DOI: 10.15386/cjmed-667

ASSESSMENT OF THE CORRELATIONS BETWEEN NICOTINE DEPENDENCE, EXHALED CARBON MONOXIDE LEVELS AND ORAL HYGIENE STATUS: AN OBSERVATIONAL STUDY

MINODORA MOGA[1], ADINA BIANCA BOSCA[2], COSMINA IOANA BONDOR[3], ARANKA ILEA[1], ONDINE PATRICIA LUCACIU[1], ANCA IONEL[1], MILENA ADINA MAN[4], RUXANDRA MIOARA RAJNOVEANU[4], RADU SEPTIMIU CÂMPIAN[1]

[1]Oral Rehabilitation Department 3, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Histology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Medical Informatics and Biostatistics Department 12, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[4]Pneumology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Background and aim. *Cigarette smoking has negative effects on general health, including oral health. The aim of our study was to assess the correlations between nicotine dependence, exhaled carbon monoxide levels and oral hygiene status.*

Methods. *Smoker and non-smoker participants were enrolled in this observational study. The Fagerström test was used to classify nicotine dependences: low (score: 0-3), medium (score: 4-6) or high (score: 7-10). The oral hygiene status was classified according to the oral hygiene indices of plaque, calculus and gingival inflammation. Lastly, the exhaled carbon monoxide levels were measured with a MicroSmokelyzer (Bedfont Scientific Ltd., Kent, United Kingdom).*

Results. *Sixty five participants (50 smokers in the study group and 15 non-smokers in the control group) were enrolled between 11th and 29th of January 2016. No statistical differences were observed between the study group and the control group in terms of age (mean age±SD 23.5±1.9 and 24.0±1.5, respectively) or gender (50% and 26.6%, respectively). A statistically significant difference was observed between the 2 groups in terms of plaque, (p=0.036), calculus (p=0.001) and gingival indices (p<0.001). A positive correlation was found between the exhaled levels of carbon monoxide and the general Fagerström score (r=0.97, p<0.001) or the Fagerström score in smokers (r=0.93, p<0.001); a negative correlation was observed between the exhaled carbon monoxide levels and the number of tooth brushings daily (r=–0.41, p=0.001). The plaque index was statistically significantly associated with the exhaled carbon monoxide levels (p=0.008), general Fagerström score (p=0.016) and number of tooth brushings daily (p<0.001). The calculus and gingival indices were statistically significantly associated with the exhaled carbon monoxide levels (p<0.001), general Fagerström score (p<0.001) and score in smoker participants (p=0.029 and p=0.001, respectively) as well as the number of tooth brushings daily (p<0.001).*

Conclusion. *Our study found a significant association between the plaque, calculus and gingival indices and smoking. Moreover, nicotine dependence was significantly associated with the number of daily tooth brushings and the gingival index.*

Keywords*:* nicotine dependence, oral health, oral hygiene, carbon monoxide

Manuscript received: 25.03.2016
Received in revised form: 04.05.2016
Accepted: 15.05.2016
Address for correspondence: biancabosca@yahoo.com

Background and aims

Cigarette smoking is harmful for almost every organ of the body and is a known cause of many diseases [1,2]. Approximately 1 in 5 deaths in the United States [1,2] and 1 in 4 cancer deaths in the UK [3] were associated with smoking. It was estimated that cigarette smoking increases the risk of coronary heart disease and stroke by 2 to 4 times [1,4], and the risk of lung cancer by 25 times in men and 25.7 times in women [1]. According to the 2015 Eurobarometer, the smoking prevalence was 26% in the European Union [5], the highest prevalence being recorded in Greece (38%), Bulgaria (35%) and Croatia (33%). In Romania, a smoking prevalence of 27% in the general population was registered [5].

