Study regarding the correlation between the salivary cotinine concentration and the Community Periodontal Index of Treatment Needs, in young smokers from Constanța District

Studiu privind corelația dintre concentrația cotininei salivare şi indicele CPITN la tinerii fumători din Județul Constanța

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Abstract:

Background: the role of the salivary cotinine in the periodontal disease is a suggested, but not fully investigated hypothesis. Aim: to evaluate the relationship between the salivary cotinine concentration and the periodontal health status, in 15 - 19 year-old smokers from Constanta District. Material and methods: a sample of 362 subjects (0.07 sampling error; 95% C.L.), participated in a clinical study for collecting 2.5 ml of unstimulated saliva, recording the periodontal status using Community Periodontal Index of Treatment Needs (CPITN Index) and completing a questionnaire regarding the smoking habit. The active smoker's saliva was tested for cotinine concentration, using NicAlert™ Saliva strip tests. Ethics approval and written consent were obtained. Statistical analyses were performed using SPSS 12. Results: 28.5% (n=103) of subjects were constant smokers, 10.5% (n=38) were occasional smokers and 61% (n=221) were non-smokers. The study found significant higher mean CPITN values in smokers than in non-smokers ($p<0.05$), and a significant correlation between the individual's CPITN score and the smoker status ($p<0.05$). The mean cotinine salivary concentration was higher in constant smokers than in occasional smokers ($p<0.05$). Significant correlations were found between the individual's salivary cotinine level and the smoker status, and also between the individual's cotinine level and the individual's CPITN score. Conclusion: The significant correlation found in our study between the active smoker's salivary cotinine level and their CPITN score needs further investigations, suggesting that measuring the salivary cotinine can give useful information for the periodontal risk assessment.

Key words: cotinine, CPITN Index, periodontal disease, smoker

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Rezumat

Rolul cotininei salivare în boala parodontală este o ipoteză sugerată, dar insuficient investigată. Scop: evaluarea corelației dintre concentrația cotininei salivare şi starea de sănătate parodontală la fumătorii cu vârste între 15 şi 19 ani din județul Constanța. Material şi metode: un eşantion de 362 subiecți (0.07 - eroare de eşantionare şi 95% interval de confidență) a participat la un studiu clinic constând în colectarea nestimulată a 2.5 ml salivă, înregistrarea stării de sănătate parodontală utilizând indicele CPITN şi completarea unui chestionar pentru evaluarea status-ului de fumător. Saliva fumătorilor activi a fost testată pentru evaluarea concentrației de cotinină utilizând testele NicAlert™ Saliva. S-au obținut avizele etice şi consimțământul liber-informat al subiecților. Analiza statistică a utilizat SPSS 12. Rezultate: 28.5% (n=103) dintre subiecți au fost înregistrați ca fumători constanți, 10.5% (n=38) - fumători ocazionali şi 61% (n=221) - nefumători. Studiul a demonstrat prezența unor valori mai crescute ale indicelui CPITN la fumători decât la nefumători ($p<0.05$) şi o corelație semnificativă între scorul individual CPITN şi status-ul de fumător ($p<0.05$). Concentrația medie a cotininei salivare a înregistrat valori mai ridicate la fumătorii constanți decat la cei ocazionali ($p<0.05$). Concentrația individuală a cotininei salivare a fost corelată atât cu status-ul de fumător cât şi cu scorul individual CPITN. Concluzie: Studiul a demonstrat o corelație semnificativă între concentrația cotininei salivare şi scorul CPITN al fumătorilor, sugerând că evaluarea concentrației cotininei salivare poate furniza informații utile pentru aprecierea riscului la boală parodontală la fumători.

Cuvinte cheie: *cotinina, indice CPITN, boala parodontală, fumător*

Introduction

Cotinine is the major metabolite resulted from the metabolism of nicotine ($C_{10}H_{14}N_2$– α 3 pyridine-N-methyl-pirrolydinyl) by the cytochrome 2A6 enzyme system in the liver.

