Published online: 2020-03-15

Original Article

Correlation between salivary cortisol levels and dental anxiety in children of smokers and nonsmokers

S. Pavani Reddy[1], M. Ghanashyam Prasad[2], A. Naga RadhaKrishna[2], Kaniti Saujanya[3], N. V. K. Raviteja[4], B. Deepthi[2]

[1]Department of Pedodontics and Preventive Dentistry, St. Joseph Dental College, Eluru, Andhra Pradesh, India,
[2]Department of Pedodontics and Preventive Dentistry, St. Joseph Dental College and Hospital, Eluru, Andhra Pradesh, India,
[3]Department of Pedodontics and Preventive Dentistry, Sree Sai Dental College and Research Institute, Srikakulam, Andhra Pradesh, India,
[4]Senior Lecturer, Department of Pedodontics and Preventive Dentistry, St. Joseph Dental College, Eluru, Andhra Pradesh, India

Correspondence: Dr. M. Ghanashyam Prasad
Email: drghanasyam@gmail.com

ABSTRACT

Objectives: The present study was undertaken to evaluate salivary cortisol levels in children of smokers and nonsmokers and thereby establishing the relationship between cortisol levels in response to anxiety in children based on their father's habit of smoking. **Materials and Methods:** The study population aged between 8 and 10 years includes two groups. Group 1 is comprised 20 children of cigarette smokers and Group 2 is comprised 20 children of nonsmokers. The passive drooling technique was used to collect unstimulated saliva from the children using a sterile container. Salivary cortisol levels were evaluated using Enzyme-linked Immunosorbent Assay method. The obtained data were subjected to statistical analysis using SPSS software and paired *t*-test. **Results:** Higher mean salivary cortisol levels were found in children of smokers compared to children of nonsmokers and the difference between them was significant statistically ($P < 0.05$). Higher salivary cortisol levels were found in females compared to males and the result was significant statistically ($P < 0.05$). **Conclusion:** This study has proved that the smoking habit of the father has a negative influence on the anxiety levels of their children.

Key words: Dental anxiety, nonsmokers, salivary cortisol, smokers

INTRODUCTION

Dental treatment involves various procedures that may be invasive or noninvasive. Pain and discomfort are the most common problems faced by patients during dental treatment. Even the fear of pain increases dental anxiety. Dental anxiety is a common problem with which dentists have to cope.[1] Dental anxiety affects people of all ages. It appears to develop mostly in childhood and adolescence.[2] Younger children exhibit more negative perceptions due to the absence

Access this article online

Quick Response Code:

Website: www.eurjdent.com

How to cite this article: Reddy SP, Prasad MG, RadhaKrishna AN, Saujanya K, Raviteja NV, Deepthi B. Correlation between salivary cortisol levels and dental anxiety in children of smokers and nonsmokers. Eur J Dent 2017;11:192-5.

DOI: 10.4103/ejd.ejd_171_16

of knowledge of the benefits of dental treatment and new environment.[3]

Smoking is common modern day epidemic and is most commonly seen in people with psychological problems. Smoking has also been linked with the incidence of mental health problems such as anxiety and alcohol abuse.[4] Smokers report that smoking helps to relieve stress. However, the stress levels of adult smokers are slightly higher than those of nonsmokers, adolescent smokers report increasing levels of stress as they develop regular patterns of smoking, and smoking cessation leads to reduced stress. Nicotine dependency seems to exacerbate stress than from acting as an aid for mood control.[5]

Smoking has severe effects on various systems of the body. It also affects the various biological and hormonal mechanisms such as pituitary, thyroid, adrenal, testicular and ovarian function, calcium metabolism and the action of insulin. An important concern is also the effect of smoking on the fetus and young children.[6] Both active and passive smoking has deleterious effects on body mechanisms. Children are more vulnerable to the harmful effects of passive smoking as they spend more time with their parents, caregivers and other adults, and are prone to high levels of exposure.[7]

