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Research Article

Estimation of cotinine level among the tobacco users and nonusers: A cross-sectional study among the Indian population

Sukhvinder Singh Oberoi[1]* and Avneet Oberoi[2]

[1]Department of Public Health Dentistry, ESI Dental College and Hospital, Sector-15, Guru Gobind Singh Indraprastha University, New Delhi, India

[2]Department of Dentistry, Oberoi Dental and Orthodontic Centre, New Delhi, India

Abstract

Background: Epidemiological studies in smokers indicate a dose-response relationship between the number of cigarettes smoked per day and the risk of developing certain smoking related diseases. The alkaloid nicotine is the major pharmacologically active substance in tobacco.

Objective: To estimate the cotinine level excretion in urine among smoked and smokeless tobacco users and nonsmokers among the Indian population.

Materials and method: The study sample consisted of 250 subjects who were apparently healthy, asymptomatic and not using any drug. The study sample was divided into smoked tobacco users (bidi and cigarette), smokeless tobacco users, both smoked and smokeless tobacco users and controls (non-users of tobacco in the past or present).

Results: The mean Cotinine level in urine was significantly (p – value < 0.05) more among smoked tobacco users in comparison to smokeless tobacco users and non-users of tobacco. Whereas, the mean Cotinine level in urine was significantly (p – value < 0.05) more among smokeless tobacco users in comparison to non-users of tobacco.

Conclusion: The mean cotinine levels among smokers and both smokeless and smoked tobacco users were found to be higher than only smokeless tobacco users and non-users of tobacco.

More Information

***Address for Correspondence:**
Dr. Sukhvinder Singh Oberoi, Reader, Department of Public Health Dentistry, Sudha College of Dental Sciences and Research, Kheri mor, Faridabad, Haryana, India,
Tel: 011-4596594; +91-96548-12568;
Email: drsukhvinder@gmail.com

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Introduction

Worldwide, tobacco-related diseases cause about 5 million premature deaths per year [1]. Most of these deaths occur in smokers, but smokeless tobacco use [2] and exposure to secondhand smoke in non-smokers also poses a significant health risk [3,4]. Most smokers in the say they want to quit, but the majority of them are unable to do so, in large part because of nicotine addiction [5].

More than 4000 compounds have been identified in tobacco smoke, and at least 50 of these have been found to be carcinogenic [6,7]. Epidemiological studies in smokers indicate a dose-response relationship between the number of cigarettes smoked per day and the risk of developing certain smoking related diseases [8]. The alkaloid nicotine is the major pharmacologically active substance in tobacco [9].

There is good evidence that most smokers are dependent on nicotine and that the severity of tobacco dependence may be related to the level of nicotine intake. Consequently, determining exposure to specific substances in tobacco and tobacco smoke is useful in epidemiological studies exploring relationships between exposure to particular toxic substances and development of disease, in assessing the outcome of tobacco dependence treatment programs, and in assessing the risks of potentially less harmful or non-addictive tobacco products.

Self-report measures in smokers, such as cigarettes smoked per day, are highly imprecise owing to individual differences in how cigarettes are smoked, with ranges of nicotine intake per cigarette from 0.3 to 3.0 mg. Self-report measures, such as hours per day exposed to environmental tobacco smoke (ETS) by nonsmokers, are also likely to be imprecise indicators of

intake of tobacco smoke owing to variations in the number of cigarettes smoked, proximity of non-smokers to smokers, room ventilation and other environmental characteristics, as well as individual differences in sensitivity to and/or concern about adverse effects of ETS. The optimal assessment of exposure to tobacco smoke would be by analysis of the concentrations of a component of smoke in the body fluids of an exposed individual-i.e., a biologic marker or biomarker [10].

One cigarette contains an average of 8.4 mg of nicotine. When tobacco is burned, nicotine is aerosolized into tar droplets that deliver 1.6 mg of nicotine per cigarette. The hydrogen ion concentration (pH) of tobacco determines how much free base is delivered. The flue-cured tobaccos used in cigarettes are acidic (pH 5.5), whereas the air-dried tobaccos used in pipe and cigar tobaccos are alkaline (pH 8.5). Burned alkaline tobacco products yield higher free-base nicotine concentrations in smoke compared with acidic tobacco products [11,12].

Factors influencing nicotine metabolism can include genetic variation, race, gender, oral contraceptive use or other oestrogen-containing hormones, kidney failure and drugs, including anticonvulsants and rifampin [13]. Cotinine concentrations in biofluids and nicotine in hair are generally higher in infants and children, compared to SHS-exposed adults; this is probably due to greater inhaled nicotine doses (closer proximity to smokers and higher minute ventilation per body mass) and slower cotinine metabolism [14].