Being a very strong toxic agent and addictive drug, nicotine is the main component of cigarettes and of the products used for nicotine substitution therapy and enters in the human body mostly by respiratory way, but also using the digestive or cutaneous routes.

It has been estimated that an average of 70-80% of the nicotine absorbed by a smoker is metabolised to cotinine and excreted mainly by kidney, but also by perspiration, maternal milk and oral fluids (1).

Assessed by quantitative (ELISA analysis, gas-chromatography etc.) or semi-quantitative methods (reagent-impregnated test strips), the salivary cotinine level is nowadays the most specific and sensitive biomarker of tobacco consumption, being correlated with the recent nicotine exposure (3-4 days), the smoking status (occasional or constant active smoker, passive smoker, non-smoker) (2-4) and the plasma and urine cotinine level (5).

Even if a lot of studies have demonstrated the dose-effect relation between smoking and the risk for a very severe periodontal disease (6-9), the correlation between the salivary cotinine level and the severity of the periodontal disease is a hypothesis suggested, but not fully demonstrated until now, approached in a very few international studies (4, 10). There are no Romanian studies on this subject carried out until the present.

Because the traditional (clinical and radiological) diagnosis methods of the periodontal disease are limited to a descriptive analysis and the studies regarding the use of biomarkers from oral fluids for the objective measurement of the onset status of a disease are in a fully ascension (11, 12), the evaluation of the role of salivary cotinine in the periodontal disease is a real necessity in dental medicine.

Against this background, the *aim* of the present study is to evaluate the relationship between salivary cotinine concentration and the periodontal health status assessed by CPITN Index, in active smokers of 15 - 19 year-olds from Constanta District.

Material and methods

Population and samples

The *clinical study* was made on a representative sample of 362 high school students aged between 15 and 19 years from Constanta District, Romania, given by a stratified multistadial sampling design (with a 0.07 sampling error and 95% C.L.).

The stratified sampling was based on three variables, which were: rural or urban domicile, geographical place of residence (random proportional selection) and the high school attended (random proportional selection). The final sample was drawn as a result of systematic sampling, using the lists of high school pupils as a sampling frame. Data regarding the localities, population and high schools were obtained from the Regional Office of the National Institute of Statistics and were used as a database for sampling (13).

The *laboratory study*, which aimed to evaluate the relationship between salivary cotinine concentration and CPITN scores, was performed amongst the active smokers (constant and occasional) found in the sample used in the *clinical study*, after collecting information regarding tobacco consumption.

Ethical permission

Ethical 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, in order to respect the ethical principles for medical research involving human subjects, given by the World Medical Association Declaration of Helsinki (revised in 2000, Edinburg) (14).

Written consent was obtained from the Local Public Health Authorities of the cities included in the study, from the local administration authorities and from the school authorities.

The school authorities acquired the written free-informed consent for the study, as a "Free-Consent Form" signed by the researcher and also by the pupils aged over 18 years or by the parents of the pupils under this age.

The consent forms offered adequate data for fully knowledge regarding the aim of the study and the methods used for clinical exam and for saliva collection.

The consent was free, the participation was optional, and the time for considering the opportunity (express the consent or refusal) was 48 hours.

Clinical examination

The children's periodontal health status was evaluated by two trained and calibrated examiners, using the World Health Organization (WHO) Community Periodontal Index of Treatment Needs (15).

The calibration of the examiners was carried out in the Department of Preventive Dentistry, Faculty of Dentistry, Ovidius University, Constanta, by Prof. C.A. The intra-examiner reliability was assessed by repetition of clinical exams in 10% of children, after 10 days interval. The inter-examiner reliability was achieved by double blind duplicate examination of 10% of children.

The clinical examination was carried out in the dental offices of the selected schools, using plain mouth mirrors, W.H.O. probes with a black band between 3.5 and 5.5 mm with respect to the tip and a spherical tip of 0.5 mm diameter and sterile gloves, under artificial optimal light, respecting the usual infection-control protocols.

No instruction in tooth brushing or oral prevention was given to the participants prior to the start of the research. Each periodontal evaluation was performed during morning hours (between 10 and 12 a.m.).

