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Possible Effect of Passive Smoking on Gingival Health of Children in Riyadh Colleges of Dentistry and Pharmacy

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Abstract

***Objectives*:** To know the level of cotinine concentration in the saliva of children exposed to secondhand smoke and know the link between smoke exposure and gingivitis. ***Materials and Methods*:** The study was conducted on 100 children from Riyadh Colleges of Dentistry and Pharmacy between the ages of 6 - 12 years. A questionnaire was conducted on the parents of the children to find out their smoking behavior. The sample (children) was separated into two categories: category (A) children exposed to secondhand smoke as a test sample and numbered 50 children (23 boys and 27 girls), category (B) children not exposed to smoking as an ideal sample and numbering 50 children (24 boys and 26 girls). Index of dental plaque (calculus), gum index, presence of pigmentation and saliva sample collection were recorded from each child from the two groups. Furthermore, the level of cotinine in saliva samples was determined using the ELISA technique. ***Results*:** The concentration of cotinine in saliva is prominently present in samples of children category (A) compared to category (B). A positive correlation was observed between the number of cigarettes consumed and the concentration of cotinine in saliva. On the other hand, no relationship was found between gingivitis and the concentration ratio Cotinine. The likelihood of pigmentation in children exposed to secondhand smoking is 1.14 times higher than in other children. ***Conclusions*:** Secondhand smoke can raise the concentration of cotinine in saliva and stimulate the presence of pigmentation in children's gums.

Keywords: Child dentistry; Gingiva; Cariology; Dental hygiene; Gingivitis; Smoking.

INTRODUCTION

Smoking is a major risk factor for morbidity and mortality, contributing to 50 out of 260 million deaths between 1950 and 2000 [1] Cigarettes produce two types of smoke: mainstream smoke exhaled by smokers and side stream smoke from burning tobacco. Research by Philip Morris in the 1980s revealed that side stream smoke is more toxic and mutagenic, affecting respiratory health [2].

According to the WHO, over 1.1 billion people smoke tobacco globally. Although smoking rates are declining overall, they are increasing in the Eastern Mediterranean and Africa [3].

According to a Bassiony [4] poll, the proportion of smokers in Saudi Arabia ranged between 2.5% to 52% during March and July 2012, the Saudi Health Interview Survey (SHIS) was conducted in 2013 included all regions of Saudi Arabia with 10,827 participants. Data of the survey were collected using questionnaire, physical examination and laboratory based biomedical examination. Statistics from surveys show that 12.1% of Saudis consume cigarettes. The prevalence rates for men and women were 23.7% and 1.5%, respectively. In addition, 11.4% of smokers consume cigarettes daily with an average of 15.0 cigarettes per day [5].

Since 1970, the number of children exposed to passive smoking has risen, with 60% of children in some studies exposed at home6. In Saudi Arabia, 17.2% of the population faces passive smoking at home, averaging 5.1 days a week, while 14.8% are exposed at work [5].

Passive smoking has been considered responsible for life threatening conditions like cancer and respiratory disorders [7]. In addition, some studies found that passive smoking affect oral health.

Smoking, mostly in the form of tobacco smoking, is recognized as the most important environmental risk factor in periodontitis [8]. Smokers have 1.6 greater times to have periodontal disease comparing with unexposed people [9].

Most of previous studies focused on the association between active smoking and periodontal disease.

Risk Factors for Periodontal Disease

Periodontal diseases are infections caused by dental plaque, but risk factors can modify the host response to microbial aggression [10]. Some of the known risk factors are diabetes, tobacco smoking, pathogenic bacteria and genetic factors.

Smoking as a Risk Factor

Tobacco smoking is recognized as the most significant environmental risk factor for periodontitis, containing numerous harmful compounds, notably nicotine [8]. Nicotine affects cardiovascular health by increasing heart rate and blood pressure [11] and impairs wound healing by reducing the proliferation of essential healing cells [12, 13].

Historical studies have linked smoking to periodontal disease, with noting its association with acute necrotizing ulcerative gingivitis [14]. Subsequent research indicates that smoking significantly increases the risk of periodontal disease, with odds ratios as high as 14.1 in young smokers [15] and a study showing that approximately Half of all instances of periodontitis have a smoking connection [16]. Smokers are found to have a 3-25 times greater risk of developing chronic periodontitis compared to non-smokers [17].