Among the negative effects smoking has on oral health, one can list increased plaque and calculus, increase in the incidence of periodontal disease or increased risk of oral cancer [6-8]. A systematic review on the relationship between smoking and periodontal disease highlighted evidence that supports the strong negative impact of smoking on periodontal health [9]. The general aspect of this finding is greatly increased by the fact that most of the studies were conducted in different parts of the world, in participants with different living style, cultural and environmental conditions, and in large cohorts (up to 7056 participants in a cross-sectional study conducted in Israel) [10]. Besides periodontal disease, increased rates of tooth loss have been observed in smoker participants in longitudinal studies [11-17].

It has been shown previously that smoking cessation has beneficial effects on overall health, including oral health [8]. Generally, the former smokers' periodontal status is intermediate to that of never smokers and active smokers. Moreover, periodontal treatment outcomes in former smokers are similar to those who had never smoked, and usually better compared to the outcomes expected for active smokers [8]. Also, a study conducted in 1462 Swedish women found that in the 12-year follow-up period, participants who had never smoked had a similar number of lost teeth compared to former smokers who quit smoking before baseline [17].

The Fagerstörm test for nicotine dependence is an instrument broadly used by physicians to document indications for prescribing medication for nicotine withdrawal. Developed by Karl-Olov Fagerström in 1978 [18] and later modified by Todd Heatherton et al. in 1991[19], the test assesses the intensity of a patient's physical dependence to nicotine.

The measurement of expired carbon monoxide (CO) levels might be useful in smoking cessation programs; by emphasizing these levels to smokers, they might become aware of the direct benefits of smoking cessation, as CO levels decrease to normal levels after cessation [20]. It is known that the odds of smoking cessation increased with the interventions in the dental setting [21].

To our best knowledge, only one retrospective study assessing the psychosocial and medical parameters associated with the vulnerability to nicotine addiction was conducted in smokers in Romania between 2009–2012 [22]. The aim of our study was to determine the correlations between nicotine dependence, exhaled carbon monoxide (CO) levels and oral hygiene status by comparing these features in smokers (study group) against a control group of non-smokers.

Methods

Study sample

This pilot observational study was conducted in students of the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Participants were enrolled if they belonged to the 20-30 years age range, were healthy, had at least 24 teeth (except the wisdom teeth), and were capable and willing to comply with the study procedures. Before enrollment, all participants signed an informed consent form. Participants were not enrolled if they were >30 years of age, had a history of chronic cardiovascular, metabolic or immunologic diseases, had less than 24 teeth or were on treatment for any chronic disease.

Nicotine dependence was assessed by filling in the modified Fagerström test [19]. The test contains 6 questions related to the time elapsed between waking and the first cigarette, the number of smoked cigarettes, as well as the moment of the day when most cigarettes are smoked. The test also inquiries about the difficulty to refrain from smoking in places where it is forbidden, which cigarette the person is less likely to give up, and smoking even in the case of sickness in bed. The score of the questionnaire was obtained by adding up the scores from each answer. Based on the obtained score, nicotine dependence was classified as low (score: 0-3), medium (score: 4-6) or high (score: 7-10).

Oral hygiene status was classified according to the oral hygiene indices of plaque (Silness-Löe plaque index [23]), calculus (Löe retention index [24]) and gingival inflammation (Löe-Silness gingival index [24]. To minimize bias, only one dentist performed all oral cavity examinations. The indices were calculated as follows [25]:

The plaque index evaluates the oral hygiene of a participant and records the presence of both soft debris and mineralized deposits on the following teeth: 1.6, 1.2, 2.4, 3.6, 3.2 and 4.4. The absent teeth were not replaced when calculating the score. For every dental surface examined (distofacial, facial, mesiofacial and lingual) a score was assigned as follows:

- 0 was assigned to no plaque;
- 1 was assigned to any visible plaque, plaque being visible only after using the probe on the tooth surface;
- 2 was assigned to soft, pellicular deposits visible with the naked eye;

• 3 was assigned to abundance of soft matter within the gingival pocket from the free gingival margin to the dental surface.

