SALIVARY COTININE - BIOMARKER OF TOBACCO CONSUMPTION IN THE ASSESSMENT OF PASSIVE SMOKING PREVALENCE

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Abstract

Existing evidence suggests there is an association between the salivary cotinine levels and passive smoking. The aim of this study was the objectively evaluation of the passive smoking prevalence in non-smoking adults from Constanta, Romania, based on their salivary cotinine levels measured by NicAlert™ Saliva tests. In a cross-sectional study made on 286 subjects, the levels of salivary cotinine were measured, together with the evaluation of the self-reported smoking status on a questionnaire basis. After analysis of the salivary cotinine, only 16.4% of all subjects were not exposed to tobacco products. Based on the self-reported smoking status (questionnaire), 44.06% of the subjects (n=126) were classified as active smokers (constant and occasional) and 55.94% (n=160) as non-smokers.

Using the salivary cotinine levels as standard, the real distribution of the subjects according to their smoker status comprised 44.06% active smokers (constant and occasional), 16.43% (n=47) non-smokers (non-exposed to tobacco smoke) and 39.50% (n=113) passive smokers.

Rezumat

Dovezile științifice existente sugerează prezența unei asocieri între nivelurile salivare de cotinina și consumul de tutun. Scopul studiului prezent a fost evaluarea obiectivă a fumatului pasiv la adulții nefumatori din Constanța, pe baza măsurării nivelurilor salivare de cotinina, utilizând testele NicAlert™ Saliva. Într-un studiu încrucișat realizat pe 286 subiecți au fost măsurate nivelurile salivare de cotinina și s-a evaluat status-ul declarat de fumător pe bază de chestionar. Pe baza auto-evaluării status-ului de fumător (chestionar), 44,06% dintre subiecți (n=126) au fost clasificați drept fumatouri activi (constanți și ocazionali), iar 55,94% (n=160) drept nefumatitori.

Utilizând nivelele de cotinina salivară ca standard, distribuția reală a subiecților, în funcție de status-ul de fumător, cuprinde 44,06% fumatouri activi (constanți și ocazionali), 16,43% (n=47) nefumatouri (neexpuși fumului de tutun) și 39,50% (n=113) fumatouri pasivi.
Keywords: tobacco consumption, cotinine, saliva, passive smoker

Introduction

The term "smoking" refers to active smoking behavior, the intentional inhalation of tobacco smoke by a smoker. Smoking does not refer to or include "passive smoking", the unintentional inhalation by non-smokers of tobacco smoke introduced into the atmosphere by smokers. Between 2000 and 2010, the number of smokers, at a global level, reached 1.4 billions, but almost everyone is a "passive" smoker at some time [33].

The term "passive smoking" was coined in the 1970s [17, 21]; till then, the evidence linking environmental tobacco smoke with impairment and illness continues to grow, and considerable work has been undertaken to identify ways of objectively measuring the extent of tobacco smoke exposure in non-smokers. Of these, measurement of nicotine and cotinine levels in the body have received the most attention.

Cotinine is the major metabolite resulted from nicotine \(\text{C}_{10}\text{H}_{14}\text{N}_2 - \alpha-(\beta`\text{-pyridine})-\text{N-methyl-pyrrolidinyl}\) metabolism [22, 23] by cytochrome P450 enzymes system (mostly CYP2A6, and also by CYP2B6 in the liver) [1]. Cytochrome P450 2A6 is a member of the P450 2A subfamily (belonging to the superfamily of cytochrome P450s). It has important roles in the metabolism of xenobiotics and metabolically activates various precarcinogens including NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone], the potent lung carcinogen. CYP2A6 is the allele encoding for cytochrome P450 2A6, and is a highly polymorphic gene. The extreme variation between individual ability to metabolize drugs, including nicotine, is due largely to the variability of the CYP2A6 allele [31].

Most of the nicotine absorbed by the body from cigarettes is metabolized by 5'-hydroxylation pathway (Figure 1), as follows: 1) Nicotine is hydroxylated at the 5' position, yielding an unstable intermediate 5'-hydroxynicotine in equilibrium with Delta\(^{1(5)}\)iminium ion. This first step is catalyzed by cytochrome P450 2A6, with cytochromes P450 2B6 and P450 2D6 also contributing to the hydroxylation. 2) 5'-Hydroxynicotine is then oxidized to cotinine, the major nicotine metabolite, by aldehyde oxidase. 3) Cotinine can then be converted either to cotinine-Gluc \(\text{(via a detoxification mechanism)}\) or trans-3'-hydroxycotinine. This compound can also be detoxified to trans-3'-hydroxycotinine-Gluc [20].
Figure 1
The 5' hydroxylation metabolism pathway of nicotine to cotinine
[Hecht, 2000]

In humans, cotinine and its metabolites represent 70-80% of the metabolism products resulted from the nicotine absorbed by a smoker, which will be excreted mainly by the kidneys, but also by perspiration, maternal milk and oral fluids [7].