Several measures have been proposed to measure anxiety in adults, among which salivary cortisol is one. Cortisol is a stress hormone, which activates the response of the body to stress conditions, produced by the pituitary gland and enters into complex interactions with the hormonal system.[8] Salivary cortisol can also be used as a psychological stress biomarker.[9] Salivary samples have the advantage of being noninvasive, stable at room temperature for up to a week, and they can be easily collected at home under stress-free conditions. Salivary cortisol represents only the bioactive fraction: Cortisol not bound to cortisol-binding-globulin or other proteins, and its concentration does not depend on salivary flow rate.[10]

In this study, we have taken salivary cortisol as a means to measure anxiety levels in children of smokers and nonsmokers.

MATERIALS AND METHODS

The present study was undertaken on children attending the outpatient Department of Pedodontics and Preventive Dentistry, St. Joseph Dental College to assess their salivary cortisol levels. Consent was obtained from the parents of the children for participation in the study. History was taken from 90 children regarding the smoking habit of their father.

Inclusion criteria

- Children with a known history of their father's smoking habit.

Exclusion criteria

- Children with psychological disorders
- Children with compromised medical health.

Forty healthy subjects of the age group 8–10 years were selected to be included in the study and were divided into two groups with equal distribution of males and females per group based on father's smoking habit.

- Group 1: Comprised 20 children of cigarette smokers with a smoking history of at least 6 years
- Group 2: Comprised 20 children of nonsmokers.

Ethical clearance was obtained from the Institutional Ethical Committee, St. Joseph Dental College.

Methodology

Later on, the children were subjected to saliva collection for cortisol estimation. The children were asked not to either eat or drink for at least 30 min before saliva collection. Saliva was collected at the same time from all the children before noon (11:00–11:30 am). Sterile containers were used to collect saliva. Unstimulated whole saliva of approximately 2 ml was collected from each subject by passive drooling method [Figure 1]. The samples were stored at −20°C and maintained at that temperature and were transferred to a nearby laboratory within 18 h of saliva collection. Estimation

Figure 1: Saliva collection by passive drooling method

of salivary cortisol levels was done by Enzyme-linked Immunosorbent Assay (ELISA) method using Beckman Coulter ELISA analyzer. The obtained data were subjected to statistical analysis using SPSS for Windows, Version 16.0. (Chicago, SPSS Inc.) and paired *t*-test.

RESULTS

After statistical analysis by calculation of mean, standard deviation and *t*-test, the obtained results were assessed. The mean age (years) in each group was calculated and the value was found to be 9.

Salivary cortisol estimation was performed in all the 40 patients and the mean salivary cortisol level was calculated. Group 1 showed a mean value of salivary cortisol levels of 0.56 with a standard deviation of 0.12, whereas the mean value of the Group 2 is 0.43 with a standard deviation of 0.07. The mean difference between Group 1 and Group 2 was 0.13 and the t value was 1.68. *P* = E-1.78. The results showed that higher mean salivary cortisol levels were recorded in children of smokers compared to children of nonsmokers. The difference between the two groups was found to be statistically significant ($P < 0.001$) [Table 1].

The mean value of salivary cortisol for females was 0.54 and males was 0.46, respectively, with a mean difference of 0.8 and standard deviation of 0.10 and 0.12, respectively. The *t* value was 1.68. $P = 0.0015$. By the obtained results, it was found out that higher mean salivary cortisol levels were observed in females than males. The difference between the two groups was found to be statistically significant ($P < 0.05$) [Table 2].