The calculus index assesses the presence or absence of supra and/or subgingival calculus by visual or tactile examination, and does not take into account the quantity of calculus. The same four dental surfaces were examined for the 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6 teeth and individual scores were assigned as follows:

• 0 was assigned to no cavities, calculus or fillings with irregular edges next to the gum;

• 1 was assigned to supragingival calculus, cavities, fillings with irregular edges;

• 2 was assigned to subgingival calculus, fillings with irregular edges;

• 3 was assigned to abundance of supragingival and subgingival calculus, deep cavities, fillings with marginal defects and retentive margins.

For the ***gingival index*** calculation, four dental surfaces (distofacial, facial, mesiofacial and lingual) of the same tooth were examined as for the plaque index (1.6, 1.2, 2.4, 3.6, 3.2 and 4.4) and the individual score were assigned as follows:

• 0 was assigned to healthy gingival tissue;

• 1 was assigned to slightly inflamed gums, discrete color modifications, discrete edema, lack of bleeding on probing;

• 2 was assigned to medium inflammation, congestion, edema, bleeding on probing;

• 3 was assigned to advanced inflammation, congestion, stasis, ulcerations, spontaneous bleeding.

The plaque, calculus and gingival indices for every participant were calculated as the sum of the individual scores for each surface divided by the total number of examined surfaces.

Finally, the exhaled CO levels were measured in all participants with a MicroSmokelyzer (Bedfont Scientific Ltd., Kent, United Kingdom), a breath CO monitor intended for multipatient use. In the case of smoker participants, the exhaled CO levels were measured 15 minutes after the participants smoked the first cigarette on the day of measurement. After automatic calibration, participants were asked to exhale completely, inhale deeply and hold their breath for 15 seconds and then exhale slowly and fully into the analyzer. Also, patients were instructed on how to exhale in the mouthpiece of the monitor to ensure that no air escaped from the measuring device while exhaling. All samples were collected in the dental cabinet where smoking is not permitted. The results were immediately displayed on the screen, showing the CO levels in parts per million (ppm).

The study was conducted according to the Good Clinical Practice Guidelines and the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy, approval no. 514/17.12.2015.

Statistical analysis

The data were analyzed using descriptive statistics. Box-plot graphs with arithmetic means, standard errors (SE) and standard deviations (SD) were calculated. Spearman or Pearson correlation coefficients were used. The correlation between 2 qualitative variables was calculated with Chi square test; normal distribution was tested using the Kolmogorov-Smirnov test. The comparison between means was done as follows: 2 normally distributed means were compared using the Student t test while 3 normally distributed means were compared using the Anova test. For non-normal distributions, 2 means were compared with the Mann-Whitney test, and 3 means with the Kruskal-Wallis test. The significance level chosen for all statistical tests was$p\leq0.05$. All statistical analyses were performed using Statistica 7.0 (StatSoft Inc., Oklahoma, USA) and SPSS 15.0 for Windows (IBM Software, New York, USA) software packages.

Results

Between 11 - 29 January 2016, 65 participants aged 20–29 years (mean age±standard deviation [SD] 23.6±1.9) were enrolled in the study. Fifty participants were smokers and were included in the study group, while 15 participants were non-smokers and were included in the control group (Table I). There were no statistically significant differences between the 2 groups in terms of age (mean age±SD 23.5±1.9 in the study group and 24.0±1.5 in the control group, $p=0.307$) or gender (50% men in the study group and 26.6% men in the control group, $p=0.11$). All participants belonged to the urban setting.

Seven out of the 50 (14.0%) participants in the study group declared that they brushed their teeth once daily, 31/50 (62.0%) twice daily, 12/50 (24.0%) three times daily. In the control group, 6/15 (40.0%) participants declared that they brushed their teeth daily, 7/15 (46.6%) three times daily while 2/15 (13.3%) four times daily, the difference between the mean number of brushings daily being statistically significant between the 2 groups (mean±SD2.1±0.6 in the study group and 2.7±0.7 in the control group, $p=0.003$). A statistically significant difference was observed between smokers and non-smokers in terms of plaque ($p=0.036$), calculus ($p=0.001$) and gingival ($p<0.001$) indices (Table I).

