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Estimation of Serum Nicotine Levels among Tobacco Users

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ABSTRACT

Determination of nicotine metabolism and pharmacokinetics provides a useful tool for estimating uptake of nicotine and tobacco related toxins, for understanding the pharmacologic effects of nicotine and nicotine addiction, and for optimizing nicotine dependency treatment during clinical encounter to improve patient compliance. Validation of self reported tobacco use status has significant clinical importance in cessation clinics, pulmonary and pediatric clinics and research settings. To estimate the serum nicotine levels among tobacco users. To understand the intake of nicotine and nicotine addiction for optimizing nicotine dependency treatment. 60 Healthy male volunteers between the age group of 18-45yrs using tobacco in either smoking or smokeless form for more than 3 months, 5 times a day were selected for the study. The smoking form consisted of use of cigarettes and bidis and smokeless form consisted of use of commercially available chewing tobacco sachets (pan masala etc). 3 blood samples were collected from each volunteer , 1st sample was collected in the early morning(first session), 30 minutes after the use of first tobacco product, the 2nd sample 60 min later, and the 3rd sample 90 min later. Serum nicotine levels were then assessed using Chemiluminescent immunoassay technique. ANOVA test was used to compare mean nicotine levels among all three groups and Post Hoc test was also undertaken to assess the difference in mean nicotine levels between two groups at a time. Average serum nicotine concentration among cigarette smokers at the end of 30 minutes was180(110-320)ngs\ml, at 60 minutes 40(30-50)ngs\ml and at 90 minutes 10(0-20)ngs\ml , among bidi smokers at the end of 30 minutes, it was 260(240-450) ngs\ml, at 60 minutes 70(50-90)ngs\ml and at 90 minutes 10(10-30)ngs\ml; among tobacco chewers at the end of 30 minutes it was 140(90-200)ngs\ml, at 60 minutes 50(40-60)ngs\ml and at 90 minutes 30(10-40)ngs\ml. Serum nicotine levels among cigarette and bidi smokers was significantly high compared to tobacco chewers. High serum nicotine levels among smokers could be attributed to high nicotine content of the tobacco product, method of consumption, rate of nicotine absorption and elimination. These findings suggest different strategies for treatment of tobacco dependence in these subjects as these three kinds of tobacco users would have different therapeutic needs.

Keywords: Serum nicotine levels, smoking tobacco, chewing tobacco, nicotine dependency treatment, Indian population

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INTRODUCTION

Tobacco use has been associated with development of oropharyngeal and upper respiratory tract cancers and is a risk factor for cardiovascular disease and adverse reproductive outcome. Because nicotine is the primary causative agent in addiction to tobacco products, assessment of nicotine metabolism and disposition should become an integral part of nicotine dependence treatment. Self report measures among tobacco users are highly imprecise owing to individual differences in how cigarettes are smoked like number of puffs, intensive puffing, occlusion of ventilation holes in the filter, length of breath holding, unsmoked butt length etc in order to obtain the desired dose of nicotine. In contrast among chewing tobacco users nicotine absorption is primarily determined by the product itself , the pH of the product, size of tobacco cuttings and not the experience or actions of the user[1-4].

The optimal assessment of tobacco exposure would be by analysis of the concentration of a component of tobacco in the body fluids of an exposed individual i.e biomarker. No marker for cumulative exposure to tobacco use is available to-date. Nicotine is a chemical found in all tobacco products and occupies a unique place as a biomarker of tobacco exposure because it is the primary addictive component and a potential toxin[3, 4]. Serum nicotine concentrations can be used to guide medical treatment to achieve biological concentration adequate to provide patient with immediate relief from nicotine withdrawal symptoms, an important factor in nicotine withdrawal success[2, 5, 6].

