Urinary levels of nicotine & cotinine in tobacco users

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Background & objectives: Of the various biochemical markers used to validate the smoking status of a person, nicotine and cotinine are considered as good markers for both active and passive smoking. In the present study an attempt was made to estimate urinary levels of nicotine and cotinine in healthy individuals from north India using different types of tobacco to identify and validate the smoking status.

Methods: Twenty four hour urine sample of 130 healthy volunteers (smokers=70, passive smokers=20, tobacco chewers=20, non smokers=20) were analyzed by high-pressure liquid chromatography (HPLC) assay. Smokers were divided into different groups, viz., cigarette, bidi and hooka smokers.

Results: The mean values of nicotine (ng/ml) and cotinine (ng/ml) in urine were highest in cigarette smokers (nicotine=703.50±304.34; cotinine=2736.20±983.29), followed by hooka smokers (nicotine 548.0±103.47 and cotinine 2379.0±424.25), and bidi smokers (nicotine=268.53±97.62, cotinine=562.60±249.38). There was no correlation of nicotine or cotinine values with smoking index. In passive smokers (nicotine=109.75±22.33, cotinine=280.75±86.30) and in nonsmokers, the values were much lower (nicotine=55.00±13.71, cotinine=7.30±2.47) compared to smokers. In tobacco chewers, the values for nicotine and cotinine were 447.75±145.09 and 2178.30±334.29 respectively.

Interpretation & conclusion: All forms of tobacco users had significantly higher values compared to passive smokers and nonusers. Thus, cotinine and nicotine levels in urine may be considered as good indicators to assess the exposure to tobacco in our population.

Key words Cotinine - nicotine - smokers - tobacco

In India, tobacco is consumed both in smoking and non-smoking forms. Smoking forms include cigarette, bidi, hooka and chutta (a reverse form of smoking in which smoking is done with the burning end inside the mouth); tobacco chewing is the main non-smoking 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 cotinine2, which could lead to an underestimation of the effect of smoking on the course of disease and could prejudicially affect decisions on patient management.

A number of biochemical markers like thiocynate, nicotine, cotinine and carbon monoxide in the expired air and carboxyhaemoglobin in blood have been used to validate claims of non-smoking3,4. Levels of thiocynate...
and carbon monoxide/carboxyhaemoglobin 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.

Cotinine is a major metabolite of nicotine but its level in the blood is not a good marker of nicotine content of blood. In contrast, urinary excretion of cotinine is a good marker as it is less influenced by the flow of urine and pH. For study of nicotine and cotinine levels, it is preferable to have non-invasive methods. The choice of body fluids for cotinine assay in smoking studies should depend on practical rather than pharmokinetics considerations. Cotinine, which is a major metabolite of nicotine is stable in body fluids, has a long half-life, low plasma protein binding (2.6%), and dose independent disposition kinetics. These factors make cotinine a good marker for estimating both active and passive exposure to tobacco smoke.

Although information about the continine and nicotine values is available for cigarette smokers from other ethnic groups, such information in the bidi and hooka smokers and tobacco chewers is not available. In the present study, we estimated the amount of nicotine and cotinine excretion in urine in a group of healthy individuals from north India who were users of tobacco in different forms and also in nonsmokers and compared the values amongst different groups of tobacco users.

Material & Methods

The study was conducted over a period of one year (1999-2000) in the Department of Pulmonary Medicine, Postgraduate Institute of Medical Education & Research, Chandigarh and included 130 healthy north Indian volunteers of either sex who were further divided into tobacco user and non-tobacco user groups. The user group included cigarette smokers (30), bidi smokers (30), hooka smokers (10) and tobacco chewers (20). The other group had passive smokers (20) and 20 nonsmokers who had not been exposed to the tobacco smoke or had not ever chewed tobacco. All the 130 volunteers were attendants of the patients, and were apparently healthy, asymptomatic and not using any drug. A smoker was a person who smoked one cigarette or one bidi/hooka smoking per day for at least one year and the tobacco chewer was one who was chewing tobacco for at least one year in most of the days. A passive smoker was one whose family members were smokers. A nonsmoker was a person who had never been exposed to the tobacco smoke either actively or passively in the home or place of work at least one week prior to the study. Smoking index was calculated as number of bidi/cigarettes smoked per day x number of years of smoking. Quantification of hooka smoking and tobacco chewing was not possible.

The individual was instructed to collect 24 h 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.

Source of chemicals/reagents: Chemicals used in the study were procured from Sigma Chemical Company, USA. For HPLC, HPLC grade chemicals were procured from Ranbaxy Laboratories Ltd., India.

High pressure liquid chromatography (HPLC) assay was used to estimate the cotinine and nicotine levels. 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 min). 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 1100g for 10 min. 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 nicotine and 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 HPLC 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 acetonitrile 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).

Nonparametric methods were used for group comparisons, using Kruskal-Wallis H test for multiple independent group and Mann Whitney U test for two independent groups. Correlations between nicotine and cotinine values were assessed using Pearson’s correlation coefficient. Linear regression analysis was performed to assess if cumulative smoking index was significantly associated with nicotine and/or cotinine levels. For all statistical procedures, significance was assessed at \( P < 0.05 \).