Effect of Smoking on Response to Treatment

Smoking not only increases the risk of periodontal disease but also negatively impacts treatment outcomes [18, 19]. Studies show that smokers have poorer responses to both surgical and non-surgical therapies, especially at plaque-positive sites [20]. This is due to smoking's role as an extrinsic factor that modifies the host's inflammatory response [8].

Passive Smoking Detection and Children Health

Passive smoking exposes individuals to over 4,000 harmful chemicals, with cotinine being the most reliable biochemical marker for tobacco use [21, 22]. Cotinine, thiocyanate and carbon monoxide are the biochemical measures most frequently used as detector for smoking status and or changes in status. Cotinine's longer half-life (10-30 hours) makes it an accurate measure of smoking status [23]. Carbon monoxide (CO) is also used to assess smoking status, demonstrating 80-90% sensitivity, but has limitations, particularly for children [24–26] and its measurement in blood, urine or saliva is considered the most accurate biochemical measure of smoking status.

Passive Smoking and Oral Health

The IARC and U.S. Surgeon General confirm a causal link between passive smoking and lung cancer but lack sufficient data for other cancers [27, 28]. Limited studies suggest a connection between passive smoking and head and neck cancers [29].

Studies show a connection between maternal smoking and dental cavities in children [30]. Cotinine levels in children are associated with caries prevalence [31]. Children in smoking households exhibit higher caries rates [32, 33]. Passive smoking may enhance the growth of cariogenic bacteria [34, 35]. Studies found a relationship between the dose of smoking and prevalence of pigmentations [27]. All of These findings indicate causal association between tobacco smoke and melanin pigmentation in gingiva [36] and performed a study on 59 children with at least one smoking parent. The findings showed that between 71% and 78% of kids had gingival pigmentation.

MATERIALS AND METHODS

A prospective this study observational was conducted with ethical approval from the Then it was reviewed by the Institutional Review Board of the college. Approval of Ethics Committee in has been obtained for the study. One hundred children aged 6-12 were selected from the pediatric dentistry clinic between March 2016 and January 2017. Parents provided informed consent, and children gave verbal agreement. Exclusion criteria included children over 12 to reduce active smoking risks. Parents completed a questionnaire on smoking habits, indicating type and location. Participants were divided into two groups: 50 children exposed to passive smoking (23 boys, 27 girls) and 50 unexposed (24 boys, 26 girls).

The study aimed to investigate the impact of passive smoking on children's oral health through a prospective observational design. It received ethical approval from the RCSDP and the Institutional Review Board, ensuring adherence to ethical standards.

A total of 100 children aged 6-12 were recruited from the pediatric dentistry clinic over nearly a year. Parents were informed about the study's purpose and confidentiality, with consent obtained before participation. To reduce the possibility of including active smokers, children older than 12 were excluded.

Saliva was collected right after the oral examination of each child. The children were asked to collect the saliva in their mouth for 2 minutes and spit into clean plastic cups. Saliva was then transferred from the cup to small 1.8 ml sterile tubes. Following samples collection, all were dispatched to King Faisal Specialist Hospital Research Center to be examined. Using a Competitive Enzyme-Linked Immunosorbent Assay, the levels of cotinine were determined (ELISA).

Statistical Analysis

The data from King Faisal Specialist Research Center was analyzed. Regarding the demographic information and salivary cotinine levels, means, standard deviations, and confidence intervals were computed. Spearman's test evaluated correlations between cotinine concentrations and smoking habits. The significance was assessed using the Mann-Whitney U test.

RESULTS

Children in group 1 (the test group) who have a positive history of being around passive smokers (Table 1). Fathers of all participants responded to questionnaire illustrating details related to smoking frequency and place (Table 2) group was further divided into: group 1(A) and group 1(B) (Table 3).

Cotinine Concentration in Test and Control Groups

The level of cotinine in saliva in samples is shown in (Tables 4 and 5). Mann-Whitney U test showed the mean (±SD) salivary cotinine concentration was significantly increased in PTS- exposed children compared with PTS-unexposed children (2.03±5.12 ng/ml, 95% CI 1.01–3.04 versus 0.02±0.14 ng/ml, 95% CI 1-1.00–1.04, *P*<0.05) (Table 6).

Cotinine Concentration in Relation to Number of Cigarettes/days

Thirty eight percent (n=19) of children's parents smoked >20 cigarettes per day followed by 34% (n=17) who smoked <10 cigarettes per day and 28% (n=14) 10-20 cigarettes per day (Figure 1). The majority of the children's parents (94%, n=47) smoked cigarettes daily in a week and only 6% (n=3) smoked in the weekend (Figure 2). Sixty four percent (n=32) of the family members reportedly smoked outside the house and 36% (n=18) smoked inside the house (Figure 2).