As per the CPITN system, the mouth was divided into sextants. Upper and lower arches were divided and numbered as follows: upper right posterior (I), upper anterior (II), upper left posterior (III), lower left posterior (IV), lower anterior (V) and lower right posterior (VI). The probe was introduced gently into the gingival sulcus in three different sites (mesial, central and distal), on both buccal and lingual aspects of each index tooth.

Only six index teeth (16, 11, 26, 36, 31 and 46) were examined. Healthy sextants (CPITN = 0) were registered when the black band of the probe remained completely visible above the gingival sulcus and no supragingival calculus or bleeding was seen upon probing. The same conditions regarding the visibility of the probe but bleeding upon probing were registered with value 1. Presence of the supra or subgingival calculus was registered value 2, the black band partially submerged in the gingival sulcus, value 3, and the black band completely submerged into the gingival sulcus, value 4.

The highest value found in each sextant was registered as the value for that sextant. When the highest value (4) was found in one tooth in a sextant, this value was recorded immediately and the remaining teeth in the sextant were not examined. The highest CPITN value found within each subject was registered as the individual's CPITN score. The clinical data were registered on individual assessment forms.

Generation and collection of the saliva

Generation and collection of the total unstimulated saliva were achieved in the same appointment with the clinical exam, but right before the oral examination, in order to avoid the blood contamination of the saliva samples.

The saliva collection was made in the same hours range for all subjects (10-12 a.m.); they were restricted from the consumption of foods and beverages 1 hour before collecting the saliva, in order to avoid its dilution and contamination.

The saliva collection was made using a standard method, compatible with the analysis of biomarkers, namely passive collection in sterile containers, with funnel and collection tube; the system for saliva collection (funnel, cap and tube container) are part of the NicAlert™ Saliva Collection Kit (NYMOX Pharmaceutical Corporation, QC Canada H4M 2V2) (16). After the funnel was placed in the saliva tube container, the subject was spiting saliva into the funnel for 5 to 10 min., for getting at least 2.5 ml.

The tube containers with the saliva samples were then locked and stored at -800C (freezer) before the analysis of the cotinine level (saliva is stable at this temperature for biomarkers evaluation for minimum 3 months (17).

Generation and collection of the tobacco consumption data (the questionnaire)

A short standard questionnaire was designed to collect information regarding the tobacco consumption in the study sample. It had been piloted before using in this main study.

The questionnaires were self-administered and answered in the same appointment with the saliva collection and clinical examination, but right after the last one.

On the basis of the questionnaire's answers, the subjects were distributed in three groups: active constant smokers, occasional constant smokers and non-smokers (18).

Evaluation of the salivary cotinine concentration

The salivary cotinine concentration was evaluated using NicAlert™ Saliva strip tests (NYMOX Pharmaceutical Corporation, QC Canada H4M 2V2) (*Figure 1).*

These tests are intended for in vitro diagnostic professional use for the semi-quantitative evaluation of cotinine in saliva, in order to determine if an individual has been exposed to tobacco products (cigarettes, pipes or chewing tobacco) in the past 48 hours.

The cutoff concentration for the NicAlert™ test is 10 ng/mL; the test is an im-

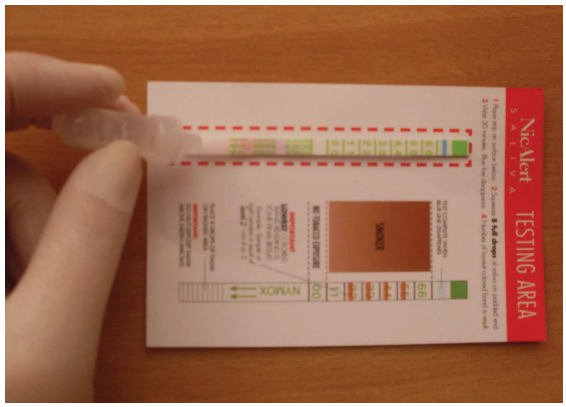

Figure.1 NicAlert Saliva Tests

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 and second-hand smokers
1	10 - 30	occasional active smokers or smokers of low level nicotine cigarettes
2	30 - 100	
3	100 - 200	constant active smokers
4	200 - 500	
5	500 - 1000	
6	> 1000	

