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Correlation of salivary biomarkers and dental caries in children exposed to passive smoking

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ABSTRACT

Background: Salivary antimicrobial peptides (AMP's) play an important role in the local defence of oral cavity and expression of these are altered by various factors. Among them cigarette smoke has known to have detrimental effects on salivary immune defence mechanisms. The effect of passive smoking on salivary AMP's and correlation to dental caries in children has not yet been reported. Thus, this study was aimed to assess the correlation between passive smoking and dental caries in exposed and unexposed children.

Materials and Methods: A randomized cross-sectional study was designed & Self-reported questionnaire was filled by the parents of the participants to obtain the data. Participants were divided into passive exposed (PE) and unexposed (UE) group based on exposure parameters. Clinical examination was performed and DMFS were marked for each subject. Unstimulated saliva was collected for 1 min and SFR was measured by analysing saliva in the graduated tubes after which the saliva was subjected for analysis of salivary cotinine and LL-37 concentration through ELISA kit.

Results: The mean DMFS and Salivary cotinine levels were elevated in Passive smoking exposed individuals, showing a positive correlation between DMFS and Salivary cotinine levels to passive smoking, but, the mean salivary LL-37 levels were reduced in passive smoking exposed individuals, showing a negative correlation between Salivary LL-37 levels to passive smoking. There was also a dose-dependent relationship between caries experience and smoking exposure.

Conclusion: Reduction of passive smoking is important not only for the prevention of various systemic ill-effects, but also for the promotion of health.

1. Introduction

A healthy oral cavity plays a major role in the child's life due to its impact on normal nutritional intake, language acquisition, and psychological behaviour. A person cannot live a healthy life until his oral cavity is free of infection like gingivitis, halitosis, periodontitis and dental caries which are not uncommon to human.[1] The innate immunity system of oral cavity provides a rapid, non-specific first line of defence against colonization of pathogenic micro-organisms causing oral diseases.[2]

The components of innate immunity include the barrier function of the skin, reduced pH of the stomach, sweeping motion of the cilia and chemical defences which includes host defence peptides. These genes encoded defence molecules are commonly known as antimicrobial peptides (AMP'S).[3] Anti-microbial peptides play an important role in wound healing and in the maintenance of the

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tissue health, particularly in environments, like the oral cavity, colonized by a microbial plethora.[4] Amongst the antimicrobial peptides found in the oral environment are a- and b-defensins, LL-37 antimicrobial peptide and histatins.[5] Besides their direct bactericidal activity, these peptides have other distinct and overlapping properties, such as chemotactic activity or induction of cytokine release.[6] These peptides have been reported to function as antimicrobial agents against Gram-negative and Gram-positive bacteria, fungi and viruses.[7]

The LL-37 antimicrobial peptide is the proteolytically processed extracellular form of human cationic antimicrobial protein of 18 kDa (hCAP-18), the only known member of cathelicidins in humans that are found in the secondary granules of neutrophils and various other cells.[8] LL-37 is present in the pulmonary and the digestive system and it has also been detected in plasma, sweat, skin, and human milk.[9] Regarding the oral cavity, LL-37 has been detected in saliva, whilst the peptide itself or its mRNA or both have been detected in salivary glands,[10] in lingual epithelium and palatal mucosa.[11] Following inflammatory stimulation, LL-37 is released at the inflamed sites mainly by neutrophils that migrate through the junctional epithelium.[12] This pattern of expression implies a possible protective role of the peptide on both hard and soft oral tissues.[13] The antimicrobial peptides present in saliva have shown to have a broad antimicrobial activity against cariogenic bacteria by destroying their cell membranes which, in turn, can be correlated to resistance to caries.[14]

However, Long term exposure to passive smoking leads to the reduction of cathelicidins.[15] Passive exposure mainly consists of the smoke released from the burning end of a smoldering cigarette, pipe, or cigar ("side-stream smoke," 85%) and, to a lesser extent, the smoke exhaled from the lungs of an active smoker nearby ("mainstream smoke," 15%). There are more than 4000 chemicals present in passive smoking, and more than 250 of these are known to be carcinogenic or toxic in some way or the other.[16]

Exposure to passive smoke begins as early as prenatal life due to parental smoking.[17] It is also seen that maternal smoking during pregnancy alters foetal blood flow and protein metabolism[18] and exposes the growing foetus to chemical toxins like nicotine. Other possible sources of PS include third-hand smoke exposure in household dust and interior surfaces, or increased bacterial load exposure of a smoking parent[19] or caretaker. Also, children are comparatively more vulnerable to second-hand smoke effects because of higher breathing rates per body weight, immature lungs and more lung surface area when compared with adults. Furthermore, infants and children are generally not capable of managing their environment & consequently unable to perform action to escape from SHS exposure because of low-socioeconomic status, under educated parents and small house[20] which constrains parents to smoke inside the house.

