Original Article

Estimation of serum nicotine by gas chromatography in smokers, passive smokers and never smokers

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Abstract

Objective: To compare the serum nicotine levels in smokers, passive smokers and those with no exposure to cigarette somke.

Methods: The comparative study spanning two years was conducted in Ziauddin Medical University, Karachi, in collaboration with HEJ Research Institute of Chemical Sciences, University of Karachi from 2009 to 2011 involved 135 self-reported smokers, never smokers and passive smokers among whom serum nicotine was analysed using 17-A gas chromatograph with flame ionisation detector equipped with Supelco SPB-5 fused silica capillary column (45m x 0.53mm id and 0.5um) attached with Class GC 10 Software. SPSS version 11.0 and Kruskal Wallis Test were used for statistical purposes.

Results: There were 43 (32%) smokers, 61 (45%) passive smokers and 31 (23%) never smokers in the study. Among the smokers 33 (77%) had serum nicotine greater than 10ng/ml. The mean nicotine concentration of smokers was 55±6.1 ng/ml and mean rank was 98.3 which was significantly higher than passive and never smokers ($p < 0.01$).

Conclusion: The method for the estimation of serum nicotine was fairly reliable. Serum nicotine was raised in smokers, and the effects of passive smoking was also seen through serum nicotine. However, more studies with increased sample size are required to establish it as an environmental tobacco smoke exposure marker.

Keywords: Smokers, Serum nicotine, Passive smokers. (JPMA 62: 790; 2012)

Introduction

During the Past three decades, there has been an increasing focus on cigarette smoking and the adverse health consequences associated with it. As nicotine is the primary causative agent in addiction to tobacco products, assessment of nicotine metabolism and disposition has become an integral part of nicotine dependence treatment.[1]

One cigarette contains an average of 8.4 mg of nicotine. When tobacco is burned, nicotine is aerosolised into tar droplets that deliver 1.6 mg of nicotine per cigarette. Burned alkaline tobacco products yield higher free-base nicotine concentrations in smoke compared with acidic tobacco products.[2,3]

Inhaled tobacco smoke reaches the small airways and alveoli of the lungs and affects the central nervous system within 20 seconds of tobacco smoke inhalation. Nicotine has short distribution (8 min) and elimination (2 hrs) half-lives. Rapid delivery of nicotine to the brain produces the intense, positive pharmacologic response and is thought to be a key factor in nicotine dependence.[4-6]

Nicotine is the primary alkaloid in tobacco products and a major tobacco-specific component in both mainstream and environmental tobacco smoke.[7] In Pakistan, one out of every two to three middle-aged men smoke cigarettes. A study on smoking behaviour revealed that 39% students of a Pakistani university had smoked a whole cigarette in their life time, whereas 25% had smoked 100 or more cigarettes in their lifetime.[8] Moreover, smoking prevalence in medical students of a private medical university was 26% among males and 1.7% in females despite awareness among them, about adverse effects on health.[9] Passive exposure to cigarette smoke is seen more in medical students as compared to the faculty and staff, so it is essential to create awareness both in health professionals and the common people to prevent them from the hazards of passive smoking.[10]

Studies on different biochemical markers of tobacco smoke exposure are lacking in Pakistan. This comparative study compared nicotine level in self-reported smokers, never smokers and passive smokers.

Subjects and Methods

This cross sectional observational study was

conducted at Ziauddin Medical University in collaboration with HEJ Institute of Chemical Sciences, University of Karachi from 2009-2011. The study comprised 135 subjects between 18 and 45 years of age. They were divided into three groups. Group one included 43 smokers. According to World Health Organisation criterion,[11] a smoker was defined as a person who, at the time of the survey, smoked any tobacco product either daily or occasionally. Group two included 31 never-smokers (a person who had never smoked and was not a passive smoker). Group three included 61 passive smokers (a person who was a never-smoker, but was exposed to cigarette smoke). Ex-smokers were excluded from the study.

A written consent form was obtained from 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 the filling up of the 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°C until analysis.

A solution of stock reference standards of nicotine was prepared in dry methanol and stored at -20°C. Five dilutions of 1, 5, 10, 25 and 50µg/ml of nicotine were prepared in dry methanol and 10 µl of each was injected into gas chromatograph (Schimarzu with flame ionisation 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 the never-smokers at five different concentrations as described earlier to establish recovery at different concentrations. The serum was extracted 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 the serum with base [a 1.5 ml aliquot of serum and 100 µl of N-ethylnorcotinine (3µg/ml aqueous solution) mixed with 1.4 ml of sodium hydroxide] and passing through the specialised extraction columns to precipitate proteins and elution of lipophilic substances. Organic phase was eluted with dichloromethane and isopropyl alcohol and methanolic HCl was added to extracted organic phase to retain nicotine, and evaporated to dryness under pressurised nitrogen and re-dissolved in 100 µl of dry methanol. The ependorff containing the sample was purged with nitrogen and capped tightly after each use.[12,13]

For gas chromatography,[14-16] nicotine was measured by 17-A gas chromatograph (Schimarzu) with flame ionisation detector (FID) equipped with Supelco SPB-5 fused silica capillary column (45m x 0.53mm id and 0.5m) attached with Class GC 10 Software. The retention time in this programme was 19.8 min for nicotine. Quantification of nicotine in serum was based on the standard and calibration curve of various known amounts of nicotine.

The above operating conditions were optimal for the specific instruments and column used in the study, and modifications were made to the method followed by Allena and Voncken. Optimisation should be achieved individually for other similar instruments.

