

Evaluation of Salivary Cotinine Levels among Adolescents in a Semi Urban Indian Town: A Cross Sectional Study

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Abstract

Introduction: Cotinine, a major nicotine metabolite is a specific biomarker for nicotine exposure. Salivary cotinine levels is an easy and effective alternative for screening large groups especially children and adolescents. The present study was aimed at evaluating prevalence of tobacco consumption habits, determine the age of initiation and correlate the findings with their salivary cotinine levels.

Material and methods: The study was conducted among 95 students from schools in Karad. A validated questionnaire was administered to collect data regarding the awareness and tobacco consumption among the study group. Unstimulated saliva sample (1ml) was also collected on the same appointment and evaluated using Calbiotech salivary cotinine assay (solid phase competitive ELISA test). Cotinine levels of > 10 ng/ml were considered as positive evidence of tobacco use.

Results: It was found that prevalence of tobacco use was 10.5% in the study group. However, an alarming 88% of these adolescents had cotinine levels between 7-10ng/ml which suggest that this group may have been exposed to passive smoking.

Conclusion: A significant proportion of the study group are active tobacco users however an alarming percentage have a significantly high level of possibly passively inhaled environmental tobacco smoke exposure.

Keywords: Saliva; Cotinine; Tobacco; Adolescents.

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INTRODUCTION

Tobacco use in children and adolescents is reaching pandemic levels. The World Bank has reported that nearly 82,000-99,000 children and adolescents all over the world begin smoking every day. About half of them would continue to smoke to adulthood and half of the adult smokers are expected to die prematurely due to smoking related diseases. If current smoking trends continue, tobacco will kill nearly 250 million of today's children.^{1,2}

Therefore it is necessary to take the steps needed



to discourage youth who start smoking before this age in order to achieve a goal of a healthier future society.

Cotinine is considered the best indicator of tobacco smoke exposure and has a high rate of sensitivity and specificity when compared to more intrusive biochemical tests.^{3,4} Extensive research has been carried out to measure cotinine using a variety of techniques assessing cotinine levels in both saliva and urine.^{5,6} Sensitivity values were consistently higher when cotinine was measured in saliva instead of urine or blood.⁷

Hence this study was planned to evaluate the prevalence of tobacco use among 95 randomly selected students from Karad. And to evaluate the association between the cotinine found in saliva with the reported use of tobacco.

Materials and methods

The study was conducted after due approval of the Institutional Ethical committee. A random sample of 95 students from schools in Karad was selected for the study. An informed consent for the same was obtained and a validated questionnaire was administered in order to collect information regarding tobacco related habits among the study group. The questionnaire was administered and answered in the same appointment with the saliva collection. Both the questionnaire and the saliva sample were coded for further evaluation. Information on the following items was obtained by the questionnaire: demographic data (age, gender), type of tobacco used, frequency, duration, reasons for use, knowledge regarding the adverse effects, attitude towards the prevalent trends and willingness and difficulties associated with quitting the habit. After instructing the subject to rinse the mouth thoroughly, unstimulated saliva sample (1ml) was collected from the study population in a sterile saliva vial (Eppendorf tubes, Fig. 1).



Fig. 1: Sterile saliva vial (ependorf tubes) used for collection of sample

The salivary cotinine level was evaluated using Cal biotech salivary cotinine assay using solid phase competitive ELISA test (Fig. 2, Fig. 3 and Fig. 4).

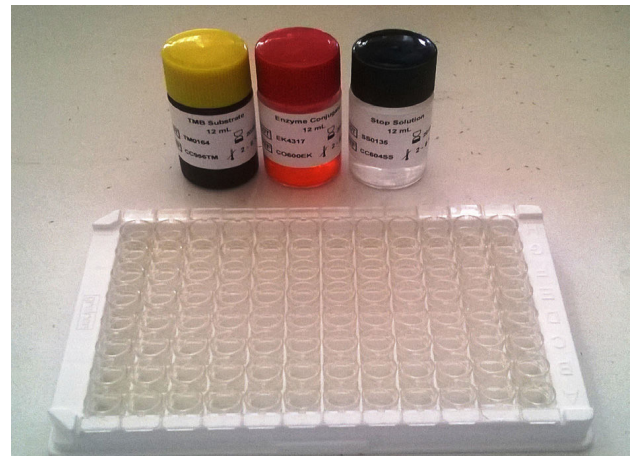


Fig. 2: Salivary cotinine kit consisting of substrate, conjugate and stop solution