SHS affects both general and oral health. Passive smoking impedes dental development through numerous mechanisms such as, interference with reciprocal induction of oral ectomesenchymal tissues, interference with tooth mineralization owing to oxidative stress and nutritional deficiency caused by unfavourable effect of SHS on appetite.[21] Also, Enamel hypoplasia in primary and permanent dentition, poor gingival attachment of teeth and supporting structures and dental caries in the primary dentition is concomitant to SHS exposure in children.[22] Nicotine furthermore boosts proliferation of cariogenic bacteria like mutans Streptococci in smoking mother's oral cavity of which gets transferred to their infants and predisposes to dental caries in children.[23] SHS exposure also predisposes children to infections through immune system suppression or modulation such as lower salivary IgA & IgG levels.

Even though dental caries has been considered as a Global Pandemic, there is rise in prevalence in the developing countries due to globalization in the urban region and in contrast lack of knowledge regarding the possible etiological factors in the rural region. India being the second largest consumer and producer of tobacco, increases the risk of dental caries among the children exposed to second hand smoke.[24] Hence, it is it biologically plausible that passive smoking could cause caries, particularly in childhood due to the suppression of immune system thus, reducing the salivary AMP. Also, due to lack of literature, this study was designed to assess the correlation between passive smoking and dental caries in exposed and unexposed children. Possible correlation to age, gender, salivary flow rate & dental caries experience was determined in the phase 1 of the study[25] and assessment of salivary LL-37, salivary cotinine levels and possible correlation of salivary biomarkers to dental caries experience were asses in the current phase 2 study.

2. Materials and Methods

2.1. Setting and population

A randomized case–control study was conducted in Bangalore (Karnataka) among 120 children aged between 3–8 years.

2.2. Ethics study consent

The study was conducted during 2019–2020 after obtaining Ethical approval for the study from the ethical clearance committee of the institution. Written informed consent was taken from parents before implementing the study.

2.3. Sample size

The sample size was calculated based on confidence level of 95%, confidence interval of 5%, and estimated population response distribution of 50%. The sample size obtained using this calculation was 120 participants. The participants were obtained from the OPD, Department of Paediatrics and Preventive Dentistry, AECS Maaruti dental college, Bangalore.

2.4. Study procedure

The study was conducted by:

1. Assessing self-reported questionnaire,
2. Clinical dental examination &
3. Salivary flow rate estimation
4. Salivary biomarkers estimation.

2.4.1. Assessing self-reported questionnaire

Self-administered close-ended questionnaire was given for parents to assess exposure to second-hand-smoking among primary caregivers of children in India (by WHO) (Table 1) which was printed both in English and regional language (Kannada). Upon assessing, the subjects were then divided into two groups with 60 samples each using simple stratified sampling methods.

1. Group 1 Passive smoke exposed (PE (n=60
2. Group 2 Unexposed (UE (n=60

For further confirmation, passive smoke exposed samples underwent screening for cotinine levels estimation using JusCheck rapid nicotine/cotinine test kits. (Rapid, self-controlled, immunoassay for the qualitative detection of Cotinine in human saliva).

2.5. Inclusion & exclusion criteria

Children who were exposed and unexposed to passive smoking, aged between 3-8 years with presence of dental caries, filled or extracted teeth due to carious lesion with prior parental consent were included in the study. The exclusion criteria included children with systemic diseases, long term medication., administration of antibiotics less than one month before and Children with filled or extracted teeth due to non-carious causes.

2.5.1. Clinical dental examination

Clinical examination was performed by a single calibrated examiner using mouth mirror and straight probe under the natural light. decayed, extracted and filled surfaces (def) in deciduous teeth & Decayed, Missing & Filled surfaces in permanent teeth were counted in each subject. [according to Gruebell. A.O in 1944]. Both groups were then further divided into subgroups based on def/DMFT scores.

2.5.2. Salivary flow rate estimation

Children were instructed not to eat or chew anything for at least 1 hour before sample collection and were instructed to spit the unstimulated saliva into the graduated polyprolene tubes for 1 min. The unstimulated salivary flow rate was determined by measuring the saliva collected in graduated tubes.

