ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 6, December - 2020, Page No. : 187 - 193

Evaluation of Salivary Cotinine Levels in Tobacco Smokers - A Case Control Study

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Citation of this Article: Dr. Laxmikanth Chatra, Dr. Saba Sayeed, Dr. Prashanth Shenoy, Dr. Veena K.M, Dr. Rachana V Prabhu, "Vestibular Screen - A Preorthodontic Treatment for Mouth Breathing Habit", IJDSIR- December - 2020, Vol. – 3, Issue - 6, P. No. 187 – 193.

Type of Publication: Case Report

Conflicts of Interest: Nil

Abstract

Aim: To evaluate salivary cotinine level in tobacco smokers"– A case control study.

Methodology: The study was performed in 94 study subjects divided into 2 groups (Group 1 and Group 2) of 47 each. The saliva samples were collected from subjects who had no previous history of tobacco smoking and subjects having habit of smoking and their cotinine contents were measured using the competitive ELISA method according to the standard curve. The results were then compared and co-related between the groups.

Results: The mean salivary cotinine levels in groups (Group 1 and Group 2)was found to be 80.5ng/ml and 6.6ng/ml respectively. When the mean values were compared between the groups the values were found to be statistically highly significant, and study also shows no co-relation between cotinine levels and duration of smoking.

Conclusion: The result of study showed the increased levels of cotinine in smokers as compared to non-smokers, with no association noted between cotinine levels and duration of smoking. This result shows that cotinine from body fluids are the marker of choice for the assessment of

absorption of tobacco related products and can be used tobacco used in any prevention and tobacco cessation programs. The present study also indicate that this estimation can be applied for routine determination of cotinine in saliva samples, not only for distinguishing between smokers and non-smokers but also assessing the exposure to environmental tobacco smoke and degree of active smoking.

Keywords: Smoker, Cotinine, Biomarker, Tobacco, Elisa.

Introduction

Tobacco use in any form, smoke or smokeless products is one of the main reason of death and can be consumed in various forms including smoking cigarettes, with hookahs, cigars, pipes and bidis[1].Tobacco smoke comprises of over 4800 different chemicals among which 69 are carcinogens, and rest are tumour promoters or co-carcinogens[2].Various oral lesions like Smoker's, Erthroplakia, leukoplakia, stomatitis nicotinia, periodontal problems, halitosis, excessive stains and calculus are related to smoking[4]. Its usage has been known as an element of danger for cardiovascular diseases, lung and other cancers, chronic respiratory diseases, stroke and complications ofpregnancy[3]. Nicotine is the main alkaloid of tobacco that causes addiction and is easily absorbed from tobacco smoke. When smoked in cigarettes, it is absorbed across buccal and nasal membranes. The drug has a fast onset of action with a half-life of 2 -3 hour and can be detected in blood, saliva and urine. More than half that is 70-80% of nicotine gets metabolised to Cotinine which is the main metabolite of nicotine[2]. As cotinine is tobacco-specific the presence of cotinine in serum is considered as the best marker of smoking.

Cotinine can be used as a marker of exposure to tobacco. In vivo it has a half-life of about 20 hours. It can be noted in urine, saliva or serum. Cotinine levels are the indicators of active smoking. Estimation of the Cotinine values help in biochemical validation and cessation outcomes[4].The biochemically estimated cotinine levels is found to be an indicator of active smoking, use of smokeless tobacco, second hand smoke exposure or use of therapeutic nicotine[5].

Therefore, this study was designed to estimate the levels of salivary cotinine in tobacco smokers and non-smokers, and to compare and associate it with duration of smoking.

Materials And Method

A 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 94 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 47 patients.

Group 1 consisted of 47 patients who do not have habit of tobacco smoking- control/study group.

Group 2 consisted of 47 patients who had habit of active smoking-experimental 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 compromised of subjects who were active smokers and group-2 compromised 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 were omitted 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, if present in the mouth. Then subjects were instructed to sit on the dental chair with the head tilted forward and asked not to speak or swallow any saliva. The subject was 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. The correlation between the cotinine levels and duration of smoking was determined by Pearson's correlation. P value was found to be < 0.05, and was considered as statistically significant.

Results

94 salivary samples were included in the study i.e 47 from each group. Cotinine level estimation was done through the Elisa cotinine kit.

Table 1: Demographic Data

	Group	N	Mean	Std. Deviation
Age	Control group	47	30.5106	7.35401
	Study group	47	34.9149	10.97790
Duration of Smoking	Case	47	4.7021	2.74982