Cotinine has become increasingly accepted as a short-term marker of nicotine exposure because of its relatively long half-life (approximately 20 hours, comparing with 2 hours for nicotine). It is also less susceptible to fluctuations during exposure to tobacco smoke and can be conveniently measured in blood, urine and saliva. Cotinine measurements from human fluids can provide an assessment of recent exposure to environmental tobacco smoke, but they do not indicate the duration of exposure nor do they indicate the intake of other components of tobacco smoke [12, 18].
Cotinine from body fluids is considered to be the marker of choice for the absorption of tobacco smoke; it has been isolated in plasma [3, 16], urine [3, 8], saliva [8, 9] and gingival crevicular fluid [6, 28]. The type of specimen and method of collection may influence the detected levels of cotinine. At the salivary level, the cotinine concentrations are significantly greater in unstimulated than in stimulated saliva, the differences being explained by changes in pH with alterations in flow rate; the transfer of cotinine from plasma to saliva is usually via passive diffusion and the pH of saliva is an important influence factor [33]. When evaluated by quantitative (ELISA analysis, gas-chromatography, high-performance liquid chromatography, etc.) or semi-quantitative methods (reagent-impregnated test strips), the cotinine level from unstimulated saliva is one of the most specific and sensitive biomarkers of tobacco exposure, giving the same information about cotinine disposition in the body and also about nicotine intake as plasma levels [7]. The salivary cotinine is correlated with recent nicotine exposure (3-4 days), smoking status (active constant or occasional smoker, passive smoker, non-smoker) [3, 14, 30] and the plasma and urinary cotinine levels [12].

Considering this background, the aim of this study was the objectively evaluation of the passive smoking prevalence in 35-44 years old nonsmoking adults from Constanta, Romania, based on salivary cotinine levels measured by NicAlert™ Saliva test strip.

Materials and Methods

In a cross-sectional study of 286 participants living in Constanta District, Romania (mean age 40.3±3.5 years old, 61.9% females), the salivary cotinine (objective biomarker of smoking status) levels were measured, together with the evaluation of the self-reported smoking status on a questionnaire basis.

Study population and sample: The study population was represented by adults aged between 35 and 44 years from Constanta District, Romania. The initial representative sample was chosen using a stratified multistadial sampling design and the data of the Regional Office of the National Institute of Statistics [29]. The initial required sample was 379, calculated for 95% C.I. (confidence interval), 5% sampling error and 50% estimated level of smokers in the targeted population.

Ethics approval: Ethical permission to conduct the study was given by the Professional Ethical Committee of Ovidius University, Constanta, Romania. Free-written informed consent was obtained from all the participants to the study; the consent was free, the participation was
optional, and the time for thinking (in order to express the consent or refusal) was 48 hours.

**Generation and collection of the tobacco consumption data (the questionnaire):** A questionnaire was designed in order to collect information regarding the tobacco consumption in the study group. It has been pilot tested before its use in this study. The questionnaire was self-administered and answered in the same appointment with the saliva collection. Information on the following items was obtained by the questionnaire: demographic data (age, gender), the number of cigarettes smoked daily (on average), the number of cigarettes smoked in the last 24 hours, the use of other forms of tobacco (cigars, pipe, chewing tobacco) and/or nicotine replacement therapy (NRT), employment-related tobacco exposure (i.e., handling tobacco). Subjects who were reported being nonsmokers of cigarettes but who reported the consumption of tobacco products by other means (such as chewing) were excluded; the subjects with work-related tobacco exposure were also excluded.

Using the answers of the questionnaire as a gold standard of the smoking status, the subjects were divided in three categories, as follows: current constant smokers (at least one cigarette/day and at least 100 cigarettes in his/her lifetime [5, 27]; current occasionally smokers (at least five cigarettes/week and at least one cigarette in the previous 7 days) [5]; nonsmokers (no smoking at least 14 days prior to the salivary sample collection) [4].