2.5.3. Salivary biomarkers estimation

The salivary samples were then centrifuged at 6000rpm for 10 min to remove cell debris. The supernatant phase was then transferred to Eppendorf tubes and stored at minus 70 degree Celsius. SalivaryLL-37 levels were quantified using a commercially available enzyme linked immunosorbent assay kit (Elabsciences, China) and salivary cotinine concentrations were quantified using a commercially available enzyme linked immunosorbent assay kit (Bioasaay Technology Laboratory) following the manufacturer's directions.

2.6. Statistical analysis

Data were analysed using the IBM Statistical Package for the Social Sciences (SPSS) (IBM, Armonk, New York) software, version 22.0.

3. Results

A total of 120 participants, participated in the present case-control study after filling the given questionnaire and thorough clinical examination. Among them, the subjects were divided into PE and UE groups.

3.1. Socio demographic analysis

Among 120 participants the number of male children were 56 (PE-26 & UE-30) and the number of female children were 64 (PE-34 & UE-30).(Table 3) The mean age of the participants in the PE group was 5.18±1.57 and 5.55±1.50 in UE group, which showed no statistical difference between the two groups (p=0.19). 70% (n=20) of the parents in PE group had education status below matriculation whereas 66.7%(n=40) of the parents in UE group were better qualified. Also, majority of the parents 78.3%(n=47) in UE group belonged to high income (>1,00,000 p.a) status, unlike PE group where 48.3% belonged to medium income status (50,000 - >1,00,000 p.a) depicting statistically significant difference in the education status & income level between two groups (p=0.001).

3.2. Analysis of distribution of smoking related characteristics in PE group

Table 3 represents the distribution of smoking related characteristics among the people at home Among 60 PE participants, 78.3% (n=47) of the participants had at least

Table 1: Questionnaire to assess self-reported exposure to second-hand-smoking among primary caregivers of children <5 years of again India (acc to who)

1. Date:
2. Name:
3. Age:
4. Sex:
5. Address:
6. Over the past 7 days, has your child been around smoke from tobacco? Do you remember smelling cigarette, bidi, hookah smoke when your child was present? YES/NO
7. Over the past 7 days, has your child been around smoke from tobacco? Do you remember smelling cigarette, bidi, hookah smoke when your child was present? YES/NO
8. Over the past 7 days, did your child visit markets, restaurants or public places? If yes, did you smell cigarette, bidis or hookah smoke? YES/NO
9. Over the past 7 days, did your child use public transportation (auto or buses)? If yes, did you smell cigarette, bidis or hookah smoke? YES/NO
10. How many people who currently live in your home smoke cigarettes or bidis?
11. Over the past 3months, has anyone smoked anywhere inside your home? YES/NO
12. Over the past 3months, has anyone smoked anywhere inside your home? YES/NO
a. Inside only
b. Inside and outside
c. Outside
d. Outside
13. How often does anyone, including visitors, smoke cigarettes or bidis inside your home?
a. Daily
b. Weekly
c. Weekly
d. Sometimes
e. Never
14. Which best describes how cigarette and bidi smoking is handled in your home?
a. No rules
b. Smoking is permitted anywhere
c. Smoking is permitted in some places
d. No one is allowed to smoke anywhere?
15. For how many years do you think, your child has been exposed to tobacco smoke?
a. 0-6 months
b. 6 months-3 years
c. 3 years-6 years
16. Mother's education:
a. No education
b. Primary school
c. Middle school
d. High school
e. Degree holders
17. Father's education:
f. No education
g. Primary school
h. Middle school
i. High school
j. Degree holders
18. Parents income:
a. <50 thousand p.a
b. 50 thousand-1 lakh p.a
c. > 1 lakh p.a
Name and signature of the volunteer:
Signature of the investigator
Date:

Table 2: Sociodemographic characteristics among 2 groups

Age and gender distribution among 2 groups						
Variable	**Category**	**Group 1**		**Group 2**		**P-Value**
		Mean	SD	Mean	SD	
Age	Mean & SD	5.18	1.57	5.55	1.50	0.19a
	Range	03 - 08		03 - 08		
			%		%	
Sex	Males	26	43.3%	30	50.0%	0.46b
	Females	34	56.7%	30	50.0%	

Comparison of Sociodemographic characteristics among 2 groups using Chi Square Test

