

## **Original article**

### **Estimation of salivary biomarkers in passive smoking children- a comparative study**

*Bhandary S<sup>1</sup>, Rao S<sup>2</sup>, Shetty S<sup>3</sup>, D'Cruz AM<sup>4</sup>*

#### **Abstract:**

**Objective:** To evaluate the relationship between passive smoking and salivary biomarkers like pH, buffering capacity and flow rate, sialic acid and amylase levels in children who were passive smokers and compare with the control group. **Materials and Methods:** Fifty children with history of passive smoking and 50 healthy age matched controls were included in the study. Details on smoking habits of household members, child's dental and dietary habits were collected using a proforma. Saliva samples were collected from both the groups and salivary analysis was done for pH, buffering capacity, flow rate, sialic acid levels and amylase levels. **Results:** The results of the study showed a lower salivary pH and flow rate with an increase in amylase activity and buffering capacity in passive smoking children when compared to healthy controls. However, sialic acid levels did not show significant differences between passive smoking children and control group. **Conclusion:** Passive smoking may reduce the protective properties of saliva which can further affect the oral health status of young children and any factor that influences the secretion rate or composition of saliva will ultimately influence caries susceptibility.

**Keywords:** passive smoking; salivary biomarkers; sialic acid; amylase; children

*Bangladesh Journal of Medical Science Vol. 15 No. 02 April'16. Page : 160-165*

#### **Introduction:**

Passive smoking or environmental tobacco smoke is a serious public health hazard. It has been estimated that at least 1 billion adults are smokers worldwide and that at least 700 million children breathe air polluted by tobacco smoke (1). Children's exposure to passive smoking is usually involuntary, arising from smoking, mainly by adults, in the places where children live and play. Children are most susceptible group for passive smoking because their bronchial tubes are much smaller and their immune systems are less developed. They also breathe faster and thus take in more harmful chemicals per kilogram of body weight than adults <sup>1</sup>.

The saliva circulating in mouth at any given time is termed as whole saliva and it comprises of a mixtures of secretions of major and minor salivary glands and traces from gingival crevicular fluid. Saliva definitely promotes oral health and hence lack of its secretion contributes to disease process. It has been long recognized that saliva serves as a mirror of the body's health as it contains proteins, hormones, antibodies and other molecules that are frequently measured in standard blood tests to monitor health and disease. However, unlike whole blood, saliva is easy to collect, less painful to patient and is less infectious for health care providers <sup>2</sup>. Passive smoking effects children's health in many

1. Srikala Bhandary , Reader, Department of Pediatric Dentistry, A. B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, Karnataka, India.
2. Shruthi Rao , A. B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, Karnataka, India.
3. Suchetha Shetty , Professor, Department of Biochemistry, K S Hedge Medical Academy, Nitte University, Mangalore, Karnataka, India.
4. Audrey Madonna D'Cruz , Reader, Department of Public Health Dentistry, A. B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, Karnataka, India.

**Corresponds to:** Dr Srikala Bhandary, Reader, Department of Pediatric Dentistry, A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore-575018, Karnataka, India.

**E-mail:** docsrikala@gmail.com, audreydcruz@yahoo.co.in

ways by predisposing them to cancer, cardiovascular diseases, asthma, lower respiratory tract infections and neurological disorders and has been found to affect child's cognitive abilities<sup>3-7</sup>. As environmental tobacco smoke, the material released into the ambient atmosphere by smoking tobacco products, contains over 4000 chemicals, it is reasonable to assume that passive smoking may also affect oral health<sup>8</sup>.

Smoking by household members could be an important risk indicator for caries in the permanent teeth of adolescents in a developing country<sup>9</sup>. Very few studies have examined the relationship between passive smoking and oral health in children have hypothesised that there are behaviours associated with tobacco use that may result in other unhealthy behaviour independent of socio-economic status, such as poor oral hygiene and diet<sup>10, 11</sup>.