Statistical Package of Social Sciences (SPSS) Ver 11.0 was used for data feeding and analysis. Kruskal Wallis Test was used for comparison of mean rank test according to the smoking status (smoker, never smoker and passive smoker), with p-value < 0.05 being considered significant.

Results

Among the smokers, 33 (77%) had nicotine levels greater or equal to10 ng/ml which was the cutoff value between smokers and never-smokers. Among the passive smokers, 6 (10%) subjects had serum nicotine greater

Table 1: Serum nicotine levels according to the smoking status n (%).

Nicotine (ng/ml)	Smoker No (%)	Never Smokers No (%)	Passive smoker No (%)
0	10 (23.3)	25 (80.6)	41 (67.2)
Under 10	0 (0)	3 (9.7)	14 (23.0)
10 and above	33 (76.7)	3 (9.7)	6 (9.8)

Table-2: Mean ± SEM, median test and Mean rank of nicotine levels according to the smoking status.

Smoking status	n	Mean ± SEM	Median test	Mean Rank
Smoker	43	55.0±6.1	> median 33 ≤ median 10	98.3 *
Never smoker	31	0.8±0.4	> median 6 ≤ median 25	48.9
Passive smoker	61	1.2±0.3	> median 20 ≤ median 41	55.1
Chi - square & (p-value)			29.1 (p<0.01)	48.8 (p<0.01)

*Significant compared with never smoker and passive smoker p<0.01.
p- value calculated by Kruskal Wallis Test.
SEM: Standard error of mean.

than 10 ng/ml (Table-1) so they were not passive smokers as they had mentioned in the questionnaire.

The mean nicotine concentration of smokers was 55 ng/ml, and the mean rank was 98.3 which was significantly higher than passive smokers and never-smokers (Table-2).

Discussion

Measurement of environmental tobacco smoke exposure by self-reports, biological markers and environmental air monitors differ considerably in terms of reliability, validity, potential biases, cost and ease of administration. Quantitative method for measuring nicotine (Gas chromatography/Flame ionisation detector) is valid and reliable though it is time-consuming and demands training to handle varions equipments.

Nicotine was one of the biomarkers to be studied for effects of passive smoking as biomarkers are becoming increasingly popular for environmental tobacco smoke exposure measurement. Moreover, it avoids many sources of bias and may provide greater sensitivity than questionnaires. This study measured serum nicotine due to its specificity to tobacco or tobacco smoke exposure. Approximately 95% of tobacco-derived nicotine is present in the gas phase. Hence, nicotine uptake is considered to be a biological marker for environmental tobacco smoke exposure.[17] In this study, mean serum nicotine concentrations in passive smokers was 1.2ng/ml which is consistent with Toumi et al and Moyer et al.[18,19] In the current study, 41 of the self-reported passive smokers had undetectable nicotine in their serum (Table-1). This is due to the fact that nicotine concentration is sensitive to recent exposure because of nicotine's relatively rapid and extensive tissue distribution and rapid metabolism. During passive smoking, non-smokers inhale nicotine proportionally to the product of concentration, exposure and duration.[20] Moreover, inhaled nicotine is absorbed into the bloodstream through the lungs and is rapidly and extensively metabolised with the half-life of the order of up to 2 hours by the liver into its metabolites. Besides, the rate of nicotine metabolism varied as much as four-fold among smokers. These subjects, therefore, are justified having no nicotine in their serum as they might not be recently exposed to tobacco smoke during the preceding two hours or so. Moreover, a given level of nicotine in the body reflects the balance between nicotine absorption, metabolism and excretion rates. Thus, in comparing two persons with the same blood concentration of nicotine, a rapid metaboliser may be absorbing up to four times as much nicotine as a slow metaboliser.[21]

Serum nicotine was found less than 10 ng/ml in 14 self-reported passive smokers. It indicated that they had recent exposure to tobacco smoke. Six self-reported passive smokers had serum nicotine 10 or > 10 ng/ml, showing that they under-reported their exposure or hid their smoking status because in our study, the reference range of serum nicotine was 10 ng/ml to distinguish between the smokers and the non-smokers.

Among the self-reported never-smokers, the mean nicotine was 0.8 ng/ml which is consistent with the study carried out by Moyer et al.[19] Three of the self-reported never smokers had serum nicotine < 10 ng/ml, showing that they had tobacco smoke exposure in the preceding two hours. Hence, passive exposure to tobacco smoke causes never-smokers to accumulate and excrete measurable nicotine and its metabolites.[19] Three self-reported never-smokers had serum nicotine >10 ng/ml, indicating that either they were misclassified or had hidden their smoking status.

Among the smokers, the mean serum nicotine was 55 ng/ml which was consistent with the values in the studies of Moyer et al[19] and Zuccaro et al.[12] In 33 smokers, the serum nicotine was >10 ng/ml and this was the cutoff value of serum nicotine to distinguish between the smokers and the non-smokers. In 10 self-reported smokers, the nicotine was not detectable indicating that they were occasional smokers or had practised abstinence in the preceding 8 hours. This finding is consistent with Zuccaro et al[12] who reported no serum nicotine in a group of six smokers who had not smoked during the night and in the early morning.

Our main study limitation was its small sample size. This led to mis-classification due to under-reporting of the smoking status in the questionnaire. This misclassification was corrected by working out a frequency table and excluding the debatable cases. The values were then calculated as Mean ± SEM and Median.

Conclusion

Serum nicotine is a fairly reliable method to observe exposure to tobacco smoke. More studies with larger sample sizes would be helpful to establish it as an environmental tobacco smoke exposure marker. Exposure among bidi and huqqa smokers is also recommended to be included in future studies.

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