Association of passive smoking with dental caries and salivary biomarkers among 5–10 years old children of Muradnagar, Ghaziabad

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ABSTRACT

Objectives: The purpose of this study is to assess the association of passive smoking (PS) with dental caries and salivary biomarkers among 5–10 years old children of Muradnagar, Ghaziabad. **Methods:** A case–control study was conducted among 160 children of age group 5–10 years who visited the outdoor patient department of a dental college. Regular smoking households were recognized and children who lived in smoking households were identified as PS subjects. Two categories of children were formed – PS (80 children) and control group (80 children). Parents completed a pretested questionnaire and clinical examination of children was done using dmft index and gingival index. This was followed by collection of stimulated saliva of children which was further subjected to determine salivary buffering capacity and pH. Inoculation on mitis salivarius-bacitracin agar for counting streptococcus colonies and Rogosa SL agar (Difco) for counting lactobacillus colonies was done. Cotinine level was then measured using enzyme-linked immunosorbent assay kit. Student's independent t-test, Mann–Whitney U test, and one-way analysis of variance test were used for analyzing data. **Results:** The mean streptococcus and lactobacillus colony count was higher in PS case subjects, that is, 348.9 ± 166.509 and 247.3 ± 15.86 in comparison to control group where the mean streptococcus and lactobacillus colony count was 63.03 ± 23.082 and 63.825 ± 12.638, respectively. The mean cotinine level among PS case subjects was 1.08 ± 0.265 which was higher than the control group, that is, 0.00 ± 0.00. The mean cotinine level was directly proportional to streptococcus colonies, lactobacillus colonies, dmft and gingival index (GI) scores, and smoking exposure. **Conclusion:** PS has deleterious impact on children which was reflected by their increased cotinine levels, streptococcus colonies, lactobacillus colonies, and poor dmft and GI scores in comparison to the control group.

Keywords: Colony count, cotinine, microbial, passive smoking, saliva

Introduction

Smoke that is released by burning the tip of cigarette and inhaled by nonsmokers is known as passive smoking (PS). In 2011, an estimated 600,000 nonsmokers were killed on global level particularly children by PS.[1] It causes several diseases such as lung cancer, heart disease, and respiratory infection.[2,3] *In utero*, PS exposure may begin and it may continue throughout childhood.[4] High breathing rates and more lung surface area of children make them more susceptible to adverse effects of PS.[5]

Paternal active smoking may damage sperm before birth[6,7] and maternal second-hand smoke *in utero* may also harm the fetus.[6,8] There are more than 4000 chemicals in environmental tobacco smoke that are released into the atmosphere which lead to PS affecting the oral health.[9] Caries in children and poor oral health could be a cause of smoking by household members.[10]

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High-exposure group and low-exposure group can be compared and recent exposure can be estimated directly using cotinine biomarker.[11] High and stable plasma concentrations are formed because cotinine that is primary metabolite of nicotine has longer half-life than nicotine.[12] Thus, PS exposure can be screened by a useful cotinine tool which is specific and highly stable with temperature change.[13] Muradnagar in Uttar Pradesh has prevalence in smoking and nonsmoking forms of tobacco consumption. Every household usually has tobacco users, leading to increase in passive smokers. Hence, this study was undertaken to study the impact of tobacco on passive smokers by associating it with their oral health status and cotinine level.

Methods

Setting and population

A case–control study was conducted in Ghaziabad (Uttar Pradesh) among 5–10 years old children visiting the outdoor patient department of a dental college.

Study consent

The study was conducted during 2017–2018. Ethical approval of the study was taken from the ethical clearance committee of the institution. Permission was taken from advanced research laboratory of the institution to conduct microbiological and salivary biomarkers' assessment. Written informed consent was taken from parents before implementing the study.

Study questionnaire

This pilot study was carried out on 30 children from March 2016 to September 2016 to check implementation before the main study was conducted. Self-administered close-ended questionnaire was used for parents to assess their demographic details, parental practice toward their children's oral health, and their smoking exposure. Content validity was assessed by Cronbach's alpha test whose value was 0.9.