**Results**

All smokers and tobacco chewers were males, their age range 25-65 yr. In the group of passive smokers, there were 19 females (22-40yr) and one male (40yr). There were 8 females (26-50 yr) and 12 males (20-60yr) in the non smokers group. Nicotine and cotinine excretion in urine was maximum in cigarette smokers followed by **hooka** smokers, tobacco chewers, **bidi** smokers and passive smokers (Table). The amount was negligible in non smokers. The smoking index in cigarette smokers was found to be 90±39.39 and the same for **bidi** smoker was 172±69.25. No correlation could be established with smoking index in both cigarette and **bidi** smokers as there was a wide scatter of values (\( R^2 = 0.002 \) for nicotine and 0.137 for cotinine, for both groups of smokers).

Correlation of nicotine to cotinine was statistically significant in cigarette smokers (\( r=0.5501, \ P<0.001 \)), **hooka** smokers (\( r=0.6836, \ P<0.05 \)), passive smokers (\( r=0.5914, \ P<0.01 \)) and non smokers (\( r=0.5077, \ P<0.05 \)). However, it was not significant in **bidi** smokers (\( r=0.1459, \ P>0.05 \)) and in tobacco chewers (\( r=-0.409, \ P>0.05 \)) a non-significant negative correlation was obtained.

**Discussion**

Nicotine and cotinine levels have earlier been used to validate the smoking status of an individual\(^{15,16}\). These

**Table.** Urinary nicotine & cotinine values in different groups of tobacco users  
(Data are mean ± SD)

<table>
<thead>
<tr>
<th>Type of tobacco users (N)</th>
<th>Age (yr) (Range)</th>
<th>Nicotine (ng/ml) (Range)</th>
<th>Cotinine (ng/ml) (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smokers (30)</td>
<td>36.93±9.15</td>
<td>703.50±304.34</td>
<td>2736.20±983.29</td>
</tr>
<tr>
<td>Bidi smokers (30)</td>
<td>36.86±7.44</td>
<td>268.53±97.62**</td>
<td>562.60±249.38**</td>
</tr>
<tr>
<td>Hooka smokers (10)</td>
<td>58.5±5.31</td>
<td>548.00±103.47</td>
<td>2379.00±424.25*</td>
</tr>
<tr>
<td>Tobacco chewers (20)</td>
<td>35.65±8.67</td>
<td>445.75±145.09**</td>
<td>2178.30±334.29*</td>
</tr>
<tr>
<td>Passive smokers (20)</td>
<td>34.05±7.16</td>
<td>109.75±22.33*†</td>
<td>280.75±86.30**†</td>
</tr>
<tr>
<td>Non smokers (20)</td>
<td>34.2±11.49</td>
<td>55.00±13.71</td>
<td>7.30±2.47**†</td>
</tr>
</tbody>
</table>

\( P<0.01, \; **<0.001 \) compared to cigarette smokers  
\( *P<0.001 \) compared to **bidi** smokers  
\( **P<0.01 \) compared to **hooka** smokers  
\( †P<0.001 \) compared to tobacco smokers  
\( ††P<0.001 \) compared to passive smokers (Mann Whitney U test)
biomarkers have also been used in epidemiological studies to assess the effects of tobacco use on human health, as measures to estimate the exposure to environmental tobacco smoking, and for assessment of the efficacy of interventional methods on cessation of smoking. While studies on nicotine and cotinine levels in cigarette smokers as well as those for passive smoking in other ethnic groups are well documented, information on bidi, hooka and tobacco used in non-smoking forms (tobacco chewing) is lacking. This is because of the fact that these peculiar forms of tobacco use are confined to certain parts of the world only. Other forms of smokeless tobacco use include snus (Snuff) in Sweden, and toombak by Sudanese people. Hooka smoking prevalent in parts of India and Pakistan, is akin to the water-pipes (narguila) used mostly in Middle-East smoking prevalent in parts of India and Pakistan, is akin to the water-pipes (narguila) used mostly in Middle-East countries. Macaron et al reported similar 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 present study, hooka smokers has lower values for both nicotine and cotinine compared to cigarette smokers. It is possible that use of water pipes, through which the smoke passes, removes some amount of nicotine. Oral intake of tobacco by chewing also increased the excretion as nicotine can be absorbed from oral mucosa. Bidi smokers had a lower value of urinary nicotine excretion than that observed in tobacco chewers and hooka smokers, though was significantly higher compared to non smokers as well as passive smokers. Bidi contains larger amount of nicotine compared to cigarette when compared for g to g. Though the bidi smokers have higher smoking index, the lower values of nicotine and cotinine may be explained by the fact that one cigarette is not equivalent to one bidi, thus the nicotine intake in terms of g will be different in both groups. Other variables like individual variation, and dietary intake of nicotine may also influence the values of nicotine and cotinine excretion, though we have not tried to find out these factors in the present study. The mean age of individuals in all the groups was comparable, except in hooka smokers because persons with this form of smoking are usually older and at present, younger population does not prefer this form of tobacco use. There is no effect of age or sex on the nicotine or cotinine excretion. Thus, the 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. One of the most frequently used biomarkers for exposure to environmental tobacco smoke is cotinine in body fluids. We found higher values of urinary nicotine and cotinine in passive smokers compared to non smokers in the present study. Our results indicate that both nicotine and cotinine may be useful markers for identifying and validating the smoking status in Indian population.

Thus, we conclude that nicotine and cotinine levels in urine may be useful markers to assess the effect of different types of tobacco use in our population.

References


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