Spearman's rho showed a positive correlation between cotinine level and number of cigarettes smoked per day and statistically significant (rho=0.351, *P*<0.05) (Table 7).

Direct Exposure to Passive Smoking

Sixty four percent of fathers smoked inside home and 36% smoked outside (Figure 3).

Cotinine Level in Subgroups of Test Group

In-subgroup 1: A 36% (n=18) of the children were exposed to tobacco smoke directly inside the house. While in-group 1: B 64% (n= 32) of children were exposed indirectly to passive smoking. Mann-Whitney U test showed the mean (±SD) salivary cotinine concentration was significantly increased in PTS-directly exposed children compared with PTS-indirect exposed children group 1 (B) 4.22 ± 5.57 ng/ml, 95% CI 1.89-6.43 versus 0.79 ± 4.48 ng/ml, 95% CI -0.94-2.53, *P*<0.05. (Table 8)

Cotinine Concentration in Relation to Clinical Parameters

The clinical characteristics of the participants according to PTS exposure are shown in (Table 9). Mann-Whitney U test showed the mean (±SD) PI significantly increased in PTS-exposed children compared with PTS-unexposed children (1.08±0.60, 95% CI 0.93–1.23 versus 0.72±0.45, 95% CI 0.57–0.87, *P*<0.05). The mean (±SD GI) too increased in PTS-exposed children compared with PTS-unexposed children (0.62±0.60, 95% CI 0.46–0.78 versus 0.42±0.54, 95% CI 0.26–0.58). However, it was statistically not significant (*P*>0.05).

Spearman's rho showed a positive correlation between cotinine level and PI (rho=0.109, *P*>0.05); and cotinine concentration and GI (rho=0.275, *P*>0.05) but it was statistically not significant. Correlation between PI and GI was positive and statistically significant (rho=0.604, *P*<0.05) (Table 10).

Relationship between Passive Smoking and Oral Pigmentation in Children

Of the children who were not exposed to passive smoking, none had oral pigmentation and of the children who were exposed to passive smoking, 6 cases (12%) had oral pigmentation (Figure 4). The Fisher's Exact test showed that this difference was statistically significant (*P*<0.05). For the children who were exposed to passive smoking, the relative risk was 1.14 (Table 11).

Table 1: Demographic data of group 1 (test group)

Age	Number	Boys	Girls
6	3	-	3
7	6	5	1
8	7	1	6
9	10	8	2
10	10	4	6
11	7	2	5
12	7	3	4
Total	50	23	27

Table 2: Demographic data of group 2 (control group)

Age	No.	Boys	Girls
6	9	5	4
7	4	2	2
8	13	7	6
9	6	3	3
10	6	2	4
11	7	3	4
12	5	2	3
Total	50	24	26

Table 3: Demographic data of sub groups 1: A and 1: B (test group)

G 1-A Direct exposed children				G 1-B indirect exposed children			
Age	Number	Boys	Girls	Age	Number	Boys	Girls
6	3	0	3	6	0	0	0
7	1	1	0	7	5	4	1
8	2	1	1	8	5	0	5
9	5	3	2	9	5	5	0
10	5	2	3	10	5	2	3
11	1	0	1	11	6	2	4
12	1	0	1	12	6	3	3
Total	18	7	11	Total	32	16	16

Table 4: Cotinine level in control group:

Sample number	Cotinine concentration	Sample number	Cotinine concentration
46	0.0000	71	0.0000
47	0.0000	72	0.0000
48	0.0000	73	0.0000
49	0.0000	74	0.0000
50	0.0000	75	0.0000
51	0.0000	76	0.0000
52	0.0000	77	0.0000
53	0.0000	78	0.0000
54	0.0000	79	0.0000
55	0.0000	80	1.0090
56	0.0000	81	0.0000
57	0.0000	82	0.0000
58	0.0000	83	0.0000
59	0.0000	84	0.0000
60	0.0000	85	0.0000
61	0.0000	86	0.0000
62	0.0000	87	0.0000
63	0.0000	88	0.0000
64	0.0000	89	0.0000
65	0.0000	90	0.0000
66	0.0000	91	0.0000
67	0.0000	92	0.0000
68	0.0000	93	0.0000
69	0.0000	94	0.0000
70	0.0000	95	0.0000

