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Effect of Smokeless Tobacco Consumption on Lipid Profile.

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ABSTRACT

Tobacco consumption is a greater source of mortality and morbidity. About 35 to 40 % of tobacco consumption in India is in smokeless forms. Tobacco in any form increases serum cotinine level. The effect of increased serum cotinine level on lipid profile was studied in adult male rural population. Studies have shown increased prevalence of cardiovascular disease risk factors like increased in triglyceride, VLDL and decreased in HDLc in study group when compared to control group. Age matched adult male of rural population in Western Maharashtra between 22 to 56 years of age were included in the study. After estimation of serum cotinine level, study group were further divided into three sub groups according to tobacco chewing duration (years), frequency (perday) and serum cotinine level. (ng/ml) Lipid profile of the study and control group was determined and compared statistically. Out of 175 subject, 95 were tobacco chewers (study group) and 80 were tobacco non-chewers (control group). Triglyceride and VLDL were significantly higher ($p<0.05$) and HDLc was found significantly decreased ($p<0.05$, $p<0.01$, $p<0.001$) in study group with respect to increased tobacco chewing duration, frequency and serum cotinine level as compared to control group. There was significantly and progressively increase in triglyceride and VLDL and decrease in HDLc in tobacco chewers according to tobacco chewing duration, frequency and serum cotinine level as compared to control group.

Keywords: adult male, serum cotinine, lipid profile, smokeless tobacco.

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INTRODUCTION

Smokeless Tobacco (ST) is an extremely addictive substance with a high rate of use in certain demographic groups. [1]. The Global Adult Tobacco Survey (GATS) showed current tobacco use in any form is 34.6% in adults. Tobacco smoking is 14% while use of smokeless tobacco is 25.9% of adults in the world [2]. Prevalence of ST users in India is 28.1% in men and 12 % in women [3]. Globally the use of smokeless tobacco has gained popularity. The smokeless tobacco is mainly used orally. Tobacco leaf production has been increasing steadily for many decades and has doubled since the 1960s [4]. About 35 – 40% of tobacco consumption in India is in smokeless forms mostly of the species Nicotiana rustica while most smoking tobacco is Nicotiana tabacum [5, 6]. Samples of N.rustica have been found to contain higher concentrations of tobacco specific nitrosamines than N. tabacum [7].

In India, the use of smokeless tobacco is common. The various forms of tobacco are chewed, sucked or applied to teeth and gums. [8]. India is a low income country, and death in middle age person increases in tobacco related deaths. The disease burden, health care cost as well as other fiscal losses resulting from premature deaths attributable to tobacco consumption will rapidly increase. [9]. Nicotine in smokeless tobacco is what gives users a buzz. It also makes very hard to quit. Every time smokeless tobacco is used, the body gets used to the nicotine and starts to crave for it. Craving is one of the signs of addiction. This is when the body adapts to the amount of tobacco needed to get a buzz. With continued use, more and more tobacco is needed to get the same feeling. Many smokeless tobacco users say it is harder to quit smokeless tobacco than cigarettes [10].

Nicotine is a principle component of all forms of tobacco use. Nicotine possesses considerable risk of diseases like CVD and cancers. Cotinine is the major metabolite of nicotine and has a much longer half-life than nicotine. For this reason cotinine is widely used as a biochemical marker of average daily intake of nicotine [11]. Tobacco is also associated with an increased risk of developing type II diabetes, which in turn is an important cardiovascular risk factor. Cigarette and smokeless tobacco is associated with changes in blood lipids, resulting in an atherogenic risk profile – primarily low HDL cholesterol. Nicotine increases lipolysis and increases free fatty acid concentrations. Increased fatty acid turnover is associated with overproduction of VLDL, triglycerides, LDLc, and lowered HDLc. In summary, multiple mechanisms are likely to contribute to tobacco induced CVD [12].

