Salivary Cotinine, Self-Reported Smoking Status and Heaviness of Smoking Index in Adults From Constanta, Romania

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Abstract

Aim: To evaluate the salivary cotinine levels and self-reported smoking status in 35-44 year olds from Constanta, and also the nicotine dependence in those identified as constant smokers, using the Heaviness of Smoking Index (HSI). ***Methods:*** In a cross-sectional study of 286 participants aged 35-44 years (6% sampling error, 95% C.L.), the unstimulated salivary cotinine levels were measured using the NicAlert™ saliva test (Jant Pharmacal Corporation, Encino, CA, USA). Self-reported smoking status and HSI were evaluated by means of a piloted self-administered questionnaire. Ethical permission and written consents were obtained. Data were entered into statistical software and analysed using the chi-square test for testing intra-group variation, ANOVA for testing between-groups variation, and the Spearman coefficient for assessing the association between variables. ***Results***: The salivary cotinine levels were: 0 for 47 subjects (16.4%), 1 for 103 subjects (36.0%), 2 for 19 subjects (6.6%), 3 for 26 subjects (9.1%), 4 for 28 subjects (9.8%), 5 for 42 subjects (14.7%), and 6 for 21 subjects (7.3%). They were higher in males than in females ($P<0.05$). When analysed, the answers to the questionnaire distributed the subjects into three categories: constant smokers (116; 40.6%), occasional smokers (10; 3.5%), and non-smokers (160; 55.9%). A higher percentage of males than females were constant smokers ($P<0.05$). The mean HSI was significantly higher ($P<0.05$) in males (4.12±1.44) than in females (2.03±1.41). Nicotine dependence levels were low for 41 subjects, moderate for 48 subjects, and high for 27 subjects. There was a significant correlation between the cotinine levels and the self-reported smoking status ($P<0.05$) and also between cotinine levels and HSI. ***Conclusions:*** In the group studied, the measurement of salivary cotinine with the NicAlert™ saliva test was a valuable method for studying the use of tobacco in a cross-sectional survey. Given the high rates of smoking prevalence and nicotine dependence demonstrated in this study, efforts have to be made to improve smoking-cessation policies in Romania.

Key Words: Cotinine, Smoking Status, Heaviness of Smoking Index, Nicotine Dependence

Introduction

The term "smoking" refers to active smoking behaviour, the intentional inhalation of tobacco smoke from any tobacco products (manufactured and hand-rolled cigarettes, cigars, pipe tobacco and cigarillos) by a smoker. Between 2000 and 2010, the number of smokers at a global level reached 1.4 billions [1]. As such, smoking is an enormous public health problem with a tremendous potential for addiction.

Cotinine is the major metabolite of nicotine and results from the metabolism of nicotine by the cytochrome 2A6 enzyme system in the liver. It is the current marker of choice for the absorption of tobacco smoke [2] because of its relatively long half-life (ten times longer than that of nicotine) [3]. Cotinine measurements from human body 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

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the intake of other components of tobacco smoke [4]. Cotinine has been isolated in plasma [5,6], urine [5,7], saliva [7,8] and gingival crevicular fluid [9,10].

In saliva, cotinine concentrations are significantly greater in unstimulated than in stimulated saliva, the differences being explained by changes in pH with alterations in salivary flow rate [11]. The cotinine from unstimulated saliva can be evaluated by quantitative (ELIZA analysis, gas-chromatography, high-performance liquid chromatography, and so on) or semi-quantitative (reagent-impregnated test strips) methods [3], which correlate with recent nicotine exposure (3-4 days), smoking status (active constant or occasional smoker, passive smoker, non-smoker) [5,12,13] and the plasma and urinary cotinine levels [14].

Salivary cotinine can be also used for measuring nicotine dependence in smokers [15]. Clinically, nicotine dependence is most often defined using the eight-item Fagerström Tolerance Questionnaire (FTQ) [16], and its modified six-item version, the Fagerström Test for Nicotine Dependence (FTND) [17].

The FTQ was designed specifically to assess physical dependence on nicotine [16]. Two of the eight original FTQ items, those that assess the time to first cigarette (TTFC) of the day after waking up and the number of cigarettes smoked per day (CPD), were found to account for most of the variance of the FTQ [18] and to be superior in predicting biochemical and behavioural indices of smoking [17,19-21]. Heatherton *et al.* (1989) [19] created a scale of just these two items. It is called the Heaviness of Smoking Index (HSI) and it has been suggested that these items may be the most useful and powerful indicators of nicotine dependence [17,19,22].