According to the Fagerström test, among the 50 smoker participants in the study group, low nicotine dependence was calculated for 16 (32.0%) participants, medium nicotine dependence for 23 (46.0%) and high nicotine dependence for 11 (22.0%).

The overall measured exhaled CO levels were in the 1–20 ppm range: 1–2 ppm in the control group and 3–20 ppm in the study group. A positive correlation was found between the exhaled levels of CO and the Fagerström score (general: $r=0.97$, $p<0.001$; in smokers: $r=0.93$, $p<0.001$;

Table I. Baseline characteristics.

		Smokers (N=50)	Non-smokers (N=15)	p
Plaque index, n (%)	0	8 (16.0)	7 (46.7)	0.036
	1	31 (62.0)	7 (46.7)	
	2	11 (22.0)	1 (6.7)	
Calculus index, n (%)	0	22 (44.0)	15 (100.0)	0.001
	1	25 (50.0)	0 (0.0)	
	2	3 (6.0)	0 (0.0)	
Gingival index, n (%)	0	17 (34.0)	15 (100.0)	<0.001
	1	24 (48.0)	0 (0.0)	
	2	9 (18.0)	0 (0.0)	
Mean age (years)		23.5	24	0.307
Gender, men (%)		50	26.66	0.11
CO (ppm)		11.46±4.76	1.27±0.46	<0.001
Fagerströmscore		4.80±2.36	0.00	<0.001
Number of brushing daily		2.10±0.61	2.73±0.70	0.003

N- total number of participants; n- number of participants in a given category; CO- carbon monoxide; ppm- parts per million.

Table II. Pearson and Spearman correlation between exhaled CO levels/ Fagerström score and other parameters.

Parameters	Pearson or Spearman coefficient of correlation (r)	p
CO- Score	0.97	<0.001
CO- Score smokers	0.93	<0.001
CO- Age	-0.06	0.614
CO- Number of brushings	-0.41	0.001
Score - Age	-0.04	0.763
Score - Number of brushings	-0.40	0.001

CO- carbon monoxide.

Table III. Relationship between nicotine dependence and oral health indices in the study group (N=50).

		Low nicotine dependence (n)	Medium nicotine dependence (n)	High nicotine dependence (n)	
Plaque index	0	4	3	1	0.69
	1	10	14	7	
	2	2	6	3	
Calculus index	0	11	9	2	0.10
	1	5	12	8	
	2	0	2	1	
Gingival index	0	11	5	1	0.001
	1	5	14	5	
	2	0	4	5	

N- total number of smoking participants; n- number of participants in a given category.

Table II). A negative correlation was found between the exhaled CO levels and the number of brushings daily (r=–0.41, p=0.001) and between the Fagerström score and the number of daily tooth brushings (Table II).

In the control group, 7/15 (46.7%) had the plaque index 1 while all of them had the calculus and gingival indices 0 (15/15, 100.0%; Table I). Only 8/50 (16.0%) of participants of the study group had the plaque index 0, the majority (31/50, 62.0%) having plaque index 1 (Table III). Half of the smoking participants (25/50, 50.0%) had the calculus index 1, while only 3/50 (6.0%) participants (2 from the medium nicotine dependence group and 1 from the high nicotine de dependence group) had the calculus index 2. The gingival index was found to be significantly associated to nicotine dependence (Table III). Additionally, statistically significant differences were observed between men and women in terms of nicotine dependence: low nicotine dependence was observed in 4 men vs 12 women; medium nicotine dependence was observed in 13 men vs 10 women; high nicotine dependence was observed in 8 men vs 3 women (p=0.036).