**Generation and collection of the saliva:** Generation and collection of the total unstimulated saliva samples were performed using a standard method, compatible with the analysis of biomarkers, namely passive collection in sterile containers (funnel, cap and tube container) provided by NicAlert™ Saliva kit [24]. The tube containers with the saliva samples were then stored at -30°C before the analysis of the cotinine level (saliva is stable at this temperature for biomarkers evaluation for minimum 3 months [26]).

**Evaluation of the salivary cotinine level:** The salivary cotinine level was evaluated using NicAlert™ Saliva strip tests (NYMOX Pharmaceutical Corporation, QC Canada, Figure 2).
NicAlert Saliva test strip zones range from zone 0 (0-10 ng/mL) to zone 6 (>1000 ng/mL). The cutoff concentration for the NicAlert™ test (an immunochromatographic assay using a monoclonal antibody), indicating a positive result, was 10 ng/mL (zones 1-6). The salivary cotinine concentration and its interpretation for each level of the NicAlert™ are shown in Table I.

Table I
Cotinine concentration and its interpretation for each level of the NicAlert™ test

<table>
<thead>
<tr>
<th>Level</th>
<th>Cotinine Concentration (ng/mL)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 - 10</td>
<td>non-smokers</td>
</tr>
<tr>
<td>1</td>
<td>10 - 30</td>
<td>occasional active smokers</td>
</tr>
<tr>
<td>2</td>
<td>30 - 100</td>
<td>constant active smokers</td>
</tr>
<tr>
<td>3</td>
<td>100 - 200</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>200 - 500</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>500 - 1000</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&gt; 1000</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analyses: All data were analysed using SPSS 12 software for Windows; kappa statistics, descriptive statistics, Chi-square tests and Spearman correlation were used.

Results and Discussion

The kappa value for test-retest of the questionnaire was 0.96 (perfect agreement). The response rate was 77.30% (86 subjects refused to take part to the study). After 7 subjects were excluded (tobacco products sellers), the final study comprised 286 subjects (6% sampling error; 95% C.I.), with a mean age of 484.01±41.80 months, 38.11% males (n=109) and 61.88% females (n=177).
The analysis of answers to the questions regarding tobacco consumption led to the distribution of subjects into three groups (Table II): current constant smokers 116 (40.56%), current occasional smokers 10 (3.50%) and nonsmokers 160 (55.94%).

Table II
The distribution of the subjects by their self-reported smoker status

<table>
<thead>
<tr>
<th>Self-reported smoker status</th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current constant smokers</td>
<td>116</td>
<td>40.56</td>
</tr>
<tr>
<td>Current occasional smokers</td>
<td>10</td>
<td>3.50</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>160</td>
<td>55.94</td>
</tr>
<tr>
<td>Total</td>
<td>286</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The distribution of the self-reported smoker’s status according to the gender showed significant differences among smoker categories (p<0.05), the percent of smokers being 38.98% for females and 52.29% for males.

Figure 3
The salivary cotinine levels in the study population
The salivary cotinine levels found in the whole study population are shown in Figure 3. The salivary cotinine level most frequently found was level 1 (10-30 ng/mL saliva); 16.43% of all subjects were not exposed to tobacco smoke.

The analysis of the possible relation between the individual’s cotinine levels and their self-reported smoking status showed a significant association between these two variables.

The distribution of the salivary cotinine levels in the self-reported non-smokers (Figure 4) showed that only 29.38% (n=47) of these subjects had a 0 level of cotinine in saliva, the others having a level 1 (61.88%) or even 2 (8.75%).

![Figure 4](image)

The salivary cotinine levels in the self-reported non-smokers

The distribution of the cotinine levels in the self-reported non-smokers showed significant differences according to the gender, with higher values in females than in males (p<0.05).

Using the salivary cotinine levels as standard, the real distribution of the study subjects by their smoker status comprised 44.06% current smokers
(constant and occasionally), 39.50% passive smokers and only 16.43% nonsmokers/not exposed to tobacco smoke (Table III).

Table III
The distribution of the study subjects by their real smoker status (based on the salivary cotinine levels)

<table>
<thead>
<tr>
<th>Real smoker status</th>
<th>Frequency (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smokers</td>
<td>126</td>
<td>44.1</td>
</tr>
<tr>
<td>Passive smokers</td>
<td>113</td>
<td>39.5</td>
</tr>
<tr>
<td>Nonsmokers/not exposed to tobacco smoke</td>
<td>47</td>
<td>16.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>286</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

A precise estimation of exposure to tobacco smoke is an important concern for the epidemiologic studies [9]. Assessment of passive exposure to tobacco smoke is even more problematic as self-reporting of the smoking habits and of the hours per day when an individual is exposed to tobacco smoke are not an accurate measurement of this parameter [2, 11, 25].