MATERIALS AND METHODS

60 Healthy male volunteers between the ages of 18-45yrs using tobacco in either smoking or smokeless form for more than 3 months, 5 times a day were selected for the study. Approval from IRB was obtained.The smoking form consisted of use of filtered and unfiltered cigarettes and bidis and smokeless form consisted of use of commercially available chewing tobacco sachets (pan masala etc). After informed consent was obtained, each participant filled out a standard checklist form, which included information on age, self-reported tobacco products usage (cigarettes, bidis, chewing tobacco), brand and type of tobacco product used, current smoking history and the number of times tobacco used per day. Of the 60 tobacco users, 20 smoked cigarettes, 20 smoked bidis and 20 used chewing tobacco.3 blood samples were collected from each volunteer at an interval of 30 minutes each. The 1st sample was collected in the early morning (first session) 30 minutes after the use of first tobacco product, 2nd sample 60 min later and 3rd sample 90 min later. These individuals were advised not to use any tobacco product for the next 90 minutes following the first morning sample. Serum nicotine levels were then estimated using Chemiluminescent immunoassay (Immilite 1000 a solid phase nicotine metabolite kit) at the Metropolis laboratory, Bangalore.

RESULTS

Average serum nicotine concentration (5% Alpha error) among cigarettes smokers at the end of 30 minutes was 180(110-320)ngs\ml, at 60 minutes 40(30-50)ngs\ml and at 90 minutes 10(0-20)ngs\ml , among bidi smokers at the end of 30 minutes, it was 260(240-450) ngs\ml, at 60 minutes 70(50-90)ngs\ml and at 90 minutes 10(10-30)ngs\ml;

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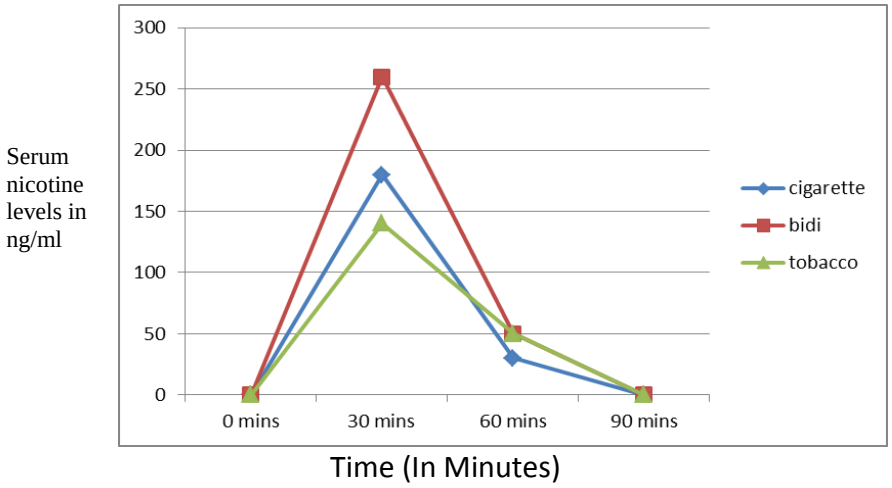
among tobacco chewers at the end of 30 minutes it was 140(90-200)ngs\ml, at 60 minutes 50(40-60)ngs\ml and at 90 minutes 30(10-40)ngs\ml.

Graph depicting the average serum nicotine levels among 20 users of each tobacco form



ANOVA test was used to compare mean serum nicotine levels among all three groups of tobacco users and Post Hoc test was used to assess the difference in mean nicotine levels between two groups at a time.

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Half_Hr	Cigarettes	20	1.9150	.23005	.05144	1.8073	2.0227	1.50	2.50
	Chewing Tobacco	20	1.4200	.13219	.02956	1.3581	1.4819	1.10	1.60
	Beedis	20	2.6650	.27391	.06125	2.5368	2.7932	2.20	3.30
	Total	60	2.0000	.55966	.07225	1.8554	2.1446	1.10	3.30
One_hr	Cigarettes	20	.3250	.11642	.02603	.2705	.3795	.20	.60
	Chewing Tobacco	20	.7150	.09881	.02209	.6688	.7612	.50	.90
	Beedis	20	.5400	.09947	.02224	.4934	.5866	.40	.80
	Total	60	.5267	.19122	.02469	.4773	.5761	.20	.90
One_half_hr	Cigarettes	20	.0600	.08826	.01974	.0187	.1013	.00	.30
	Chewing Tobacco	20	.3250	.04443	.00993	.3042	.3458	.30	.40
	Beedis	20	.1100	.05525	.01235	.0841	.1359	.00	.20
	Total	60	.1650	.13254	.01711	.1308	.1992	.00	.40