Table 5: Cotinine level in test group

Sample number	Cotinine concentration	Sample number	Cotinine concentration
1	0.0000	26	8.5592
2	0.0000	27	12.8175
3	0.0000	28	25.3670
4	0.0000	29	0.0000

Sample number	Cotinine concentration	Sample number	Cotinine concentration
5	0.0000	30	0.0000
6	0.0000	31	0.0000
7	0.0000	32	0.0000
8	0.0000	33	0.0000
9	0.0000	34	0.0000
10	0.0000	35	7.3574
11	0.0000	36	18.6533
12	0.0000	37	0.0000
13	7.8507	38	0.0000
14	0.0000	39	0.0000
15	0.0000	40	7.1685
16	0.0000	41	0.0000
17	5.2124	42	0.0000
18	0.0000	43	0.0000
19	0.0000	44	0.0000
20	0.0000	45	0.0000
21	0.0000	96	0.0000
22	0.0000	97	0.0000
23	0.0000	98	0.0000
24	0.0000	99	8.2615
25	0.0000	100	0.0000

Table 6: Mean Cotinine levels in test and control group

Body fluid	PTS-exposed (n=50)			PTS-unexposed (n=50)			p value
	Mean (SD)	95% CI		Mean (SD)	95% CI		
		Lower Bound	*Upper Bound*		*Lower Bound*	*Upper Bound*	
Saliva (ng/ml)	2.03 (5.12)	1.01	3.04	0.02 (0.14)	-1.00	1.04	0.007*

Table 7: Relationship between cotinine level and number of cigarettes smoked per day

Spearman's rho		Cotinine level	Number of cigarettes smoked per day
Cotinine level	*Correlation Coefficient*	1.000	.351*
	Sig. (2-tailed)	.	.013
	n	50	50
Number of cigarettes smoked per day	*Correlation Coefficient*	.351*	1.000
	Sig. (2-tailed)	.013	.
	n	50	50

Table 8: Cotinine level in saliva of directly exposed children

Body fluid	PTS-Directly exposed group 1 (A) (n=18)			PTS-indirectly unexposed group 1 (B) (n=32)			p value
	Mean (SD)	95% CI		Mean (SD)	95% CI		
		Lower Bound	*Upper Bound*		*Lower Bound*	*Upper Bound*	
Saliva (ng/ml)	4.22 (5.57)	1.894	6.537	0.79 (4.48)	-0.948	2.534	0.001*

Table 9: Cotinine concentration in relation to clinical parameters

Clinical characteristics	PTS-exposed (n=50)			PTS-unexposed (n=50)			p value
	Mean (SD)	95% CI		Mean (SD)	95% CI		
		Lower Bound	*Upper Bound*		*Lower Bound*	*Upper Bound*	
PI	1.08 (0.6)	0.93	1.23	0.72 (0.45)	0.57	0.87	0.002*
GI	0.62 (0.60)	0.46	0.78	0.42(0.54)	0.26	0.58	0.091

Table 10: Relationship between cotinine level, GI, and PI in PTS exposure

Spearman's rho		Cotinine level	GI	PI
Cotinine level	*Correlation Coefficient*	1.000	.275	.109
	Sig. (2-tailed)	.	.053	.452
	n	50	50	50
GI	*Correlation Coefficient*	.275	1.000	.604*
	Sig. (2-tailed)	.053	.	.000
	n	50	50	50
PI	*Correlation Coefficient*	.109	.604*	1.000
	Sig. (2-tailed)	.452	.000	.
	n	50	50	50

Table 11: Oral pigmentation in both groups:

	Oral pigmentation		95% CI		OR	p value
	Presence n (%)	Absence n (%)	*Lower Bound*	*Upper Bound*		
PTS-exposed (n=50)	6 (12.0)	44 (88.0)	1.03	1.26	1.14	0.027*
PTS-unexposed (n=50)	0 (0.0)	50 (100.0)				