Smoking is at present the principal avoidable cause of premature death in the world as it is involved in the aetiology of numerous systemic diseases [23,24]. Because it has an enormous addictive potential and a growing prevalence, epidemiological studies of smoking prevalence and nicotine dependence have an increasing importance in public health policies for smoking cessation.

Against this background, the aim of this study was the evaluation of salivary cotinine levels and self-reported smoking status in 35-44 year old adults from Constanta, Romania, and also the evaluation of the nicotine dependence in daily smokers assessed using the HSI.

Methods

In a cross-sectional study, the salivary cotinine (objective biomarker of smoking status) levels were measured during a questionnaire-based study, which involved the evaluation of the participants' self-reported smoking status and HSI.

Study population and sample

The study population was drawn from adults aged between 35 and 44 years who lived in the Constanta district of Romania. The initial representative sample was chosen using a stratified multistage sampling design and population data from the Regional Office of the National Institute of Statistics [25]. The initial target sample of 379 was calculated so that it met the criteria of 95% confidence level, 5% sampling error, and 50% estimated level of smokers in the targeted population.

Salivary cotinine evaluation

The salivary cotinine evaluation was performed in two stages.

Stage 1: Generation and collection of the saliva

The first stage involved the generation and collection of total unstimulated saliva samples. This was achieved using a standard method, compatible with the analysis of biomarkers, namely passive collection of unstimulated saliva in sterile containers using a funnel and a tube which was capped, which were part of the NicAlert™ saliva collection system [26]. The tubes with the unstimulated saliva samples were then sealed and stored at -30°C prior to analysis of the cotinine level (saliva is stable at this temperature for evaluation of biomarkers for at least three months [27]).

Stage 2: Evaluation of the salivary cotinine level

The salivary cotinine level was evaluated using NicAlert™ saliva strip tests (Nymox Pharmaceutical Corporation, St.-Laurent, QC, Canada). The system *(Figure 1)* provides a semi-quantitative measure of cotinine in saliva for the purpose of determining whether an individual has been exposed to tobacco products within the past 48 hours. NicAlert™ saliva test strip zones range from level 0 (0-10 ng/mL, non-user of tobacco products) to level 6 (>1000 ng/mL, user of tobacco products). The cut-off concentration for the NicAlert™ saliva test (an immunochromatographic assay using monoclonal antibody), indicating a positive result, was 10 ng/mL (zones 1-6). The salivary cotinine concentration and the interpretation for each level of the NicAlert™ test are shown in *Table 1.*

Figure 1. *NicAlert™ saliva test equipment.*

Table 1. *Cotinine concentration and its interpretation for each level of the NicAlert™ test*

Level	Cotinine concentration (ng/mL)	Interpretation
0	0-10	Non-smokers
1	10-30	Occasional active smokers
2	30-100	
3	100-200	Constant active smokers
4	200-500	
5	500-1000	
6	>1000	

Salivary cotinine level was recorded after squeezing eight drops from the saliva-containing tubes (after bringing it to room temperature) directly onto the white padded end of the strip. Results were read after allowing the strip to develop by laying it on the marked area of the plastic laminated instruction card for 15 to 30 minutes [26]. The lowest numbered zone displaying a red colour was documented as the NicAlert™ saliva test result.

Self-reported smoking status and HSI evaluation

A questionnaire *(Figure 2),* designed in order to collect information regarding the tobacco consumption and to evaluate smoking status and the HSI of the participants, was given to the participants to complete during the same appointment as that for the saliva collection. The questionnaire had been pilot tested before its use in this study. It sought information on the following items:

- Demographic data (age, gender).
- The number of cigarettes smoked in the last 24 hours.
- The number of cigarettes smoked per day (CPD).
- The time to first cigarette in the morning (TTFC).
- The lifetime smoking period.
- 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).

Participants who reported being non-smokers of cigarettes but who reported the consumption of tobacco products by other means (such as chewing) were excluded; the participants who reported work-related tobacco exposure were also excluded.

Using the answers from the questionnaire as a gold standard of smoking status, the subjects were divided in three groups. These were:

1. Current constant smokers: subjects who reported current, daily use of cigarettes (at least one cigarette per day) and who had smoked at least 100 cigarettes in their lifetime [28,29].

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Studies for evaluation of cotinine and other biomarkers in the oral fluids, as a base for the development of a non-invasive diagnosis method and a prognosis model of the periodontal disease in smokers

SCRRENING QUESTIONNAIRE for ASSESSING TOBACCO CONSUMPTION

This questionnaire is aimed to assess the tobacco consumption among the population as part of the researches provided in the above mentioned project.

