

A STUDY OF EFFECTS OF SMOKING ON LIPID AND VITAMIN C METABOLISM. A PILOT STUDY IN CENTRAL BIHAR.**KSHITISH KUMAR KSHITIZ*¹ , RAM BINAY SINHA² AND JAYASHREE BHATTACHARJEE¹**¹ Department of Biochemistry, Lady Hardinge Medical College, New Delhi-110001, India.² Department of Biochemistry, Patna Medical College, Patna -800020, India.** Corresponding Author* drkshitiz08@yahoo.co.in**ABSTRACT**

Smoking is a established cardiovascular disease risk factor. But the mechanism how it cause cardiovascular risk is still not properly understood. This study was aimed to evaluate lipid and vitamin C metabolism in chronic smokers from central Bihar, India for public enlightenment. 60 male cigarette smokers and 20 male non-smokers were enrolled. It was found that mean serum Triglycerides (TG), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C) and urinary vitamin C were significantly higher in smokers as compared to non-smokers. Mean serum High density lipoprotein cholesterol (HDL-C) was significantly lower in smokers as compared to non-smokers. All these adverse effects may lead to serious metabolic diseases like atherosclerosis.

KEY WORDS

Smoking, Lipid profile, coronary Heart Disease, Cholesterol, vitamin C.

INTRODUCTION

Cigarette smoking is the most common type of tobacco use . By 2030, if current trends continue, smoking will kill more than 9 million people annually ¹

Smoking is considered as a major cardiovascular risk factor ² .There is a dose-response relationship between the number of cigarettes smoked and cardiovascular morbidity and mortality ³ . The mechanism by which cigarette smoking causes atherosclerosis remains obscure, but cigarette smoking have been found to alter the level of lipoproteins ⁴⁻⁸ . Plasma lipoprotein abnormalities are said to be the underlying major risk factors and may even be essential

for the common occurrence of atherosclerotic vascular diseases ⁹ .

Nicotine stimulates sympathetic adrenal system leading to increased secretion of catecholamine resulting in increased lipolysis and increased concentration of plasma free fatty acids (FFA) which further result in increased secretion of hepatic FFAs and hepatic triglycerides along with VLDL-C in the blood stream ⁵ . These changes contribute to the atherosclerotic potential of cigarette smoke.

Blood of cigarette smokers routinely displays decreased antioxidant capacity and increased oxidized lipids compared to non-smokers ¹⁰ . Oxidative pathway appears to be one important mechanism for modifying LDL,

because a wide variety of structurally unrelated antioxidants inhibit atherosclerosis in animal models of hypercholesterolemia¹¹. Evidence suggests that oxidatively modified LDL contribute

to the pathogenesis of atherosclerosis¹². Cigarette smoke contains approximately 10¹⁷ oxidant molecules per puff¹ that can cause damage to lipids, proteins, DNA, carbohydrates, and other bio-molecules¹³. It is becoming increasingly evident that a pro-oxidant / antioxidant imbalance largely contributes to atherosclerosis processes¹⁴. It has been postulated that many of the adverse effect of smoking may result from oxidative damage to critical biological substances¹⁵. Previous reports have demonstrated abnormal endothelial function in chronic smokers¹⁶. Endothelial dysfunction in turn has been proposed to play a pathogenic role in the initiation of vascular disease¹⁷. Although smoking induced endothelial dysfunction is very likely multifactorial, more recent clinical and experimental observations strongly point to a potential role of oxygen derived free radicals in mediating this phenomenon¹².

In vivo, antioxidant nutrients, including vitamin C play crucial roles in defending against oxidant damage¹³. Vitamin C protects against oxidation of isolated LDL by different types of oxidative stress, including metal ion dependent and independent processes¹⁸.

Preliminary reports have indicated adverse affects of smoking on vitamin C metabolism¹⁹. Cigarette smoking is associated with decreased serum vitamin C concentration^{13,14,15,19} and this association was seen despite correction for factors which independently affected serum vitamin C levels such as age, gender, race, and BMI¹⁹. Changes in serum concentration of vitamin C in chronic smokers might be due to changes in urinary excretion of vitamin C. But no study to date has confirmed

it. It is interesting to study the effects of smoking on urinary excretion of vitamin C to get some clue to decreased serum concentration of vitamin C among them.