munochromatographic assay using monoclonal antibody - coated gold particles and a series of avidity traps that allow quantification. The sample collection end of the strip contains gold particles coated with monoclonal antibodies to cotinine. The distance the gold particles migrate on the strip is shown by a clear color change and provides an accurate measure of the saliva cotinine concentration (16). The salivary cotinine concentration and its interpretation for each level of the NicAlert™ are shown in *Table 1.*

Statistical analyses

Statistical analyses were performed using SPSS 12 *for Windows*. The intra- and inter-examiner reliability in the CPITN registration and the test-retest reliability of the questionnaire were tested using *kappa statistics*. Descriptive statistics was used for analysis of CPITN Index and proportion of the questionnaire answers. Analysis of variance (*ANOVA*) and *Chi-square* statistics were used for testing intra-group variation. *Pearson coefficient* was used for measuring the association between variables.

Results

The study sample comprised 181 (50.0%) males (114 from urban and 67 from rural places) and 181 (50.0%) females (109 from urban and 72 from rural places). Analysis of the questionnaire's answers regarding the tobacco consumption shared the subjects of the studied sample in three different groups: active constant smokers (28.5%), active occasional smokers (10.5%) and non-smokers (61%).

The distribution of the smoker's status according to the gender shows that there are significant differences in active constant smokers ($p<0.05$), more frequently boys (n=60) than girls (n=43), and also in non-smokers ($p<0.05$), more frequently girls (n=124) than boys (n=97); there are no gender differences ($p>0.05$) in active occasional smokers (n=38).

There are no significant differences in active constant smokers and in non-smokers according

Table 2. The CPITN values according to the subject's smoker status

Smoker status	N	CPITN score Mean ± S.D.	p (ANOVA)
Active constant smokers	103	1.68 (± 0.782)	0.002
Active occasional smokers	38	1.76 (± 0.786)	0.026
Non-smokers	221	1.26 (± 1.033)	0.000
Total	362	1.43 (± 0.966)	

Table 3. The distribution and correlation of the individual's CPITN score according to the smoker status

Smoker status	CPITN score					Pearson Correlation/ Sig. (2-tailed)	Total
	0	1	2	3	4		
Active constant smokers	15	8	75	5	0	0.161(**) 0.002	103
Active occasional smokers	5	2	28	3	0	0.117(*) 0.026	38
Non-smokers	73	39	88	20	1	-0.222(**) 0	221
Total	93 (25.69%)	49 (13.53%)	191 (52.76%)	28 (7.73%)	1 (0.27%)	362	

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

Table 4. The cotinine concentration according to the CPITN score in smokers

Active smoker status	CPITN score	N	Cotinine concentration (ng/mL) Mean ± S.D.	p (ANOVA)
Constant	0	15	4.107 ± 1.5813	0.001
	1	8	4.538 ± 1.3711	
	2	75	4.928 ± 1.4050	
	3	5	4.400 ± 2.2000	
	Total	*103*	*4.752 ± 1.4791*	
Occasional	0	5	2.860 ± 1.2542	
	1	2	1.650 ± 0.7778	
	2	28	4.007 ± 1.2421	
	3	3	5.500 ± 0.0000	
	Total	*38*	*3.850 ± 1.3949*	
Total	0	20	3.795 ± 1.5750	
	1	10	3.960 ± 1.7354	
	2	103	4.678 ± 1.4177	
	3	8	4.813 ± 1.7578	
	Total	*141*	*4.509 ± 1.5066*	

to their life environment ($p>0.05$), but only in the active occasional smokers, found more frequently in the urban than in the rural areas ($p<0.05$).

