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Evaluation of the relationship between passive smoking and salivary electrolytes, protein, secretory IgA, sialic acid and amylase in young children

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ABSTRACT

Objective: To evaluate the relationship between passive smoking as determined by salivary cotinine levels and salivary electrolytes, protein, secretory IgA, sialic acid and amylase in children.

Design: Saliva was collected from 90 passive smoker (PS) subjects (the study group) and 90 healthy age-matched children (the control group). The study group was divided into three subgroups according the number of cigarettes smoked. Socio-economic status, dental and dietary habits were recorded by questionnaire. Stimulated salivary calcium (Ca), phosphate (P), sodium (Na), potassium (P), total protein, amylase activity, sialic acid level, secretory IgA concentration and cotinine level were analysed. All data were analysed using SPSS, version 13.0.

Results: Socio-economic status, dental and dietary habits were similar between the two groups. The salivary electrolytes concentrations did not reveal significant difference between the two groups ($p > 0.05$). The mean cotinine levels of PS children were 1.58 ± 4.3 ng/mL. The salivary concentrations of protein were similar between the two groups ($p > 0.05$). The salivary secretory IgA concentration was significantly lower in the PS group than controls. The sialic acid level and amylase activity in PS group were found significantly higher compared with the controls ($p < 0.05$). No difference was observed for all these parameters with sex ($p > 0.05$). When saliva samples were analysed for output, the sialic acid level and amylase activity increased significantly in PS subjects ($p < 0.05$). Further, the output of secretory IgA concentration was found significantly lower compared with the controls ($p < 0.05$).

Conclusion: In conclusion, we show that passive smoking was associated with a decrease in secretory IgA concentration, whereas with increase in amylase activity and sialic acid level of stimulated whole saliva in young children.

1. 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

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children live and play.[2,3] In a study carried out in elementary school children in Turkey, the prevalence of passive smoking was 81.3%.[4] Children are the most susceptible group for passive smoking because their bronchial tubes are smaller and their immune systems are less developed.[1,5] There have been many investigations regarding passive smoking exposure and health status during childhood.[6–10] On the other hand, very few studies have examined the relationship of passive smoking to oral health in children.[11–15] However, a majority of these investigations were evaluated only with questionnaire surveys.[11,13–15]

There are several methods available for measuring children's exposure to passive smoking, including well-validated laboratory techniques.[8,16–18] Cotinine is a breakdown product of nicotine with a half-life of 20 h; it is stable with temperature change, or current infection, and has high specificity and sensitivity.[19] Salivary cotinine concentration is the preferred assay for assessing passive smoking exposure as it has a simple collection procedure, a longer half-life than plasma nicotine, and is specific to tobacco.[2,7]

Although the association between passive smoking and caries has been accepted in the literature,[11–15,20] its relationship with the composition of saliva is unclear. Saliva plays a major part in the health of the mouth and any changes in its quantity or quality may alter the oral health status.[21,22] Therefore, the aim of this study was to investigate the association between passive smoking (as determined by salivary cotinine levels) with electrolytes, salivary protein, secretory IgA, sialic acid and amylase compared to healthy controls.

2. Materials and methods

This study was approved by the Ethical Committee of Ondokuz Mayıs University. After a complete and detailed explanation about the nature of the research, its objective, methods, anticipated benefits, and the inconvenience may the methodology cause, written informed consent for each child's participation was obtained from a parent.

2.1. Participants

Four hundred and fifty parents were asked to fill out a questionnaire about their smoking habits whilst waiting for their child's first 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 ($n = 152$). The children who lived in the smoking household were identified as passive smoker (PS) subject. If nobody smokes at home, then the home was categorized as non-smoking household ($n = 140$). An additional 136 current household smokers who did not smoke for the entire period since the birth of the child and 22 who used nicotine replacement therapy were excluded from the study.

Three categories were formed with respect to the children's daily exposure at home according to the total sum of the number of cigarettes smoked per day by each smoking household member: (1) <10 cigarettes ($n = 58$), (2) 10–20 cigarettes ($n = 41$), and (3) >20 cigarettes ($n = 53$).

A sample of 30 PS subjects from each exposure group (15 boys and 15 girls) (4–6 years of age; $n = 90$) were selected randomly and enrolled in the study as the study population. For each PS subject, children of the same gender and age were selected randomly from patients who lived in a non-smoking household and received their dental treatment at the same facility enrolled in the study as the control subjects ($n = 90$).

Socio-economic status, dental and dietary habits were recorded by questionnaire by the same interviewer. All of the participants were life-long inhabitants of Samsun, which has a water fluoridation level of 0.3 ppm. Children who attended kindergarten and did not have stay-at-home mothers were excluded from the study because of all the possible sources of exposure to PS e.g. outside the home. The exclusion criteria that were used for the sample also included the presence of systemic conditions that could influence the salivary gland physiology, such as Sjögren's syndrome, obesity, cachexia, diabetus mellitus, using additional fluoride prophylaxis, other than with the use of fluoride toothpaste, and antibiotics or antimicrobial agents in the previous 3 months.

2.2. Saliva collection protocol

Saliva samples were collected from all subjects between 09:00 and 12:00 h to minimize the effects of circadian rhythms. The subject was asked to refrain from eating or drinking for 2 h before the assessment. Whole saliva was stimulated by chewing paraffin for 30 s and was collected for 5 min into ice-chilled test tubes. Saliva samples (1 mL) were stored at $-80\ ^{\circ}C$ until used for analysis of cotinine. The remaining saliva was used to measure phosphate (P), calcium (Ca), sodium, (Na), potassium (P), total protein, secretory IgA, sialic acid and amylase.

2.3. Assessment of salivary cotinine level

To determine the salivary cotinine level, on the day of testing, samples were brought to room temperature, centrifuged at 3000 rpm for 15 min, and the clear top phase of the sample was pipetted into appropriate test tubes. The cotinine level was measured using a microplate enzyme immunoassay without modification of the manufacturer's protocol (Salimetrics, State College, PA, USA) and expressed as ng/mL. The test used 20 μL of sample, had a lower limit of sensitivity of 0.05 ng/mL, a range of sensitivity from 0.05 to 200 ng/mL, and average intra- and inter-assay coefficients of variation of <5.8 and 7.9%, respectively.

2.4. Assessment of salivary flow rate and electrolytes

Total phosphate was determined spectrophotometrically at 700 nm after the molybdic reaction.[23] Total calcium was determined by atomic absorption spectrophotometer at 422.7 nm in an air–acetylene flame. Saliva sodium and potassium concentrations were determined by atomic absorption spectrophotometry as described previously.[24]

2.5. Assessment of salivary flow rate, protein, enzymes and secretory IgA

To estimate the salivary flow rate, the amount of saliva collected was recorded and the results were converted to mL/