India is a very large and vast country with much regional variation. Currently Bihar, an Indian state, is facing epidemiological and economic transition with the extension of chronic diseases notably cardiovascular and smoking habit. To date no data is available regarding lipid profile and vitamin C status of smokers from Bihar. Since dietary and environmental factors influence lipid profile and vitamin C status, this study was undertaken to evaluate lipid profile and urinary excretion of vitamin C of chronic smokers in Bihar, India for further translation of the results for public awareness.

MATERIAL AND METHOD

The study was conducted on 60 healthy male cigarette smokers in age group of 25-35 years and it was compared with 20 healthy age, diet and Body mass index(BMI) matched non smokers selected from patient attendants, undergraduates, postgraduates and paramedical staffs. Informed consent was obtained from all subjects. Only those persons were included in the study, who had no other existing diseases. Persons taking vitamin C or any other antioxidants were also not included in the study. Persons smoking 5 cigarettes or more per day continuously for a year were considered as smokers. Smokers were divided into 3 different categories according to the no. of cigarettes smoked per day. A detailed physical examination of the subjects of both groups was done.

After 12 hrs overnight fasting, venous blood samples were drawn and subjected to laboratory investigations including blood glucose, blood urea, serum alkaline phosphatase, haemogram and lipid profile. Just before taking the blood sample, the subjects were instructed to collect about 15-20 ml urine in a dry beaker. It was used for the estimation of urinary vitamin C. Various lipid fractions were done by enzymatic method using kits manufactured by Randox

Laboratories Ltd., Antrim, U.K on an auto-analyzer of Beckman Coulter model, 'Synchron-CX-9.'

For estimation of total cholesterol and HDL-cholesterol CHOD-PAP method which is an enzymatic method were used. Serum triglyceride was estimated by GPO-PAP method. LDL-C & VLDL-C were determined by calculation method. For the estimation of urinary vitamin enzymatic C, a method given by Harris and Ray²⁰, was used. Each urine sample was immediately titrated after collection, to prevent loss of ascorbic acid

on standing as a result of oxidation of ascorbic acid to dehydro-ascorbic acid.

Statistical Analysis

All variables are shown as the mean \pm standard error of mean (SEM). The data between control and test groups was compared using unpaired student's t test. Correlation was determined by Pearson's correlation coefficient. A two tailed p value less than 0.05 was considered to be statistically significant. All analyses were performed using SPSS (version 17.0).

RESULTS

Table 1
Showing clinical characteristics (Mean \pm SEM) in non-smokers and smokers.

Parameters	Non –smokers (n= 20)	Smokers (n= 60)
Age (yrs)	31.20 \pm 1.33	31.23 \pm 1.35
Body weight (kg)	58.90 \pm 1.91	59.20 \pm 1.91
Height (cm)	167.40 \pm 1.1	167.90 \pm 0.90
BMI (kg/m ²)	20.96 \pm 0.60	20.99 \pm 0.60

All parameters were not significantly different between groups.

Table 2
Showing lipid profile and urinary excretion of vitamin C (Mean \pm SEM) in smokers in comparison to non-smokers.

Tests	Non smokers (n=20)	Smokers (n=60)	p value
TG(mg/dl)	123.95 \pm 6.55	143.37 \pm 4.66	0.032*
TC(mg/dl)	159.10 \pm 4.74	175.21 \pm 3.33	0.013*
HDL-C(mg/dl)	50.00 \pm 1.88	42.33 \pm 1.16	0.001*
LDL-C(mg/dl)	84.31 \pm 4.29	104.21 \pm 4.04	0.009*
VLDL-C(mg/dl)	24.79 \pm 1.31	28.67 \pm 0.93	0.033*
Urinary Vitamin C(mg/dl)	1.00 \pm 0.06	1.69 \pm 0.06	0.000*

*P value <0.05 is considered as statistically significant.