Fig. 3: Colour change after step 2



Fig. 3a: Colour change after step 3

A cut of >10 ng/ml of salivary cotinine was considered as positive evidence of active smoking. Data obtained was statistically analysed using SPSS version 17.0.

Results

Out of 95 students that participated in the study, 16(36.8%) were of 16 years, 23(24.2%) were of 17 years. 18(22.1%) were of 18 years, 19(13.7%) were of 19 years and only 3(3.2%) were of 20 years. Of these 52(54.7%) were of females, and 43(45.3%) were of males.

Out of 8 subjects who agreed on their tobacco usage, 1 first tried a cigarette at the age of 12 years, 1 first tried cigarette at the age of 14 years and 6 first tried cigarettes at the age of >16 years. All the participants confirming to the use were males and used smoked tobacco.(Chart 1)

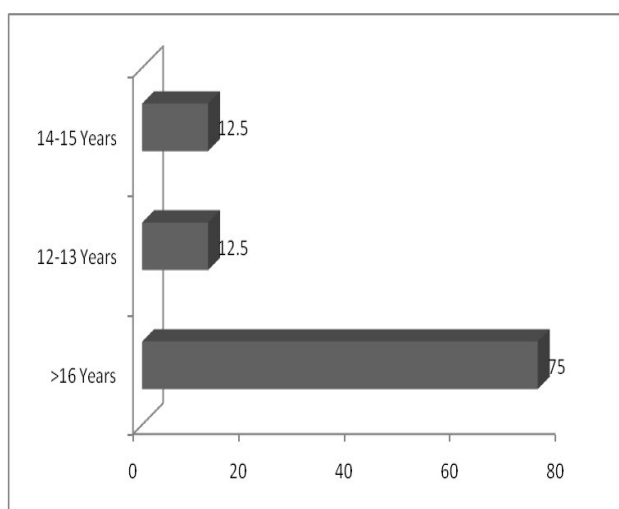


Chart 1: Time of initiation of Tobacco use

3(37.5%) smokers smoked 2 cigarettes per day, 2 (25%) smokers smoked 1 cigarette per day.1 (12.5%) smoked 30 cigarettes per day.(Chart 2)

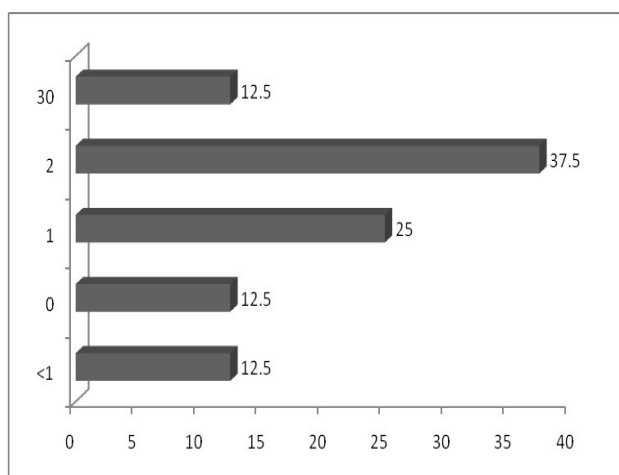


Chart 2: Frequency of Cigarette smoking

The findings of our study show that there is a

relationship (association) between standard of living and tobacco use. It was seen that the students in upper class were more addicted than students in other classes.

92(96.8%) students were aware about the ill effects of tobacco usage while 3 (3.2%) were unaware. 69 (72.6%) have seen anti-tobacco advertisements or promotions at stores, shops, etc. which means health warning on packs are effective. 26 (27.4%) had not seen such advertisements.