DISCUSSION

Dental anxiety is common in all the individuals undergoing treatments, but the level of anxiety varies. The hormonal analysis is one of the measures for finding out the anxiety levels and salivary cortisol was used to assess anxiety levels in this study. Cortisol levels can be assessed in serum, urine, and saliva. However, serum cortisol involves an invasive procedure to which children do not cooperate. Salivary cortisol is used for assessing the anxiety levels of the children in our study. Aardal and Holm compared the concentrations of free cortisol in saliva to those of total cortisol in serum with a radioimmunoassay and concluded that the simple, noninvasive sampling procedure suggests that saliva may be used for cortisol measurements in situations where blood sampling is difficult to perform.[11]

Saliva was collected at the same time in all children in this study as to overcome the variations of salivary cortisol levels as stated by Kirschbaum and Hellhammer. They suggested that salivary cortisol is stimulated by waking up in the morning and the cortisol levels in saliva are increased by 50%–100%, peaking about 30 min after awakening. After the morning peak, salivary cortisol levels decrease until meals and after meals, it is 1.5 times greater than the normal value. During the afternoon and evening hours, it continues to decrease with no major episode.[12]

Saliva was collected before noon to avoid diurnal variations as there are very complicated daily fluctuations of cortisol in the saliva of healthy persons of both sexes and the middle value of cortisol concentration before noon is statistically significantly higher in comparison to the middle value of cortisol concentration in the afternoon as stated by Ljubijankic *et al*.[13]

Salivary cortisol is used for the assessment of anxiety in children in the present study as suggested by Martí-Álamo *et al*. that salivary analysis can be used as a measure to stress and anxiety.[14] The saliva samples were stored at −20°C soon after collection till the samples have reached the laboratory. Kirschbaum and Hellhammer have confirmed that salivary cortisol is stable at room temperature for 2–4 weeks. However, the sampling devices will start to mold within 4–7 days at room temperature. As a result, this produces a very bad smelling sample but does not appear to affect the cortisol levels. So whenever possible, saliva samples should be stored at −20°C or lower.[12]

Salivary cortisol levels were found higher in the children of smokers than the children of nonsmokers indicating higher anxiety levels in children of smokers than children of nonsmokers. Talukdar *et al*. conducted

Table 1: Comparison of salivary cortisol levels (µg/ml) between Group 1 and Group 2

Groups	Cortisol (µg/ml)					
	Mean	SD	SEM	Mean difference	*t*	*P*
Group 1 children of smokers	0.56	0.12	0.02	0.13	1.68	<0.001*
Group 2 children of nonsmokers	0.43	0.07	0.017			

SD: Standard deviation, SEM: Standard error of mean, $P < 0.001$* - Highly significant, $P < 0.05$* - Significant

Table 2: Comparison of salivary cortisol levels (µg/ml) among males and females

Gender	Cortisol (µg/ml)					
	Mean	SD	SEM	Mean difference	*t*	*P*
Males	0.46	0.12	0.02	0.8	1.68	<0.05*
Females	0.54	0.10	0.02			

SD: Standard deviation, SEM: Standard error of mean, *P*< 0.001* - Highly significant, *P* < 0.05* - Significant

a study on the evaluation of salivary cortisol level in cigarette smokers and concluded that salivary cortisol levels were significantly higher in smokers when than nonsmokers. Higher mean anxiety and stress score was recorded in smokers compared to nonsmokers and the difference between them was found to be statistically significant.[15]

Fathers play a key role in the transmission of dentist fear from mothers to their children. Increase or reduction of anxiety and transmission of fear from the mother to the child were influenced by the reactions that the father displays in the dentist as confirmed by Lara *et al*.[16] In the present study, higher anxiety levels were found in the children of smokers where smokers have high levels of anxiety and it influences their children's anxiety.

Higher mean salivary cortisol levels were found in females in the present study. Kanegane *et al*. found that adult females reported higher dental anxiety than males.[17] Carrillo-Diaz *et al*. stated that gender influences dental anxiety with higher levels in female children compared with male children.[18]

CONCLUSION

By the above study, it can be concluded that the children of smokers show more anxiety than children of nonsmokers and the anxiety levels were more observed in the female population than males. Further research can be directed on comparing the salivary cortisol levels and anxiety levels of children of smokers and nonsmokers by longitudinal studies in a large population. Moreover, if the early detection of factors leading to anxiety were known during recording case history, it can help in the early management of anxiety.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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