Present work is an attempt to determine the alteration in fasting serum triglyceride, total cholesterol, LDLc, VLDL, HDL and the atherogenic indices as indicated by various risk ratios in a group of tobacco chewers. In addition we have determined the serum cotinine level in both groups and the possible interaction of serum cotinine with lipid profile.

MATERIALS AND METHODS

The present comparative study was carried out in the department of Physiology and Biochemistry, Krishna Institute of Medical Sciences Deemed University, Karad during 2012–2013. Study protocol was approved by Institutional Ethical Committee of KIMSDU, Karad. Apparently healthy tobacco chewers and tobacco non-chewers class four male employees of tertiary health care center of age group between 22 to 56 years were included in the study. The subjects having any serious disorder like hypertension, cardiovascular diseases, cancer, any other marked disability or any major endocrinological disorders were excluded from the study. Experimental protocol was fully explained to all eligible subjects and written consent was obtained from subjects expressed willingness to participate in the study. Detailed medical history, family history and personal history with special reference to the history of tobacco consumption at present and past was recorded in structured pro-forma.

Total 175 subjects participated in present study. They were categorized into tobacco chewers (study group) and tobacco non chewer (control group). Serum cotinine level was estimated by cotinine ELISA CALBIOTECH kit method on Elisa reader [13]. Participants from study group were further divided into sub groups according to tobacco chewing duration in years, tobacco chewing frequency per day and serum cotinine concentration level (ng/ml).

Collection of Blood Sample

5ml venous blood samples were collected after an overnight fast of 12 to 14 hours, in plain bulb. After two hours samples were centrifuged at 3000 rpm for 5 minutes, serum from plain blood was separated. Serum was used for estimation of lipid profile fractions and cotinine level.

Investigation

All biochemical parameters were measured by using Erba Mannheim XL system packs [14].

- Serum total cholesterol was measured by CHOD-PAP method.
- Serum triglyceride was measured by GPO method.
- Serum HDL cholesterol was measured by Immunoinhibition method.
- LDL c was estimated by using Fredrickson-Fried-Wald formula.

The tests were carried out according to the manufacturer's instructions. Measurements of serum total cholesterol, triglycerides and HDL cholesterol were done on the ERBA 360 fully automated analyzer [GERMANY] [15].

STATISTICAL ANALYSIS

Descriptive statistics (minimum, maximum, mean and standard deviation) was determined for each study variable. Comparison of study variables between tobacco chewers and tobacco non- chewers was done by using unpaired t test. ANOVA test was used for comparison of inter-groups of study variables. In case of significant F value, Tukey Kramer multiple comparison test, the post-hoc test was applied. p value less than 0.05 was considered as significant.

RESULTS

Table 1:Tobacco chewing duration wise comparison of lipid profile in tobacco chewers and tobacco non chewers.

Sr. No	Parameters	Control Group tobacco non-chewers	Study group (Tobacco chewing duration in years)			ANOVA F value (p value)
		(N=80) M±SD (Min-Max)	0-10 Yrs (N=30) M±SD (Min-Max)	11-20 Yrs (N=33) M±SD (Min-Max)	21-30 Yrs (N=32) M±SD (Min-Max)	
1	Total cholesterol (mg/dl)	163.88±33.73 (89-232)	156.57±37.61 (98-265)	162.63±28.48 (104-230)	170.14±33.26 (119-227)	0.626 (0.599)
2	Triglyceride (mg/dl)	123.38±41.67 (64-269)	123.33±39.21 (65-181)	140.20±60.34 (59-339)	150.96±68.47* (80-370)	2.35 (0.074)
3	LDLc (mg/dl)	100.24±30.61 (36-162)	91.97±31.26 (42-153)	100.39±23.47 (50-159)	106.73±31.69 (61-166)	0.939 (0.423)
4	VLDLc (mg/dl)	24.81±8.27 (12.8-53.8)	24.68±7.89 (13-36.2)	28.02±12.08 (11.8-68)	30.14±13.65* (16-74)	2.223 (0.008)
5	HDLc (mg/dl)	38.29±6.10 (24.3-57)	38.69±6.37 (25-50)	34.56±5.47** (24.5-43)	34.14±5.89*** (22-46)	5.691 (0.001)
6	Cotinine (ng/ml)	0.23±1.0 (0-5)	209.43±154.20*** (18-600)	196.70±107.5*** 6(10-457)	212±90.51 *** (35-417)	80.01 (<0.001)

Significance by Tukey Kramer multiple comparison test * p< 0.05, ** p<0.01, ***p<0.001.

