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Association between Passive Smoking, Salivary Calcium Level, and Periodontal Health: A Cross-Sectional Study

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Background and objectives: Due to the lack of definitive conclusions about the effects of passive smoking on periodontal health especially in adults and the lack of information on the relationship between salivary calcium levels and periodontitis, this study aimed to compare salivary calcium levels and periodontal status of passive smoking and non-smoking adults.

Materials and methods: This cross-sectional study was conducted on 60 adult females in two groups of passive smokers and non-smokers (n = 30), who were selected using consecutive sampling. The salivary calcium levels were measured using an enzymatic colorimetric method with a kit, and their periodontal health was evaluated by measuring clinical attachment loss (CAL), bleeding index (BI), and probing depth (PPD). The values were then compared between the two groups. Data were analyzed using t-tests, Mann-Whitney tests, Pearson and Spearman's correlation coefficients ($\alpha = 0.05$).

Results: The mean CAL was significantly higher in passive smokers than in non-smokers (P = 0.013). The mean values of BI (P = 0.446), PPD (P = 0.066), and salivary calcium levels (P = 0.248) were not significantly different between the two groups, although slightly higher values were recorded in the passive group. In passive smokers, the mean PPD had a significant correlation with the salivary calcium levels (P = 0.037). In non-smokers, CAL and salivary calcium levels were significantly correlated (P = 0.010).

Conclusion: Passive smokers had significantly greater CAL than non-smokers. Additionally, higher salivary calcium levels were significantly correlated with higher mean PPD in passive smokers and CAL in non-smokers, indicating a possible association between elevated salivary calcium levels and periodontal problems, regardless of smoking status.

Keywords: Calcium, Saliva, Periodontics, Tobacco smoke pollution.

1. Introduction

Tobacco smoke has adverse effects not only on smokers but also on individuals around them who do not smoke (1). Passive inhalation of environmental tobacco smoke, known as passive smoking, poses a risk to public health (2). According to the World Health Organization, passive smoking is defined as non-smokers being exposed to tobacco smoke from at least one cigarette daily or spending a minimum of 2 hours per day in an environment contaminated with tobacco smoke (3,4). Passive smoking has negative health consequences for individuals of all ages (5).

Periodontal disease is the second most prevalent oral disease after dental caries, affecting 5-30% of the adult population (6). Periodontitis is an inflammatory disease caused by biofilm bacteria that can compromise the integrity of the structures supporting the teeth, including the gingiva, periodontal tissue, and alveolar bone (7).

The correlation between smoking and periodontal disease has been widely studied, with cross-sectional and longitudinal studies providing strong evidence of the harmful effects of smoking on periodontal health (8). It has been reported that elevated levels of inorganic ions such as calcium and phosphorous may increase the incidence of periodontitis (7). Saliva is the primary source of mineralization of supra-gingival plaque, and saliva minerals, particularly calcium and phosphorous, are absorbed by dental plaque surrounding the gingival third of the tooth (9). Dental plaque is the main cause of periodontal disease, with calcium, phosphorous, and other minerals being the primary inorganic components of dental plaque. Salivary calcium and phosphorous can be absorbed by dental plaque, resulting in the formation of calculus and eventually causing periodontitis (6). Smokers have been found

to have significantly higher levels of salivary calcium compared to non-smokers (10). Additionally, elevated salivary calcium levels have been significantly correlated with greater bone loss and reduced bone mineral density in smokers compared to non-smokers (8).

Given the limited number of studies available on periodontal status in passive smokers, particularly adults, the existing controversy surrounding the effects of passive smoking on periodontal health, and the lack of information regarding the potential correlation between salivary calcium levels and periodontitis, this study aimed to compare the salivary calcium levels and periodontal health status of passive smokers and non-smokers.