Variable	**Category**	**Group 1**		**Group 2**		**P-Value**
			%		%	
Income Level	< 50, 000	2	3.3%	0	0.0%	0.002*
	> 50, 000 & < 1, 00, 000	29	48.3%	13	21.7%	
	> 1, 00, 000	29	48.3%	47	78.3%	
Education	Up to Matriculation	42	70.0%	20	33.3%	0.001*
	Above Matriculation	18	30.0%	40	66.7%	

single smoker at home whereas 21.7% of the participants had two or more smokers at home. In 71.7%(n=43) of the participants the parents smoked both inside & outside. In 65% of houses there were no rules with respect to smoking. 61.7%(n=37) of the participants had history of being exposed to smoke for >3 years and 41.7% (n=25) of the parents had history of smoking 5-10 cigarettes/day.

3.3. Comparison of mean values of different parameters between 2 groups.

Table 4 & Graph-4A, 4B & 4C shows the comparison of mean values of DMF scores, SFR, Salivary Cotinine levels & Salivary LL-37 levels between PE & UE groups. The mean DMF score of PE group were high (mean DMF score 5.58±4.66) than UE group (mean DMF score 3.15±3.26), also salivary cotinine levels were higher in PE group (mean Sal. Cotinine level 3.80±0.32) when compared to UE group (mean Sal. Cotinine level 2.18±0.26), but the salivary LL-37 levels were low in PE group (mean Sal.LL-37 level 114.72±42.71) over the UE group (mean Sal.LL-37 level 158.67± 78.12), which showed a statistical difference in DMF scores, Salivary Cotinine levels & Salivary LL-37 levels between PE & UE groups. However, no statistical difference was found in the SFR between the PE & UE groups, where, (p=0.16). 3.4. Analysis of distribution of smoking related characteristics in PE group.

3.4. Comparison of mean values of different parameters based on the caries status in PE group

Table 5 implies the Comparison of mean values of SFR, Sal. Cotinine levels & Sal.LL-37 levels based on the caries status in PE group. The mean SFR (2.74±0.37) & Sal. LL-37 (158.08±12.10) was greater in the children with no dental caries than in children with dental caries, but the Sal. Cotinine level (3.50±0.47) was low in children without dental caries than in children with caries which showed statistically significant difference among 2 groups.

3.5. Comparison of mean values of different parameters based on the caries status in UE group

Table 6 implies the Comparison of mean values of SFR, Sal. Cotinine levels & Sal.LL-37 levels based on the caries status in UE group. The mean SFR (2.42±0.23) & Sal. LL-37 (223.41±36.96) was greater in the children with no dental caries than in children with dental caries, but the Sal. Cotinine level (1.97±0.14) was low in children without dental caries than in children with caries which showed statistical significant difference among 2 groups.

3.6. Correlation between Caries Scores, Sal. Cotinine & LL-37 levels in both PE & UE group.

In both PE and UE group, there was a very strong positive correlation between the dental caries and Sal. Cotinine levels however there is strong negative correlation between Sal. Cotinine levels and Sal.LL-37 levels and very strong negative correlation between Sal.LL-37 and dental caries, showing statistically significant difference (Table 8).

3.7. Comparison of smoking exposure to caries

It was seen that as smoking exposure and DMFS had a positive correlation. The mean DMF score was 9.38±4.23 who were exposed to two or more smokers at home than one smoker with DMF score 4.53±4.24. Also DMF score was significantly higher (6.93±4.48) in children, whose parents smoked both inside & outside and (6.62±4.64) in families

Table 3: Distribution of smoking related characteristics among the people at home in PE group

Variable	Category	n	%
No. of smokers at house	One	47	78.3%
	Two	13	21.7%
Smoking location	Inside only	1	1.7%
	Outside only	3	5.0%
	Inside & Outside	43	71.7%
	Based on Season	13	21.7%
Smoking rules	Smoking is permitted in some places	9	15.0%
	Smoking is permitted everywhere	12	20.0%
	No rules	39	65.0%
Duration of Smoking	< 6 Months	4	6.7%
	> 6 Months & < 3 years	19	31.7%
	> 3 years	37	61.7%
Frequency of Smoking	< 5 nos.	24	40.0%
	> 5 & < 10 nos.	25	41.7%
	> 10 nos.	11	18.3%

Table 4: Comparison of mean values of different parameters between 2 groups using Mann Whitney Test