We found no significant difference in total cholesterol & LDLc in tobacco chewers group as compared to tocbacco non chewers according to duration of tobacco chewing in years.

TG and VLDLc were significantly high (Tukey Kramer p<0.05) in study group with tobacco chewing duration 21 to 30 years as compared to controls, while HDLc was significantly decreased (Tukey Kramer

p<0.001) in study group with tobacco chewing duration 11 to 20 years and very significantly decreased (Tukey Kramer p<0.001) in study group with tobacco chewing duration 21 to 30 years as compared to controls.
Serum cotinine level was significantly high (Tukey Kramer p<0.001) in all study groups according to tobacco chewing duration as compared to controls.

Table 2: Tobacco chewing frequency wise comparison of lipid profile in tobacco chewers and tobacco non chewers.

Sr. No	Parameters	Control Group	Study group			ANOVA F value (p value)
			tobacco chewing frequency per day			
		tobacco non-chewers (N=80) M±SD (Min-Max)	1-4 times/day (N=31) M±SD (Min-Max)	5-7 times/day (N=34) M±SD (Min-Max)	8 -10 (& above) times/day (N=30) M±SD (Min-Max)	
1	Total cholesterol (mg/dl)	163.88±33.73 (89-232)	163.89±39.86 (106-230)	160.67±32.54 (98-265)	164.91±31.50 (104-224)	0.110 (0.954)
2	Triglyceride (mg/dl)	123.38±41.67 (64-269)	134.44±52.17 (65-330)	138.53±67.71 (59-339)	157.67±44.32* (115-250)	1.734 (0.162)
3	LDLc(mg/dl)	100.24±30.61 (36-162)	98.95±31.09 (57-159)	97.66±27.70 (42-154)	101.89±29.05 (50-166)	0.125 (0.945)
4	VLDLc(mg/dl)	24.81±8.27 (12.8-53.8)	26.88±10.41 (13-74)	27.67±13.56 (11.8-68)	31.55±8.84* (23-50)	1.632 (0.184)
5	HDLc (mg/dl)	38.29±6.10 (24.3-57)	36.16±5.83 (24-47.7)	35.30±6.54* (22-50)	33.36±5.96* (24.5-41.1)	3.324 (0.021)
6	Cotinine(ng/ml)	0.23±1.0 (0-5)	73.11±68.67** (10-213)	191.65±91.93*** (35-457)	252.82±123.42*** (74-600)	117.30 (<0.001)

Significance by Tukey Kramer multiple comparison test * p< 0.05, ** p<0.01, ***p<0.001.

There was no significant difference in total cholesterol, LDLc in tobacco chewers as compared to tobacco non chewers according to tobacco chewing frequency per day. Triglyceride and VLDL were found significantly high (Tukey Kramer p<0.05) in study group with frequency of tobacco chewing 8 to 10 times / day.

Table 3: Serum cotinine level wise comparison of lipid profile in tobacco chewers and tobacco non chewers.