Sample size

The sample size was calculated to be 160 (80 as PS case group and 80 as control group).

Study procedure

Eighty parents were asked to complete a pretested questionnaire about smoking habits [Figure 1]. According to answers, children
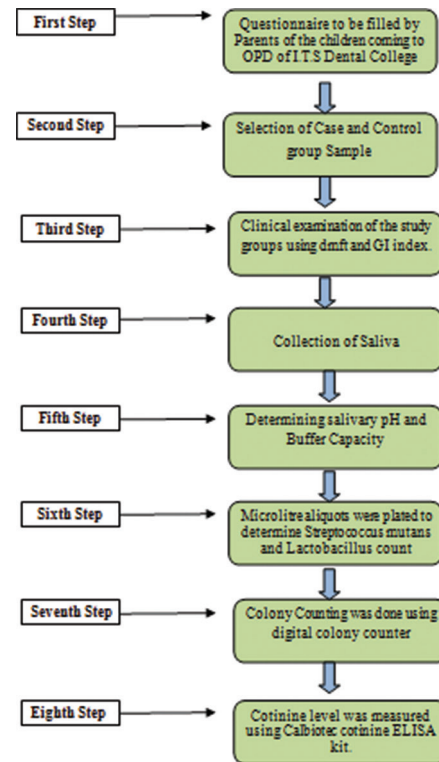

Figure 1: Study Procedure Step wise Step

of regular smoking household were identified as PS subjects. Two groups of children – PS and control group –were screened. Depending on the number of cigarettes smoked by parent per day, the frequency and duration of smoking were divided into three categories [Table 1]. Clinical examination of children was done using dmft scores and gingival index (GI).

Stimulated saliva of screened children was collected in sterile plastic disposable containers. Precipitation was removed by centrifugation at 2000–3000 rpm for 20 min. It was stored frozen at −20°C, salivary pH was determined using narrow range pH strip system (Himedia, Mumbai), and its buffer capacity was measured following collection by standard method of Ericsson.

Saliva was transported to the laboratory in sterile tubes, vortexed for 30 s, and diluted four-fold in 0.05 M sodium phosphate buffer. Fifty-microliter aliquots were plated on mitis salivarius-bacitracin

Table 1: Interpretation of smoking exposure colony count				
Smoking Exposure		**Low**	**Medium**	**High**
No. of cigarettes smoked/day		1-2 cigarettes	More than 2 cigarettes but less than one 1 packet	1 packet and more
Frequency of smoking		once	Two-three times	More than three times
Duration of smoking		1-3 years	4-6 years	More than 6 years
Bacterial Colonies	**No activity**	**Low colony**	**Moderate colony**	**High colony**
Streptococcus colonies		0-200	201-400	Above 400
Lactobacillus colonies	0-100	101-200	201-300	Above 300

agar supplemented with 15% sucrose, 1% potassium, and 0.2 units/mL bacitracin for *Streptococcus mutans* count, and on Rogosa SL agar (Difco, Gurugram, Haryana) for lactobacilli count [Figure 2]. All plates were incubated at 37°C in an atmosphere containing 10% CO_2 for 48 h. Colony counting was done using digital colony counter and they were divided into three categories [Table 1]. The cotinine level was then measured using Calbiotec (California, USA) cotinine enzyme-linked immunosorbent assay (ELISA) kit.

ELISA procedure

Ten standard wells on ELISA plates were set and coated [Figure 3]. The total volume in the well was 50 μm and concentration was 2400, 1600, 800, 400, and 200 pg/mL, respectively [Figure 4]. The blank well and sample well were set. Sample diluent 40 μm was added to testing sample (10 μm).
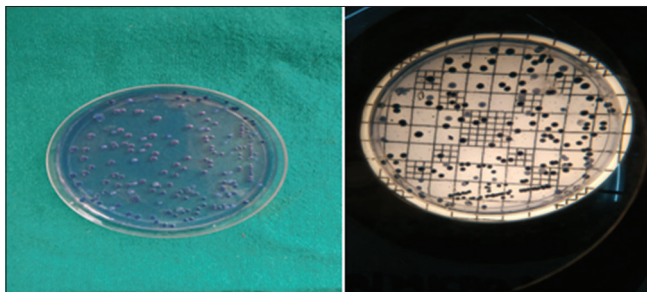