Parameters	Groups	N	Mean	SD	Mean Diff	P-Value
DMFS	Group 1	60	5.58	4.66	2.43	0.003*
	Group 2	60	3.15	3.26		
Salivary flow rate	Group 1	60	1.824	0.775	-0.16	0.16
	Group 2	60	1.983	0.545		
Sal. Cotinine	Group 1	60	3.805	0.329	1.62	<0.001*
	Group 2	60	2.185	0.265		
Sal. LL-37	Group 1	60	114.726	42.710	-43.95	0.001*
	Group 2	60	158.675	78.128		

Table 5: Comparison of mean values of different parameters based on the caries status in PE Group using Mann Whitney Test

Parameters	Caries	N	Mean	SD	Mean Diff	P-Value
Salivary flow rate	No caries	20	2.740	0.373	1.374	<0.001*
	With Caries	40	1.366	0.442		
Sal. Cotinine	No caries	20	3.505	0.147	-0.450	<0.001*
	With Caries	40	3.955	0.290		
Sal. LL-37	No caries	20	158.089	12.109	65.044	<0.001*
	With Caries	40	93.045	35.239		

Table 6: Comparison of mean values of different parameters based on the caries status in UE Group using Mann Whitney Test

Parameters	Caries	N	Mean	SD	Mean Diff	P-Value
Salivary flow rate	No caries	30	2.427	0.239	0.887	<0.001*
	With Caries	30	1.540	0.376		
Sal. Cotinine	No caries	30	1.971	0.144	-0.428	<0.001*
	With Caries	30	2.399	0.165		
Sal. LL-37	No caries	30	223.412	36.969	129.475	<0.001*
	With Caries	30	93.937	48.794		

Table 7: Spearman's correlation test to assess the relationship between caries scores, Sal. Cotinine & LL-37 levels in each group

Groups	Parameters	Values	Caries Scores	Sal. Cotinine	Sal. LL-37
Group 1	Caries scores	rho	1	0.81	-0.96
		P-Value	.	<0.001*	<0.001*
	Sal. Cotinine	rho	0.81	1	-0.76
		P-Value	<0.001*	.	<0.001*
	Sal. LL-37	rho	-0.96	-0.76	1
		P-Value	<0.001*	<0.001*	.
Group 2	Caries scores	rho	1	0.91	-0.90
		P-Value	.	<0.001*	<0.001*
	Sal. Cotinine	rho	0.91	1	-0.79
		P-Value	<0.001*	.	<0.001*
	Sal. LL-37	rho	-0.90	-0.79	1
		P-Value	<0.001*	<0.001*	

* - Statistically Significant
The correlation coefficients are denoted by 'rho'
Correlation coefficient range
0.0 - No Correlation
0.01 - 0.20 - Very Weak Correlation
0.21 - 0.40 - Weak Correlation
0.41 - 0.60 - Moderate Correlation
0.61 - 0.80 - Strong Correlation
0.81 - 1.00 - Very Strong Correlation

who had no rules regarding smoking habits. There was also positive correlation in DMF score & duration of smoking. Children who had history of exposure to smoke >3 years had mean DMF score of 8.22±3.17 than children exposed to smoke 10 cigarettes/day. (Table 9)

3.8. Comparison of smoking exposure to Salivary LL-37 levels

The Sal.LL-37 levels in participants who had single smoker at home was high (123.08±39.26), when compared to the Sal.LL-37 levels in the participants had two smokers at home (84.53±42.43). In case of the participants whose parents smoked only inside mean Sal.LL-37 levels was least (108.39) followed by inside & outside (102.860±41.60), based on the season (140.92±32.02) and (159.37±14.22) in houses where they smoked only outside. Also, the mean Sal.LL-37 levels was least in the houses, where there were no rules with respect to smoking (105.72±42.52) than in the houses where smoking was permitted in some places (152.90±17.10) The mean Sal.LL-37 levels was in-directly proportional to no of cigarettes/day 139.96±27.93 (10 cigarettes/day) and duration of exposure. i.e 153.70±10.21 (3 years) (Table 9).

3.9. Comparison of smoking exposure to salivary cotinine levels

The Sal. Cotinine levels in participants who had single smoker at home was lesser (3.73±0.27), whereas Sal. Cotinine levels was (4.060±0.39) in the participants had two smokers at home. In case of the participants whose parents smoked only inside mean Sal. Cotinine levels was higher (3.940) followed by inside & outside (3.86±0.35), based on the season (3.67±0.22) and (3.52±0.08) in houses where they smoked only outside. Also, the mean Sal. Cotinine levels was highest in the houses, where there were no rules with respect to smoking (3.87±0.3) than in the houses where smoking was permitted in some places (3.55±0.13). The mean Sal. Cotinine levels was directly proportional to no of cigarettes/day 3.63±0.23 (10 cigarettes/day) and duration of exposure. i.e 3.48±0.04 (3 years) (Table 10).