Sr. No	Parameters	Control Group tobacco non-chewers	Study group of tobacco chewing according to cotinine concentration			ANOVA F value (p value)
		(N=80) M±SD (Min-Max)	10-200ng/ml (N=41) M±SD (Min-Max)	201-400 ng/ml (N=32) M±SD (Min-Max)	401-600 ng/ml (N=22) M±SD (Min-Max)	
1	Total cholesterol (mg/dl)	163.88±33.73 (89-232)	166.20±35.60 (98-265)	157.84±27.72 (111-224)	166.83±37.30 (104-213)	0.421 (0.738)
2	Triglyceride (mg/dl)	123.38±41.67 (64-269)	128.25±57.64 (65-339)	142.73±54.43* (59-370)	168.83±83.73* (72-306)	2.509 (0.061)
3	LDLc (mg/dl)	100.24±30.61 (36-162)	102.74±31.63 (42-159)	95.61±21.92 (52-166)	99.76±37.17 (59-151.5)	0.354 (0.787)
4	VLDLc (mg/dl)	24.81±8.27 (12.8-53.8)	25.63±11.56 (13-68)	28.53±10.87* (11.8-74)	33.70±16.74* (14-61)	2.390 (0.071)
5	HDLc (mg/dl)	38.29±6.10 (24.3-57)	36.55±6.75 (25-50)	34.92±5.44** (24-47.7)	33.45±7.44*** (22-43)	3.474 (0.018)

Significance by Tukey Kramer multiple comparison test * p< 0.05,** p<0.01,***p<0.001.

HDLc was found significantly decreased (Tukey Kramer p<0.05) in study group with frequency of tobacco chewing 5 to 7 times / day and 8 to 10 times / day as compared to controls. Serum cotinine level found significantly high (Tukey Kramer p<0.001) and increasing with increased tobacco chewing frequency in study group as compared to controls.

There was no significant difference in total cholesterol and LDLc in tobacco chewers as compared to tobacco non chewers according to serum cotinine level. TG and VLDL found significantly higher (Tukey Kramer

p< 0.05) in study group with cotinine level 201 to 400 ng/ml and 401 to 600 ng/ml as compared to control group.

HDLc was found significantly decreased (Tukey Kramer p<0.01) in study group with cotinine level 201 to 400 ng/ml and very significanly decreased (Tukey Kramer p<0.001) in study group with cotinine conc level 401 to 600 ng/ml as compared to controls.

DISCUSSION

Tobacco chewing in any form is one of the leading public health problems in the world and especially in India. When a smokeless tobacco is chewed, nicotine is absorbed from the tobacco and spread throughout the body via the blood stream in seconds. Cotinine is a major metabolite of nicotine and have longer half-life than nicotine [16] it reflects tobacco exposure very clearly. For this reason cotinine is widely used as a biochemical marker of average daily intake of nicotine (tobacco) [17]. Smoking or oral use of tobacco introduces nicotine into the circulation. It was previously shown that tobacco induces an enhancement of circulating adrenaline and nor-adrenaline levels, which was associated with an increased lipolysis [18]. Nicotine effect on cardiovascular disease is by affecting lipid metabolism, coagulation and hemodynamic status [19].

In present study the effect of tobacco consumption shows association between higher serum cotinine level with increased triglycerides and VLDL whereas decreased HDLc. Whereas no significant change observed in total cholesterol and LDL in tobacco chewers group when compared with tobacco non chewers. It was found that triglyceride and VLDL progressively increased according to tobacco chewing duration in years. TG and VLDL were significantly increased (Tukey Kramer p<0.05) in study group for duration of 21 to 30 years in tobacco chewers as compared to tobacco non-chewers. Serum cotinine level is significantly high (Tukey Kramer p<0.001) in tobacco chewers according to tobacco chewing duration of 0 to 10 years, 11 to 20 years and 21 to 30 years as compared to tobacco non chewers (Table No 1).

Also it was found that the triglyceride and VLDLc progressively increased and HDLc progressively decreased in tobacco chewers according to tobacco chewing frequency per day. TG and VLDL were found significantly high (Tukey Kramer p<0.05) in tobacco chewers group of 8 to 10 times / day of tobacco chewing frequency. HDL significantly decreased (Tukey Kramer P<0.05) in tobacco chewers group of 5 to 7 times and 8 to 10 times per day of tobacco chewing frequency. Whereas serum cotinine level was progressively and significantly increased in tobacco chewers according to tobacco chewing frequency (Tukey Kramer p<0.001) (Table No. 2).