Figure 2: Saliva inoculated on mitis salivarius bacitracin agar and streptococcus mutans under colony counter
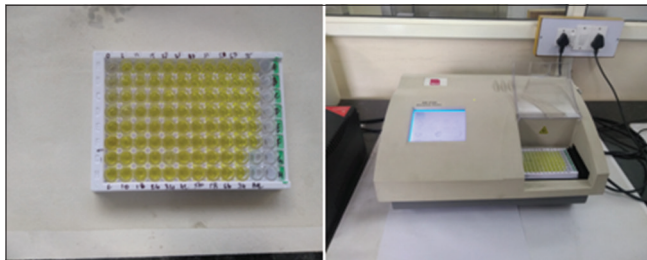

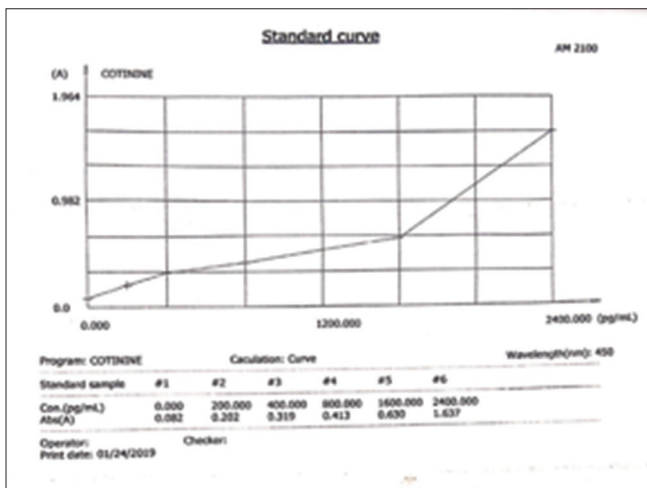**Figure 3:** Cotinine positive ELISA wells

Figure 4: Standard Curve

After closing plate, it was incubated for 30 min at 37°C. Thirty-fold washing solution was prepared and diluted with distilled water until 600 mL, washing buffer was added to each well, kept still for 30 s, drained, repeated for 5 min, and dried by pat. Horseradish peroxidase enzyme-conjugate reagent 50 μm was added to each well, except blank well to produce change in color which is used as signal with the presence of antibody or antigen.

Tetramethylbenzidine chromogen solution A 50 μm was added and mixed gently, and light preservation was evaded for 15 min at 37°C. Stop solution 50 μm was then added to each well to stop reaction. Blue color changed to yellow immediately. The optical density of blank well was set as zero, and absorbance was read at 450 nm after adding stop solution within 15 min. The optical density of the microplate reader was taken as a standard, used dual wavelength to assay, and the reference wavelength was taken as 630 nm. The concentration of human cotinine in samples was then determined by comparing optical density of samples with standard curve. The standard density was taken as horizontal, and optical density value for vertical and standard curve was obtained. The corresponding density according to sample optical density value was then found and multiplied with dilution multiple.

Inclusion and exclusion criteria

Only children of age group 5–10 years and PS subject children from smoking household were included. Parents on nicotine replacement therapy or those who have quit smoking, children with rampant caries, nursing bottle caries, and children with poor oral hygiene were excluded.

Statistical analysis

All data were analyzed using Statistical Package for the Social Sciences Version 18.0 (SPSS Inc., Chicago, IL, USA).