Overall, the plaque index was significantly associated with the exhaled CO levels (p=0.008), general Fagerström score (p=0.016) and the number of teeth brushings daily (p<0.001; Table IV, Figure 1, Figure 2). The calculus and gingival indices were significantly associated with the exhaled CO levels (p<0.001), general Fagerström score (p<0.001) and the number of teeth brushings daily (p<0.001) in the overall population; also, the calculus and gingival indices were significantly associated with the Fagerström score in smoker participants (p=0.029 and 0=0.001, respectively). Age was not found to have a significant impact on the oral health indices.

Table IV. Relationship between oral health indices and Fagerström score, brushing and age in the overall population (N=56).

		Mean ±SD		
		Fagerström score	**Brushings daily**	**Age**
Plaque index	0 (n=15)	1.93±2.60	3.13±0.35	24.20±1.61
	1 (n=38)	3.95±2.87	2.16±0.37	23.50±1.91
	2 (n=12)	5.08±2.50	1.42±0.51	23.08±2.02
p		0.016	<0.001	0.412
Calculus index	0 (n=37)	2.22±2.53	2.62±0.59	23.86±1.86
	1 (n=25)	5.52±2.14	1.80±0.41	23.24±1.7
	2 (n=3)	6.67±2.08	1.33±0.58	23.00±3.00
p		<0.001	<0.001	0.400
Gingival index	0 (n=32)	1.75±2.29	2.72±0.58	24.03±1.84
	1 (n=24)	5.00±1.98	1.88±0.34	23.13±1.87
	2 (n=9)	7.11±1.62	1.56±0.53	23.22±1.86
p		<0.001	<0.001	0.192

N- number of participants in the overall population; n- number of participants in a given category; SD- standard deviation.