4. Discussion

Since decades, smoking is known as a potential risk factor and a major preventable cause of morbidity and mortality. SHS not only affects the general health of the child who is exposed to tobacco smoke, but also studies by Hong et al 26 and Lowe et al.[26] have proposed a positive association between SHS exposure and oral health. Among the various ill effects on oral health, cigarette smoke has known to have detrimental effects on salivary immune defence mechanisms, thereby reducing the expression of salivary AMP's (ll-37) which is known to have bactericidal property against the cariogenic microorganisms.

Goncalves et al[27] and Hagiwara et al[28] have shown that both innate immunity and adaptive immunity are susceptible to cigarette smoke, which interrupts immunological homeostasis, causes various diseases and paradoxical effects on immune cells. However, very limited literature has been available which shows the possible correlation between passive smoking and dental caries in children.

Table 8: Comparison of Mean Dental caries scores based on the smoking related characteristics in PE Group

Variable	Category	DMFS		P-Value
		Mean	SD	
No. of smokers at house	One	4.53	4.24	0.001*
	Two	9.38	4.23	
Smoking location	Inside only	5.00	.	0.003*
	Outside only	0.00	0.00	
	Inside & Outside	6.93	4.48	
Smoking rules	Based on Season	2.46	3.43	0.008*
	Smoking is permitted in some places	1.22	2.44	
	Smoking is permitted everywhere	5.50	4.32	
	No rules	6.62	4.64	
Duration of Smoking	< 6 Months	0.00	0.00	<0.001*
	> 6 Months & < 3 years	1.63	3.67	
	> 3 years	8.22	3.17	
Frequency of Smoking	< 5 nos.	2.75	3.14	<0.001*
	> 5 & < 10 nos.	5.52	3.86	
	> 10 nos.	11.91	2.55	

Table 9: Comparison of Sal. LL-37 levels based on the smoking related characteristics in PE Group

Variable	Category	Sal. LL-37		P-Value
		Mean	SD	
No. of smokers at house	One	123.080	39.260	0.004*
	Two	84.530	42.430	
Smoking location	Inside only	150.390	.	0.003*
	Outside only	159.370	14.220	
	Inside & Outside	102.860	41.600	
Smoking rules	Based on Season	140.920	32.020	0.007*
	Smoking is permitted in some places	152.900	17.100	
	Smoking is permitted everywhere	115.360	42.960	
	No rules	105.720	42.520	
Duration of Smoking	< 6 Months	153.700	10.210	<0.001*
	> 6 Months & < 3 years	148.660	32.030	
	> 3 years	93.090	34.780	
Frequency of Smoking	< 5 nos.	139.960	27.930	<0.001*
	> 5 & < 10 nos.	115.700	35.540	
	> 10 nos.	57.460	28.280	

Hence, our study was designed to assess the correlation between passive smoking and dental caries in exposed and unexposed children and possible correlation to salivary flow rate, salivary AMP concentration and salivary cotinine levels were determined.

Phase I of our study[25] results showed that there was a dose-response relationship between levels of exposure to tobacco use and dental caries in children which was in accordance with various authors. However, the limitation was validation of SHS exposure was which was obtained by questionnaire reports and was not performed by measurements of biomarkers. Phase 2 of our study included quantitative assessment of salivary biomarkers and correlation of the same with dental caries experience.

The most sensitive way to assess exposure is by measurement of biomarkers in body fluids. Various procedures have been explained for assessment of biomarkers, among which measurement in saliva becomes a non-invasive, easy and well tolerated collection procedure when multiple samples are required.[29] A widely used biomarker is cotinine, which is Nicotine's major metabolite. It has a longer half-life and is considered a reliable biomarker when screening for passive exposure to tobacco use. It is less prone to instabilities and can be easily measured in body fluids.[30] Cotinine estimation from body fluids provide an estimation of recent exposure to tobacco products but not the duration of exposure.[31] The cotinine levels are found to be significantly higher in unstimulated

Table 10: Comparison of Sal. Cotinine levels based on the smoking related characteristics in PE group