Table 2: Gender Distribution

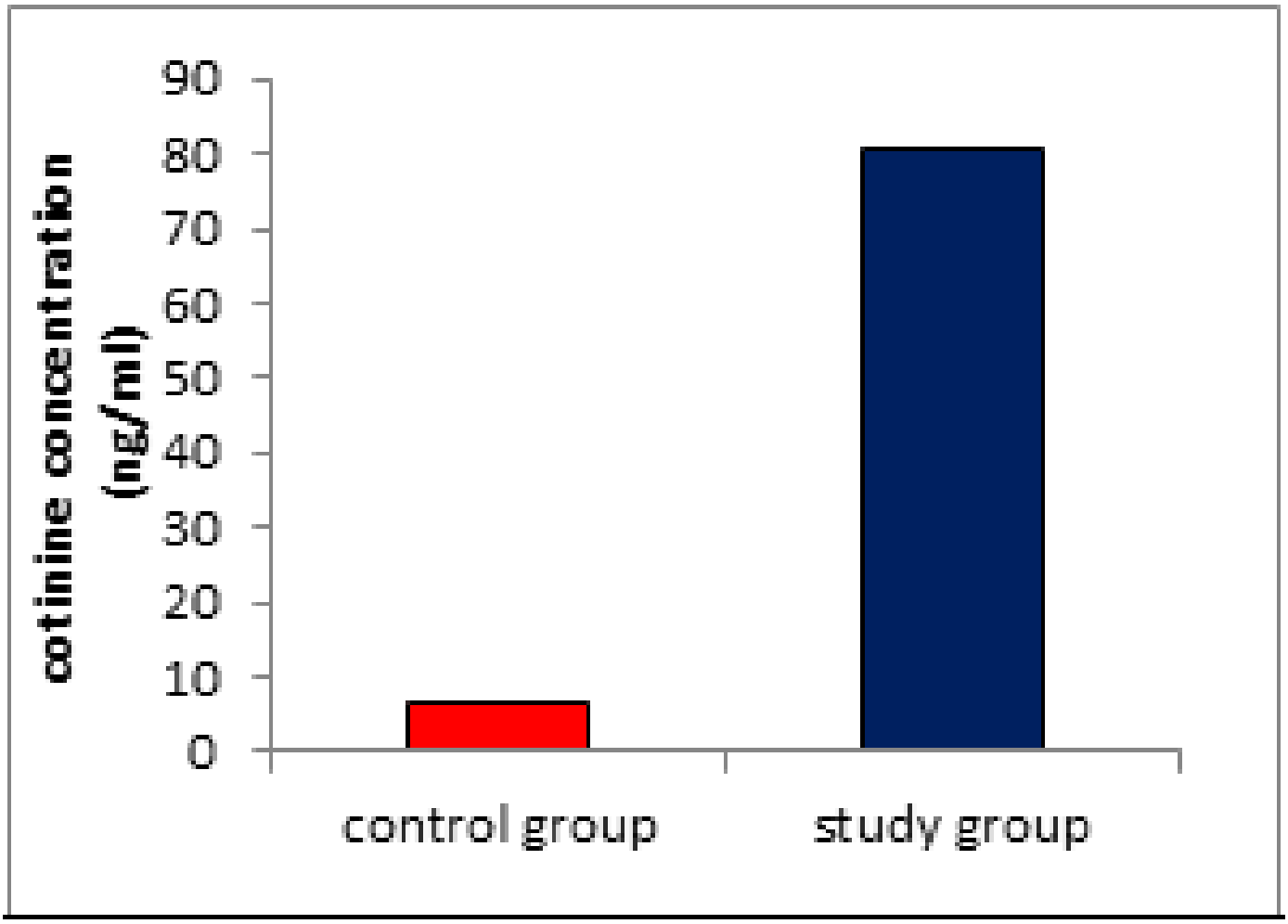
Group	gender	
	Female	Male
Control group	44	3
	93.6%	6.4%
Study Group	0	47
	0.0%	100.0%

Table 3: Correlation between the duration of smoking and cotinine concentration

		Cotinine value
Duration of smoking	Pearson Correlation	-0.287
	p-value	0.050
	N	47

Table 4: Comparison of continine concentration between case and control:

	G	N	Mean	Std. Deviation	p-value
Cotinine value	Control group	47	6.6515	3.12472	<0.001
	Study group	47	80.5617	9.68130	



Observations

Table 1 shows the demographic data analysis of control group and study group. The mean age of the control group was 30.5 years with a standard deviation of 7.9 and for case group was 34.9 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 2.7.

Table 2 shows the gender distribution in the study. In the control group, 44 females (93.6%) and 3 male (6.4%) subjects were included. In the study group, all the subjects were males(100%).

Table 3 shows the co-relation between duration of smoking and cotinine concentration. Here, Pearson co-relation was used to determine the co-relation and was found to be -0.287. The P value was 0.05, hence no significant co-relation between duration of smoking and continue concentration was observed.

Table 4 shows the comparison in cotinine concentration in both groups. In case group, the mean concentration was

6.6ng/ml with a standard deviation of 3.12 and in control group, the mean concentration 80.5ng/ml with a standard deviation of 9.6.Independent 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

Nicotine is the principal tobacco alkaloid, which constitute about 95% of the total alkaloid content and weight about 1.5% in commercial cigarette tobacco. About 1–1.5 mg of nicotine is absorbed systemically during smoking. As nicotine enters the bloodstream after absorption, 69% gets ionized while 31% remains unionized. The ability of nicotine to bind with plasma proteins is less than 5%. It gets distributed extensively to body tissues with a steady-state volume of distribution. The highest affinity for nicotine has been seen in the liver, kidney, spleen, and lung and lowest in adipose tissue[6].