2. Materials and methods

This cross-sectional study was conducted on 60 females in two groups: passive smokers and non-smokers (n=30). The participants were selected from the adult female population of Yazd city, Iran, who referred to the Dental School of Yazd, Shahid Sadoughi University of Medical Sciences. The study protocol was approved by the university's ethics committee (IR.SSU.DENTISTRY.REC.1401.099).

2.1. Sample size

The minimum sample size was calculated to be 30 in each group based on a previous study [11], assuming a 95% confidence interval, 80% study power, a standard deviation of 0.96 for the salivary concentration of calcium in the two groups, and mean salivary calcium levels of 1 in the passive smoker group and 1.8 in the non-smoker group. The participants were selected using consecutive sampling.

2.2. Eligibility criteria

The main inclusion criteria for the passive smokers group were living with an active smoker who smoked at least 1 cigarette per day or was exposed to secondhand smoke pollution in an enclosed area for at least 2 hours per day (4,12). Other inclusion criteria were the same for both the passive smoker and non-smoker groups and included: no history of smoking cessation, age between 18 and 40 years (To avoid the bias of both puberty and menopause on the periodontal condition and to exclude people under the age of 18, who were mainly focused on in previous studies about passive smoking, and that the occurrence of periodontitis in that period is unlikely), female gender (As the probability of a non-smoking adult woman living with a smoking spouse is much higher than the opposite probability in Iranian society), no intake of antibiotics in the past 6 months, no calcium supplementation, no intake of medications that might affect periodontium (such as hypotensive drugs, anticonvulsants, and immune-suppressants), no systemic diseases affecting the gingiva (such as diabetes mellitus), no pregnancy or nursing, no history of periodontal therapy in the past year, no heavy calculus, and no exposure to other tobacco products (such as hookah smoking or chewing snuff). Participants who were uncooperative, unwilling to participate in the study, or did not consent to have their salivary calcium levels measured for any reason were excluded.

2.3. Data collection

After obtaining written informed consent, the participants were assigned to two groups: passive smokers and non-smokers based on self-report (n = 30). The two groups were standardized in terms of mean age and socioeconomic status (financial status and level of education). Additionally, the two groups were matched in terms of mean baseline plaque index. Furthermore, the two groups were standardized regarding baseline oral hygiene behavior and enrolling only those who self-reported regular tooth brushing at least once a day. This was done to minimize the effect of plaque on periodontal status. Next, clinical attachment loss (CAL), pocket probing depth (PPD), and bleeding index (BI) were measured in the two groups and compared.

PPD was measured in all fully erupted teeth at three points in the buccal and three points on the palatal surface (6 points), and the mean value was recorded as the PPD of each tooth. Then, the mean PPD was calculated for each participant and recorded. CAL for each tooth was recorded as the largest measured value and the mean of the recorded values was calculated and reported as the mean CAL for each participant (13).

BI was measured according to Bay and Ainamo in 1975. A periodontal probe was gently inserted into the gingival sulcus at the mesiobuccal, mid-buccal, distobuccal, mesiolingual, midlingual, and distolingual surfaces, and the bleeding areas after 10 seconds were recorded. The number of bleeding surfaces was divided by the total number of surfaces multiplied by 100 and the percentage of BI was recorded (14).

All measurements were performed by a senior dental student who was blinded to the participants' group allocation. Participants' demographic information and results related to para-clinical tests were recorded by another operator.

2.4. Measurement of salivary calcium level

Unstimulated saliva samples were collected from all participants under standard conditions (6). To collect samples, 2 ml of unstimulated saliva was collected by the spitting method from each participant. participants were instructed to avoid eating, drinking, and tooth-brushing for 90 minutes prior to saliva collection to minimize the effects of daily changes in saliva composition. Saliva samples were collected while the participants were seated. They were asked to hold their saliva in their mouth and spit into a sterile test tube every 60 seconds for a total of 5 minutes. The saliva samples were then sent to a laboratory where they were centrifuged and frozen at -24°C. The

salivary calcium level was measured manually using the enzymatic colorimetric method with a kit (Delta Darman, Iran) and a spectrophotometer (Ray Leigh, Italy). The optical density of the samples was measured and the salivary concentration of calcium in milligrams/deciliters (mg/dL), was calculated using the following formula:

Calcium level (mg/dL) = $\frac{\text{optical density of test sample}}{\text{Optical denisty of calibrator}}$ x calibrator concentration

2.5. Statistical analysis

Normal distribution of data was evaluated by the Kolmogorov-Smirnov test and the Shapiro-Wilk test. PPD and BI had a normal distribution ($P > 0.05$) and thus, were analyzed by independent t-test. Their correlations were also analyzed by the Pearson's correlation coefficient. CAL and salivary calcium level had non-normal distribution ($P < 0.05$). Therefore, they were analyzed using Mann-Whitney U test and Spearman's correlation coefficient. All statistical analyses were performed by SPSS version 23 (SPSS Inc., IL, USA) at a 0.05 level of significance.

3. Results

A total of 60 female participants were evaluated with a mean age of 29.25 ± 7.57 years (range 18 to 40 years). No significant difference was found in the mean age of passive smokers (29.27 ± 7.62 years) and non-smokers (29.27 ± 7.62 years) ($P = 0.987$). Table 1 presents the mean salivary calcium level, PPD, CAL, and BI in the two groups. As shown, the mean CAL was significantly higher in passive smokers than non-smokers ($P = 0.013$). The mean BI ($P = 0.446$), PPD ($P = 0.066$), and salivary calcium level ($P = 0.248$) were not significantly different between the two groups although slightly higher values were recorded in the passive smoker group.

Correlations between the variables are shown in Table 2. As indicated, by an increase in salivary calcium level, PPD significantly increased ($P < 0.05$). No other significant correlations were found ($P > 0.05$). In the non-smoker group, CAL had a significant correlation with elevated salivary calcium level ($r = 0.464$, $P = 0.010$), therefore by an increase in salivary level of calcium, CAL significantly increased. No other significant correlations were found ($P > 0.05$).

Table 1. Mean salivary calcium level (mg/dL), PPD, CAL, and BI in the groups.

Variable	Passive smokers	Non-smokers	P-value
Salivary calcium	5.37 + 3.91	1.15 + 3.12	0.248
PPD	0.38 + 1.80	0.32 + 1.62	0.066
CAL	0.29 + 0.13	0.29 + 0.05	0.013
BI	16.67 + 37.32	18.77 + 33.81	0.446

Table 2. Correlations between the variables.

Periodontal parameters correlated with salivary calcium level	Age	PPD	CAL	BI
Passive smokers	r = 0.093 P = 0.626	r = 0.383 P = 0.037*	r = -0.096 P = 0.615	r = 0.111 P = 0.560
Non-smokers	r = 0.130 P = 0.492	r = 0.109 P = 0.567	r = 0.464 P = 0.010*	r = 0.119 P = 0.533

4. Discussion

The effects of passive smoking on oral health are still a matter of question (15,16). This study compared the salivary calcium level and periodontal health status of passive smokers versus non-smokers. The results showed significantly higher CAL in passive smokers, compared to non-smokers, indicating the adverse effect of passive smoking on periodontal health. Consistent with the present result, Akinkugbe et al, (17) and Arbes et al. (18) found a positive correlation between environmental exposure to tobacco smoke and periodontitis in passive smokers. However, Kanmaz et al. (19) reported insignificantly higher CAL in passive smokers, compared with non-smokers. Tanaka et al. (20) reported a significant correlation between active smoking and periodontal disease but did not detect any correlation between passive smoking and periodontal disease. Variations in the reported results in the literature can be attributed to differences in the adopted methods for periodontal health assessment. For instance, Tanaka et al, (20) only evaluated six teeth, and all female patients with a minimum of 1 tooth with ≥ 3.5 of PPD were assigned to the periodontitis group. Unlike the present results, Alan et al. (15) compared periodontal parameters in passive smoker and non-smoker children and found no evidence supporting the correlation between passive smoking and the development of periodontal disease in children. The difference between their results and the current findings might be attributed to the fact that they only examined the periodontal status of four teeth due to poor cooperation of children, which cannot be a good representative of their overall periodontal health. Moreover, they did not use a standard criterion for selection of passive smokers, and only asked about parental smoking. Also, unlike the present study, they did not pay attention to the household surface area and duration of exposure to secondhand smoke (15).