Table 2: Distribution of study population on the basis of age, formal education and socio economic class

Socio demographic variables	Case		Control	
	n	%	*n*	%
Age				
5-6 years	14	17.5	28	35
7-8 years	23	28.8	26	32.5
9-10 years	43	53.8	26	32.5
Formal Education				
Primary school	12	15	11	13.8
Middle school	23	28.8	24	30
High School	21	26.3	27	33.8
Intermediate School	9	11.3	13	16.3
Graduate	3	3.8	8	10
Socio economic class				
Lower	24	30	29	36.3
Upper Lower	33	41.3	37	46.3
Lower Middle	15	18.8	8	10
Upper Middle	8	10	6	7.5

Table 3: Comparison of Parental Practice towards their child's oral health and Bacterial Colonies between Case and Control group

| | Parental Practice | | | | | | Bacterial Colonies | | | |
| | Good Practice | | Fair Practice | | Poor Practice | | *Streptococcus colonies* | | *Lactobacillus colonies* | |
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Mean±Std Dev	16.6±25.48	21.2±27.01	31.4±23.26	34.6±26.71	32.0±24.04	34.6±23.33	348.9±166.50	63.03±23.082	247.3±15.86	63.825±12.638
Mann Whitney U	41.000		48.000		37.000		316.000		272.500	
P	0.475		0.880		0.325		<0.001		<0.001	

Numerical data were analyzed by *t*-test and Mann–Whitney *U* test for normal and non-normal data, respectively. Pearson's correlation test was used for determination of correlations. One-way analysis of variance (ANOVA) test was used to compare dichotomized caries prevalence variable for more than two groups.

Results

Sociodemographic analysis

The number of male children was 76 (47.5%) and the number of female children was 84 (52.5%) [Table 2]. The cumulative population of PS case subjects was 17.5% in 5–6 years, 28.8% in 7–8 years, and 53.8% in 9–10 years, whereas the cumulative population of control subjects was 28% in 5–6 years, 26% in 7–8 years, and 26% in 9–10 years. The maximum percent of the study population belonged to upper lower class, that is, 41.3% of PS case subjects and 46.3% of control subjects, and the minimum belonged to upper middle class, that is, 10% of PS case subjects and 8.8% of control subjects.

Parental practice analysis

The mean of the study population frequency with good parental practice was 16.6 ± 25.487 among the PS subjects and 21.2 ± 27.009 among the control subjects [Table 3]. The difference between the means was found to be nonsignificant using Mann–Whitney *U*-test with *P* = 0.475. Similarly, the mean of the study population frequency with fair parental practice was 31.4 ± 23.258 among the PS subjects and 34.6 ± 26.713 among the control subjects which demonstrated difference in means to be nonsignificant using Mann–Whitney *U*-test with *P* = 0.880. However, the mean of the study population frequency with good parental practice was 32.0 ± 24.042 among the PS subjects and 34.6 ± 23.332 among the control subjects with again difference in means being insignificant with *P* = 0.325. It was noted that there was not much difference in the parental practice toward their child's oral health between case and control groups.

Bacterial colony analysis

The mean number of study population with low streptococcus colony count was 16.3%, whereas in the control group it was 100% [Table 3]. Similarly, the mean number of study population with moderate streptococcus colony count was 53.8%, whereas in the control group it was nil (0). Also, the mean number of the study population with high streptococcus colony count was 30%, whereas in the control group it was nil (0). The mean number of the study population with no activity range of lactobacillus count was 0 in the case group and 100% in the control group. The mean number of the study population with low lactobacillus colony count was 87.5% in the case group, and in the control group it was nil (0). Similarly, the mean number of the study population with moderate lactobacillus colony count was 12.5% in the case group, and in the control group it was nil (0). However, the mean number of high lactobacillus colony count was nil in both the case and control groups. Statistically, a high significant difference between the mean colony count of case and control groups was seen using Student's *t*-test where *P* < 0.001.

Salivary pH and buffering capacity analysis

The mean salivary pH among PS case subjects was 6.49 ± 0.27 and among the control group was 7.2 ± 0.38. The salivary buffering capacity was also compared between the two groups, and a low buffering capacity was seen among 48.75% of the PS case group subjects and 27.5% among the control group. Similarly, a moderate buffering capacity was seen among 35% of the PS case group subjects and 32.5% among the control group. A high buffering capacity was seen among 37.5% of the PS case group subjects and 40% among the control group. The difference in means between both the groups was seen to be statistically significant.