A widely used approach for measuring exposure is determination of tobacco derived biomarkers in biologic fluids [15-17]. In this regard, the nicotine metabolite cotinine is the most widely used, and has excellent specificity for both active use of tobacco and for secondhand smoke exposure [10,15,18], except in individuals using nicotine-containing medications [16]. Cotinine concentrations have been determined in a variety of biological matrices, including plasma, serum, urine, saliva, hair, and nails [19-23]. Saliva concentrations are highly correlated with plasma concentrations [24,25], and since obtaining saliva does not require venipuncture, saliva is the preferred biofluid for many studies. Urine concentrations are generally much higher than those in plasma or saliva [26], and for this reason urine analyses can provide greater sensitivity for assessing low level exposure.

Nicotine can be measured in various biological specimens including plasma, saliva, and urine [27]. Its specificity for tobacco use is excellent except for persons using nicotine-containing medications. There are dietary sources of nicotine, but they are insignificant compared to tobacco use [28]. Nicotine concentrations are moderately expensive to measure, and a variety of methods are applicable, including gas chromatography [29], high performance liquid chromatography [30] and immunoassays [31].

Plasma levels, especially taken in the afternoon of a smoking day, correlate well with nicotine intake and may be used to estimate the extent of tobacco use [32]. Urine levels also correlate fairly well with nicotine intake [33,34]. Because of the short half-life of nicotine (about 2 hours) [35,36]. nicotine levels are not useful in assessing tobacco use that occurred more than 8–12 hours previously. Thus, the present study was conducted to estimate the cotinine level excretion in urine among smoked and smokeless tobacco users and nonsmokers among the Indian population.

Materials and methods

Study setting

The study was conducted over a period of one year from March 2015 to March 2016 at oberoi dental clinic and orthodontic centre, New Delhi. The study sample consisted of 300 subjects who were apparently healthy, asymptomatic and not using any drug. The study sample included Cigarette smokers (50), Bidi smokers (50), smokeless tobacco users (50), both smoked and smokeless tobacco users (50) and controls – non-users of tobacco (50).

Sample collection

The individual was instructed to collect 24 hours urine in a clean glass bottle. Urine collection was started at 8 am in the morning after passing and discarding the first urine and collecting the whole urine till 8 am of the next morning. The total volume was noted and after mixing the urine properly, the sample was taken for testing.

Inclusion and exclusion criteria

The study sample consisted of the healthy subjects in the age group of 30-45 years.

The subjects with any existing systemic conditions were excluded which can affect the metabolism of nicotine.

Analysis of the sample

Gas choromatography (GC) assay was used to estimate the cotinine levels [13,14]. For extraction, 1 ml of urine sample was taken and added to 1 ml of trichloacetic acid (TCA), kept in vortex for 30 seconds and the mixture was centrifuged at 1100 g (10-20 minutes). The supernatant was transferred to another tube. To the supernatant, 0.5 ml of KOH and 6 ml of dichloro methane (DCM) were added, shaken in a water bath for 30 seconds, followed by centrifugation at 1100 g for 10 minutes.

In the upper layer, 3 ml of HCl (50 mmol) was added and was shaken for 30 seconds followed by centrifugation. To the upper layer, 0.5 ml KOH and 5 ml of DCM were added and shaken for 30 seconds, and centrifuged again. To the upper layer, 200 µl of methanolic HCl was added and dried under N2 gas, 30 µl of it was injected in the HPLC column and values of cotinine were read at the wavelength of 256 and 262 nm respectively. The assay was performed using reversed

phase C-18 ion pair column in an isocratic mode. The GC unit consisted of a pump (model 510, Waters, India), a variable-wavelength ultraviolet detector (model 481, Waters, India) with a deuterium lamp. We used a 15 x 0.2 cm column of ODS Hypersil, 3 µm particle size, from Shandon Inc., Pittsburgh, PA, an injector with a 200 µl loop. Mobile phase used was a mixture of citrate and dibasic phosphate (30 mmol of each/litre) containing 1 mmol of sodium heptanesulphonate and 50 ml of acetonitrite per litre (pH 6.1). The flow rate of the mobile phase was 0.3 ml/min and the column pressure was 3000 psi. Respective nicotine and cotinine standards (Sigma, USA) were used (20 nmol/200 µl methanol).