Table 2 - showing the mean, standard deviation and standard error, of the serum nicotine values at the end of 30mins, 60mins and 90mins, for the various forms of tobacco

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DISCUSSION

Self reports on tobacco use through questionnaires and interviews may be inaccurate leading to bais. Nicotine levels increase slowly and progressively during the period of exposure and fall during periods of non exposure. The half life of nicotine is 2 hrs whereas cotinine a principal metabolite of nicotine has a half-life of about 16 hrs. During the first 2 minutes of nicotine absorption, the arterial concentration exceeds the venous concentration by 6-10 folds. Self administration of nicotine is driven by the need to maintain serum nicotine concentration. Serum nicotine levels are preferred over cotinine levels during the tobacco cessation programme(TCP) to guide medical treatment to achieve biological concentration adequate to provide patient with immediate relief from nicotine withdrawal symptoms, an important factor in smoking cessation success. Absence of nicotine metabolite cotinine and anabasine can be used to document abstinence from tobacco products, an indicator of treatment success [6, 7].

Quantification and accuracy are adequate for clinical evaluation of patients undergoing treatment for tobacco dependency. The serum nicotine concentration can be used to guide dosage in nicotine replacement therapy to achieve the concentrations present when the patient was actively using a tobacco product allowing a greater opportunity for withdrawal success. These measurements should become an integral part of the clinical treatment and management of patients who wish to overcome tobacco dependence.

Cigarette

With cigarettes, virtually any brand can readily provide the user with the desired dosage; thus, nicotine intake from a cigarette is mainly determined by the smoking pattern of the user.

Table 1 Tobacco weight, Nicotine content per tobacco product and Average serum nicotine concentration among cigarette ,bidi and chewing tobacco users -

Tobacco type	Typical weight of a single dose of tobacco product(mg)	Nicotine content (mgs/g)tobacco	Average serum nicotine conc 30 min after single use of the product (ng/ml)
Cigarettes	712.2	14.5 (11.6-15.9)	180
Bidis	187.5	26.9 (25.6-29.1)	260
Chewing tobacco	3696 fill wt	3.4 (2.6-4.1)	140

(Fill wt means actual wt of the tobacco product present minus the wt of the tobacco pouch\filter)

Cigarette delivers about 1mg (0.3-3.2mgs) of nicotine to the circulation of the smoker, representing a bioavailability of about 3-40% varying from person to person[3, 4]. In our study, it was found that the average serum nicotine concentration among cigarette smokers at the end of 30 minutes was 180(110-320)ngs\ml, at 60 minutes 40(30-50)ngs\ml and at 90 minutes 10(0-20)ngs\ml. Several studies have reported different values of actual nicotine intake from cigarettes,2.5mg\cigarette was reported by Benowitz et al(1994),1.4mg\cigarette was reported by Jacob et al (1997),1mg\light cigarette was

reported by Shiener (1988),0.6,1.0,1.3,and 1.4mg for smokers of ultra- low, low, medium and high yield cigarettes was reported by Ueda et al(2002).The difference in the serum nicotine levels among various studies could be due to the type of tobacco used, processed (flue-cured ,air-dried),nicotine content of the product etc.

