitative measure which is more reliable than the smoking history or counting the number of cigarettes smoked per day. Cotinine also allows the measurement of secondary exposure to tobacco smoke.[12]

The assessment of the smoking status of a subject is needed for documenting the extent of the exposure to tobacco smoke, both for monitoring the progress of tobacco control programs and because smoking is a major confounding factor in the assessment of exposure to important occupational and environmental pollutants such as benzene, formaldehyde, polycyclic aromatic hydrocarbons, and some heavy metals. For example,

Figure 1: JusChek urinary cotinine test kit

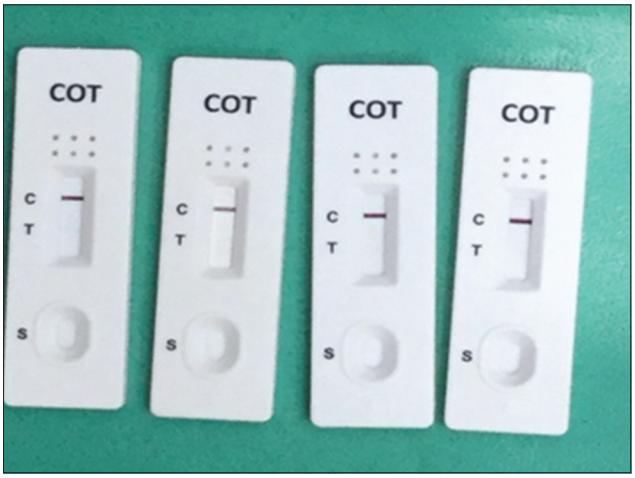

Figure 2: Cotinine test kit results

the median urinary value of S-phenyl- mercapturic acid, the most specific human metabolite of benzene, is about ten times higher in smokers than in nonsmokers, and its concentration is linearly correlated to that of urinary cotinine in smokers.[13] A strong influence of smoking on the urinary biomarkers of the polycyclic aromatic Hydrocarbons 1 and 2 naphthalene and pyrene (1-OH-Naphthalene, 2-OH-Naphthalene, and 1-OH-Pyrene) was observed in a biomonitoring study on 200 volunteers conducted using high-performance liquid chromatography-mass spectrometry (MS)/MS.[14]

Self-reported smoking status may not always represent a subject's true smoking status, and for this reason, the level of urine cotinine is used as a biomarker of tobacco smoke exposure. In humans, cotinine is one of the most important metabolites of nicotine, a major component of tobacco smoke, metabolized in the liver by the enzyme cytochrome P450 2A6. Cotinine can be excreted in the urine as an N-glucuronide conjugate, and it accounts for about 10%–15% of the sum of the nicotine excreted unchanged plus the other metabolites; it could also be measured in blood, urine, saliva, hair, or nails. The dosage of cotinine concentration in the body fluids indicates a recent exposure to tobacco smoke, while a long-term exposure information can be obtained from the dosage of nicotine concentration in nails and hair.[15]

It is possible to set an optimal cutoff value for the urinary cotinine concentration to distinguish smokers from nonsmokers and to validate their self-reported smoking status: The urinary cotinine concentration depends on the smokers' behavior or on the extent of exposure to secondhand smoke. In passive smokers, the cotinine concentration is lower than 50 g/L of urine, while in smokers, the cotinine concentration is generally more than 100 g/L.[16] Assessing smoking status is important in epidemiological studies, clinical studies of smoking-related diseases, and the monitoring of smoking cessation interventions. Hence, the aim of the study was to assess the urinary coitinine level and to correlate with the level of tobacco dependence among smokers.

Materials and Methods

It is a cross-sectional study conducted among company employees, Chennai. Informed consent was obtained from the study participants. Before the start of the study, ethical clearance was obtained from the institutional Ethics committee, Saveetha university. Sample size $n = 44$. Sample size was calculated based on the study done by Hong *et al*., 2018.[17-21]

Study group

Smokers $n = 44$.

The term "Smoker" defined a participant who smoked over 10 cigarettes a day for at least 3 years. Parameter of "pack years" was calculated (cigarettes per day multiplied by smoking years).

The term "nonsmoker" defined a participant who had never smoked or did not smoke for minimum 5 years.[22]

The term "Healthy" defined a participant who was free of any compromised medical condition did not receive any treatment known as promoting oral candidiasis (antibiotics, steroids, high blood pressure medication, anemia due to iron deficiency, diabetes, AIDS etc.).[23]

Inclusion criteria

- Study subjects with age group between 20 and 55 years with a habit of tobacco usage were included in the study
- Subjects who have smoking habit for a period of 2 year
- Subjects who were willing to participate in the study were included.

Exclusion criteria

- Study participant who has systemic disease such as diabetes mellitus, hepatitis B and C infection
- Participant who is not willing to participate in the study were excluded.

Survey instrument

The first section includes the collection of demographic information such as name, age, gender, occupation, and marital status.