Saliva plays a major part in the health of the mouth and any changes in its quantity may alter the oral health status<sup>12, 13</sup>. Although the association between passive smoking and caries have been accepted in the literature<sup>2-7</sup>, its relationship with the composition of saliva is unclear and very few studies are done to evaluate the relationship between passive smoking and saliva, there was a need to do this study. Therefore the aim of this study was to investigate the association of passive smoking and related salivary biomarkers such as pH, buffering capacity, flow rate, sialic acid and amylase in young children.

#### **Material and Methods:**

A cross sectional comparative study was undertaken on the patients visiting the Department of Pedodontics and Preventive Dentistry, A. B. Shetty Memorial Institute of Dental Sciences, Deralakatte Mangalore. Prior to commencement of the study, consent was taken from the concerned authorities; approval for the study was obtained from the Nitte Research and Ethical Committee. The procedure was explained to the patient and the patient's parent or guardian and informed consent was obtained from them.

#### **Based on the pilot study conducted, the sample size was calculated by the formula**

patients per group =  $f(\alpha, \beta) \times [(2 \times SD^2) / (d)^2]$   
where,  $f(\alpha, \beta) = 7.85$  for 80% power with 5% significance.

A sample size of 44 was required per group which was rounded off to 50.

#### **Study population**

Fifty passive smokers and 50 controls of the same

age group reporting to the OPD were enrolled as the study population. The participants in the study and control group were matched for age and gender before including them in the study.

#### **Inclusion criteria:**

Based on the tobacco smoking habits (3 – 50 cigarettes/ beedis) of the family members, the children were grouped into passive smokers (study group) or non passive smokers (control group).

#### *Criteria for selection of passive smoker subjects:*

One hundred parents were given a questionnaire to fill about their smoking habits while waiting for the child's dental treatment. Based on these answers, if someone at home was reported to be a regular smoker since the birth of the child, then the home was categorized as a regular smoking household. The children who lived in such a household were identified as passive smoker subject.

#### *Criteria for selection of controls:*

If nobody smoked at home then it was categorized as non-smoking household and the children living in such environment was included as the controls. For each passive smoker subject, children of the same gender and age were selected randomly from patients who lived in a non-smoking household and received their dental treatments<sup>14, 15</sup>.

#### **Exclusion criteria:**

- Subjects with any systemic diseases or taking any medications for the past three months
- Uncooperative children
- Children without parental consent.

#### **Saliva collection protocol**

Saliva assessment included pH, buffering capacity, flow rate, sialic acid and amylase levels. Tests were done during the second appointment because the subjects were asked to refrain from eating for two hours before the assessment. Whole stimulated saliva samples were collected after chewing paraffin wax for 30 seconds between 9 and 11am to minimize the circadian rhythm effects. The salivary samples were collected by the second author and sent immediately for analysis to the biochemistry laboratory for determination of various salivary parameters. The salivary determination was undertaken by the third author without freezing the salivary samples.

#### a) Estimation of pH-

The collected saliva was stored in refrigerator for no longer than 30sec and then using manual pH meter (Presto Stantest Pvt. Ltd.), the pH was measured. Based on the degree of colour change

pH was then estimated.

b) Estimation of buffering capacity by Ericsson's method-1959<sup>16</sup>

About 0.5 ml of saliva was added to 1.5 ml of 5mmol/L HCl. The mixture was vigorously shaken and then centrifuged for 1 minute and allowed to stand for 10 minutes. The final pH of supernatant was measured by using manual pH meter.

