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ASSOCIATION OF SERUM COTININE LEVEL WITH CARDIOVASCULAR RISK FACTORS IN SMOKELESS TOBACCO USERS .

Physiology

Alka Srivastava Assistant Professor, Department of Physiology, HIMS Lucknow (U.P.); Corresponding Author.

Gaurav garg Assistant Professor, Department of Medicine, LLRM Medical College,Meerut (U.P.)

ABSTRACT

Nicotine is a major constituent of smokeless tobacco and its addiction in smokeless form is as dangerous as smoking. As burn product of nicotine is absent but absorption from buccal mucosa leads to accumulation of metabolites which affects the cardiac parameters. To assess these effects case control study was designed. 50 ST consumers (cases) and 50 age and sex matched tobacco non users (controls) were enrolled . Anthropometry , pulse rate, bp and E.C.G .were recorded and serum cotinine level was assessed .

Strong positive relation (r>0.7) was observed between S. cotinine levels and duration of tobacco use, SBP, DBP and MAP. All correlations were significant statistically (p<0.001). Moderate positive correlation (r=0.5 to 0.7) between S. cotinine levels and pulse rate and QTc interval was observed which is significant statistically (p<0.001).

KEYWORDS:

cotinine, ST-smokeless tobacco, SBP-(systolic blood pressure) DBP –(Diastolic blood pressure) MAP-(mean arterial pressure)

INTRODUCTION

The most recognized health risk associated with any tobacco product is carcinogenesis. However, tobacco has deleterious effects, on cardiovascular system also. It has been well established that cigarette smoking is strongly related to cardiovascular morbidity and mortality. In contrast, limited data are available concerning the effects of smokeless tobacco [ST][4,5]

Effect of smokeless tobacco consumption on cardiovascular events has been studied in detail in western population. Results from these studies paint a mixed picture with some showing increased incidence of these adverse events[6,9]while others showing no such association[10,20]. Similarly, contradictory results have been seen in studies evaluating increased risk factors for cardiovascular diseases in smokeless tobacco consuming population [2,12]. In India, limited studies have shown that tobacco chewing is associated with increased prevalence of cardiovascular risk factors like hypertension and ECG abnormalities as compared to non tobacco users[18,19].

Gutkha, a form of smokeless tobacco, is manufactured through a wide variety of processes and contain many additives. Some are added for flavour (sugar, nuts, spices, and oils), and some alkaline buffers are applied to increase the pH. These salts also enhance the level of unprotonated nicotine. Unprotonated or free base nicotine is more readily absorbed than protonated nicotine.

Nicotine is the major addicting substance in tobacco is metabolized in the liver by cytochrome P450 enzymes (mostly CYP2A6, and also by CYP2B6). A major metabolite is cotinine, concentration of which can be measured in urine and serum.13Since half life of cotinine is more than nicotine, the level of cotinine is used to assess the tobacco exposure. Because of the pharmacological properties of nicotine and other constituents of smokeless tobacco there is concern that smokeless tobacco products may lead to cardiovascular disease or death from cardiovascular causes.[3]

Blood pressure levels are affected by the high sodium content of smokeless tobacco. Licorice inhibits the metabolism of minera locorticoids and indirectly causes sodium retention, thereby increasing blood pressure levels.7Paper presented at the 56th Annual Meeting of the Biophysical Society (BPS), suggest that nicotine, no matter how it's delivered, can damage blood vessels.[14]

In short, nicotine modifies cell structure in a way that facilitates migration and invasion of cells that line the blood vessels. This enables a change in structures called podosomes, which lead to poor vessels and can cause the formation of plaque. Over time, plaque can cause arteries to harden, a form of heart disease called atherosclerosis. It can also block blood flow to the heart or brain, keeping oxygen from reaching those organs and causing heart attack or stroke.[14]

In view of all these facts this study is design to assess the effect of smokeless tobacco [mainly gutkha] on various cardiovascular parameters.

MATERIAL AND METHOD

SETTINGS

The study was conducted at KGMU Lucknow . Ethical clearance and informed consent was taken . 100 subjects were included in the study of which 50 were cases and remaining 50 were controls.

INCLUSION CRITERIA

1. Subjects of 20 to 60 years of age having history of tobacco chewing and gutkha eating (only smokeless tobacco).
2. Control group was comprised of age and sex matched subjects who did not use tobacco in any form

EXCLUSION CRITERIA

1. Age <20 and >60 years.
2. Smokers [cigarettes, beedi, hookah etc.]
3. Smokeless tobacco chewers but smokers too.
4. Chronic systemic disease.
5. Diabetes mellitus.
6. Obese persons (WHO Criteria: BMI)
7. Family history of hypertension and diabetes mellitus.
8. Pregnancy
9. Oral carcinoma

STUDY DESIGN

This is a case control study. The cases are exclusively smokeless tobacco users and controls did not consume tobacco in any form.