The second section comprises questionnaire to assess the oral hygiene practices, about the tobacco usage, duration of usage, reason for tobacco usage and Fagestron nicotine dependence scale were used to assess the level of dependence of study participant.

The third section includes the collection of urine samples. Urine samples are to be collected in empty plastic sterile containers. Subject code to be marked in the sterile container. It is frozen at 20°C. The coitinine content was assessed using COT rapid test caseette (urine) (product name: JusChek). It is a rapid chromatographic immunoassay for the detection of coitinine in human urine at a cutoff concentration of 200 ng/mL.

Procedural steps to use JusChek kit

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within 1 h
2. Place the test cassette on clean and level surface. Hold the dropper vertically and transfer 3 drops of

urine to the specimen well of test cassette and then start the timer
3. Wait for the colored lines to appear. The result should be read at 5 min.

Statistical analysis

Statistical analysis to be done using (Statistical Package for Social Sciences) SPSS software version 20. Descriptive statistics were done to assess the correlation of urinary cotinine level with tobacco dependence among study participants.

Results

Table 1 depicts the distribution of study participants based on age group. Study subjects comprised 44 participants. There are about 34 (77.3%) study subjects belong to the age group of 20–30 years. About 4 (9.09%) participants belong to the age group of 31–40 years and 41–50 years. About 1 (2.3%) of participants belong to the age group of 51–60 years and 61–70 years. Majority of the study participants belong to 20–30 years of age group. Table 2 depicts the distribution of occupation of study participants based on age group. In 20–30-year age group, 75% (33) and 2.3% (1) belong to skilled and semi-skilled worker. About 2.3% of 51–60-year age group of participant belong to skilled worker. In 61–70-year age group, 2.3% (1) belong to semi-skilled worker. Table 3 depicts the association of number of cigarettes with fagestron nicotine dependence level of study participants. Low nicotine dependence level was found in 40.9% (18), 13.6% (6), and 4.5% (1) of study participants, who smokes 1–3, 4–10, and 11–15 cigarettes. High dependence level was found in 2.3% (1) of study participants, who smokes 4–10 cigarettes per day. Table 4 depicts the association of urinary coitinine level with the age group of study subjects. Urinary coitinine level was found to be more than 200 ng among all study participants. It shows the chronic nature of smoking habit of study subjects. Table 5 depicts the parameters evaluated among smokers in the study. The mean age and smoking duration of study subjects were 28.8 years and 6.6 years. Table 6 depicts the Association of urinary coitinine level with number of ` be > 200ng among 11-15 cigatettes smoked per day. Figure 1 depicts the JusChek urinary cotinine test kit. Figure 2 depicts Cotinine test kit results.

Table 1: Distribution of study participants based on age group

Age group (years)	*n* (%)
20-30	34 (77.3)
31-40	4 (9.09)
41-50	4 (9.09)
51-60	1 (2.3)
61-70	1 (2.3)
Total	44 (100)

Table 2: Distribution of occupation of study participant based on age group

Age group (years)	Skilled worker, *n* (%)	Semiskilled worker, *n* (%)
20-30	33 (75)	1 (2.3)
31-40	4 (9.09)	0
41-50	4 (9.09)	0
51-60	1 (2.3)	0
61-70	0	1 (2.3)
Total	42 (95.48)	2 (4.6)

Discussion

Tobacco is used in both smoke and smokeless forms. Smoking forms are more common in western countries while India stands first in the use of smokeless forms of tobacco.[24] Smoking has many negative effects on the oral cavity. Staining the teeth and restorations, decreasing the ability to smell and taste and the formation of oral lesions such as smokers' palate, smokers' melanosis, hairy tongue, and possibly oral candidiasis, tooth decay, periodontal disease, failure of implant treatment, cancer, and precancerous lesions of oral cavity are some of the effects that can be noted.[25]

Oral diseases are the most common of noncommunicable diseases affecting varied population. It remains as a significant public health problem because of its high prevalence, expensive treatments, and lack of awareness.[26] To measure a population's oral health status, epidemics, health behavior, and risk factors and to assess the quality of oral health-care services received

Table 3: Association of number of cigarettes with fagestron nicotine dependence level of study participants

Number of cigarettes	Fagestron nicotine dependence level				Total
	Low dependence, *n* (%)	Moderate dependence, *n* (%)	High dependence, *n* (%)	Very high dependence, *n* (%)	
1-3	18 (40.9)	12 (27.3)	0	0	30
4-10	6 (13.6)	3 (6.8)	1 (2.3)	1 (2.3)	11
11-15	2 (4.5)	1 (2.3)	0	0	3
Total	26 (59)	16 (36.4)	1 (2.3)	1 (2.3)	44

Fischer's exact test. *F* value: 7.60. *P*: 0.32

Table 4: Association of urinary coitinine level with age group of the study subjects

