ORIGINAL ARTICLE

Relation of Serum Cotinine with Passive Smoking

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ABSTRACT

Background: Cotinine is a major metabolite of nicotine and retains for a substantial time in different body fluids. It is considered as a passive smoke exposure marker. Studies on different biochemical markers of tobacco smoke exposure are lacking in Pakistan and serum continine levels in nonsmokers provide a comprehensive measure of passive smoking from all sources in the last two to three days.

Objective: To compare the frequency of serum cotinine detection in smokers, passive smokers and never smokers.

Methods: Serum cotinine was analyzed on 135 self reported smokers, never smokers and passive smokers by 17-A gas chromatograph with flame ionization detector (FID) equipped with Supelco SPB-5 fused silica capillary column attached with Class GC 10 Software. Association between the qualitative variables (cotinine present/absent) between groups (smoker, never smoker and passive smoker) was taken out using Pearson chi-square test. The mean difference for serum cotinine between the groups (smoker, never smoker and passive smoker) was evaluated by using kruskal wallis test. P-value less than 0.05 was taken as significant.

Results: Serum cotinine was detectable in 72.1% of passive smokers which was a significantly higher proportion than smokers and never smokers ($p< 0.001$).

Conclusion: Cotinine was detectable in the serum of smokers and passive smokers. Passive smoking may contribute to increased serum cotinine levels. Large sample size may be required to establish it as a marker for environmental tobacco smoke exposure marker and for validation of smoking status.

KEY WORDS: *Cotinine, Passive Smoking, Environmental Tobacco Smoke (ETS), Secondhand Smoke (SHS), Gas Chromatography.*

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INTRODUCTION

Passive smoking occurs when non-smokers are exposed to tobacco smoke of smokers in a closed environment and the health risks associated with secondhand smoke exposure (SHS) have been documented since 1970s.[11] Environmental tobacco smoke (ETS) also known as secondhand smoke (SHS) is a complex mixture of more than 4000 chemical compounds that are generated during the burning of tobacco products. This mixture contains numerous irritants and toxicants with acute health effects as well as toxicants with carcinogenic effects in humans and is known to increase morbidity and mortality risks in infants, children, and adult non-smokers.[2] It has been estimated that secondhand smoke is responsible for 22,000 hospitalizations, between 150,000 and 300,000 cases of bronchitis and pneumonia, and between 8000 and 26,000 cases of asthma each year.[3]

Cotinine is the major metabolite of nicotine and persists for a considerable time period in body fluids ; only a minor fraction of the generated cotinine is excreted by the kidneys. So cotinine is generally regarded as the best marker for monitoring tobacco exposure in both active and passive smokers.[4] Moreover, cotinine has a much larger half life of 18-20 hours, making it more appropriate for use as an exposure marker. [5] Lung cancer and heart disease have been associated with cotinine levels in passive smokers. Cotinine is the most widely used biological marker of ETS exposure and can be detected in saliva, blood, urine, semen, and hair.[6]

It is essential to create awareness about passive exposure to cigarette smoke in both the health professionals and common people to prevent them from hazards of passive smoking.[7] Studies on different biochemical markers of tobacco smoke exposure are lacking in Pakistan and serum cotinine levels in nonsmokers provide a comprehensive measure of passive smoking from all sources in the previous two to three days period.

The study was conducted to compare the serum cotinine detection in self reported smokers, never smoker and passive smokers.

METHODOLOGY

The study was conducted at Ziauddin University in collaboration with HEJ Research Institute of Chemical Sciences, University of Karachi from January, 2009 to October, 2011.Subjects between 18 to 45 years of age were divided into three groups, group one included 43 smokers ; according to World Health Organization, (a smoker was defined as a person who, at the time of the survey, smoked any tobacco product either daily or occasionally).[8] Group two included 31 never-smokers, (defined as a person who had neither actively and nor a passive smoker). Group three included 61 passive smokers who were never-smokers but exposed to cigarette smoke. Ex-smokers were excluded from this study. A written consent form was signed by each participant. The study was approved by ethical committee. There was no bias in the study. A questionnaire was administered regarding environmental and health history, smoking status and passive exposure of the participants.

After filling up of questionnaire, blood was drawn in proper biochemistry laboratory, free of tobacco smoke. Blood samples (10 ml) were obtained by venipuncture with vacutainers. The blood was allowed to clot and all samples were centrifuged at 1000 g for 5 minutes. Serum was collected and stored at -20^{0}C until analysis.