Data from your responses will be confidential and used exclusively for scientific purposes; these data will have access only the research team members.

I. Check with 'x' the age, gender and life environment that are appropriate for you:

a. Age

35-44 years	
65-74 years	

b. Gender

female	
male	

c. life environment

urban	
rural	

II. Check with 'x' the appropriate answer, after reading carefully:

Do you smoke cigarettes?	yes	constantly/daily [you are a constant smoker of cigarettes, meaning you are smoking at least 1 cigarette/day and you smoke at least 100 cigarettes (5 packs) till now]?	
		occasionally/every few days [you are smoking cigarettes routinely, but not every day (at least 5 cigarettes/week and at least 1 cigarette in the last week)]?	
	no	[you was a smoker but you quit and you didn't smoke any cigarette in the last 14 days or you have never smoked]?	

III. If the answer at the IInd question was 'no', check with 'x' the appropriate answers:

1.Have you used the last 14 days (two weeks) nicotine containing products (gum, drops or other nicotine replacement products)?	yes	
	no	
2. Are you using other tobacco products than cigarettes (cigars, pipe, chewing tobacco, etc.)?	yes	
	no	
3.Do you have a tobacco work-related job (handling, manufacture, sale, distribution, etc.)?	yes	
	no	

IV. If the answer at the IInd question was 'yes/constantly', check with 'x' the appropriate answers:

1.How soon after you wake up do you smoke yours first cigarette?	within 5 min.	
	6 - 30 min.	
	31 - 60 min.	
	after 60 min.	
2. How many cigarettes/day do you smoke?	10 or less	
	11 - 20	
	21 - 30	
	31 or more	
3. How many cigarettes did you smoke in the last 24 hours?		

THANK YOU VERY MUCH FOR YOUR COLLABORATION!

Figure 2. *The questionnaire for assessing tobacco consumption.*

2. Current occasional smokers: subjects who reported current, but not everyday use of cigarettes (at least five cigarettes per week, almost every week, and at least one cigarette in the previous seven days) [28],
3. Non-smokers: subjects who reported that they had not smoked during at least 14 days prior to the salivary sample collection, including former smokers and also people who had never smoked [30].

Time to first cigarette in the morning (TTFC) and number of cigarettes smoked per day (CPD) were used to assess participants' HSI. They were coded as ordinal variables. TTFC was classified in four categories:

1. Smoking less than 5 minutes after waking up (code 3).
2. Smoking between 6-30 minutes after waking up (code 2).
3. Smoking between 31–60 minutes after waking up (code 1).
4. Smoking more than 60 minutes after waking up (code 0).

CPD was also categorised into four categories, which were:

1. 10 or fewer CPD (code 0).
2. 11-20 CPD (code 1).
3. 21-30 CPD (code 2).
4. 31 CPD or more (code 3).

Adding together the TTFC and CPD codes produced the HSI, which has a three-level scale, as follows:

1. Low nicotine dependence (0-2 points).
2. Moderate nicotine dependence (3-4 points).
3. Heavy nicotine dependence (5-6 points) [17,31].

Ethical approval

Permission to conduct the study was given by the Professional Ethical Committee of Ovidius University, Constanta, and by the Ethical Committee of the Medical College of Constanta District. Written informed consent (including patient information on the aim and methods of the study) was obtained from all participants. Participation was optional, and the time for deciding whether to take part or not (express consent or refusal) was 48 hours.

Statistical analyses

Data were analysed using statistical software (SPSS version 12 for Windows; SPSS Inc, Chicago, USA). The test–retest reliability of the questionnaire was tested using kappa statistics. Descriptive statistics were used for the analysis of the questionnaire's answers. The chi-square test was used for testing intra-group variation, ANOVA was used for testing between-groups variation, and Spearman's coefficient was used for measuring the association between variables.

Results

The kappa value for test–retest of the questionnaire was 0.96 (perfect agreement).

The response rate was 77.3% (86 subjects refused to participate in the study and a further seven subjects were excluded because they were sellers of tobacco products). The final study sample comprised 286 subjects (6% sampling error; 95% C.L.), with a mean age of 40 +4 years, of whom 109 (38.1%) were males and 177 (61.9%) were females. One hundred and fifty-six were urban and 130 were rural dwellers *(Table 2).*

Table 2. *Distribution of the subjects by gender and place of residence*

Place of residence	Males	Females	Total
Urban	62	94	156
Rural	47	83	130
Total	109	177	286