There are significant differences (ANOVA) in the mean values of the CPITN score in the active smokers (constant and occasional) and non-smokers ($p<0.05$), with higher values in both smoker categories than in the non-smoker category (*Table 2*).

The analysis of the correlation between the individual's CPITN score and the smoker status (*Table 3)* shows that there is a positive relationship between these variables in both smoker categories ($p<0.05$).

The mean cotinine concentration in active smoker groups (*Table 4*), according to the CPITN score, shows significant differences ($p<0.05$) between the constant and occasional

Table 5. The CPITN values according to the cotinine level in smokers

Active smoker status	Cotinine level ng/mL	N	CPITN values (Mean ± S.D.)	p (ANOVA)
constant	1.1	5	1.40 ± 1.342	
	2.2	3	1.33 ± 1.155	
	3.3	20	1.80 ± 0.616	
	4.4	23	1.26 ± 0.915	
	5.5	30	1.87 ± 0.681	
	6.6	22	1.86 ± 0.560	
	Total	*103*	*1.68 ± 0.782*	
occasional	1.1	3	1.00 ± 1.000	0.58
	2.2	4	1.25 ± 0.957	
	3.3	14	1.71 ± 0.726	
	4.4	5	1.60 ± 0.894	
	5.5	12	2.25 ± 0.452	
	Total	*38*	*1.76 ± 0.786*	
Total	1.1	8	1.25 ± 1.165	
	2.2	7	1.29 ± 0.951	
	3.3	34	1.76 ± 0.654	
	4.4	28	1.32 ± 0.905	
	5.5	42	1.98 ± 0.643	
	6.6	22	1.86 ± 0.560	
	Total	*141*	*1.70 ± 0.781*	

smokers, with higher salivary cotinine concentrations in the first group.

On the other side, the analysis of the CPITN mean values according to the cotinine level in the two groups of active smokers (*Table 5*) shows that there are no significant differences ($p>0.05$) in the mean CPITN values between the constant and occasional smokers.

The analysis of the correlation between the individual's salivary cotinine level and the active smoker status (Pearson coefficient) shows that there is a positive significant relationship ($p=0.001$) between these two variables (*Table 6).*

In the same time, there is a positive significant correlation ($p=0.008$) between the individual's salivary cotinine level and the individual's CPITN score (*Table 7)*.

Discussions

According to the World Health Organisation (W.H.O.) (19), smoking is at the present moment the principal avoidable cause of premature death in the whole world, being involved in the etiology of numerous systemic diseases and also in the appearance of the principal oral-dental diseases: dental caries, periodontal diseases and oral cancer.

With a very complex and multifactorial etiology, the periodontal disease tends to become the most important problem of the oral health at global level; it has a very strong association with smoking (6-8), recognized as the most important behavioral risk factor of periodontal disease, and it has a bi-directional relation with the general health status (20).

As mentioned in the introduction of this

Tabel 6. Distribution and correlation of the individual's cotinine level according to the active smoker status

Active smoker status	Cotinine level (ng/mL)						Total
	1.1	2.2	3.3	4.4	5.5	6.6	
Occasional	3	4	14	5	12	0	38
Constant	5	3	20	23	30	22	103
Total	*8*	*7*	*34*	*28*	*42*	*22*	*141*
Pearson Correlation/ Sig. (2-tailed)	0.267(**)/ 0.001						

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

Table 7. Distribution and correlation of the individual's cotinine level according to the individual's CPITN score

CPITN score	Cotinine level (ng/mL)						Total
	1.1	2.2	3.3	4.4	5.5	6.6	
0	3	2	3	8	3	1	20
1	1	1	3	3	0	2	10
2	3	4	27	17	34	18	103
3	1	0	1	0	5	1	8
Total	*8*	*7*	*34*	*28*	*42*	*22*	*141*
Pearson Correlation/ Sig. (2-tailed)	0.221(**)/0.008						

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

paper, nicotine is a chemical found in all tobacco products that is metabolized to cotinine in the liver.

Because the half lives of salivary and plasma cotinine are similar and the clearance and distribution values in saliva are directly proportional to the corresponding values in plasma, the salivary cotinine has been first used as an important biomarker in the environmental research, to document the tobacco smoke exposure.