The arterial/venous concentration ratio is greater after the first cigarette, which increases the amount of nicotine delivered to the brain. If a smoker consumes tobacco until bedtime, significant nicotine concentrations persist throughout the night. When the number of cigarettes available to the smoker is reduced from an average of 40 to 5 per day, intake of nicotine increases threefold, a figure consistent with the maximal absolute bioavailability cited at 40%. It is estimated that an intake of 5 mgs of nicotine per day is a threshold level that can readily establish and sustain addiction [4,6-9].

Bidis

Bidi, a product of cottage industry, is the most prevalent form of tobacco smoked in India. A bidi contains crude tobacco wrapped in a dry Tendu leaf (Dyospyros melonaxylon). Bidis have a high nicotine concentration 26.9mgs\gm(Table 1) as compared to cigarettes. Bidi is smoked in a way different from cigarettes. Here bidis have to be inhaled more frequently to prevent them from being extinguished. Moreover there is also absence of filter in bidis[5]. In our study the average serum nicotine concentration among bidi users at the end of 30 minutes was 260(240-450) ngs\ml, at 60 minutes 70(50-90)ngs\ml and at 90 minutes 10(10-30)ngs\ml (Fig.1).

Chewing tobacco

Nicotine is absorbed in substantial quantities from smokeless tobacco; pH being the crucial determinant of nicotine absorption from the oral mucosa. Average nicotine content of an Indian smokeless tobacco sachet of 4gms was 3.4mg\gm[5]. In our study the average serum nicotine concentration among chewing tobacco users at the end of 30 minutes was 140(90-200)ngs\ml, at 60 minutes 50(40-60)ngs\ml and at 90 minutes 30(10-40)ngs\ml. Various studies among tobacco chewers have reported serum nicotine levels ranging from 0.14mg\ml to 3.6mg/ml. Benowitz et al(2002) in his study found 0.14mg\ml average serum nicotine levels among chewing tobacco users.2 mg\ml after using high pH product and 0.4mg\ml after use of lower pH product was reported in a study by Ayo-yusaf et al (2004). 3.6mg of nicotine absorption was observed when 2.5gm of smokeless tobacco was held in mouth for 30min (Benowitz et al 1988)[10, 11].

Amount of nicotine absorption and speed of transfer across the oral mucosa is determined by a number of factors like pH value, nicotine concentration of the product, surface area exposed , time kept and handled in the mouth, size of the tobacco cuttings, chemical coatings etc. Only un-ionized (uncharged or free) nicotine will be readily absorbed from the oral mucosa. If the amount of un-ionized nicotine is low, the rate of absorption will be greatly diminished. The ionized nicotine remains in the mouth until swallowed or until free nicotine is liberated by converting ionized nicotine to the un-ionized form .At pH above 7.0, half of the nicotine is un-ionized and readily absorbed and the other half gets ionized. At pH below 7.0, more than 90% of nicotine is ionized, so there is very little absorption.