The salivary cotinine levels found in the entire study sample were as follows: 0 for 47 subjects (16.4%), 1 for 103 subjects (36.0%), 2 for 19 subjects (6.6%), 3 for 26 subjects (9.1%), 4 for 28 subjects (9.8%), 5 for 42 subjects (14.7%), and 6 for 21 subjects (7.3%) *(Table 3)*. The results showed significant differences ($P<0.5$) in cotinine levels according to the subjects' gender *(Table 4),* with higher values in males than in females. There were no statistically significant differences in cotinine levels according to place of residence (urban vs. rural) *(Table 5).*

Table 3. *The salivary cotinine levels in the study sample*

Salivary cotinine levels	Frequency (n)	%*
0	47	16.4
1	103	36.0
2	19	6.6
3	26	9.1
4	28	9.8
5	42	14.7
6	21	7.3
Total	286	100

* Rounded to one decimal place, as in all the following tables

Table 4. *Distribution of the salivary cotinine levels by gender*

Salivary cotinine levels	Males		Females	
	n	%	n	%
0	9	8.3	38	21.5
1	36	33.0	67	37.9
2	7	6.4	12	6.8
3	3	2.8	23	13.0
4	9	8.3	19	10.7
5	28	25.7	14	7.9
6	17	15.6	4	2.3
Total (n=286)	109 (38.1%)		177 (61.9%)	
	P (chi-square test)=0.05			

Table 5. *Distribution of the salivary cotinine levels according to place of residence*

Place of residence	Rural		Urban	
	n	%	n	%
0	29	22.3	18	11.5
1	43	33.1	60	38.5
2	8	6.2	11	7.1
3	13	10.0	13	8.3
4	13	10.0	15	9.6
5	13	10.0	29	18.6
6	11	8.8	10	6.4
Total (n=286)	130 (45.5%)		156 (54.5%)	
	P (chi-square test)=0.131			

The self-reported smoking status and analysis of the answers to the questions regarding tobacco consumption led to the distribution of subjects into three categories *(Table 6).* These were: current constant smokers (116; 40.6%), current occasional smokers (10; 3.5%) and non-smokers (160; 55.9%). The distribution of the self-reported smoking status of the subjects according to gender *(Table 7)* showed significant differences between the smoker categories ($P<0.05$). Sixty-nine (39.0%) of the females and 57 (52.3%) of the males were smokers. There were no significant differences in the smoking status categories related to the subjects' place of residence *(Table 8).*

Table 6. *Distribution of the subjects by their self-reported smoker status*

Self-reported smoker status	Frequency (n)	%
Current constant smokers	116	40.6
Current occasional smokers	10	3.5
Non-smokers	160	55.9
Total	286	100.0

Table 7. *Distribution of the self-reported smoking status by gender*

Self-reported smoking status	Males		Females	
	n	%	n	%
Non-smokers	52	47.7	108	61.0
Current occasional smokers	0	0	10	5.7
Current constant smokers	57	52.3	59	33.3
Total (n=286)	109 (38.1%)		177 (61.9%)	
	P (chi-square test)=0.001			

Table 8. *Distribution of the self-reported smoking status according to place of residence*

Place of residence	Rural		Urban	
	n	%	n	%
Non-smokers	77	59.2	83	53.2
Current occasional smokers	4	3.1	6	3.8
Current constant smokers	49	37.7	67	42.9
Total (n=286)	130 (45.5%)		156 (54.5%)	
	P (chi-square test)=0.588			

The HSI, as mentioned previously, was computed only for the current constant smokers (daily smokers), as the sum of TTFC and CPD. The mean HSI value was significantly higher ($P<0.05$; ANOVA) for males (4.1±1.4) than for females (2.0±1.4). The distributions of TTFC and CPD codes are shown in *Tables 9* and *10.* Both TTFC and CPD codes had significantly higher values for males than for females ($P<0.05$; ANOVA) but there were no differences in these variables related to the place of residence of the smokers ($P>0.05$; ANOVA).

Table 9. *Distribution of TTF codes in current constant smokers*

TTF codes	Frequency (n)	%
0 (>60 min.)	17	14.7
1 (31-60 min.)	24	20.7
2 (6-30 min.)	44	37.9
3 (<5 min.)	31	26.7
Total	116	100.0

Table 10. *Distribution of CPD codes in current constant smokers*

CPD codes	Frequency (n)	%
0 (=10)	23	19.8
1 (11-20)	43	37.1
2 (21-30)	39	33.6
3 (=31)	11	9.5
Total	116	100.0

The distribution of HSI values *(Table 11)* grouped the current constant smokers in three nicotine dependence levels, as follows: 41 (35.4%) subjects with low nicotine dependence, 48 (41.4%) subjects with moderate nicotine dependence, and 27 (23.2%) subjects with heavy nicotine dependence.