Solution of stock reference standards of cotinine was prepared in dry methanol and stored at -20^{0}C. Five dilutions of 1, 5, 10, 25 and 50µg/ml of cotinine were prepared in dry methanol and 10 µl of each was injected into gas chromatograph (Schimarzu with flame ionization detector (FID) equipped with Supelco SPB-5 fused –silica capillary column (45 m x 0.53 mm i.d., 0.5um) attached with Class GC-10 Software.) to obtain standard curve in the range of 10-500ng/ml Serum standards were prepared by adding known amounts of stock standards to human serum collected from never-smokers at five different concentrations as described above to establish recovery at different concentrations. The serum was extracted as described in the Sample preparation before adding the known concentrations of stock standards. The extracted serum was tested before addition of the known concentrations of the standards to confirm that samples were tobacco free.

Sample preparation involved mixing of serum with base (A 1.5 ml aliquot of serum and 100 µl of N-ethylnorcotinine mixed with 1.4 ml 0.5 M sodium hydroxide) and passing through the specialized extraction columns.Organic phase is eluted with dichloromethane and isopropyl alcohol and methanolic HCl was added to extracted organic phase to retain cotinine and evaporated to dryness under pressurized nitrogen and redissolved in 100 µl of dry methanol.[9-10]

Cotinine was measured by 17-A gas chromatograph (Schimarzu) with flame ionization detector (FID) equipped with Supelco SPB-5 fused silica capillary column (45m x 0.53mm id and 0.5□m) attached with Class GC 10 Software. The retention time in this programme was 33.8 minutes for cotinine. Quantification of cotinine in serum was based on the standard and calibration curve of various known amounts of cotinine. [11-13]

Data was entered on Statistical Package for Social Sciences (SPSS) version 16.0. Frequencies and percentages were taken out for the qualitative data; mean and standard deviation were taken out for the numerical variable. Association between the qualitative variables (cotinine present/absent) between groups (smoker, never smoker and passive smoker) was taken out using Pearson chi-square test. The mean difference for serum cotinine between the groups (smoker, never smoker and passive smoker) was evaluated by using kruskal wallis test. p- value less than 0.05 was taken as significant.

RESULTS

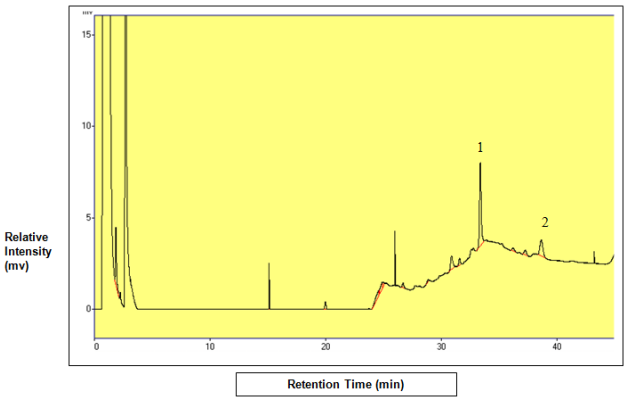
The study included 135 subjects with the age range of 18-45 years. Among 43 self reported smokers, 30 were males and 13 were females. There were 33 males and 28 females passive and 9 males and 22 females were never smokers .The mean age± SEM was 26±1.3 years for smokers, 25±0.8 years for passive and 24±0.8 years for never smokers. Presence of cotinine were significantly less in never smokers 8 (25.8%) as compared to smokers 30 (69.8%) and passive smokers 44 (72.1%, p<0.001). Among thirty one never smokers, eight subjects had cotinine in their serum indicating that they were exposed to tobacco smoke. Evaluation of the mean difference between the groups by kruskal-wallis test revealed no significant difference between the groups as the mean rank (41.50) for smokers, never smokers and passive smokers was the same (Table 1). Figures 1 and 2 show the GC ion chromatogram of serum from a passive smoker exposed to cigarette smoke after two and seventeen hours. The peak height of cotinine was higher in figure 1 as compared to figure 2. Cotinine was present in the blood of passive smoker even after 17 hours of exposure indicating its long half life and stability in the blood.

Table 1. Evaluation of serum cotinine according to smoking status

Serum Cotinine	Smoking Status			P-Value
	Smoker (n=43)	Never Smoked (n=31)	Passive Smoker (n=61)	
Absent	13 (30.2%)	23 (74.2%)	17 (27.9%)	<0.001
Present	30 (69.8%)	8 (25.8%)	44 (72.1%)	
Mean Rank*	41.50	41.50	41.50	