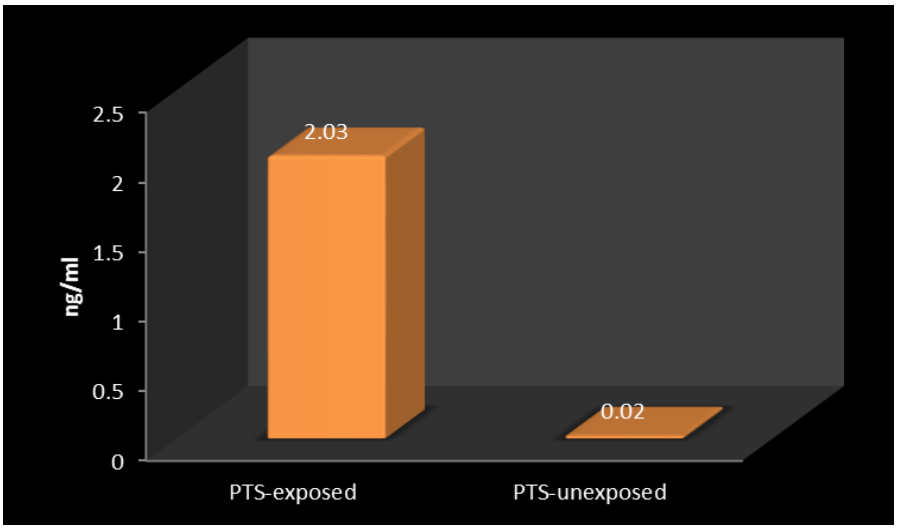

Figure 1: Cotinine levels in saliva of children

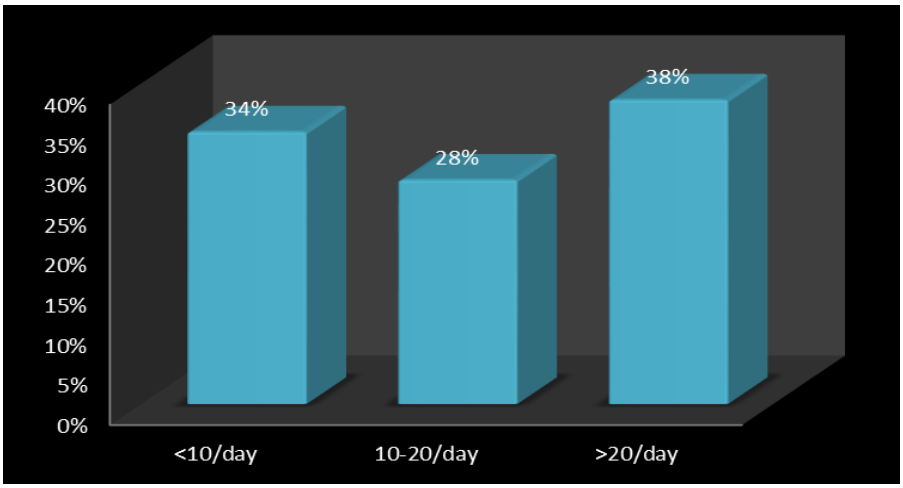

Figure 2: Number of cigarettes smoked per day

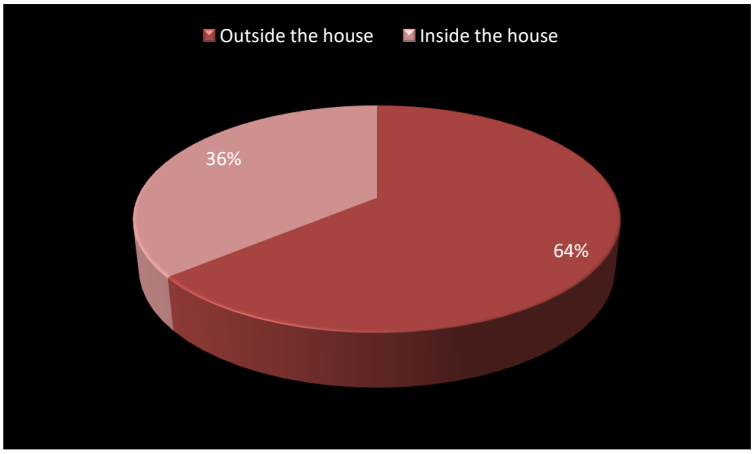

Figure 3: Location of cigarette smoking

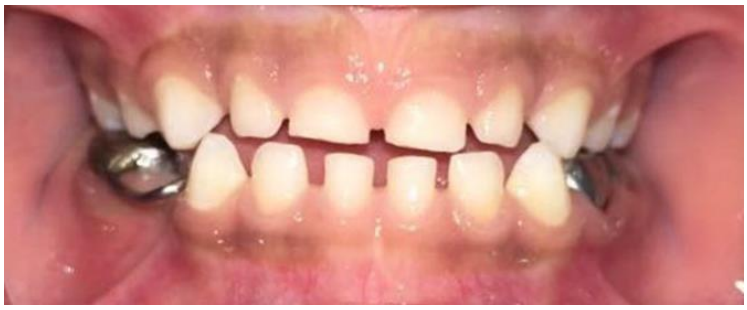

Figure 4: Gingival pigmentation in direct exposed child

DISCUSSION

In the present study, cotinine concentration was used as a marker for exposure of passive smoking. It is more stable with longer half-life and remain relatively constant [37], reporting that the serum levels of cotinine were more closely correlated with unstimulated saliva (>by 4%) than with stimulated saliva (> by 41%). According to that, unstimulated salivary cotinine levels were used in the current study.