Variable	Category	Sal. Cotinine		P-Value
		Mean	SD	
No. of smokers at house	One	3.730	0.270	0.008*
	Two	4.060	0.390	
Smoking location	Inside only	3.940	.	0.09
	Outside only	3.520	0.080	
	Inside & Outside	3.860	0.350	
Smoking rules	Based on Season	3.670	0.220	0.02*
	Smoking is permitted in some places	3.550	0.130	
	Smoking is permitted everywhere	3.780	0.290	
	No rules	3.870	0.350	
Duration of Smoking	< 6 Months	3.480	0.040	<0.001*
	> 6 Months & < 3 years	3.560	0.200	
	> 3 years	3.970	0.300	
Frequency of Smoking	< 5 nos.	3.630	0.230	<0.001*
	> 5 & < 10 nos.	3.780	0.230	
	> 10 nos.	4.240	0.330	

than in stimulated saliva. In saliva, values between 1 ng/mL and 30 ng/mL may be associated with light smoking or passive exposure, and levels in active smokers typically reach 100 ng/mL or more.[32]

Studies by Aligne et al.[33] and Avçar et al.[34] have used cotinine levels to quantify passive exposure to tobacco use. Also, study by Ipseeta et al.[35] assessed the Association of passive smoking with dental caries and salivary biomarkers among 5-10 year children which showed that the mean cotinine level was directly proportional to DMFS, and smoking exposure. Similarly, our study results showed that salivary cotinine levels were higher in PE group (mean Sal. Cotinine level 3.80±0.32) when compared to UE group (mean Sal. Cotinine level 2.18±0.26). Intragroup comparison from the study also revealed that, the mean salivary cotinine was greater in children with dental caries than in children without caries in both PE & UE groups. The results of the present study showed that the Sal. Cotinine levels in participants who had single smoker at home was lesser (3.73±0.27), whereas Sal. Cotinine levels was (4.060±0.39) in the participants had two smokers at home which is in line with a previous study conducted in England[36] that compared the cotinine concentration in children who live in smoke-free homes, according to the smoking status of their parents, showing that cotinine concentration was high among children of smoking parents (nonsmoking parent GM: 0.22 ng/ml; one smoking parent GM: 2.37 ng/ml; two smoking parents GM: 4.71 ng/ml).

Winkelstein et al,[37] assessed cotinine concentration in individuals who had parents who smoked outside versus inside the house. He found reduced cotinine levels among smoking practices done outdoor thus, decreasing children's exposure to ETS. Similarly, in line with the above mentioned study, our results showed that, in case of the participants whose parents smoked only inside mean Sal. Cotinine levels was higher (3.940) followed by inside & outside (3.86±0.35), based on the season (3.67±0.22) and (3.52±0.08) in houses where they smoked only outside. Also, the mean Sal. Cotinine levels was highest in the houses, where there were no rules with respect to smoking (3.87±0.3) than in the houses where smoking was permitted in some places (3.55±0.13). The mean Sal. Cotinine levels was directly proportional to no of cigarettes/day 3.63±0.23 (10 cigarettes/day) and duration of exposure. i.e. 3.48±0.04 (3 years). In accordance to our results, Yoko Guto et al.[38] also, observed a significant dose-response relationship for pack and years of smoking by all family members, exposure to smoking after childbirth and cotinine levels which may have an effect on the development of dental caries. Blackford, et al.[39] studied the quantitative relationship between number of cigarettes consumed and level of salivary cotinine, a biomarker of nicotine dose in China, Brazil, Mexico and Poland. In addition, increased cotinine concentration in children has been associated with socioeconomic factors and education status of the parents. Dell'Orco et al. and Cook et al.[40] have showed that cotinine increases with decrease in the social class. Other studies conducted by Castelino et al.[41] and Gilman et al.[42] 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.[43] and Harper et al.[44] smoking rates are higher among low socioeconomic status groups. We can substantiate our results similar to them as in our study it was seen that salivary cotinine levels were greater in under educated parents (3.9±0.32) and parents from low socio-economic status (4.23±0.31).

Across the literature, various biomarkers have been studied which are the potential predictors of SHS. However, not much data exists between the correlation of passive smoking, so in our current study along with salivary cotinine assessment, salivary LL-37 levels were also assessed.