A working proforma (case sheet) was filled for every subject. It included demographic data [particulars of subject like name, age, sex, address, contact number], past history and family history of chronic illness. Further addiction history was taken which included type, amount, and duration of consumption of smokeless tobacco

ANTHROPOMETRIC MEASUREMENTS

Various anthropometric measurements were taken.

Weight measured on calibrated balance scale [within 100 grams] without heavy clothings.

Height measured by rigid stadiometer to the nearest centimetre while bare foot.

BMI was calculated as the weight in kg divided by meter square of height.

Waist to hip ratio is calculated.

CARDIOVASCULAR SYSTEM EXAMINATION –

1] Blood Pressure– Blood pressure was measured twice while subject was sitting, after 5 minutes of rest using a standardized random zero sphygmomanometer.
Pulse pressure = Systolic pressure – Diastolic pressure
Mean blood pressure= Diastolic pressure +1/3 pulse pressure

2] Pulse rate– After rest of 10 minutes, pulse rate was calculated. Three readings were taken and mean was recorded.

3] E.C.G.- E.C.G. of all subjects was recorded with the help of 12 lead E.C.G. machine. QT interval and RR interval is counted in lead II recording.

Corrected QT [QTc] –Calculated by
Bazett's formula: $QTC = QT / \sqrt{RR}$

Blood Sample collection –
After overnight fasting, 5 ml of blood samples were collected in a syringe from all the subjects in the morning. Serum was separated by centrifugation at 3000 rpm.

BIOCHEMICAL INVESTIGATION
SERUM COTININE LEVEL
Serum cotinine levels were measured by COTININE ELISA KIT [Blue Gene Biotech], Catalogue Number E01C0050.

RESULTS.
Table 1 shows comparison of haemodynamic parameters in two groups and were found to be higher in Group I as compared to Group II, however, the difference between two groups was not significant statistically for mean pulse pressure . For all the other parameters, the difference between two groups was significant statistically .mart in.jeff@hhp.ufl.edu

SN	Parameter	Group I (n=50)		Group II (n=50)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	Pulse	98.18	11.42	78.27	9.17	9.669	<0.001
2.	SBP	134.68	6.59	123.33	5.97	9.076	<0.001
3.	DBP	91.52	6.07	80.75	4.73	9.962	<0.001
4.	MAP	105.91	5.80	94.94	4.75	10.405	<0.001
5.	Pulse pressure	43.16	4.92	42.59	4.37	0.618	0.538
6.	QTc interval	0.44	0.01	0.42	0.01	8.028	<0.001

SBP –systolic blood pressure ,DBP- diastolic blood pressure ,MAP- mean arterial pressure
"p" is level of significance and "t" is student 't' test was used

Table 2 shows comparison of serum cotinine levels (ng/ml) between two groups. Serum cotinine levels ranged from 56.9 to 210.5 ng/ml in Group I and from 23.1 to 68.7 ng/ml in Group II. Mean serum cotinine level in Group I was 146.89±33.21 ng/ml and 38.66±10.66 ng/ml in Group II. Difference between groups were found significant statistically (p<0.001).

SN	Variable	Group I (n=50)	Group II (n=50)
1.	Minimum	56.9	23.1
2.	Maximum	210.5	68.7
3.	Mean	146.89	38.66
4.	SD	33.21	10.66

=22.14; p<0.001

Table 3 shows bivariate correlation between serum cotinine and other hemodynamic parameters Overall a strong positive relation (r>0.7) was observed between S. cotinine levels and duration of tobacco use, SBP, DBP and MAP. All correlations were significant statistically too (p<0.001). A moderate positive correlation (r=0.5 to 0.7) between S. cotinine levels and pulse rate and QTc interval was observed which is significant (p<0.001). A mild positive correlation between S. cotinine levels and age was observed (r=0.302; p=0.002). All the other correlations were weak (r<0.3).