c) Sialic acid estimation using Diphenylamine method by Winzler BJ<sup>17</sup>

d) About 0.2 ml of saliva and 0.2ml of sialic acid standard was pipetted in separate 15×150mm test tubes marked as test and standard respectively. To each tube 4.8ml of 5% trichloroacetic acid was added and shaken. The tubes were covered with aluminium foil and placed in a water bath. After 15 min, the test tubes were immersed in cold water. The cooled sample is filtered through Whatman No 1 filter paper. Two 15×150mm tubes were set up and 2ml filtrate was pipetted into each of the pair of tubes. The tubes containing filtrate from the standard were marked standard and 'standard-blank' respectively. A reagent blank containing 2ml of 5% trichloroacetic acid was prepared. About 4ml of diphenyl amine reagent was pipetted into the tubes marked test and standard. All the tubes were gently mixed on a vortex mixture, covered with aluminium foil and returned to boiling water for 30 minutes. The test tubes were cooled in water and absorbance was determined at 530nm in a suitable calorimeter or spectrophotometer set at zero with reagent blank and then calculated.

d) Estimation of salivary amylase was done by using the CNP-G3 method<sup>18</sup>

About 1000µl of Amylase reagent and 25µl of sample is mixed and incubated at 37°C. The release of 2-chloro-4-nitrophenol (CNP) from the substrate and the resulting absorbance increase per minute is directly related to the α-Amylase activity in the sample. The resulting increase in absorbance after 15 sec were measured spectrophotometrically at 410/480nm. Two additional absorbance at 30 seconds interval were measured. The mean absorbance change per minute was calculated.

Estimation of flow rate-

Since the flow rate is calculated as ml/min, the saliva was collected separately to estimate the flow rate. Unstimulated saliva was collected for 1 minute in a 5 ml bottle. The amount of saliva collected in 1 minute was noted.

**Statistical analysis**

The results obtained were tabulated in Microsoft

Excel 2007 and analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The mean score and standard deviations were calculated for all the study variables in study and control groups. Chi square test was used to find the significance of categorical variables among both the groups. Unpaired t test was used to find compare the mean of study variables among the study and control groups. A 'p' value less than 0.05% was considered as significant.

**Results:**

The present study comprised of 100 children, out of which 50 belonged to passive smoking group and 50 belonged to control group. The age of the subjects ranged from 5 years to 13 years (Table 1). Among the study and the control group, 50% were males and 50% were females (Table 2). There was no significant difference between the study and the control groups with respect to the age and gender ( $p > 0.05$ , not significant).

Table 3 shows the mean salivary pH, salivary sialic acid levels, salivary amylase and salivary buffering capacity in the study and control groups. The results of the present study showed that buffering capacity and amylase activity was higher in passive smoking children ( $p < 0.05$ , significant) whereas salivary pH and flow rate was lower in passive smoking children when compared to their healthy controls ( $p < 0.05$ , significant). Salivary sialic acid levels did not show any much significant difference between the two groups ( $p > 0.05$ ).

**Table 1:** Distribution of the study subjects according to the age.

Group	Age			Total
	5-7 years	8-10 years	11-13 years	
Control	13	19	18	50
Study	13	19	18	50
Total	26	36	36	100

$p > 0.05$ , not significant

**Table 2:** Distribution of the study subjects according to the gender.

Group	Gender		Total
	Male	Female	
Control	25	25	50
Study	25	25	50
Total	50	50	100

$p > 0.05$ , not significant

**Table 3:** Mean salivary pH, salivary sialic acid levels, salivary amylase and salivary buffering capacity in the study and control groups.

	Group	N	Mean	Std. Deviation	Std. Error Mean	95% confidence interval		Sig. (2-tailed)
						Lower limit	Upper limit	
pH	Control	50	7.20	.380	.061	0.497	0.902	.000*
	Study	50	6.50	.580	.082			
Sialic Acid mg/100 ml	Control	50	13.2080	4.37506	.61873	-1.855	1.433	.800
	Study	50	13.4188	3.89911	.55142			
Amylase IU/L	Control	50	45.4728	18.81020	2.66016	-17.39	-4.89	.001*
	Study	50	56.6222	11.92187	1.68601			
Buffering Capacity	Control	50	4.290	.2493	.0353	-1.074	-0.866	.000*
	Study	50	5.260	.2718	.0384			
Flow rate ml/min	Control	50	1.176	.4424	.0626	.1303	.4337	.000*
	Study	50	.894	.3106	.0439			