Statistical analysis

The data was tabulated and entered into the Microsoft excel. The data was analyzed using the SPSS version 21.0. The statistical test used was one-way ANOVA test with post-hoc Tukey HSD test for inter-group comparisons. The *p* - value was taken significant when less than 0.05 ($p < 0.05$) and Confidence interval of 95% was taken.

Results

The study population consisted of the male subjects only and smoked tobacco group had cigarette smokers only with mean age of the study population being 38.91 ± 2.89.

The mean cotinine levels among smoked tobacco users was 39.84 ± 3.01, smokeless tobacco users was 28.91 ± 2.01, both smokeless and smoked tobacco users was 51.11 ± 4.62 and non-smokers was 3.71 ± 0.63. The mean cotinine level in urine was significantly *(p* – value < 0.05*)* more among both smoked and smokeless tobacco users in comparison to smoked tobacco users which was significantly more than smokeless tobacco users which was significantly more than non-users of tobacco (Table 1).

Table 1: Urinary Cotinine values among Cigarette smokers, Cigarette chewers, Bidi chewers, Both Tobacco Smokers and Chewers and Non-smokers.

		Cotinine levels (ng/dl)	
Type of tobacco use		**Mean**	**S.D.**
1.	Cigarette smokers	56.84	3.01
2.	Bidi smokers	42.98	4.09
3.	Tobacco chewers	28.91	2.18
4.	Both Tobacco Smokers and Chewers	51.11	7.62
5.	Non-smokers	3.71	0.63
ANOVA (*F* - value)		1234.677	
p - value[a]		< 0.001*	
Post-hoc comparisons[b]	1 *vs.* 2	< 0.001*	
	1 *vs.* 3	< 0.001*	
	1 *vs.* 4	< 0.001*	
	1 *vs.* 5	< 0.001*	
	2 *vs.* 3	< 0.001*	
	2 *vs.* 4	< 0.001*	
	2 *vs.* 5	< 0.001*	
	3 *vs.* 4	< 0.001*	
	3 *vs.* 5	< 0.001*	
	4 *vs.* 5	< 0.001*	

[a]One-way ANOVA test
[b]Post-hoc tukey test
**p* - value is significant at < 0.001 level

Discussion

Nicotine and cotinine levels have earlier been used to validate the smoking status of an individual [28,29]. These biomarkers have also been used in epidemiological studies [30-33], to assess the effects of tobacco use on human health [34,35], as measures to estimate the exposure to environmental tobacco smoking, and for assessment of the efficacy of interventional methods on cessation of smoking [36].

The half-life of nicotine averages about 2-3 hours. With intermittent exposure of tobacco, nicotine levels in the body rise and fall throughout the day. The half-life of cotinine averages about 17 hours. Because of the longer half-life, cotinine levels tend to build up throughout the day, and cotinine is eliminated over a much longer period of time compared with nicotine. With intermittent nicotine exposure such as occurs with cigarette smoking, cotinine levels remain relatively constant throughout the day and remain at near steady-state values [10].

The presence of cotinine in a biologic fluid indicates exposure to nicotine. There is some individual variation in the quantitative relation between cotinine levels in the blood (or saliva or urine) and the intake of nicotine. This is because different people convert different percentages of nicotine to cotinine (usual range 55% - 92%) and because different people metabolize cotinine at different rates (usual range of cotinine clearance, 19-75 ml/min) [37].

Considerable between-individual variability exists in the rate and pattern of nicotine metabolism, possibly affecting cotinine concentration resulting from a given nicotine exposure. Factors influencing nicotine metabolism can include genetic variation, race, gender, oral contraceptive use or other oestrogen-containing hormones, kidney failure and drugs, including anticonvulsants and rifampin [13]. The advantages of determining cotinine concentrations in urine are that cotinine concentrations and other metabolites are higher than in other biological fluids; it represents relatively acute exposure; and collection is non-invasive [38].

In the present study, the mean cotinine level in urine was significantly (p – value < 0.05) more among smokers in comparison to smokeless tobacco users and non-users of tobacco. Whereas the mean cotinine level in urine was significantly (p – value < 0.05) more among smokeless tobacco users in comparison to non-users of tobacco. This was similar to the study by Jacob III, et al. [16] in which, mean nicotine concentrations were significantly (p – value < 0.05) lower in smokeless tobacco users than in cigarette smokers. Mean cotinine concentrations ranged from 1790 to 2420 ng/ml, and were significantly higher among smokeless tobacco users than in smokers. The ratio of nicotine:cotinine in urine of smokeless tobacco users (subjects from studies 1 and 2 combined) and cigarette smokers averaged 0.67 and 1.24, respectively. The difference between the two groups was significant.