An estimation of tobacco consumption in people is an important concern especially in monitoring cessation programmes. Assessment of tobacco exposure is extremely beneficial in youth population and can be done by evaluating its biomarkers from body fluids[4]. A number of biochemical markers have been used to identify the use of tobacco, including measures based on thiocyanate, carbon monoxide, nicotine, cotinine[7]etc.

Cotinine is the major metabolite resulting from nicotine which is a by-product of tobacco and results from the metabolism of nicotine by the cytochrome 2A6 enzyme system in the liver[8]. Cotinine and its metabolites represent about 80% of the metabolic products resulting from the nicotine absorbed by a smoker[4]. It is most frequently used marker to differentiate between tobacco users and non-users due to its greater sensitivity and specificity than other biochemical tests. It is stable in body fluids, have low plasma protein binding, and a long half-life 15-20 hour, It is directly proportional to the quantity of nicotine absorbed and dose independent disposition kinetics[9]. Thus, Cotinine can be used as useful marker in estimation of exposure to active as well as passive smoke[10]. Saliva collection is considered as best method over blood and urinary measures as it is easy to obtain and non-invasive. Therefore, collecting saliva is non invasive, easy and well tolerated procedure when multiple samples are required over a limited period[8].

Cotinine concentrations are viably used in epidemiological studies as a biomarker. Cotinine levels have been used to mark out and compare patterns of tobacco usage in smokers in different countries, to rule out if addiction and smoking patterns differ across the populations[2.]

In this study, unstimulated saliva from the subjects was collected as the cotinine levels are found to be significantly higher in unstimulated than in stimulated saliva[6]. Also, various evaluation methods have shown that the cotinine levels from un-stimulated saliva is the most specific and sensitive biomarker of tobacco exposure. The type of specimen and method of collection also impacts the levels of cotinine during detection[12].

In the present study, salivary Cotinine levels were estimated in tobacco smokers and non-smokers in order 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.

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 while for smokers, it Is <15ng/ml and

above. Based on these recommendations given by the SRNT subcommittee similar values were taken into consideration in the present study.

The mean salivary cotinine levels in control group and study group was found to be 6.6 ng/ml and 80.5 ng/ml respectively. Independent t test was used to compare cotinine concentration between the groups. And the mean values were found to be statistically significant which was in accordance with the study done by Etzel RA et al and Lorina castelino et al.

In the control group the lowest level of cotinine concentration estimated was 1.3ng/ml and 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 difference in food related habits and exposure to environmental tobacco smoke.

In the Study group, the lowest level of cotinine concentration was 60.5 ng/ml and highest was 99.8 ng/ml. This variation in cotinine level can be attributed to the time gap between the consumption of tobacco and time of saliva collection as cotinine's half-life is 19 h, providing a short window of detection to evaluate use that occurs over longer periods of time. Factors potentially influencing cotinine levels relate to the product smoked (filter or non-filter and nicotine yield), the way the product is smoked (depth of inhalation and butt length), and smoker characteristics (age, gender, and phenotype of nicotine metabolism), different type of brand and type of measurement method[13]. However, in present study salivary cotinine concentration and its association with duration of smoking was considered.

The mean duration of smoking in case group was 4.7 years. The Pearson's correlation was used to estimate the correlation between duration of smoking and salivary cotinine concentration. No correlation was found between the duration of the smoking and the salivary cotinine levels, which was in accordance with the study of Figueiredo et al. who had found no significant association between cotinine concentration and duration. Although a study done by Etter et al, showed a positive association between cotinine concentration with duration, which, however, disappeared after multiple adjustments. Patel et al conducted a study which showed a positive co-relation between level of cotinine level and duration of smoking. Therefore, duration of the habit was not found to be significant predictors of cotinine levels.

The present study's limitations included its reliance on information provided by subjects with regard to the tobacco smoking. Further studies should try to validate subject information with objective measures, such as determination of nicotine yield using smoking machines.

Conclusion

This study was designed to estimate the levels of salivary cotinine in tobacco smokers and non-smokers and also to associate possible variation in Cotinine level to duration of smoking. The result of our study showed the increased levels of cotinine in smokers as compared to non-smokers. No association was noted between cotinine levels and duration of smoking. This result shows that cotinine from body fluids are the marker of choice for the assessment of absorption of tobacco related products. Salivary cotinine level proved to b a useful biomarker of recent smoking and can be in epidemiological studies and smoking cessation programs. Since the use of tobacco is increasing among youth, especially among educated people and, furthermore, as many studies have shown a relationship between tobacco consumption and mouth cancer, so it

becomes a great responsibility of healthcare organizations to raise awareness of people, especially of young people. The present study also indicate that this estimation can be applied for routine determination of cotinine in saliva samples, not only for distinguishing between smokers and non-smokers but also assessing the exposure to environmental tobacco smoke and degree of active smoking.

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