\*p < 0.05, significant

### **Discussion:**

Human saliva is a unique secretion of major and minor salivary glands to maintain normal physiological functions of oro-biological structures. Saliva consists of over 99% water and provides the body's own natural protection against tooth decay and gum disease. Saliva helps to protect the teeth from caries by means of, both, cleansing and buffering action of saliva. It also controls the calcium and phosphate concentration in the saliva and around the teeth <sup>2</sup>.

Sialic acid is a very important structural component of glycoprotein's, playing a part in enhancing bacterial agglutination. Previous studies have reported that passive smoking is a risk factor for cancer in children, which has also been linked to elevated sialic acid level <sup>19</sup>. The salivary sialic acid levels of control subjects (non-passive smoking) and study subjects (passive smoking) did not show any significant difference in our study. The difference in results amongst the study may be due to age, sample size, cultural differences or length of child's exposure to passive smoking.

Salivary amylase may play an important part in the colonisation and metabolism of streptococci, leading to the formation of dental plaque and caries. It is a constituent of the acquired pellicle and could, therefore, be available to act as receptor for the adhesion of micro-organism to tooth surfaces <sup>20-22</sup>. According to Lindemeyer et al, nicotine promotes the growth of carcinogenic S. mutans bacteria; thus,

mother who smoke may be likely than non-smokers to transmit these bacteria to their children <sup>23</sup>. Previous studies reported that smokers and passive smoking children may have a higher number of S. mutans in saliva than non-smokers. Amylase is produced primarily by the parotid gland and its activity has been proposed as a marker for parotid saliva <sup>24</sup>. GranGer et al reported that tobacco smoke exposure was associated with lower salivary amylase activity for mothers, but not for infants <sup>25</sup>. In disagreement with this study, our results showed that the salivary amylase activity of passive smoking children was found to be statistically higher compared to that in control subjects (Table 3). A study conducted by Avsar et al showed similar results <sup>15</sup>.

Under resting conditions without the exogenous stimulation that is linked with feeding, there is a slow flow of saliva which keeps the mouth moist and lubricates the mucous membrane. The unstimulated saliva flow rate is 0.3-0.4 ml/min whereas stimulated whole saliva is 1-2ml/min. Saliva dilutes and removes substances (oral debris, noxious agents) from the oral cavity which is referred to as salivary or oral clearance. Both the act of swallowing and the salivary flow rate are important to this process, and these are the principal ways by which oral bacteria and injurious, noxious agents are eliminated from the mouth <sup>2</sup>. In our study, it was seen that flow rate was significantly low in the passive smoking children when compared to the control subjects (Table 3).

Saliva has a pH varying from 6.7 to 7.4. It was seen in our study that the salivary pH was significantly lower in the passive smoking subject group when compared to the control subjects (Table 3).

The buffering capacity of both unstimulated and stimulated saliva involves three major buffer systems: the bicarbonate ( $\text{HCO}_3^-$ ), the phosphate, and the protein buffer systems. These systems have different pH ranges of maximal buffer capacity, the bicarbonate and phosphate systems having pK values of 6.1-6.3 and 6.8-7.0, respectively. It was seen in our study that the salivary buffering capacity was significantly lower in the passive smoking subject group compared with the control subjects. (Table 1) as concluded by Avsar et al in their study<sup>14</sup>. According to Aligne et al<sup>26</sup> passive smoking may reduce the protective properties of saliva that operate against caries, with saliva acting as a buffering agent.