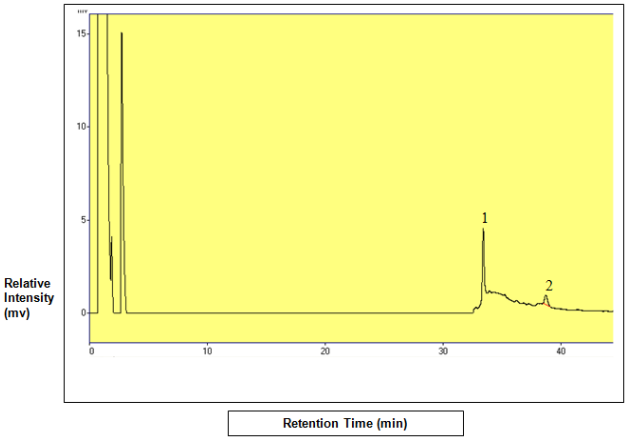
P Value <0.01 calculate by Chi-square test
*Calculated by Kruskal-Wallis test

Figure 1. GC on chromatogram of serum from a passive smoker exposed to cigarette smoke in the last 2 hours



Peak # 1→ cotinine
Peak # 2→ N-ethylnorcotinine (internal standard)

Figure 2. GC on chromatogram of serum from a passive smoker exposed to cigarette smoke in the last 17 hours



Peak # 1→ cotinine
Peak # 2→ N-ethylnorcotinine (internal standard)

DISCUSSION

Cotinine is the major metabolite of nicotine. It is the most widely used biological marker of environmental tobacco smoke exposure and can be detected in blood, saliva, urine, semen and hair.[6, 14-15]. We considered it due to its specificity for exposure to nicotine from tobacco smoke. It is chemically stable. In addition, its longer half life (17 hrs in blood compared with 2 hrs for nicotine) reflects long term exposure whereas nicotine reflects recent exposure. So cotinine is an indicator of environmental tobacco smoke exposure over the previous 1-2 days.[4]

The mean levels of serum cotinine in smokers were higher than passive smokers. This finding is in accordance with a cohort study[16] done in 2011 which showed clear evidence that tobacco smoking as well as involuntary smoking showed increased Cotinine levels. In thirteen self reported smokers, cotinine was not detected (Table 1). These findings are consistent with Vartiainen et al, 2002 who reported that small proportion of daily smokers does not have cotinine in their serum for an unknown reason.[12] Moreover, most of this was explained by the fact that persons who reported to have smoked 'today or yesterday' or occasionally smoked in relation to the time of completing the questionnaire did not have cotinine in their serum. There is also intra individual variation that how well serum cotinine is describing nicotine intake. Different people convert different amount of nicotine to cotinine. Usually, this varies between 55% and 92% and also the cotinine clearance varies from 19-75 ml/min.[4] In 1997, Benowitz and colleagues reported a person with different c-oxidation of nicotine[17]. This is associated with a long half life of nicotine and low level of cotinine in plasma compared with nicotine. In addition to this, cotinine undergoes further biotransformation[14] apart from its good stability and usefulness as environmental tobacco smoke exposure marker.

In our study, Seventeen of self reported passive smokers showed no serum cotinine (Table 1). This is explained by the Surgeon General Report that the quantification of a non- smoker's exposure to environmental tobacco smoke is affected by the type of cigarette (filter or nonfilter, low tar or nicotine and so forth), smoking rate, room size, ventilation rates, duration of exposure and many other factors.[18]

In this study, eight self reported never smokers had detectable serum cotinine in their serum (Table 1). The finding is in full agreement with Jarvis et al and Moyer et al who reported detectable serum cotinine in self reported never smokers[5, 19]. In comparison, studies of active and passive smoking, a truly 'unexposed' comparison group is frequently lacking. Thompson et al, found that unexposed persons have measurable cotinine in urine.[20] Since virtually the only source of cotinine in body fluid is tobacco products, primarily through exposure to the smoke, it follows that unexposed persons are exposed to the environmental tobacco smoke exposure. This shows how difficult it is to avoid tobacco smoke altogether.

As Cotininine is an immediate metabolite of nicotine, it should be higher in smokers as is obvious from the results. In passive smokers it will be present as its half life is 1-2 days. So cotinine may be used as marker for both passive smoke exposures as well as for validating the smoking status.

Our main study limitation was its small sample size. This leads to the misclassification as subjects hesitate to report the smoking status. This misclassification was corrected by making frequency table and excluding the outliers. Then the values are given as Mean $\pm$ SEM.

CONCLUSION

Serum cotinine was present in smokers and passive smokers. Passive smoking may contribute to increased serum cotinine levels; however, more studies with increased sample size may be required to establish it as an environmental tobacco smoke exposure marker in our settings. Serum cotinine may be used as marker for validation of smoking status. Moreover, Environmental tobacco smoke exposure can be measured in bidi , huqqa and sheesha smokers as a next prospect.

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