Numerous techniques, including chromatography, colorimetry, radioimmunoassay, ELISA, etc., have been used to detect the amount of cotinine present in the biologic matrices [38]. 38. It has been demonstrated that ELISA using monoclonal antibodies is effective at detecting cotinine in saliva [39].

In the present study, the cotinine in saliva was reported in 8 out of 18 children who directly exposed to smoking at home (44.4%). Other investigators [9] found that overall prevalence of passive smoking exposure in his sample was 32.8%. Higher percentage of detected cotinine in saliva (76.4%) was reported in Turkey for children exposed to passive smoking by [40].

In children exposed to passive smoking, salivary cotinine levels varied greatly, ranging from 0 to 25.3670 ng/ml. This variation can be due to differences in tobacco smoke concentration, proximity to the smoker, and other environmental factors [41].

A result that leads to a conclusion that harmful effects of smoking is not related to dental plaque [42]. Such controversy could be related to environmental factors like socioeconomic status and hygiene levels.

400 kids, 200 boys and 200 girls, between the ages of 10 and 11 were investigated in two groups: control and passive smokers. They found that children exposed to passive smoking had a 1.23 relative risk of oral pigmentation compared to children who were not exposed to it [28].

Result of the present study revealed that self-reported smoking was almost similar to result obtained by analysis of cotinine. This is in agreement with other studies which have documented increasing cotinine levels with increasing levels of self-reported PTS exposure [43, 44].

Limitation of the Study

1) The study included limited number of participants, because many of parents refused to let their children to participate in such study or to admit weather, they are smokers or no.
2) In the present study clinical attachment loss (CAL) was not used as clinical parameter, this was done according the result of [40], who reported inferior CAL to passive exposed children in the same age group due to reduced growth rate of the periodontal tissues for such age.

CONCLUSIONS

Within the limitation of present study, it can be concluded that:

- Children who were directly exposed to passive smoking have high cotinine concentration in their saliva.
- Exposure to passive smoking could be associated with decrease in clinical manifestations of gingival inflammation.
- Passive smoking can be associated with gingival pigmentations
- The amount of salivary cotinine can be utilized to detect passive smoking.
- Studies with larger sample size are recommended to clarify harmful effects of passive smoking on children.
- Population awareness concerning exposure to PTS need to be emphasized to protect children especially from indoor smoking.