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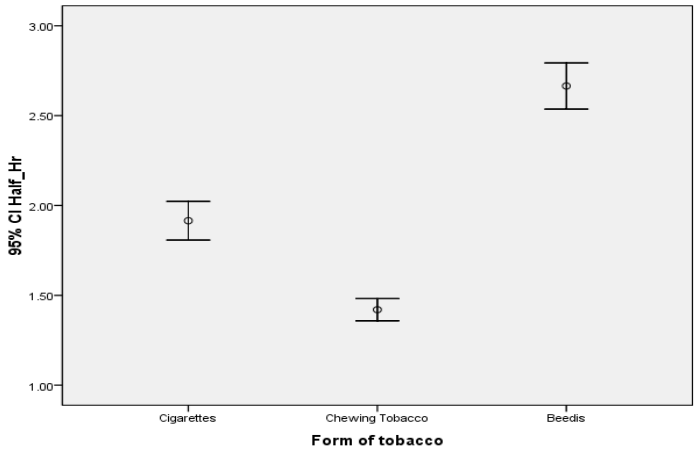
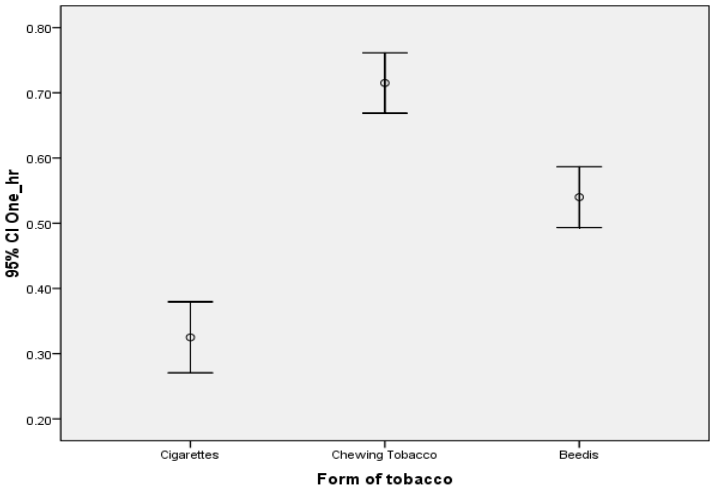
Saliva might also alter the nicotine absorption because the buffers it contains could alter the pH of the tobacco product. Bioavailability of nicotine from chewing tobacco is lower than with smoking because considerable amounts of nicotine are swallowed and thus not available in the bloodstream beyond the liver where it is metabolized to cotinine[11-13].

Serum nicotine levels among smokers is high compared to chewers and this can be attributed to a number of factors like nicotine content of the tobacco product, method of consumption, rate of nicotine absorption and elimination. Time taken to smoke a cigarette is 7 minutes and nicotine absorption is primarily by pulmonary vasculature whereas time taken for chewing tobacco is as much as 7 minutes or lesser in case the tobacco juices are spit out and nicotine is absorbed both through buccal mucosa and gastrointestinal tract. Variations in the serum nicotine level among different individuals chewing the same tobacco product and brand can be due to differences in absorption, metabolism and excretion of nicotine which can be attributed to genetic mechanism, diet and environmental factors[14, 15].



Box Diagram 1- showing mean nicotine levels of the various forms of tobacco at the end of 30 mins



Box Diagram 2- showing mean nicotine levels of the various forms of tobacco at the end of 60 mins

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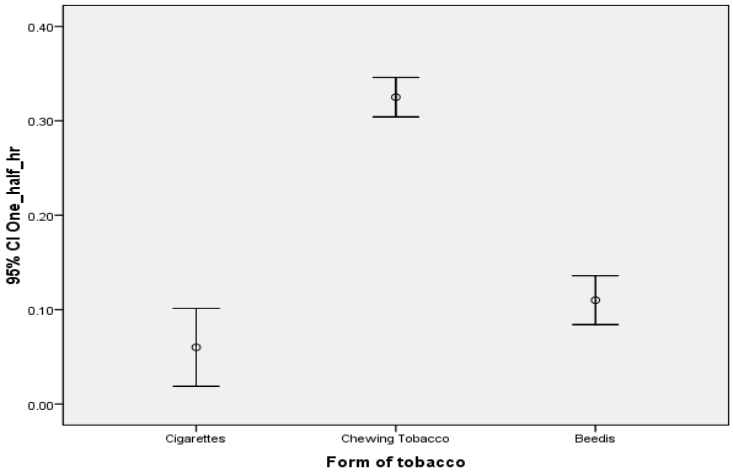

Box Diagram3- showing mean nicotine levels of the various forms of tobacco at the end of 90 mins

CONCLUSION

In spite of enforced education and prevention strategies, tobacco use remains a major health risk. Tobacco sales are known, but how much of nicotine is extracted and absorbed by individual users remains largely unknown. These measurements should be an integral part of the clinical treatment and management (dose-response relationship) of patients who wish to overcome tobacco dependence since nicotine replacement is one of the main pharmacological therapy for tobacco dependence. Serum nicotine values can be used for individualized nicotine therapy for successful outcome.

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