Cotinine level analysis

The mean cotinine level was 1.08 ± 0.265 in the PS case subjects and 0.00 ± 0.00 in the control subjects [Table 4]. The difference in means was compared using Student's *t*-test and the results were highly significant with *P* < 0.001.

Caries prevalence analysis

The mean dmft score among the PS case groups was 3.24 ± 2.302 and among the control group was 0.38 ± 0.753 [Table 4]. Similarly, GI scores among case groups was 2.44 ± 1.281 and among control group was 0.25 ± 0.436. The difference in means was compared using Student's *t*-test and the results were highly significant with *P* < 0.001.

Comparison of smoking exposure to caries prevalence, bacterial colonies, and cotinine level among PS subjects

It was seen that as smoking exposure increased, dmft and GI scores among children became poor [Table 5]. Similarly, with

Table 4: Comparison of cotinine Level and caries prevalence between Case and Control group

	Cotinine Level		dmft Score		GI Score	
	Case	**Control**	**Case**	**Control**	**Case**	**Control**
Mean±Std Dev	1.08±0.265	0.00±0.00	3.24±2.302	0.38±0.753	2.44±1.281	0.25±0.436
Std. Mean Error	0.030	0.00	0.257	0.084	0.143	0.049
t	36.276		10.573		14.457	
P	<0.001		<0.001		<0.001	

Table 5: Comparison of smoking exposure with caries prevalence, bacterial colonies and cotinine level amongst passive smoking subjects

	Smoking Exposure		
	Low exposure	**Medium exposure**	**High exposure**
dmft score (Mean±Std Dev)	0	3.58±2.452	4.055±1.744
GI Score (Mean±Std Dev)		2.57±0.789	2.98±1.39
Streptococcus Mutans			
Low	0	4 (15.4%)	9 (16.7%)
Moderate	0	13 (50%)	30 (55.6%)
High	0	9 (34.6%)	15 (27.8%)
Lactobacillus			
Low	0	20 (76.9%)	50 (92.3%)
Moderate	0	6 (23%)	4 (7.4%)
High	0	0	0
Cotinine level (mean±std dev)	0	0.946±0.352	1.212±0.221

increasing smoking exposure, there was an increase in bacterial colonies and cotinine level.

Correlation of cotinine level with bacterial colonies and caries prevalence

There was a positive Pearson's correlation between cotinine level and streptococcus colonies ($r = 0.596$), lactobacillus colonies ($r = 0.603$), dmft scores ($r = 0.818$), and GI scores ($r = 0.711$).

Comparison of smoking exposure to educational status of the parents

With increase in the educational level, the level of smoking exposure decreased in household thereby reducing cotinine level, and the mean difference between different educational levels was found to be statistically significant using one-way ANOVA test for the number of cigarettes/day ($P = 0.044$), frequency of smoking ($P = 0.037$), duration of smoking ($P = 0.025$), and cotinine levels ($P = 0.028$).

Discussion

Since decades, smoking is known as a potential risk factor and a major preventable cause of morbidity and mortality.[14] Thousands of chemicals are present in complex aerosol of cigarette which contains volatile gases with suspension of particulate matter. Second-hand smoke inhaled by other members is 85% composed of side stream and the remaining 15% is mainstream smoke.[15] Hence, biomarkers were developed for epidemiological research

relative to significance of PS exposure. Reliable and major biomarker of recent nicotine intake and second-hand smoke exposure has been referred to as cotinine.[16-18]