REFERENCES

1. Smejkalova J, Jacob V, Hodacova L, Fiala Z, Slezak R, Vellappally S. *The Influence of Smoking on Dental and Periodontal Status*. www.intechopen.com
2. Schick S, Glantz S. Philip Morris toxicological experiments with fresh sidestream smoke: more toxic than mainstream smoke. *Tob Control*. 2005;14(6):396-404. doi:10.1136/tc.2005.011288
3. European Organization for Research on Treatment of Cancer. Symposium on the diagnosis of liver metastases; held in Brussels, November 29--December 1, 1978. Abstract. *J Belge Radiol*. 1979;62(6):625-632.
4. Bassiony MM. Smoking in Saudi Arabia. *Saudi Med J*. 2009;30(7):876-881.
5. Moradi-Lakeh, M., El Bcheraoui, C., Tuffaha, M., Daoud, F., Al Saeedi, M., Basulaiman, M., ... & Mokdad, A. H. (2015). Tobacco consumption in the Kingdom of Saudi Arabia, 2013: findings from a national survey. *BMC public health*, *15*, 1-10. doi:10.1186/s12889-015-1902-3
6. Mackay J, Amos A. Women and tobacco. *Respirology*. 2003;8(2):123-130. doi:10.1046/j.1440-1843.2003.00464.x
7. Moravej-Salehi E, Moravej-Salehi E, Hajifattahi F. Relationship of Gingival Pigmentation with Passive Smoking in Women. *Tanaffos*. 2015;14(2):107-114.
8. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors--tobacco smoking. *J Clin Periodontol*. 2005;32 Suppl 6:180-195. doi:10.1111/j.1600-051X.2005.00786.x
9. Arbes SJ, Agústsdóttir H, Slade GD. Environmental tobacco smoke and periodontal disease in the United States. *Am J Public Health*. 2001;91(2):253-257. doi:10.2105/ajph.91.2.253
10. Calsina G, Ramón JM, Echeverría JJ. Effects of smoking on periodontal tissues. *J Clin Periodontol*. 2002;29(8):771-776. doi:10.1034/j.1600-051x.2002.290815.x
11. Primatesta P, Falaschetti E, Gupta S, Marmot MG, Poulter NR. Association between smoking and blood pressure: evidence from the health survey for England. *Hypertension*. 2001;37(2):187-193. doi: 10.1161/01.hyp.37.2.187
12. Bain CA, Moy PK. The association between the failure of dental implants and cigarette smoking. *Int J Oral Maxillofac Implants*. 1993;8(6):609-615.
13. Bornstein MM, Halbritter S, Harnisch H, Weber HP, Buser D. A retrospective analysis of patients referred for implant placement to a specialty clinic: indications, surgical procedures, and early failures. *Int J Oral Maxillofac Implants*. 2008;23(6):1109-1116.
14. PINDBORG JJ. Tobacco and gingivitis: statistical examination of the significance of tobacco in the development of ulceromembranous gingivitis and in the formation of calculus. *J Dent Res*. 1947;26(3):261-264. doi:10.1177/00220345470260030901
15. Linden GJ, Mullally BH. Cigarette smoking and periodontal destruction in young adults. *J Periodontol*. 1994;65(7):718-723. doi:10.1902/jop.1994.65.7.718
16. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *J Periodontol*. 2000;71(5):743-751. doi:10.1902/jop.2000.71.5.743
17. Bergström J. Tobacco smoking and chronic destructive periodontal disease. *Odontology*. 2004;92(1):1-8. doi:10.1007/s10266-004-0043-4
18. Preber H, Bergström J. Occurrence of gingival bleeding in smoker and non-smoker patients. *Acta Odontol Scand*. 1985;43(5):315-320. doi:10.3109/00016358509046515
19. Scabbia A, Cho KS, Sigurdsson TJ, Kim CK, Trombelli L. Cigarette smoking negatively affects healing response following flap debridement surgery. *J Periodontol*. 2001;72(1):43-49. doi:10.1902/jop.2001.72.1.43
20. Bunaes DF, Lie SA, Enersen M, Aastrøm AN, Mustafa K, Leknes KN. Site-specific treatment outcome in smokers following non-surgical and surgical periodontal therapy. *J Clin Periodontol*. 2015;42(10):933-942. doi:10.1111/jcpe.12462
21. Avşar A, Darka O, Topaloğlu B, Bek Y. Association of passive smoking with caries and related salivary biomarkers in young children. *Arch Oral Biol*. 2008;53(10):969-974. doi: 10.1016/j.archoralbio.2008.05.007
22. Armitage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, Turner DM. Absorption and metabolism of nicotine from cigarettes. *Br Med J*. 1975;4(5992):313-316. doi:10.1136/bmj.4.5992.313
23. Benowitz NL, Kuyt F, Jacob P, Jones RT, Osman AL. Cotinine disposition and effects. *Clin*