One limitation of this study is that we did not collect data on the body mass index of the children. It has been shown by previous studies that body size has a

positive correlation to passive smoking<sup>27</sup>. Also, the dental caries status of the children was not assessed in the present study. Conclusion

In conclusion, the study showed a lower salivary pH and lower flow rate and higher amylase activity and higher buffering capacity in passive smoking children when compared to control group. However, sialic acid levels did not show significant differences between passive smoking children and control group. Passive smoking may reduce the protective properties of saliva which can further affect the oral health status of young children and any factor that influences the secretion rate or composition of saliva will ultimately influence caries susceptibility.

#### **Acknowledgement:**

The authors acknowledge support of the Indian Council of Medical Research for providing financial assistance for carrying out this study as a part of Short Term Student project (STS-ICMR) in the year 2011.

**Conflicts of interest:** Nil

#### **References:**

- Williams SA, Kwan SY, Parsons S. Parental smoking practices and caries experience in pre-school children. *Res* 2000;**34**:117-22. <http://dx.doi.org/10.1159/000016578>
- John T, Mc Devitt. Saliva as the next best diagnostic tool. *J biochem.* 2006;**45**:23-5.
- Chang JS, Selvin S, Metayer C, Crouse V, Golembesky A, Buffler PA. Parental smoking and the risk of childhood leukaemia. *Am J Epidemiol* 2006;**163**:1091-100. <http://dx.doi.org/10.1093/aje/kwj143>
- Tyc VL, Throckmorton-Belzer L, Klosk JL, Greeson FL, Lensing S, Rai SN, et al. Smoking among parents of paediatric cancer patients and children's exposure to environmental tobacco smoke. *J Child Health Care* 2004;**8**:288-300. <http://dx.doi.org/10.1177/1367493504047319>
- Kosecik M, Erol o, Sevinc E, Selek S. Increased oxidative stress in children exposed to passive smoking. *Int J Cardiol* 2005;**100**:61-4. <http://dx.doi.org/10.1016/j.ijcard.2004.05.069>
- Goodwin RD. Environmental tobacco smoke and the epidemic of asthma in children: the role of cigarette use. *Ann Allergy Asthma Immunol* 2007;**98**:447-54. [http://dx.doi.org/10.1016/S1081-1206\(10\)60759-4](http://dx.doi.org/10.1016/S1081-1206(10)60759-4)
- Julvez J, Ribas-Fito N, Torrent M, Fornes M, Gracia-Esteban R, Sunyer J. Maternal smoking habits and cognitive development of children at age 4 years in a population based birth cohort. *Int J Epidemiol* 2007;**36**:825-32. <http://dx.doi.org/10.1093/ije/dym107>
- Florek E, Piekoszewski W, Wrzosek J. Relationship between the level and time of exposure to tobacco smoke and urine nicotine and cotinine concentration. *Pol J Pharmacol* 2003;**55**:97-102.
- Ayo-Yusuf OA, Reddy PS, van Wyk PJ, van den Borne BW. Household smoking as a risk indicator for caries in adolescent's permanent teeth. *J Adolesc Health* 2007;**41**:309-11. <http://dx.doi.org/10.1016/j.jadohealth.2007.04.012>

