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EVALUATION OF SALIVARY COTININE LEVEL IN SECOND-HAND SMOKERS - A CASE CONTROL STUDY

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ABSTRACT Understanding SHS exposure is important in measuring and preventing exposure. Estimation of the Cotinine values help in biochemical validation and cessation outcomes. The biochemically estimated cotinine levels is found to be an indicator, second hand smoke exposure or use of therapeutic nicotine.

AIMS: To evaluate salivary cotinine level in second-hand smokers

MATERIALS AND METHODS : 78 study subjects divided into 2 groups (Group 1 and Group 2) of 39 each. The saliva samples were collected from subjects who had no previous history of tobacco smoking and subjects who are second-hand smokers. Their cotinine contents were measured using the competitive ELISA method according to the standard curve.

STATISTICAL ANALYSIS: Data analysed by using descriptive analysis. Independent t-test was used to compare the cotinine concentration between the groups.

RESULTS: The mean salivary cotinine level in groups (Group 1 and Group 2) was found to be 20.37 ng/ml and 6.78 ng/ml respectively..

CONCLUSIONS: salivary cotinine level was significantly high in second hand smokers compared to nonsmokers.

KEYWORDS : Second hand smoke, Cotinine, Biomarker, Tobacco, Elisa

INTRODUCTION

Second-hand smoke(SHS) is a mixture of the side stream smoke emitted into the environment from the smouldering of cigarettes and other tobacco products and from the mainstream smoke exhaled by the smoker.[1] This exposure to SH smoke frequently named secondhand smoking. Second hand smoking is a known risk factor for asthma, bronchitis, and coronary artery disease ,Sudden infant death syndrome(SIDS), increase in respiratory illness in children's and lung cancer in adults . Understanding SHS exposure is important in measuring and preventing exposure. Exposure to SHS can take place in the home, workplace or other environments that are accessible to the public. Second hand smokes contain more than 4000 chemicals which are toxic and carcinogenic[1].

Cotinine, a metabolite of nicotine is the most common biomarker of second hand exposure. Saliva cotinine concentration of non-smokers was influenced by the smoking status of close friends or spouse and in children was strongly related to the smoking habit of their parents. In vivo it has a half-life of about 20 hours. It can be noted in urine, saliva or serum.. Estimation of the Cotinine values help in biochemical validation and cessation outcomes[3].The biochemically estimated cotinine levels is found to be an indicator, second hand smoke exposure or use of therapeutic nicotine[4] Therefore, this study was designed to estimate the levels of salivary cotinine in second hand smokers and non-smokers.

MATERIALS AND METHODS:

This case control study was conducted on subjects reporting to the Department of Oral Medicine and Radiology. After obtaining the institutional ethical clearance, the nature and purpose of the study was explained and informed written consent was acquired from the subjects who were to be included in the study. On the basis of convenience sampling method, a sample size of 78 were found to be fit to be included in the study as per strict inclusion and exclusion criteria.

The subjects were divided into Group 1 and Group 2, each group had 39 patients.

Group 1 consisted of 39 patients who are second hand smokers (case group)

Group 2 consisted of 39 patients who do not have habit of tobacco smoking (control/study group).

INCLUSION AND EXCLUSION CRITERIA:

Strict inclusion and exclusion criteria were followed. Both the groups in the study included subjects between the ages of 18-70 years. The group-1 composed of subjects who were second hand smokers and group-2 composed of subjects who do not have a history of smoking. Individuals with history of any other substance abuse other than smoking and pan chewing with tobacco products, recent infection, subjects with systemic illness and subjects on any medication, nicotine replacement therapy were excluded from the study.

Saliva collection: From above patients, unstimulated saliva was collected through "Spit Technique". The subject was asked to rinse the mouth with water in order to remove any debris in the mouth and instructed to spit into a sterile graduated container. The collected sample was then transferred to laboratory for further process. With the help of micro-centrifuge tubes, samples were centrifuged at 3000 rpm for 10 minutes and the supernatant collected was stored in -20C. For processing, the samples were taken out from the deep freezer and brought to room temperature. Cotinine Direct Elisa kit was used to analyse the salivary samples and levels were measured and were given in ng/ml. The values collected after analysis, were entered into Microsoft excel spreadsheet. Descriptive data was presented in the

form of mean and standard deviation. The cotinine levels were compared between the study and control group using independent t test. P value was found to be < 0.05, and was considered as statistically significant

RESULTS

78 salivary samples were included in the study i.e. 39 from each group. Cotinine level estimation was done through the Elisa cotinine kit.

Table 1: Demographic Data.

	Group	N	Mean	Std. Deviation
Age	**Case**	39	25.4103	2.78830
	Control	39	29.4872	7.20333

Table 2: Gender Distribution.