Pharmacol Ther. 1983;34(5):604-611. doi:10.1038/clpt.1983.222
24. Preshaw PM, Heasman L, Stacey F, Steen N, McCracken GI, Heasman PA. The effect of quitting smoking on chronic periodontitis. *J Clin Periodontol*. 2005;32(8):869-879. doi:10.1111/j.1600-051X.2005.00779.x
25. Dukellis D, Zuk J, Pan Z, Morrison JE, Friesen RH. Exhaled carbon monoxide screening for environmental tobacco smoke exposure in preanesthetic children. *Paediatr Anaesth*. 2009;19(9):848-853. doi:10.1111/j.1460-9592.2009.03083.x
26. Florescu A, Ferrence R, Einarson T, Selby P, Soldin O, Koren G. Methods for quantification of exposure to cigarette smoking and environmental tobacco smoke: focus on developmental toxicology. *Ther Drug Monit*. 2009;31(1):14-30. doi: 10.1097/FTD.0b013e3181957a3b
27. Araki S, Murata K, Ushio K, Sakai R. Dose-response relationship between tobacco consumption and melanin pigmentation in the attached gingiva. *Arch Environ Health*. 1983;38(6):375-378. doi:10.1080/00039896.1983.10545823
28. Hajifattahi F, Azarshab M, Haghgoo R, Lesan S. Evaluation of the Relationship between Passive Smoking and Oral Pigmentation in Children. *J Dent (Tehran)*. 2010;7(3):119-123.
29. Warren CW, Lea V, Lee J, Jones NR, Asma S, McKenna M. Change in tobacco use among 13-15 year olds between 1999 and 2008: findings from the Global Youth Tobacco Survey. *Glob Health Promot*. 2009;16(2 Suppl):38-90. doi:10.1177/1757975909342192
30. Bolin AK, Bolin A, Jansson L, Calltorp J. Children's dental health in Europe. *Swed Dent J*. 1997;21(1-2):25-40.
31. Aligne CA, Moss ME, Auinger P, Weitzman M. Association of pediatric dental caries with passive smoking. *JAMA*. 2003;289(10):1258-1264. doi:10.1001/jama.289.10.1258
32. Shenkin JD, Broffitt B, Levy SM, Warren JJ. The association between environmental tobacco smoke and primary tooth caries. *J Public Health Dent*. 2004;64(3):184-186. doi:10.1111/j.1752-7325.2004.tb02750.x
33. Hanioka T, Nakamura E, Ojima M, Tanaka K, Aoyama H. Dental caries in 3-year-old children and smoking status of parents. *Paediatr Perinat Epidemiol*. 2008;22(6):546-550. doi:10.1111/j.1365-3016.2008.00950.x
34. Lindemeyer RG, Baum RH, Hsu SC, Going RE. In vitro effect of tobacco on the growth of oral cariogenic streptococci. *J Am Dent Assoc*. 1981;103(5):719-722. doi: 10.14219/jada.archive.1981.0372
35. Kum-Nji P, Meloy L, Herrod HG. Environmental tobacco smoke exposure: prevalence and mechanisms of causation of infections in children. *Pediatrics*. 2006;117(5):1745-1754. doi:10.1542/peds.2005-1886
36. Hanioka T, Tanaka K, Ojima M, Yuuki K. Association of melanin pigmentation in the gingiva of children with parents who smoke. *Pediatrics*. 2005;116(2):e186-90. doi:10.1542/peds.2004-2628
37. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev*. 1996;18(2):188-204. doi: 10.1093/oxfordjournals.epirev.a017925
38. Dhar P. Measuring tobacco smoke exposure: quantifying nicotine/cotinine concentration in biological samples by colorimetry, chromatography and immunoassay methods. *J Pharm Biomed Anal*. 2004;35(1):155-168. doi: 10.1016/j.jpba.2004.01.009
39. Bernert JT, Harmon TL, Sosnoff CS, McGuffey JE. Use of continine immunoassay test strips for preclassifying urine samples from smokers and nonsmokers prior to analysis by LC-MS-MS. *J Anal Toxicol*. 2005;29(8):814-818. doi:10.1093/jat/29.8.814
40. Erdemir EO, Sönmez IS, Oba AA, Bergstrom J, Caglayan O. Periodontal health in children exposed to passive smoking. *J Clin Periodontol*. 2010;37(2):160-164. doi:10.1111/j.1600-051X.2009.01510.x
41. Yamamoto, Y., Nishida, N., Tanaka, M., Hayashi, N., Matsuse, R., Nakayama, K., ... & Shizukuishi, S. (2005). Association between passive and active smoking evaluated by salivary cotinine and periodontitis. *Journal of clinical periodontology*, *32*(10), 1041-1046. doi:10.1111/j.1600-051X.2005.00819.x
42. Apatzidou DA, Riggio MP, Kinane DF. Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. *J Clin Periodontol*. 2005;32(9):973-983. doi:10.1111/j.1600-051X.2005.00788.x
43. Jarvis M, Tunstall-Pedoe H, Feyerabend C, Vesey C, Salloojee Y. Biochemical markers of smoke absorption and self reported exposure to passive smoking. *J Epidemiol Community Health (1978)*. 1984;38(4):335-339. doi:10.1136/jech.38.4.335
44. Tunstall-Pedoe H, Woodward M, Brown CA. The drinking, passive smoking, smoking deception and serum cotinine in the Scottish Heart Health Study. *J Clin Epidemiol*. 1991;44(12):1411-1414. doi:10.1016/0895-4356(91)90102-f