SN	Parameter	Overall (N=100)		Tobacco users (n=50)		Non Users (n=50)	
		r	p	r	p	r	P
1.	Pulse	0.673	<0.001	0.308	0.030	-0.509	<0.001
2.	SBP	0.742	<0.001	0.462	0.001	0.457	0.001
3.	DBP	0.790	<0.001	0.591	<0.001	0.296	0.035
4.	MAP	0.803	<0.001	0.587	<0.001	0.388	0.005
5.	Pulse pressure	0.050	0.619	-0.110	0.445	0.303	0.031
6.	QTc interval	0.675	<0.001	0.335	0.017	0.326	0.019

r is Pearson correlation coefficient
Among cases strong positive correlation between S. cotinine levels and duration was observed. A moderate positive correlation between S. cotinine levels and age, DBP and MAP was observed. A mild positive correlation between S. cotinine levels and pulse rate, SBP and QTc intervals was observed. A mild negative correlation between S. cotinine levels and BMI was observed. All the other correlations were weak (r<0.3).

Among non-users, moderate positive association between S. cotinine levels and age was observed whereas moderate negative correlation between S. cotinine levels and pulse rate was observed. A mild positive correlation between S. cotinine levels and SBP, MAP, Pulse pressure and QTc interval, was observed.Rest correlations were weak (r<0.3).

DISCUSSION
Here statistically no significant difference was found between two groups for any demographic characteristic. High mean serum cotinine level was found in cases (146.89±33.21 ng/ml) as compared to controls (38.66±10.66 ng/ml).

Mean duration of smokeless tobacco use was 17.34±7.64 years, maximum cotinine level was 178.04 ng/ml for >20 years of duration and minimum level was found to be 85.58 ng/ml for <5 years duration. Significant association was observed between higher mean serum cotinine level and longer duration of use (p<0.001). A strong positive relation was observed with duration of tobacco use.

Longer duration of ST exposure resulted in high level of serum cotinine levels. Cotinine is accumulated in hair, brain and probably other parts of body.11On comparing the daily intake of ST amount and mean serum cotinine level no significant association was found whereas Siegel *et al.*[15] suggested that nicotine intake may be more efficient with shorter periods of daily use than with longer periods of use.

Since nicotine is a water soluble chemical, most of its daily consumed amount (80-90%) is excreted out leaving small amount in the body. Therefore, longer duration of ST exposure leads to accumulation of this amount. It could be a probable explanation of our finding. Secondly the difference in result could be due to difference in type of ST used by the subject. Our findings were based on gutkha consumption, which contain different concentration of tobacco in different brands, whereas findings of Siegel *et al.*[15] were based on snuff and chewing tobacco.

In the control group mean serum cotinine level was 38.66±10.66 ng/ml. Maximum level was 68.7 ng/ml and minimum level was 23.1 ng/ml. Since our controls were not using tobacco in any form, it is assumed that the level above the expected value was due to passive exposure of tobacco smoke. It is also in accordance with the significant association of cotinine level with age (p<0.001).

A significant association is found between mean serum cotinine level and blood pressure in both groups. After exclusion of various risk factors of hypertension 24 subjects had increased systolic as well as diastolic blood pressure. The mean cotinine level were higher in cases. Statistically significant association is found between mean serum cotinine level and MAP and pulse rate.

Similar results were found in studies done by Siegel *et al.*[20] who documented higher diastolic blood pressure in ST users. In study of

Westman EC[17], it was observed that ST use has been associated with raised systolic blood pressure, raised diastolic blood pressure, tachycardia and finally chronic hypertension. Also Bolinder G[6] and Arabi Z[1] reported strong positive correlation between cotinine level and blood pressure. Gupta B K *et al.*[8] also reported hypertension in ST users.

ECG recording of both the groups were analysed and QTc interval was compared. Mean value in cases was 0.44±0.01 which is higher than control (0.42±0.01). The difference was found statistically significant. A moderate positive correlation was also found with mean serum cotinine level.

Gupta BK *et al*.[8] reported similar findings in ST users. They observed ECG changes and resting tachycardia. These findings were also supported by the study of Sougut O *et al*.[16] who reported paroxysmal atrial fibrillation in ST users.

Above findings could be explained by the fact that nicotine after absorption binds to acetylcholine receptors on endothelial cell surface where it promotes atherogenesis thrombotic and vascular occlusion by promoting formation of plaque in vessel wall. It causes direct injury to endothelial cells which act as a nidus for plaque formation.[14]

The present study on smokeless tobacco is based especially on gutkha consumption. High serum cotinine levels were found in gutkha consumers as compared to non users. Also these values correlate positively with various cardiovascular risks viz. chronic hypertension, resting tachycardia and prolonged QTc interval. These findings support many of the previous studies done on snuff users and tobacco chewers.[12,15, 17]Therefore, consumption of gutkha on a long term basis imposes serious threat to overall health precisely to cardiovascular health.

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