The lower ratio of nicotine, cotinine in smokeless tobacco users (0.67) compared with smokers (1.24) is most likely a result of more nicotine being swallowed by smokeless tobacco users, which then undergoes presystemic metabolism to cotinine in the liver [39].

Whereas in the study by Behera, et al. [40] bidi smokers had a lower value of urinary nicotine excretion (cotinine levels) than that observed in tobacco chewers whereas the cigarette smokers had more than the tobacco chewers. In the present study, no differentiation was made between the cigarette and bidi smokers.

In the present study, the mean Cotinine level in urine was significantly (p – value < 0.05) more among patients with both chewing and smoking habit in comparison to the smokers only, smokeless tobacco users only and Non-smokers. The similar findings were also reported in the study by Behera, et al. [40].

The mean cotinine levels among smoked tobacco users was 37.92 ± 2.35. Whereas Macaron, et al. [41] reported that urinary levels of cotinine for the smokers of cigarette (median 30 cigarettes per day) and narguila (median 2 pipes per day or around 40 g of tobacco).

In the study by Chen, et al. [42] cigar smokers had higher cotinine, NNAL, and lead concentrations than nontobacco users. The geometric mean concentration 95% confidence interval (CI) of cotinine for primary cigar smokers (i.e., current cigar/never cigarette smokers) was 6.2 (4.2-9.2) ng/mL *vs.* 0.045 (0.043-0.048) ng/mL for non-tobacco users, and the NNAL concentration was 19.1 (10.6-34.3) pg/mg creatinine for primary cigar smokers versus 1.01 (0.95-1.07) pg/mg creatinine for non-tobacco users.

However, Blackford, et al. [43] studied the quantitative relationship between number of cigarettes consumed and level of salivary cotinine, a biomarker of nicotine dose in China, Brazil, Mexico and Poland. Overall, saliva cotinine concentrations averaged approximately 200 ng/mL across countries, with Poland having the highest and Mexico the lowest median concentration. This median cotinine value is similar to that seen in smokers in the United States and the United Kingdom [44,45]. In all countries, the level of salivary cotinine increased with increasing numbers of cigarettes smoked, up to approximately 20 cigarettes per day, and from that number, either the smoothed curve flattened or the slope dropped. The most likely explanation for this observation is that smokers titrate to their intake of nicotine, with an average preferred intake for heavy smokers corresponding to approximately 200 ng/mL cotinine. At lower levels of cigarette consumption (up to 20 per day), the slope of the saliva cotinine per cigarette smoked curves averaged approximately 11.3 ng/mL per cigarette.

Age variation may not be an attributable factor for the variation seen. The adverse effects of passive smoke exposure on the respiratory tract are well established [34,46]. One of the most frequently used biomarkers for exposure to environmental tobacco smoke is cotinine in body fluids [33].

In the study by Benowitz, et al. [47], among a large, nationally representative group of US smokers and nonsmokers indicated that the optimal overall cutpoint for minimizing the rate of misclassification of self-reported smoking status is a serum cotinine concentration of 3 ng/mL. This cutpoint had a high degree of sensitivity and specificity for adults, giving it excellent discriminative ability. The cutpoint was similar, though sensitivity was substantially lower, in adolescents. This was most likely due to a higher prevalence of occasional smoking and potentially more underreporting of smoking among adolescents. However, this represents a large change from the value of 14 ng/mL determined by Jarvis, et al. [26].

Saliva and plasma cotinine concentrations are quite similar, so the optimal cutpoint in saliva would be the same as that recommended for blood [15]. Urinary cotinine concentrations based on unconjugated cotinine alone are approximately 5 times those of plasma cotinine [11]. Therefore, a urinary cotinine concentration of 15 ng/mL would be the appropriate cutpoint corresponding to our serum estimate. This level contrasts with urinary cutpoints proposed in various other studies ranging from 20 ng/mL to 550 ng/mL [48].

Conclusion

Urine cotinine was our main criterion for assessing the validity of self reported exposure to tobacco use. However, cotinine level is influenced by factors independent of exposure to cigarette smoke, including metabolism, imprecision in laboratory measurement, and the hour of the day when urine is collected. Nicotine can be found in food, but at usual levels of food consumption, nicotine intake from food is trivial [10]. A single spot evaluation of cotinine level may not reflect its long term average [49], which may attenuate associations with self reported measures of exposure to smoke. Finally, only a minority of participants provided a urine sample, but smoking related variables were similar in those who provided and did not provide a urine sample. Further studies of self reported exposure to tobacco use could use other indicators, such as expired carbon monoxide, or more specific markers such as anatabine or anabasine [50].

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