Children age 4–11 years approximately 1511 in number whose data were obtained from U.S. National Health and Nutrition Examination Survey (NHANES) were studied in a cross-sectional study by Mattheus *et al*.[19] The results revealed that caries prevalence was 1.59 times more in children who were exposed to smoke inside the house in comparison to smoke outside. In agreement with the study mentioned and a study conducted by Tanaka *et al*.,[20] this study also found that dmft score of PS children was significantly higher (3.24 ± 2.302) than that for control subjects (0.38 ± 0.753) and the results are found to be highly significant (<0.001). Similarly, GI scores in this study are significantly higher in the PS subjects (2.44 ± 1.281) in comparison to the control group (0.25 ± 0.436) which is in accordance with a study conducted by Erdemir *et al*.[21]

The growth of *S. mutans* and *Streptococcus sanguis* as an effect of cigarette smoke was studied by Zonuz *et al*.[22] and the results reflected that growth was increased in the presence of cigarette smoke. The results are in accordance with this study that found higher salivary *S. mutans* scores in PS children (348.9 ± 167.5) compared with the control subjects (63.03 ± 23.082) and the results were found to be highly significant (<0.001). Kumar *et al*.[23] reported results similar to this study that found higher *Lactobacillus* scores in PS children (3247.3 ± 1615.86) in comparison to the control subjects (563.825 ± 312.638) and the results were found to be highly significant.

In a study conducted by Castelino *et al*.,[24] salivary cotinine levels were assessed in different tobacco groups. The salivary cotinine level in the PS subjects was higher than in the control group which is similar to the results in this study where cotinine level is higher in the PS subjects (1.08 ± 0.265) in comparison to the control group (0) and the results were found to be highly significant (<0.001). Methods of self-reported smoking and serum cotinine test ≥10 mg/mL complement each other, are strongly correlated to patient's metabolism,[25] and are effective methods to assess the patient's smoking status.[26] There is a definite association of circulating cotinine concentration with lung cancer risk for current smokers.[27] Studies focusing on the amount, type of tobacco consumed, environmental factors to nicotine, and its metabolism are needed to view the comprehensive relationship between smoking and cotinine levels.[28]

Studies conducted by Castelino *et al.*[24] and Gilman *et al.*[29] have illustrated that the number of pack-years smoked was higher among individuals with less than high school education. According to studies conducted by Hitchman *et al.*[30] and Harper *et al.*,[31] smoking rates are higher among low socioeconomic status groups. The results of the studies are in contrast to this study where there is no significant difference between smoking exposure and cotinine level of different socioeconomic groups ($P = 0.203$).

The study has some major strengths as cotinine concentration in the PS subjects only added predictive power when it was at variance with their claimed control subjects. Then cigarette smoking behavior was reported directly by the parents and is therefore likely to be more accurately measured. This study shows long-term impact of smoking in household on their children which serves as an important motivating factor for their parents to quit smoking. This study highlights PS as health hazard which is not known by many people in study setting and serves as an important enlightening message. It provides glimpse of oral health knowledge, oral hygiene practice, and oral hygiene status of children affected by smoking of other people in house. However, the study has some limitations too. First, the study is not based on large sample size. Second, other factors such as vitamin C concentration, smoking of mothers while they were pregnant, and body mass index that could affect the level of cotinine were not evaluated. Third, the salivary flow rate was not measured in the study which can also be an important factor in caries prevalence.

Frequent interactions with identified smoking subjects and their family members can be made by dentists and primary healthcare workers to have long-term assessment data on the impact of passive smoke so that more appropriate ways for creating awareness on subject can be made. Training programs can also be conducted to circulate flow of knowledge on PS and to be able to motivate active smokers to quit. The establishment of systematic oral healthcare program for community is needed which may serve as a role model for promotion of best practice of oral health habits. Routine biochemical assessment of tobacco smoke exposure and intensified smoking education and prevention activities in school is essential for more effective interventions to prevent adverse effects of PS.[32,33] These recommendations can bring about a major change in oral health status of the children affected by passive smoke.

Conclusion

To conclude, PS has an adverse impact on children which can be evidently reflected by an increase in cotinine level, bacterial colonies, and poor dmft and GI scores among the PS subjects in comparison to the control subjects who reflected comparatively low cotinine level and bacterial count and better dmft and GI scores.

Conflicts of interest

There are no conflicts of interest.

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