In this context, the optimal assessment for quantitating the recent exposure of an individual to cigarette smoke is the analysis of cotinine in the human body fluids (blood, urine or saliva) [2, 13, 24]. Even if there are individual variations in the quantitative relation between cotinine levels in the body fluids and the intake of nicotine, because of individual’s differences in converting nicotine to cotinine (usual range 55-92%) and in the rates of cotinine metabolism (usual range of cotinine clearance, 19-75 mL/min.) [19], the presence of cotinine in a biologic fluid surely indicates the exposure to nicotine [2].

In the present study we used the questionnaires as the standard for the initial classification of the subjects in smokers (constant and occasional) and nonsmokers. Following the analysis of the answers, 44.06% of the subjects were classified as current smokers (constant and occasional) and 55.94% were classified as nonsmokers. The total percent of active smokers (44.06%) is a little higher than the percent reported by the WHO (World Health Organisation) for the adult population from our country in 2000 (43.5%); comparing with the WHO data, the prevalence of smoking habit decreased in males and increased in females, also in agreement with the WHO predictions for 2000-2010 [35].

After the initial classification of the subjects, the NicAlert Saliva Test Strips (Nymox) were used for the objective assessment of the passive smoking prevalence. The results of the present study showed that only approximately 1/3 of the subjects that were reported as nonsmokers in the initial classification were not exposed to tobacco smoke (level 0 of salivary
cotinine) in the last few days, while more than 2/3 had a salivary cotinine level that reflects an important passive tobacco smoke exposure. Even if, based on the self-reported smoking habit, more than a half of the subjects participating in this study were nonsmokers, after the objective assessment of the tobacco exposure, this percent decreased dramatically. The salivary cotinine levels found in this study (11 - 100 ng/mL saliva) in the most part of non-users of tobacco products (self-declared as nonsmokers) are similar to those of previous studies [3, 9, 10, 24, 32] and indicate a passive smoking status. The higher percent of self-reported nonsmokers from this study, but with detectable levels of cotinine in their unstimulated saliva leads to increasing the anti-smoking educational programs and public policies for smoking cessation in our country.

Because smoking is at present the principal avoidable cause of premature death in the whole world, involved in the aetiology of numerous systemic diseases [34], public health initiatives are needed for increasing the quality and length of the people lives.

Conclusions

Based on the self-reported smoking status (questionnaire), 44.06% of the subjects were classified as active smokers (constant and occasional) and 55.94% (n=160) as nonsmokers; the total percent of smokers was higher (p<0.05) for males (52.29%) than for females (38.98%).

The salivary cotinine levels found in the entire study population were as follows: 0 - 16.43% of subjects (n=47); 1 - 36.01% (n=103); 2 - 6.64% (n=19); 3 - 9.09% (n=26); 4 - 9.79% (n=28); 5 - 14.69% (n=42); 6 - 7.34% (n=21); the salivary cotinine level found most frequently was level 1 (10-30 ng/mL).

The distribution of the salivary cotinine levels in the self-reported non-smokers showed that only 29.38% of these subjects had a 0 level of cotinine in saliva, the others having a level 1 (61.88%) or even 2 (8.75%).

Using the salivary cotinine levels as standard, the real distribution of the subjects by their smoking status comprised 44.06% active smokers (constant and occasional), 16.43% (n=47) non-smokers/unexposed to tobacco smoke and 39.50% passive smokers.

The salivary cotinine levels are higher in females than in males in our study, meaning that females are more strongly exposed to the passive smoking than males; this fact may be the result of the current smoking prevalence, higher in males than in females.
Limitations of the study

This study has several limitations. The first may be linked to the fact that we considered the answers to the questionnaires as the gold standard for assessing the smoking status of individuals, assuming that all the answers were sincere; the previous studies demonstrated that the underestimation of current smokers by self-reporting the smoking status may be possible [15], but it is higher in younger people than in adults [2, 34, 4, 32]. Second, we didn’t include data regarding the environmental tobacco smoke exposure in our questionnaire (the reported hours per week that subjects were exposed to tobacco smoke), that could improve the understanding of the passive smoking prevalence demonstrated in this study.

Acknowledgements

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References


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