Age group (years)	*n*	Urinary coitinine level (ng)
20-30	34	>200
31-40	4	>200
41-50	4	>200
51-60	1	>200
61-70	1	>200

Table 5: Parameters evaluated among smokers in the study

Parameters	Mean±SD
Age	28.8±4.12
Smoking initiation age	22.3±4.78
Smoking duration	6.6±8.3
Carbon monoxide level	5.4±4.1

SD: Standard deviation

Table 6: Association of urinary coitinine level with number of cigarettes smoked

Number of cigarettes	Urinary coitinine level (ng)
1-3	>200
4-10	>200
11-15	>200

oral health surveys are used as a judgmental tool. India is the second highest populated country with more than 1030 million populations. The dentist to population ratio is 1:10,000 in urban areas, whereas 1:150,000 in rural areas.[27]

In the present study, about 77.3% of study subjects belong to the age group of 20–30 years. In a similar study done by Levine *et al*. (Isreal, 2013),[19] about 74.7% of the smokers belong to the age group of 20–44 years.

In the present study, in 20–30 years age group, 75% and 2.3% belong to skilled and semi-skilled worker. About 95% of the study participants are skilled workers. In a similar study done by Hong *et al*. (Korea, 2017),[17] about 30% of the study subjects are skilled workers. In the present study, study participants are graduates, and they are company employers.

Smoking initiation age is essential to record, to assess the person behavior, his surroundings and to assess the pack years. In the present study, the mean smoking initiation age among study subjects was 22.3 years. In a similar study study done by Sonti Sri Harsha (Chennai, 2013),[28] the study showed that the initiation age of smoking was found that more than 50% of the population started smoking between 21 and 25 years of age.

In the present study, the mean smoking duration was found to be 6.6 years. In a dissimilar study done by Ana maria vlăsceanu,[29] the duration of smoking was long with an average of 30.2 years. In the present study, majority of the study subjects are young adults, because of young age, the duration of smoking habit was less among the study participants.

In the present study, low, medium, and high dependence was found among 59.1%, 36.2%, and 4.6% of the study subjects. In a similar study done by Umesh Raj Aryal (Nepal, 2015),[30] low, medium, and high dependence was found among 16.2%, 31.9%, and 20.4% of the study subjects. In the present study, years of smoking among study subjects was majorly 1–5 years. Hence, the nicotine dependence level was found to be low.

In the current study, the urinary coitinine level was evaluated to be >200 ng among all study subjects. The coitinine test results were positive implying the chronic nature of smoking habit of study participants. The coitinine test kit valilidy was checked among five healthy individuals, the results showed as a control for them. Later, then it was used to assess the urinary coitinine level among smokers in this study. In a study done by Hong *et al*. (Korea, 2017), about 52% of 19–44-year age group of study subjects showed a urinary coitinine level of >100 ng/ml.

In the present study, the mean carbon monoxide level was found to be 5.4 ppm. In a similar study done by Zhang *et al*. (China, 2013),[31] the mean carbon monoxide level was 7.8 ppm; it was higher compared to the current study. The mean smoking duration years was found to be less in the present study.

Tobacco affects oral health adversely. The willingness/need to quit the habit and their level of nicotine dependence must be assessed by the dental practioner. People like to quit the habit knowing the ill-effects of tobacco. The concept of need is essential for planning and evaluation of oral health care.[32] After assessing the level of dependence, the modalities of treatment/intervention varies from behavioural management to pharmacological management among population. Data of treatment need to provide curative treatment to vast and diverse population as access and affordability to the dental facilities become a constraint for the majority of the population.[33] The patient's comfort is more vital to the acceptance of any intervention.[34] Tobacco cessation measures are undertaken only when the person accepts to the intervention to quit the habit.

Health education is provided about the harmful effects of tobacco on general health and oral health. Oral health

education is not only directed at reducing disease and injury to the teeth and their supporting structures rather, it influences general health and promotes a feeling of well– being.[35] In order to assess the magnitude of the preventive task, it is necessary to know the extent and severity of the disease.[36] Knowledge about ill effects of tobacco use must be imparted to tobacco users so that they can refrain from using tobacco products. It is important to instill good oral health practices from young age to ensure long-term dental health and hygiene.[37]

Conclusion

In the present study, low, medium, and high nicotine dependence was found among 59.1%, 36.2%, and 4.6% of the study subjects. Urinary coitinine level was found to be more than 200 ng among all study participants. It shows the chronic nature of smoking habit of study subjects. National tobacco survey should consider biochemical verification using urinary cotinine as well as self-report to estimate more accurate smoking prevalence. Younger age, male sex, low education level, service and sales workers, low household income, and high-risk alcohol drinking were associated with the risk of smoking. These factors related to smoking should be focused in intervention-based studies including smoking-cessation program in workplaces and health education for public to reduce the smoking rate and smoking attributable disease.

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Conflicts of interest

There are no conflicts of interest.

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