A multitude of studies has been done and demonstrated important correlations between the tobacco smoke exposure and the salivary cotinine levels.

On the other side, the association between tobacco smoke exposure and the severity of periodontal disease was also fully demonstrated in a lot of clinical and laboratory studies, but the role of cotinine, that is always present in smoker's oral fluids, have been only suggested, as a possible determining factor in terms of development of periodontitis.

In this order, the present study had two parts: a cross-sectional study aimed to identify the smokers in the study group and to evaluate their periodontal health by CPITN Index, and a laboratory study for evaluating the salivary cotinine concentration in the active smokers.

Regarding the prevalence of smoking, our study found that 39% of the subjects are active smokers (shared in constant and occasional smokers), the most part of the subjects (61%) being non-smokers. According to our results, the smoking habit is more frequently in boys than in girls. This percent of active smokers is higher than the results found in other articles (21), or than the W.H.O. data, where the smoking in the ranks of the youth reaches 20 - 29% in U.S.A., over 30% in Russian Federation, about 30% and over in Ukraine and 20 -

29% in Poland (22).

Regarding the CPITN values given by this study, they show the same conclusion as the most other studies related to this subject (6-9, 10, 21, 22), namely higher mean CPITN values in smokers comparing with non-smokers, and a positive relationship between CPITN individual score and the smoking status.

The distribution of the CPITN scores shows that 25.69% of subjects have as a highest CPITN score the value 0, meaning healthy periodontal conditions. This percent is higher than the data given by W.H.O. for Europe (19 - 20% of CPITN value 0 in people aged 15 - 19 year old), showing a little better periodontal health in the study's subjects. However, the most prevalent CPITN score in our study is 2, in accordance with the W.H.O. data (20).

The results of the laboratory study, regarding the relationship between the individual's salivary cotinine level and the active smoker status showed a positive direct relationship between these two variables, with significant higher salivary cotinine levels in active constant smokers comparing with the active occasional smokers.

Even if there are differences in smoker groups according to the cotinine level, there are no significant differences in the mean CPITN values between the constant and occasional smokers, meaning that smoking, even occasional, gives the same risk for periodontal disease as the constant smoking.

Finally, the most important results of this study obtained a direct relationship between the individual's salivary cotinine level and the individual's CPITN score.

The results of our study have to be discussed and interpreted with caution, as time as a comparison of these results with other similar studies is very difficult, given the low number of studies on this theme even at international level, the different methods used in the studies and also the conflicting results given by some of these studies. The results of McGuire et al. (23) showed that the presence of cotinine in the saliva reflects the extent of the systemic distribution of nicotine in smokers and may possibly affect the pathogenesis of periodontal disease. The conclusion of the study made by Y. M. Gonzales et al. (24) was that the serum cotinine levels are correlated with the severity of periodontal attachment loss. The study of Eramo S. et al. (25) showed that the presence in saliva of cotinine explains the increased susceptibility to periodontal disease in smokers.

On the other side, the study made by Chen X. et al. (10) did not find any relationship between the salivary cotinine level and periodontal disease status, being in contradiction with the results of our study and the other studies cited before.

The significant correlation found in the current study between salivary cotinine level and individual CPITN score in smokers needs further investigation as the results of the study suggest that measuring the salivary cotinine level can give useful information for periodontal risk assessment.

Given the importance of using salivary biomarkers for monitoring the periodontal disease, the increasing prevalence of this disease and its appearance at young ages often in association with smoking habit, it can be considered that studies on this subject have to be repeated in extended samples of subjects and in all age groups, in order to clarify the role of the salivary cotinine in the periodontal disease pathogenic mechanism.

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Competing interests disclosure

The authors of this article declared that

there are no potential conflicting interests regarding this research, in which they may be involved.

Abbreviations

CPITN - Community Periodontal Index of Treatment Needs
WHO - World Health Organization
SPSS - Statistical Package for the Social Sciences

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