90(94.7%) were in favour of a ban on tobacco usage, while 3 (3.2%) were against it and 2(2.1%) did not answer the question.

In the questionnaire the participants were asked about passive smoking exposure. 46.3% students responded that someone had smoked in their presence within the last 7 days and atleast 14.7% responded that the exposure was within a day. 25.3 % did not report of passive exposure. The rest had been exposed between 2-6 days.

8 subjects in the questionnaire confirmed their tobacco use of which 7 had cotinine levels above 10 ng/ml.

10% (10/95) participants had cotinine levels above 10 ng/ml indicative of positive evidence of tobacco use of which 7% admitted in the questionnaire about using tobacco, the rest indicate the hidden cases who did not admit of using tobacco with a $\chi^2 = 67.8$, P value = 0.0 suggesting a significant association between reported use and cotinine levels. 1 participant who agreed to the use of tobacco in the questionnaire had a cotinine level of less than 7 ng/ml. (Table 1)

Table 1: Salivary Cotinine levels

Salivary Cotinine Levels	Tobacco use		Total
	No	Yes	
< 7	0	1	1
7.0-10	84	0	84
>10	3	7	10
Total	87	8	95

$\chi^2 = 67.8$, P value = 0.0*

However the most disturbing finding was that an alarming 84% subjects had salivary cotinine levels between 7-10ng/ml.(Chart 3)

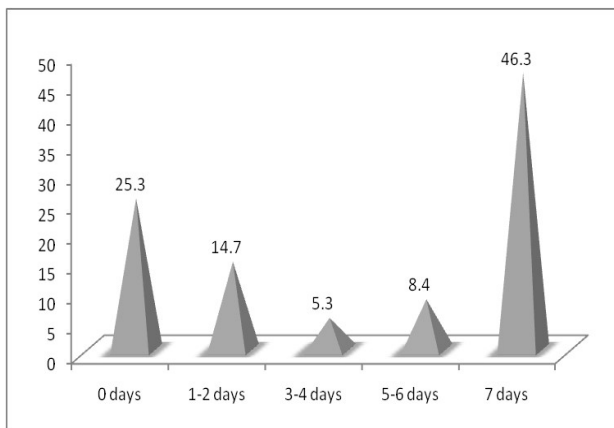


Chart 3: Time of exposure to passive smoking

Discussion

Self-reports are widely used in different research settings as a measure of smoking status. The validity of self-reports has long been debated as there is concern that smokers may misconstrue or underestimate the amount they smoke. For this reason, validation of self-reports with biochemical measurements is recommended when feasible.⁸⁻⁹

Cotinine is the major metabolite of nicotine and has become the standard marker of nicotine exposure. The Society for Research on Nicotine and Tobacco (SRNT) Subcommittee on Biochemical Verification published guidelines for use of biomarkers in tobacco control studies, and one of the recommendations is to use cotinine as an indicator of addiction severity.¹⁰⁻¹¹

Cotinine is considered the best indicator of tobacco smoke exposure and has a high rate of sensitivity and specificity when compared to more intrusive biochemical tests. Extensive research has been carried out to measure cotinine using a variety of techniques assessing cotinine levels in both saliva and urine. Sensitivity values were consistently higher when cotinine was measured in saliva instead of urine or blood.¹²⁻¹⁴

Self-reported smoking status using saliva cotinine has been validated several times in Western countries, but its use in low and middle income countries is limited. In India, only a very few validation studies could be identified and that was among adolescents.¹⁵

Ab Manan N et al. in their study found that self-reporting had 67.6% sensitivity and 99.3% specificity as compared to those of urine cotinine strip testing and had 92% positive predictive value and 96.2% negative predictive value. They also concluded that self-reporting can be used to assess smoking status

but should be used with care among adolescents. Urine cotinine strip test validation of self-reporting enabled the measurement of the true prevalence of smoking among adolescents.¹⁶

Hence the current study was chosen to find the prevalent scenario among the adolescent age group, in a semiurban location of Maharashtra. Due to a limitation of the cost factor associated with the biochemical analyses 95 randomly selected subjects were included in the study and hence this can be considered a pilot project for further larger prospective and cross-sectional studies.