	Female	Male
Case	8	31
	20.5%	79.5%
control	36	3
	92.3%	7.7%

Table 3: Comparison Of Cotinine Concentration Between Case And Control

	G	N	Mean	Std. Deviation	p-value
Cotinine concentration ng/ml	**Case**	39	20.3669	8.43274	<0.001
	Control	39	6.7779	3.16911	

Graph 1:

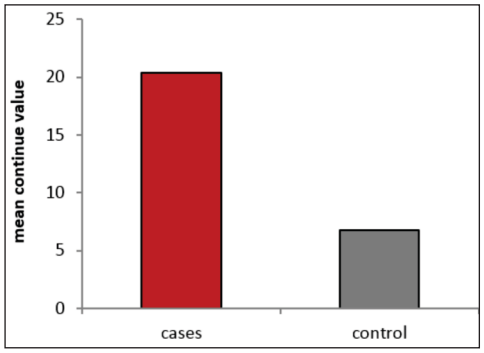

Table 1 shows the demographic data analysis of control group and study group. The mean age of the control group was 29.49 years with a standard deviation of 7.20333 and for case group was 25.4103 years with a standard deviation of 10.9. The mean duration of smoking in case group was 4.7 years with a standard deviation of 2.79.

Table 2 shows the gender distribution in the study. In the control group, 36 females (92.3%) and 3 male (7.7%) subjects were included. In the study group, 8 female (20.5%) and 31 male (79.5%)were included.

Table 3 shows the comparison in cotinine concentration in both groups. In case group, the mean concentration was 20.37ng/ml with a standard deviation of 8.43274 and in control group, the mean concentration 6.78ng/ml with a standard deviation of 3.17Independent t test was used to compare cotinine concentrations. Significant difference in mean cotinine concentration between the groups with p<0.001. Graph 1 show there is significant difference between cotinine concentrations between the groups. Cotinine concentration in study group was found to be more as compared to control group.

DISCUSSION

An estimation of second-hand exposure in people is an important concern, especially in monitoring cessation programs. So, assessment of second-hand exposure can be done by evaluating its biomarkers from the body fluids. The most common biomarker of second-hand exposure is cotinine, a major metabolite of nicotine.

Cotinine, a major metabolite of nicotine, is the most commonly used marker to distinguish between tobacco users and no nusers because of its greater sensitivity and specificity than other biochemical tests. It is stable in body fluids, has low plasma protein binding, long half-life of 15-20 hours, it is directly proportional to the quantity of nicotine absorbed and dose-independent disposition kinetics. Thus, cotinine is a useful marker as it helps in the estimation of exposure to active as well as passive smoke. Cotinine levels <10 ng/mL in saliva are considered to be consistent with no active smoking while values between 1 ng/mL and 30 ng/mL in saliva may be associated with light smoking or passive exposure, and levels in active smokers typically reach 100 ng/mL or more. Saliva collection is considered as best method is non invasive, easy and well tolerated procedure when multiple samples are required over a limited period[8].

In this study, unstimulated saliva from the subjects was collected. Also, the quantitative and semi-quantitative evaluation methods have revealed that the cotinine levels from un-stimulated saliva are the most specific and sensitive biomarker of tobacco exposure. The type of specimen and method of the collection also impacts the levels of cotinine during detection[6].

The subjects were asked to rinse the mouth with water and were instructed spit into a sterile graduated container. The samples were transferred immediately to the laboratory and were centrifuged at 3000 rpm for 10 minutes and the supernatant collected was stored in a deep freezer at a temperature of -20C. Before processing, the samples were thawed at room temperature and cotinine analysis was done using a cotinine ELISA kit, absorbance was read on an ELISA reader.

In the present study, salivary cotinine levels were estimated in second hand smokers and non-smokers to assess the cotinine values in both groups. In the control group, the subjects were between 18 to 50 years of age and the mean age was 30.5 years. In the study group, the subjects were between 20 to 60 years of age and the mean age was 34.9 years.

The mean cotinine concentration in second hand smokers was found to be 20.37 ng /ml, and in non-smokers, it was 6.78ng/ml. An independent t-test was used to compare cotinine concentration between the groups. Hence the significant difference in mean cotinine concentration between the groups with p<0.001 was noted. The result of the study is accordance to the studies done by Jarvis et al [14],Etzel RA et al[7] and Sharma et al [10]in which results showed passive smokers had significantly higher cotinine levels than nonsmokers. However these studies also estimated the cotinine level in urine and serum which showed the significantly higher cotinine levels in passive smoker than non-smokers.

In the control group, the lowest level of cotinine concentration estimated was 1.3ng/ml and the highest was 12.04ng/ml. According to The Society for Research on Nicotine and Tobacco Subcommittee (SRNT) on biochemical verification, the salivary cotinine level in a non-tobacco user is <15ng/ml, so in the present study, all the subjects in the control group were having cotinine concentration within the normal limit. The variation in cotinine concentration can be due to differences in food-related habits and exposure to environmental tobacco smoke.

In the Study group, the lowest level of cotinine concentration was 11 ng/ml and the highest was 36.3 ng/ml. This variation in cotinine level can be attributed to the time gap between the exposure to second hand smoke and the time of saliva collection as cotinine's half-life is 19 h, providing a short window of detection to evaluate the use that occurs over long periods.

The present study's limitations included its reliance on information provided by subjects concerning the independent variables. Whenever possible, further studies should try to validate subject information with objective measures, such as the determination of nicotine yield using smoking machines.

CONCLUSION

There is significantly higher level of salivary cotinine in secondhand smokers than in non smokers. Periodic assessment of salivary cotinine can play a major role in helping the subjects by making them aware, monitering of their Second hand smoke exposure and the cotinine content. This may be considered as a baseline value to help in further preventive measures for Second hand exposure and tobacco cessation.

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Conflicts of interest
There are no conflicts of interest.

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