Table 11. *Distribution of the HSI values and nicotine dependence levels in current smokers*

HSI values (TTF+CPD)		**Frequency (n)**	**%**
Low nicotine dependence (n=41; 35.3%)	0	13	11.2
	1	11	9.5
	2	17	14.7
Moderate nicotine dependence (n=48; 41.4%)	3	25	21.6
	4	23	19.8
Heavy nicotine dependence (n=27; 23.3%)	5	17	14.7
	6	10	8.6
Total		116	100.0

The analysis of the possible correlations between the analysed variables showed the following:

- There was a significant correlation between the individual's cotinine level and self-reported smoking status (*Table 12; P*<0.05) in the entire study group.
- The individual's salivary cotinine levels in the whole study sample were also correlated with age (*P*<0.05).
- There was a significant positive correlation in current constant smokers between the salivary cotinine levels and TTF *(Table 13),* CPD *(Table 14),* and also with HSI codes *(Table 15).*

Discussion

A precise estimate of tobacco consumption and nicotine dependence in people is an important concern in epidemiologic studies. The present study was carried out on adults aged 35-44 years, this being the adult age group suggested by the World Health Organization (WHO) to be involved in cross-sectional studies and also the age group with the highest prevalence of smoking.

Table 12. *Distribution/correlation of the individuals' cotinine levels according to/with their self-reported smoker status*

Self-reported smoking status		**Non-smokers**		**Current occasional smokers**		**Current constant smokers**	
		n	%	n	%	0	0
Cotinine levels	0	47	29.4	0	0	0	0
	1	99	61.9	3	30.0	1	0.9
	2	14	8.8	4	40.0	1	0.9
	3	0	0	3	30.0	23	19.8
	4	0	0	0	0	28	24.1
	5	0	0	0	0	42	36.2
	6	0	0	0	0	21	18.1
Total (n=116)		160	55.9	10	3.5	116	40.6
Spearman correlation/Sig. (2-tailed)		*0.877"/0.000*					

** Correlation is significant at the 0.01 level (2-tailed).

Table 13. *Distribution/correlation of the current constant smokers' cotinine levels according to/with TTF*

TTF		**0 (≤5 min)**		**1 (6-30 min)**		**2 (31-60 min)**		**3 (=60 min)**	
		n	%	n	%	n	%	n	%
Cotinine levels	1	0	0	0	0	0	0	1	3.2
	2	0	0	0	0	0	0	1	3.2
	3	11	64.7	6	25.0	6	13.6	0	0
	4	4	23.5	13	54.2	10	22.7	1	3.2
	5	2	11.8	4	16.7	23	52.3	13	41.9
	6	0	0	1	4.2	5	11.4	15	48.5
Total (n=116)		17	14.7	24	20.7	44	37.9	31	26.7
Spearman correlation/Sig. (2-tailed)		*0.604"/0.000*							

** Correlation is significant at the 0.01 level (2-tailed).

Table 14. *Distribution/correlation of the current constant smokers' cotinine levels according to/with CPD*

CPD		**0 (=10)**		**1 (11-20)**		**2 (21-30)**		**3 (=31)**	
		n	%	n	%	n	%	n	%
Cotinine levels	1	0	0	0	0	0	0	1	9.1
	2	0	0	0	0	1	2.6	0	0
	3	16	69.6	6	14.0	1	2.6	0	0
	4	5	21.7	21	48.8	2	5.1	0	0
	5	2	8.7	13	30.2	25	64.1	2	18.2
	6	0	0	3	7.0	10	25.6	8	72.7
Total (n=116)		23	19.8	43	37.1	39	33.6	11	9.5
Spearman correlation/Sig. (2-tailed)		*0.666"/0.000*							

** Correlation is significant at the 0.01 level (2-tailed).

Table 15. *Distribution/correlation of the current constant smokers' cotinine levels according to/with their HSI (nicotine dependence)*

HSI		**Low nicotine dependence**		**Moderate nicotine dependence**		**Heavy nicotine dependence**	
		n	%	n	%	0	0
Cotinine levels	1	0	0	0	0	1	3.7
	2	0	0	0	0	1	3.7
	3	19	46.3	4	8.3	0	0
	4	17	41.5	11	22.9	0	0
	5	4	9.8	27	56.3	11	40.7
	6	1	2.4	6	12.5	14	51.9
Total (n=116)		41	35.3	48	41.4	27	23.3
Spearman correlation/Sig. (2-tailed)		*0.636"/0.000*					

** Correlation is significant at the 0.01 level (2-tailed).

Cross-sectional studies of tobacco use generally employ questionnaires to assess smoking and nicotine dependence. The answers from those who complete the questionnaires may be subjective. It is far more objective to assess and quantify the recent exposure of an individual to cigarette smoke by analysing cotinine in his or her body fluids (blood, urine or saliva) [32-34].