Triglyceride and VLDL progressively increased and it was highly significant (Tukey Kramer p<0.001) in tobacco chewers according to serum cotinine level. Whereas HDLc was decreased highly significant (Tukey Kramer P<0.01) in tobacco chewers group according to cotinine level 201 to 400 ng/ml and (Tukey Kramer p<0.001) in tobacco chewers group according to cotinine level 401 to 600 ng/ml as compared to tobacco non chewers (Table No. 3). Brischetto et al [20] proposed a mechanism to explain dyslipidemia in tobacco users. Nicotine stimulates release of adrenaline by the adrenal cortex, leading to the increased serum concentration of free fatty acids observed in smokers and tobacco users. Free fatty acids are well known stimulus of hepatic secretion of VLDL and hence TG. HDLc concentration varies inversely with VLDL concentration in serum. Any type of tobacco use is associated with changes in blood lipids, resulting in atherogenic risk profile-primarily low HDLc cholesterol [21].

Serum nitric oxide concentration is increased in tobacco users and smokers, which may be involved in the development of vascular diseases [22]. Tobacco is one of the most important exogenous factors, which cause three fold higher incidence of oxidative stress in tobacco users. Free radical mediated oxidative stress appears to play a central role in tobacco mediated athrothrombotic diseases. The atherogenic effects of tobacco are mediated in part by free radical damage to lipids. Which may manifest itself as coronary heart disease, atherosclerosis and cancer [23].

Human studies have demonstrated that if nicotine is administered orally to non smokers, this will result in change in the plasma concentration of triglycerides [24]. Tobacco use exerts effect on lipids at least in part, by the sympathomimetic effects of nicotine. Nicotine increases lipolysis and increases free fatty acid concentrations [25]. Increased fatty acid turnover is associated with overproduction of VLDL and triglycerides,

increased LDLc and lowered HDLc. Thus the tobacco use develops the dyslipidemia. Two recent studies showed that the tobacco use in any form have similar effects on lipid profile and therefore, raising cardiovascular risk in the same proportion [26]. Also the Framingham offspring study showed that smoking was significantly associated with lower HDLc levels of 4 mg/dl in men and 6 mg/dl in women [27].

Non HDLc cholesterol has been found to be a better tool for screening and assessing the risk for atherosclerosis [28]. In the present study the level of non HDLc cholesterol level was significantly elevated in tobacco chewers when compared to controls. The constellation of these altered lipoproteins suggests that ST chewers at a high risk for development of coronary heart disease. In the present study the effect of Tobacco consumption showed an association with higher serum cotinine level in all tobacco chewers when compared with Tobacco non-chewers ($p<0.001$).Our finding suggest that thobacco chewing alters the lipid profile adversely & causing dislipidemia in ST users and the alteration become more significant with the increased tobacco chewing duration in years, tobacco chewing frequency per day and serum cutinie level.

Mero N et al [29], Mathew S et al [30], Yusuf et al [31] Hazarika N c et al [32] where supported the present study, that nicotine affects lipid metabolism and causes increase in non HDL cholesterol and decreases HDL cholesterol resulting in cardiovascular diseases. Arslan et al [33] reported increased TG, LDLc and VLDLc levels and decreased HDL level which was consistent with our findings. However some studies have not showing the similar effects of tobacco chewing on lipid profile like present study [34]. This could be due to difference in study population, study design, composition of smokeless tobacco used and difference in predisposition to lipid profile in population of different origin.
Our findings was similar with the findings of Campbell SC et al [35].

This is a small scale study, however to confirm these findings more detailed and large scale study is required. It appears that there is increased need of education to the rural population for making them aware about cancer and cardiovascular risk factors associated with tobacco consumption.

CONCLUSION

An increased prevalence of triglyceride, VLDL and decreased level of HDLc is seen in tobacco chewer adults male. Smokeless tobacco produces adverse effects on lipid profile and the changes become more significant with the increased tobacco chewing duration in years, frequency per day and serum cotinine level.

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