Sotrio Davidopoulo et al[45] have showed that significantly lower concentrations of LL-37 were found in children with high caries activity, compared to caries free children or to children with low to moderate caries activity. Similarly, study by Karsiyaka et al[46] have showed the mean salivary LL-37 concentration of PS-exposed children was significantly lower than that of PS-unexposed children. In accordance to the above mentioned studies, our results have also revealed that the mean salivary LL-37 levels were significantly lesser (114.726±42.71) in PE in comparison to UE group (158.67±78.12). Also, intragroup comparison has shown that expression of LL-37 is greater in children without dental caries as opposed to the children with dental caries. Comparison of Sal.LL-37 levels based on the smoking related characteristics in PE group have shown that, the Sal.LL-37 levels in participants who had single smoker at home was high (123.08±39.26), when compared to the Sal.LL-37 levels in the participants had two smokers at home (84.53±42.43). In case of the participants whose parents smoked only inside mean Sal.LL-37 levels was least (108.39) followed by inside & outside (102.860±41.60), based on the season (140.92±32.02) and (159.37±14.22) in houses where they smoked only outside. Also, the mean Sal.LL-37 levels was least in the houses, where there were no rules with respect to smoking (105.72±42.52) than in the houses where smoking was permitted in some places (152.90±17.10) The mean Sal.LL-37 levels was in-directly proportional to no of cigarettes/day 139.96±27.93 (10 cigarettes/day) and duration of exposure. i.e 153.70±10.21 (3 years).

Our study shows that there is strong negative correlation between Sal. Cotinine levels and Sal.LL-37 levels and very strong negative correlation between Sal.LL-37 and dental caries, showing statistically significant difference, similar to the study by Takeuchi et al and Hendek et al.[47]

Exposure to tobacco smoke, contains numerous chemical toxins which might predispose children to infection through suppression or modulation of the immune system.[48] Numabae et al[49] showed that the phagocytic activity of salivary PMN's intensifies after exposure to smoking, where as an another invitro study[50] demonstrated that nicotine inhibited phagocytic activity, which could be the plausible reason for reduced LL-37 levels. In normal condition soon after the colonization of the cariogenic pathogens, they are identified by pathogen recognition receptor of the oral cells, which signals for the secretion of LL-37. Once the LL-37 reaches the site, it binds onto the bacterial cell wall and causes cellular lysis by carpet or barrel sieve model, which maintains the tooth in healthy condition. Whereas, in case of passive smoke exposed individuals, there will reduced Salivary LL-37 concentration, which alters the protective and antibacterial effects of LL-37 increasing the risk of dental caries.[51]

Multitude of mechanisms have been described by the various authors, regarding the association of passive smoking on dental caries in children,[52–54] yet epidemiological shows inconsistent results because of unknown factors related to passive smoking which may have confounded the observed relationship. However, study results have concluded that, the mean DMF score and salivary cotinine levels were higher in PE group when compared to UE group, but the salivary LL-37 levels were low in PE group over the UE, showing a statistical difference between both the groups. Hence, it is plausible from our results that, passive smoking was positively associated with the prevalence of dental caries, due to the suppression of Salivary AMP, which predisposes to dental caries.

Smoking is injurious to health and its contribution to oral cancer is well known. However, not much is known regarding its relationship to dental caries. The annual world no Tobacco Day (31st May) is an opportunity to raise awareness on the harmful effects of tobacco use.[55] Yet, lesser is discussed about the ill effects of passive smoking on oral health and its association to Dental caries.

The study has some major strengths as cotinine concentration was measured in the PS subjects after reported directly by the parents, which reduced the bias among selection of PE & UE groups. This study also shows long-term impact of smoking in household on their children which serves as an important motivating factor for their parents to quit smoking and the study also highlights PS as health hazard which is not known by many people in study setting and hence, serves as an important enlightening message. 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. 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 Passive Smoking.[56,57] These recommendations can bring about a major change in oral health status of the children affected by passive smoke.

5. Conclusion

Dental caries is a common public health problem among children due to multi factorial aetiological agents. Prevalence of Dental Cancer among smokers are 20

in 1,00,000, 158 but, possibilities of acquiring dental caries due to the ill effects of environmental smoke is higher. History recording about parental smoking practices have also been neglected during routine case history documentation, which could be one of the confounding etiological factor in caries etiology. Our results revealed that passive exposure to tobacco smoke had independent relationship with dental caries in children, due to the suppression of Salivary AMP, which predisposes to dental caries. Hence, it is the need of hour to document parental smoking practices and create guidelines by the necessary authorities to ensure that the ill effects of passive smoking reaches out to the masses.

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None.

7. Conflict of Interest

The authors declare no conflict of interest.

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