In the present study, the questionnaire was used to classify the subjects as smokers (constant and occasional) and non-smokers. The assessment of salivary cotinine levels, measured by the NicAlert™ saliva test, then provided an objective technique to determine the level of subjects' exposure to tobacco.

The results regarding smoking prevalence indicated that the subjects included a slightly higher percentage of active smokers (44.1%) than the one reported by the WHO (43.5%) for the adult population of Romania in 2000 [35]. In comparison with these WHO data, the prevalence of smoking decreased among Romanian males and increased among Romanian females, in agreement with the WHO predictions for 2000-2010 [35].

After the initial classification of subjects, the NicAlert™ saliva test was used for the objective assessment of tobacco exposure. When based on the self-reported information, more than half of the subjects participating in this study claimed to be non-smokers. However, after objective assessment of their exposure to tobacco, this percentage decreased dramatically, indicating that they had not been truthful when completing the questionnaire and were either active or passive smokers.

The salivary cotinine levels founded in this study (11-100 ng/mL saliva), in the most part among those who claimed to be non-users of tobacco products (self-declared as non-smokers), are similar to those of previous studies [5,8,33,36-38] and indicated either a passive smoking status or deception. In our study, the salivary cotinine levels were higher in females than in males, indicating that females are more exposed to passive smoking than males; this finding may reflect the results of the current smoking prevalence, higher in males than in females.

In the current study, the HSI mean values were higher in males than in females and at higher values than in other previous studies on this topic [39-41]. The HSI distributed the current constant smokers

into three nicotine dependence levels, as follows: 41 (35.3%) with low nicotine dependence, 48 (41.4%) with moderate nicotine dependence, and 27 (23.3%) with heavy nicotine dependence. In agreement with previous studies, the individual's salivary cotinine level measured by the NicAlert™ saliva test was well correlated with the self-reported smoking status evaluated by questionnaire [5,12,13,42].

In self-reported current constant smokers, the salivary cotinine levels were significantly correlated both with items of the HSI, TTFC and CPD, and also with nicotine dependence, measured by HSI. These results are in accordance with other studies that have assessed tobacco exposure and nicotine dependence [18,19,22,39]. They confirmed that measuring salivary cotinine by a semi-quantitative method such as NicAlert™ saliva test is a very valuable method for assessing the use of tobacco in cross-sectional surveys.

In order to increase the quality and length of people's lives, further epidemiological studies on tobacco consumption and nicotine dependence in a social context should be performed. Their results will inform anti-smoking educational programmes and public policies for smoking cessation in Romania.

Conclusions

- The salivary cotinine levels found in the entire study sample were as follows: 0: 47 subjects (16.4%); 1: 103 subjects (36.0%); 2: 19 subjects (6.6%); 3: 26 subjects (9.1%); 4: 28 subjects (9.8%); 5: 42 subjects (14.7%); 6: 21 subjects (7.3%); The results showed significantly higher values of cotinine levels in males than in females.
- The self-reported smoking status analysis (the answers to the questions regarding tobacco consumption) distributed the subjects into three categories of smokers: 116 (40.6%) current constant smokers, 10 (3.5%) current occasional smokers, and 160 (55.9%) non-smokers; there was a higher percentage of smokers among males (52%) than females (39%).
- The HSI values distributed the current constant smokers in three nicotine dependence levels, as follows: 41 (35.3%) with low nicotine dependence, 48 (41.4%) with moderate nicotine dependence, and 27 (23.3%) with heavy nicotine dependence.
- Salivary cotinine levels measured by the NicAlert™ saliva test were well correlated with individuals' self-reported smoking status.
- Salivary cotinine levels were also significantly correlated with TTFand CPD, and also with nicotine dependence evaluated by HSI.

Acknowledgements

The authors would like to thank to all subjects for their unconditional cooperation and support. This work was supported by CNCSIS–UEFISCSU, project number PNII–IDEAS 1216/2008: "Studies for evaluation of cotinine and other biomarkers in the oral fluids, as a base for the development of a non-invasive diagnosis method and a prognosis model of the periodontal disease in smokers".

Contribution of each author

CIN planned the conceptual model for the study and its design, analysed the results, drafted and redrafted the paper, and approved the final version.