As a relative summary, according to a meta-analysis of eight cross-sectional studies by Oliveira et al. in 2022, extremely uncertain evidence suggests that children and adolescents exposed to secondhand smoke have poorer periodontal status, possibly due to higher gingival index scores. In this study, there was no significant difference in PPD in the two groups (21). On the other hand, Rafieyan et al. in 2022 in a systematic review on both children and adults, concluded that scientific evidence showing the effect of passive smoking on periodontitis is limited and inconclusive (4).

In the present study, PPD, BI and salivary calcium level were not significantly different between the two groups although slightly higher values were recorded in the passive smoker group. Similarly, Erdemir et al., (22) Alan et al., (15) and Kanmaz et al. (19) found no significant difference in bleeding on probing and PPD between passive smokers and non-smokers. Some studies reported higher salivary calcium level in active smokers compared with matched non-smokers. It is believed that smoking, as an independent factor, increases the salivary level of calcium by decreasing the skeletal bone mass density and release of calcium ions (23,24). Studies on salivary calcium level of passive smokers are scarce. Avşar et al. (22) reported that the serum calcium level of children exposed to secondhand smoke had no significant difference with non-smoker healthy controls, which was in line with the present results. Acharya et al. (9) reported that high salivary level of calcium can serve as a risk factor for periodontal disease in all individuals. In the present study, the mean PPD had a significant correlation with the salivary calcium level in the passive smoker group. In the non-smoker group, CAL and salivary calcium level were significantly correlated. Sutej et al. (8) demonstrated that by an increase in salivary level of calcium, the incidence of periodontal disease increased in both smoker and non-smoker groups, which was in agreement with the present results.

It should be noted that no significant correlation has been reported between daily calcium intake through diet and salivary level of calcium in different individuals, including smokers. In other words, the salivary calcium level is determined by the salivary gland secretions, and does not reflect the daily calcium intake (11,25). Therefore, assessment and comparison of calcium intake through diet does not seem to affect the present results; however, no calcium supplementation was among the inclusion criteria of the present study.

5. Limitations

Passive smoking status of the participants was determined based on their self-report since taking serum samples for research purposes was not ethical. Due to the higher prevalence of smoking in males in Iran, and the higher chance of females being a passive smoker, only females were included in this study. However, this situation may be different in other communities, and therefore, the present results may not be generalizable to all populations. Moreover, it was not possible to get the exact time of exposure to cigarette smoke in this study as it was not possible to monitor the participants around the clock.

Future studies are recommended to assess para-clinical parameters indicating the presence of inflammation such as C reactive protein and alkaline phosphatase. Also, salivary cotinine can be measured to ensure exposure to secondhand smoke instead of self-reporting by the participants. It is also suggested to investigate the effect of passive smoking on periodontal conditions in a dose-dependent manner in future studies.

6. Conclusion

Passive smokers had significantly greater CAL than non-smokers. Also, higher salivary calcium level was significantly correlated with higher mean PPD in passive smokers, and CAL in non-smokers, indicating a possible association between elevated salivary calcium levels and periodontal problems irrespective of passive smoking or non-smoking status.

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Authors' contribution
Susan Taheri: Data curation, Formal analysis,
Adele Pouyafard: Conceptualization, Methodology, Writing – Review & editing
Fahimeh Rashidi Maybodi: Conceptualization, Methodology, writing – Original draft, Writing – Review & editing

Conflict of Interest
The authors declare no conflict of interest.

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