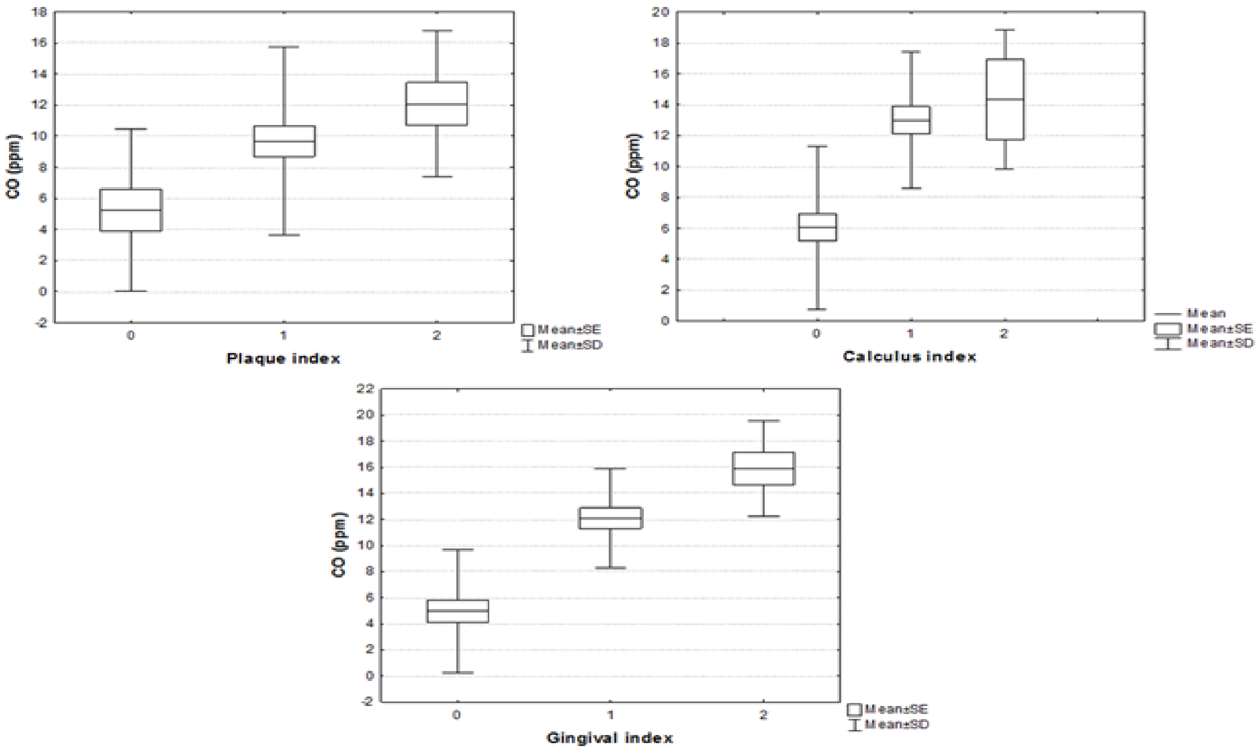

Figure 1. Correlation between exhaled CO levels and oral hygiene (plaque, calculus and gingival indices) in the overall population (N=65).

N- total number of participants; CO- carbon monoxide; ppm- part per million; SD- standard deviation; SE- standard error.

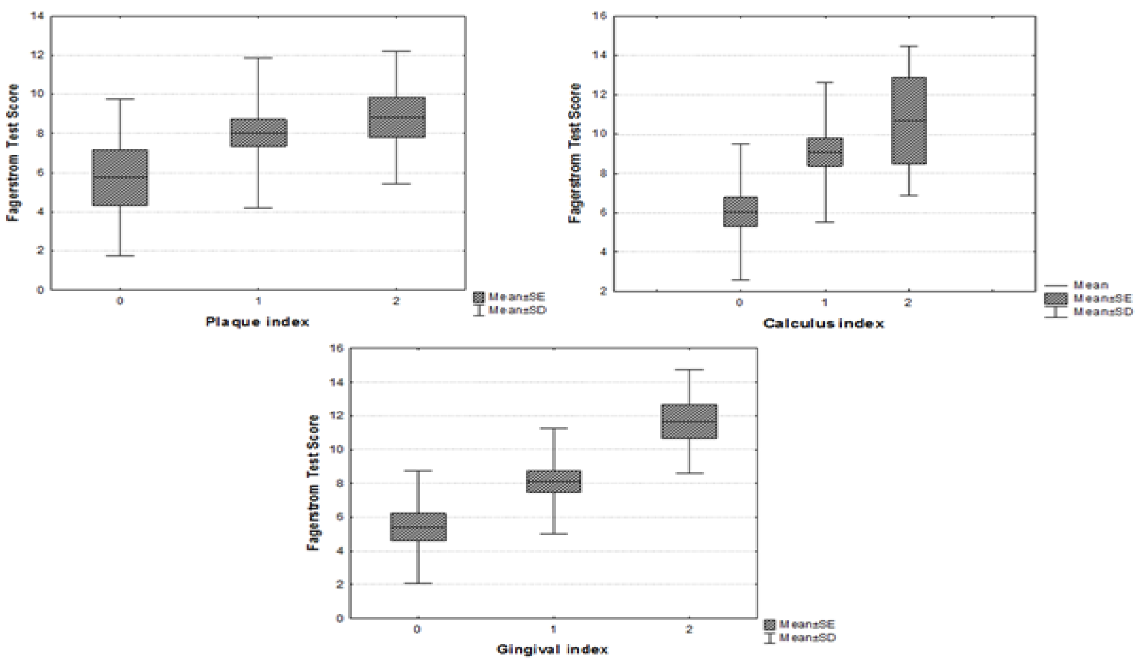

Figure 2. Correlation between nicotine dependence and oral hygiene (plaque, calculus and gingival indices) in the overall population (N=65).

N- total number of participants; SD- standard deviation; SE- standard error.