10. Tanaka K, Hanioka T, Miyake Y, Ojima M, Aoyama H. Association of smoking in household and dental caries in Japan. *J Public Health Dent* 2006;**66**:279-81. <http://dx.doi.org/10.1111/j.1752-7325.2006.tb04083.x>
11. Shenkin JD, Broffitt B, Levy SM, Warren JJ. The association between environmental tobacco smoke and primary tooth caries. *J Public Health Dent* 2004;**64**:184-6. <http://dx.doi.org/10.1111/j.1752-7325.2004.tb02750.x>
12. Herrera JL, Lyons IIMF, Johnson LF. Saliva: its role in health and disease. *J Clin Gastroenterol* 1988;**10**:569-78. <http://dx.doi.org/10.1097/00004836-198810000-00019>
13. Mandel ID. The functions of saliva. *J Dent Res* 1987;**66**:623-7. <http://dx.doi.org/10.1177/00220345870660S203>
14. Avsar A, Darka O, Topaloglu B, Bek Y. Association of passive smoking with caries and related salivary biomarkers in young children. *Arch Oral Biol*. 2008 Oct;**53**(10):969-74. <http://dx.doi.org/10.1016/j.archoralbio.2008.05.007>
15. Avsar A, Darka O, Bodrumha EH, Bek Y. Evaluation of relationship between passive smoking and salivary electrolytes, proteins, secretory IgA, sialic acid and amylase in young children. *Arch Oral Biol*. 2009;**54**:457-63. <http://dx.doi.org/10.1016/j.archoralbio.2009.01.017>
16. Ericsson Y. Clinical investigation of salivary buffering action. *Acta Odontol Scand* 1959;**17**:131 - 65. <http://dx.doi.org/10.3109/00016355908993928>
17. Winzler BJ. Determination of serum glycoprotein In: Glick D, ed. *Methods of biochemical analysis*. New York: Interscience Publishers Inc; 1955; p. 279-311. <http://dx.doi.org/10.1002/9780470110188.ch10>
18. Winn Dean ES, David H, Sigler G, Chavez R. Development of a direct assay for  $\alpha$ -amylase. *Clin Chem* 1988;**34**:2005-8.
19. Mendel MA, Dvorak K, DeCosse J. Salivary immunoglobulins in patients with oropharyngeal and bronchopulmonary carcinoma. *Cancer* 1973;**31**:1408-11. [http://dx.doi.org/10.1002/1097-0142\(197306\)31:6<1408::AID-CNCR2820310616>3.0.CO;2-S](http://dx.doi.org/10.1002/1097-0142(197306)31:6<1408::AID-CNCR2820310616>3.0.CO;2-S)
20. Orstavik D, Kraus PW. The acquired pellicle: immunofluorescent demonstration of specific proteins. *J Oral Pathol* 1973;**2**:68-76. <http://dx.doi.org/10.1111/j.1600-0714.1973.tb01675.x>
21. AI-Hashimi I, Levine MJ. Characterization of in vivo salivary derived enamel pellicle. *Arch Oral Biol* 1989;**34**:289-95. [http://dx.doi.org/10.1016/0003-9969\(89\)90070-8](http://dx.doi.org/10.1016/0003-9969(89)90070-8)
22. Scannapieco FA, Torres CI, Levine MJ. Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite. *J Dent Res*. 1995;**74**:1360-6. <http://dx.doi.org/10.1177/00220345950740070701>
23. Lindemeyer RG, Eaum RH, Hsu SC, Going RE. In vitro effect of tobacco on the growth of oral cariogenic streptococci. *J Am Dent Assoc* 1981;**103**:719-22. <http://dx.doi.org/10.14219/jada.archive.1981.0372>
24. Sas R, Dawes C. The intraoral distribution of unstimulated and chewing gum stimulated parotid saliva. *Arch Oral Biol* 1997;**42**:469-74. [http://dx.doi.org/10.1016/S0003-9969\(97\)00045-9](http://dx.doi.org/10.1016/S0003-9969(97)00045-9)
25. Granger DA, Blair C, Willoughby M, Kivlighan KT, Hibell LC, Fortunato CK, et al. Individual differences and alpha-amylase in salivary cortisol and alpha amylase in mothers and their infants relation to tobacco smoke exposure. *Dev psychobiol* 2007;**49**:692-701. <http://dx.doi.org/10.1002/dev.20247>
26. Aligne AC, Moss ME, Auinger P, Weitzman M. Association of pediatric dental caries with passive smoking. *JAMA* 2003;**289**:1258-64. <http://dx.doi.org/10.1001/jama.289.10.1258>
27. Kwok MK, Schooling CM, Lam TH, Leung GM. Paternal smoking and childhood overweight: evidence from the Hong Kong "Children of 1997". *Pediatrics* 2010;**126**:46-56. <http://dx.doi.org/10.1542/peds.2009-2642>