While cotinine is commonly used as a biomarker to validate self-reported smoking status, the selection of an optimal cotinine cutoff value for distinguishing true smokers from true nonsmokers shows a lack of standardization among studies. A review conducted on English language literature search in PubMed using the keywords "cotinine" and "cutoff" or "self-reported" and "smoking status" and "validation" for the years 1985–2014, found that the saliva cotinine cutoff value range of 10–25 ng/mL, serum and urine cotinine cutoff of 10–20 ng/mL and 50–200 ng/mL, respectively, have been commonly used to validate self-reported smoking status using a 2 × 2 table or a receiver operating characteristics (ROC) curve. The authors also reported that they also found that a recent large population-based studies in the U.S. and UK reported a lower cutoff value for cotinine in serum (3 ng/mL) and saliva (12 ng/mL), compared to the traditionally accepted ones (15 and 14 ng/mg, respectively).¹⁷ Based on the recommendation of the manufacturer and a few more studies a cut off of 10ng/ml was chosen in our study.

Recently, Jarvis et al. reported a new salivary cotinine cutoff value of 12 ng/mL using an ROC curve, with a sensitivity of 96.7% and specificity of 96.9% for distinguishing cigarette smokers from never smokers among participants aged 16 years or older in the Health Survey for England (HSE) for the years 1996–2004.¹⁸

From ROC curve analysis of the present study population, it was found that 12.45ng/mL as a maximum value for cut off is recommended to distinguish between hidden cases of tobacco in tobacco user and non-user group. And hence this value may be used for further studies.

Dhavan et al. conducted a study during the baseline household survey of a community-based tobacco prevention and cessation intervention trial for youth (10-19 years old) residing in slum communities in Delhi, India in 2009. They reported, 8.33% (n=102) of the youth were identified as

tobacco users at 15 ng/ml as the cut-point. Changing the cut point to 20, 12 and 10 ng/ml did not make much difference in prevalence rates (7.92%, 8.58% and 8.91%, respectively).¹⁵

10% participants in the present study had cotinine levels above 10 ng/ml indicative of positive evidence of tobacco use of which 7% of these admitted in the questionnaire about using tobacco, the rest indicate the hidden cases of using tobacco. This was much higher than 8.91% for a semi urban locality in comparison to a metropolitan like Delhi. In another recent study among Malaysians adolescents, it was found that the prevalence of self-reported smoking was 8% and that of urine cotinine strip testing was 10.8%.^{15,16}

The results of the study show that though the study area was a semi urban area still a sizeable and alarming percentage of adolescents are indulging in smoking and tobacco consumption.

But the most significant finding noticed in the study sample was that 84% subjects had a cotinine value between 7-10ng/ml which is considerable higher for a non-smoking group indicative of high environmental tobacco smoke and/or passive smoking.

The findings of our study also emphasize the use salivary diagnostics for cotinine analysis level of second-hand smoke exposure as a compelling alternative in conventional diagnostics using bio-fluids, such as blood and urine, owing to its simple and non-invasive collection method. In a study by Lidón-Moyano C et al. no differences in cotinine levels were found between those exposed to second-hand and third-hand smoke at home.^{19,20}

Hence steps need to be taken in this direction to evaluate the effect of ban on smoking and tobacco consumption in public places and also to reduce exposure to third-hand smoke.

Conclusion

In the present study it was observed that a high percentage of our adolescent population in indulging in adverse tobacco habits. Majority of this group used smoked tobacco and there was an increased trend in the higher socioeconomic group. However an increased amount of cotinine levels in non-smokers was the most alarming finding in the study and we need to further evaluate the true reasons for this observation.

The trend of using tobacco in its various forms in alarmingly and on the rise among children and adolescents and there is an urgent need to take

steps in this direction if we wish that the future generation of Indians lead a healthy and productive life. Also the effectiveness of ban on tobacco use in public places needs to be evaluated and policies and actions that would further enhance the “No Tobacco” drive need to be implemented.

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