Discussion

Our study conducted in healthy smoker and non-smoker participants found a positive correlation between the plaque, calculus and gingival indices, as part of oral health evaluation, and smoking. Also, we found that the Fagerström score assessing nicotine dependence was significantly correlated to the number of daily tooth brushings and the gingival index. Additionally, in our study we found nicotine dependence to be gender related.

A 1-year follow-up study conducted in Switzerland found a good correlation between exhaled CO levels and self-reported smoking habits [26]. Considering that the measurement of exhaled CO levels is a non-invasive, objective and simple technique to monitor and document smoking cessation and reduction, we used this tool to study the association of exhaled CO levels with nicotine dependence. The measurement of exhaled CO levels shows immediate results, with high specificity (96%) and sensitivity (94%) [27]. We found that exhaled CO levels were positively correlated to nicotine dependence as assessed by the Fagerström score. Brügger et al. found that the CO levels were influenced by the number of cigarettes smoked daily ($p<0.001$) and by the pack-year values ($p=0.005$ at the initial visit, and $p=0.006$ at the follow-up visit) [26]. Also, the authors recorded a 0.15 ppm increase in the exhaled CO value after 1 pack-year more (95% confidence interval [CI] 0.05–0.26 ppm, both at the baseline [$p=0.005$] and 1-year follow-up [0.006] examinations). For current smokers, an increase of 0.77 ppm (95% CI 0.70–0.98 ppm, $p<0.0001$) in the exhaled CO levels was observed at the 1-year follow-up visit when cigarette consumption increased by 1 cigarette per day. Frei et al. found a mean difference in exhaled CO values between smokers and non-smokers of 13.95 ppm (95% CI 11.60–16.30 ppm, $p<0.001$), while the consumption of 1 pack-year more was similar to that reported by Brügger et al.: 0.16 ppm (95% CI 0.06–0.26 ppm, $p=0.003$) [26].

Our study found a statistically significant difference between smokers and non-smokers in terms of calculus ($p=0.001$) and gingival ($p<0.001$) indices. Bergström observed prevalence rates for supragingival calculus of 86%, 66%, and 65%for current smokers, former smokers, and nonsmokers, respectively [28]. The occurrence and severity of calculus in former smokers who quit smoking in the distant past was similar to participants who had never smoked. Also, our findings are in line with a recent study conducted in healthy smokers and non-smokers with or without periodontal disease, which found that the plaque control record was significantly higher in the smoker groups compared to the non-smokers, independent of their periodontal condition [29].

Our pilot study enrolled a relevant number of participants to obtain significant associations between the studied parameters. However, for an accurate determination of the correlations between nicotine dependence, oral hygiene and social status, future research is planned on a higher sample size, enrolling patients with a broader age range (18–80 years) and belonging to both rural and urban settings.

Conclusion

Our study found a positive correlation between the plaque, calculus and gingival indices and smoking. Moreover, the Fagerström score assessing nicotine dependence was significantly associated with the number of daily tooth brushings and the gingival index.