CIA planned and supervised the study, critically reviewed its drafts, and approved the final version.

VVB collected the saliva samples and processed them, assisted in the literature review, and checked and approved the final version of the paper.

ANZ collected the saliva samples, distributed and collected the questionnaires, helped produce the tables, and approved the final version of the paper.

CTA designed the questionnaire, helped in the collection of samples and completed questionnaires, analysed the results, and approved the final version of the paper.

Statement of conflict of interests

The authors of this article are not aware of any conflicts of interests regarding this study.

References

1. Woodward A, Konrad J. Passive smoking: what are the limits to liberty? *Medical Journal of Australia* 1996; **164**: 260.

2. National Research Council, Committee on Passive Smoking. *Environmental Tobacco Smoke: Measuring Exposure and Assessing Health Effects*. Washington, DC: National Academy Press; 1986: pp. 133-160.

3. Curvall M, Elwin C-E, Kazemi-Vala E, Warholm C, Enzell CR. The pharmacokinetics of cotinine in plasma and saliva from non-smoking healthy volunteers. *European Journal of Clinical Pharmacology* 1990; **38**: 281-287.

4. Hawamdeh A, Kasasbeh FA, Ahmad MA. Effects of passive smoking on children's health: a review. *Eastern Mediterranean Health Journal* 2003; **9**: 441-447.

5. Binnie V, McHugh S, Macpherson L, Borland B, Moir K, Malik K. The validation of self-reported smoking status by analysing cotinine levels in stimulated and unstimulated saliva, serum and urine. *Oral Diseases* 2004; **10:** 287-293.

6. Haley N, Axelrad C, Tilton K. Validation of self-reported smoking behaviour: biochemical analyses of cotinine and thiocyanate. *American Journal of Public Health* 1983; **73**: 1204-1207.

7. Davis R, Stiles M, DeBethizy J, Reynolds J. Dietary nicotine: a source of urinary cotinine. *Food and Chemical Toxicology* 1991; **29**: 821-827.

8. Etter JF, Vu Due T, Perneger TV. Saliva cotinine levels in smokers and nonsmokers. *American Journal of Epidemiology* 2000; **151**: 251-258.

9. Chen X, Wolff L, Aeppli D, Guo Z, Luan W, Baelum V. Cigarette smoching, salivary/gingival crevicular fluid cotinine and periodontal status. A 10-year longitudinal study. *Journal of Clinical Periodontology* 2001; **28**: 331-339.

10. McGuire JR, McQuade MJ, Rossmann JA, Garnick JJ, Sutherland DE, Scheidt MJ, *et al*. Cotinine in saliva and gingival crevicular fluid of smokers with periodontal disease. *Journal of Periodontology* 1989; **60**: 176-181.

11. Schneider N, Jacob P, Nilsson F, Leishcow S, Benowitz N, Olmstead R. Saliva cotinine levels as a function of collection method. *Addiction* 1997; **92**: 347-351.

12. Glover ED, Glover PN, Laflin MT, Nochur SV, Strnad JV. Sensitivity and specificity of a reagent-impregnated test strip in identifying smokeless tobacco users. Nicotine Tobacco Research 2007; **9**: 499-503.

13. Nishida N, Yamamoto Y, Tanaka M, Maeda K, Kataoka K, Nakayama K, *et al*. Association between passive smoking and salivary markers related to periodontitis. *Journal of Clinical Periodontology* 2006; **33**: 717-723.

14. Figueiredo VC, Szklo M, Szklo AS, Benowitz N, Lozana JA, Casado L. Determinants of salivary cotinine level: a population-based study in Brazil. *Revista de Saúde Pública* 2007; **4**: 954-962.

15. Ferketich AK, Wee AG, Shultz J, Wewers ME: A measure of nicotine dependence for smokeless tobacco users. Addictive Behaviors 2007; **32**:1970-1975.

16. Fagerström KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addictive Behaviors* 1978; **3**: 235-241.

17. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerström Test for Nicotine Dependence, a revision of the Fagerström Tolerance Questionnaire. *British Journal of Addiction* 1991; **86**: 1119-1127.

18. Lichtenstein E, Mermelstein RJ. Some methodological cautions in the use of the tolerance questionnaire. *Addictive Behaviors* 1986; **11**: 439-442.

19. Heatherton TF, Kozlowski LT, Frecker RC, Rickert WS, Robinson J. Measuring the heaviness of smoking using self-reported time to first cigarette of the day and number of cigarettes smoked per day. *British Journal of Addiction* 1989; **86**: 1119-1127.