References

1.U.S. Department of Health and Human Services. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. 2014 Surgeon General's Report: The Health Consequences of Smoking—50 Years of Progress. Available from: http://www.cdc.gov/tobacco/data_statistics/sgr/50th-anniversary/index.htm.
2.U.S. Department of Health and Human Services. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. Consumer Booklet. Available from: http://www.cdc.gov/tobacco/data_statistics/sgr/2010/consumer_booklet/index.htm.
3.Cancer Research UK. How smoking causes cancer. Available from: http://www.cancerresearchuk.org/about-cancer/causes-of-cancer/smoking-and-cancer/how-smoking-causes-cancer.
4.U.S. Department of Health and Human Services. Rockville (MD): U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. Reducing the Health Consequences of Smoking: 25 Years of Progress: A Report of the Surgeon General: 1989 Executive Summary. Available from: http://profiles.nlm.nih.gov/NN/B/B/X/S/.
5.Special Eurobarometer 429. Attitudes of Europeans towards tobacco and electronic cigarettes. Available from: http://ec.europa.eu/public_opinion/archives/ebs/ebs_429_en.pdf.
6.Johnson NW, Bain CA. Tobacco and oral disease. EU-Working Group on Tobacco and Oral Health. Br Dent J. 2000;189:200-206.
7.Reichart PA. Identification of risk groups for oral precancer and cancer and preventive measures. Clin Oral Investig. 2001;5:207-213.
8.Warnakulasuriya S, Dietrich T, Bornstein MM, Casals Peidró E, Preshaw PM, Walter C, et al. Oral health risks of tobacco use and effects of cessation. Int Dent J. 2010;60:7-30.
9.Bergström J. Periodontitis and smoking: an evidence-based appraisal. J Evid Based Dent Pract. 2006;6:33-41.
10.Vered Y, Livny A, Zini A, Sgan-Cohen HD. Periodontal health status and smoking among young adults. J Clin Periodontol. 2008;35:768-772.
11.Krall EA, Dawson-Hughes B, Garvey AJ, Garcia RI. Smoking, smoking cessation, and tooth loss. J Dent Res. 1997;76:1653-1659.
12.Krall EA, Dietrich T, Nunn ME, Garcia RI. Risk of tooth loss after cigarette smoking cessation. Prev Chronic Dis. 2006

Oct;3:A115.
13.Eklund SA, Burt BA. Risk factors for total tooth loss in the United States; longitudinal analysis of national data. J Public Health Dent. 1994;54:5-14.
14.Holm G. Smoking as an additional risk for tooth loss. J Periodontol. 1994;65:996-1001.
15.Jansson L, Lavstedt S. Influence of smoking on marginal bone loss and tooth loss--a prospective study over 20 years. J Clin Periodontol. 2002;29:750-756.
16.Dietrich T, Maserejian NN, Joshipura KJ, Krall EA, Garcia RI. Tobacco use and incidence of tooth loss among US male health professionals. J Dent Res. 2007;86:373-377.
17.Ahlqwist M, Bengtsson C, Hollender L, Lapidus L, Osterberg T. Smoking habits and tooth loss in Swedish women. Community Dent Oral Epidemiol. 1989;17:144-147.
18.Fagerstrom KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict Behav. 1978;3:235-241.
19.Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict. 1991;86:1119-1127.
20.Jarvis MJ, Belcher M, Vesey C, Hutchison DC. Low cost carbon monoxide monitors in smoking assessment. Thorax. 1986;41:886-887.
21.Needleman IG, Binnie VI, Ainamo A, Carr AB, Fundak A, Koerber A, et al. Improving the effectiveness of tobacco use cessation (TUC). Int Dent J. 2010;60:50-59.
22.Postolache P, Dima Cozma C, Cojocaru D. Assessment of nicotine dependence in a large cohort of smokers - social and medical aspects. Revista de cercetare si interventie sociala. 2013;41:106-117.
23.Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22:121-135.
24.Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol. 1967;38(6):Suppl:610-616.
25.Dumitriu H. Parodontologie. Bucuresti: Viata Medicala Romaneasca; 1997.
26.Brugger OE, Frei M, Sendi P, Reichart PA, Ramseier CA, Bornstein MM. Assessment of smoking behaviour in a dental setting: a 1-year follow-up study using self-reported questionnaire data and exhaled carbon monoxide levels. Clin Oral Investig. 2014;18:909-915.
27.Middleton ET, Morice AH. Breath carbon monoxide as an indication of smoking habit. Chest. 2000;117:758-763.
28.Bergstrom J. Tobacco smoking and supragingival dental calculus. J Clin Periodontol. 1999;26:541-547.
29.Torkzaban P, Hedayatipanah M. Comparison of Periodontal Conditions Between Smokers and Nonsmokers. Avicenna J Dent Res. 2016:e28019. Epub 20 January 2016. doi: 10.17795/ajdr-28019