20. Breslau N, Johnson EO. Predicting smoking cessation and major depression in nicotine-dependent smokers. *American Journal of Public Health* 2000; **90**: 1122-1127.

21. Prokhorov A V, De Moor C, Pallonen UE, Hudmon KS, Koehly L, Hu S. Validation of the modifed Fagerström Tolerance Questionnaire with salivary cotinine among adolescents. *Addictive Behaviors* 2000; **25**: 429-433.

22. Haddock CK, Lando H, Klesges RC, Talcott G W, Renaud E A. A study of the psychometric and predictive properties of the Fagerström Test for Nicotine Dependence in a population of young smokers. *Nicotine & Tobacco Research* 1999; **1**: 59-66.

23. World Health Organization. *Framework Convention on Tobacco Control.* Geneva: WHO; 2003.

24. Tauras A, Chaloupka FJ. Public health initiatives for smoking cessation. *Current Cardiovascular Risk Reports* 2007; **1**: 391-395.

25. National Institute of Statistics, Romania. Accessed (2010 Jun 01) via: http://www.insse.ro

26. Nymox Pharmaceutical Corporation. Indications use statement for NicAlert™. Accessed (2010 Jun 01) at: http://www.nymox.com/default.action?itemid=46

27. Koh DS-Q, Koh GC-H. The use of salivary biomarkers in occupational and environmental medicine. *Journal of Occupational and Environmental Medicine* 2007; **64**: 202-210.

28. Caraballo RS, Giovino GA, Pechacek TF, Mowery PD. factors associated with discrepancies between self-reports on cigarette smoking and measured serum cotinine levels among persons aged 17 years or older. Third National Health and Nutrition Examination Survey, 1988–1994. *American Journal of Epidemiology* 2001; **153**: 807-814.

29. Mannino DM, Holguin F, Greves HM, Savage-Brown A, Stock AL, Jones RL. Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey. *Thorax* 2004; **59**: 194-198.

30. Bramer SL, Kallungal BA. Clinical considerations in study designs that use cotinine as a biomarker. *Biomarkers* 2003; **8**: 187-203.

31. Lacchetti C, Cohen J, Ashley MJ, Ferrence R, Bull S, de Groh M, *et al*. Is nicotine dependence related to smokers' support for restrictions on smoking? *Nicotine & Tobacco Research* 2001; **3**: 257-260.

32. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiologic Reviews* 1996; **8**: 188-204.

33. Jarvis MJ, Tunstall-Pedoe H, Feyerabend C. Comparison of tests used to distinguish smokers from nonsmokers. *American Journal of Public Health* 1987; **77**: 1435-1438.

34. Glasgow RE, Mullooly JP, Vogt TM. Biochemical validation of smoking status: pros, cons, and data from four low intensity intervention trials. *Addictive Behaviors* 1993; **18**: 511-27.

35. World Health Organisation. Tobacco statistics. Accessed (2011 Jan 30) at: http://www.who.int/tobacco/statistics/tobacco_atlas/en/

36. Etzel RA. A review of the use of saliva cotinine as a marker of tobacco smoke exposure. *Preventive Medicine* 1990; **19**: 190-197.

37. Patrick DL, Cheadle A, Thompson DC, Diehr P, Koepsell T, Kinne S. The validity of self-reported smoking: a review and meta-analysis. *American Journal of Public Health* 1994; **84**: 1086-1093.

38. Murray RP, Connett JE, Lauger GG, Voelker HT. Error in smoking measures: effects of intervention on relations of cotinine and CO to self-reported smoking. *American Journal of Public Health* 1993; **83**: 1251-1257.

39. Pérez-Ríos M, Santiago-Pérez MI, Alonso B, Malvar A, Hervada X, de Leon J. Fagerström test for nicotine dependence vs heavy smoking index in a general population survey. *BMC Public Health* 2009; **9**: 493.

40. Borland R, Yong HH, O'Connor RJ, Hyland A, Thompson ME. The reliability and predictive validity of the Heaviness of Smoking Index and its two components: Findings from the International Tobacco Control Four-Country study. *Nicotine & Tobacco Research* 2010; **12** (suppl 1); S45-S50.

41. Mizoue T, Fujino Y, Yamato H, Tokunaga S, Kubo T, Kari Reijula K. Overtime work, cigarette consumption, and addiction to cigarette among workers subject to mild smoking restrictions. *Industrial Health* 2006; **44**: 244-249.

42. Parker DR, Lasater TM, Windsor R, Wilkins J, Upegui DI, Heimdal J. The accuracy of self-reported smoking status by cotinine test strips. *Nicotine Tobacco Research* 2002; **4**: 305-309.