Table 3
Showing lipid profile and urinary excretion of vitamin C (Mean \pm SEM) in relation to no. of cigarettes smoked per day.

Tests	5- 10 Cigarette/ Day (n=20)		P value	>15 cigarettes/ Day(n=20)	
	(a)	(b)		(c)	(a)-(c)
TG(mg/dl)	134.37 \pm 4.64	143.38 \pm 4.71	0.181	152.34 \pm 5.19	0.014*
TC(mg/dl)	167.45 \pm 5.09	173.60 \pm 5.93	0.436	184.65 \pm 6.27	0.039*
HDL(mg/dl)	44.03 \pm 1.41	42.80 \pm 1.55	0.562	40.20 \pm 0.95	0.030*
LDL(mg/dl)	96.55 \pm 4.51	102.13 \pm 5.41	0.433	113.99 \pm 5.63	0.020*
VLDL(mg/dl)	26.87 \pm 0.92	28.67 \pm 0.94	0.181	30.46 \pm 1.03	0.013*
Urinary vitaminC(mg/dl)	1.39 \pm 0.09	1.67 \pm 0.11	0.073	2.01 \pm 0.11	0.000*

*P value <0.05 is considered as statistically significant

Table 4
Showing lipid profile and urinary excretion of vitamin C in smokers in relation to number of cigarettes and duration of smoking

Tests	5-10 cigarettes(n=20)			11-15 cigarettes(n=20)			> 15 cigarettes(n=20)		
	1-10 Yrs (n=10)	> 10 Yrs (n=10)	p value	1-10 Yrs (n=10)	> 10 Yrs (n=10)	p value	1-10 Yrs (n=10)	> 10 Yrs (n=10)	p value
	Mean \pm SEM	Mean \pm SEM		Mean \pm SEM	Mean \pm SEM		Mean \pm SEM	Mean \pm SEM	
TG(mg/dl)	126.15 \pm 6.72	142.60 \pm 7.98	0.132	135.28 \pm 6.41	151.46 \pm 7.48	0.118	143.00 \pm 6.83	161.70 \pm 6.57	0.0641
TC(mg/dl)	158.93 \pm 7.64	176.00 \pm 7.80	0.135	163.44 \pm 7.60	183.68 \pm 7.52	0.074	176.25 \pm 7.77	193.05 \pm 7.93	0.147
HDL(mg/dl)	46.13 \pm 1.86	41.93 \pm 1.42	0.090	44.80 \pm 2.05	40.80 \pm 1.70	0.151	41.60 \pm 1.33	38.80 \pm 1.22	0.139
LDL(mg/dl)	87.57 \pm 5.14	105.55 \pm 5.96	0.034*	91.59 \pm 5.98	112.59 \pm 6.38	0.027*	106.05 \pm 5.46	121.91 \pm 5.12	0.048*
VLDL(mg/dl)	25.23 \pm 1.34	28.52 \pm 1.59	0.132	27.05 \pm 1.28	30.29 \pm 1.49	0.117	28.60 \pm 1.61	32.34 \pm 1.56	0.114
Urinary vitaminC(mg/dl)	1.28 \pm 0.09	1.50 \pm 0.11	0.154	1.50 \pm 0.14	1.84 \pm 0.15	0.134	1.83 \pm 0.13	2.19 \pm 0.15	0.101

*P value <0.05 is considered as statistically significant.

DISCUSSION

In the study, the two groups of subjects (smokers and non-smokers) were of comparable sex, age, BMI and diet. They were non-diabetic, non-alcoholic, normotensive subjects, mostly (70%) belonging to middle socioeconomic status. Serum TG, TC, LDL-C, & VLDL-C are significantly higher in smokers as compared to non-smokers. The serum anti-atherosclerotic HDL level is significantly lower in smokers as compared to non-smokers. The above findings are in accordance with the findings of most of workers worldwide⁴⁻⁸. The change in the serum lipoprotein levels became more marked with the number of cigarettes & duration of smoking. This finding has been substantiated by N S Neki⁵. Contrary to the above findings Diricana M et al did not find significant differences in serum TC, TG, LDL-C and HDL- and serum-free and- ester cholesterol levels between smokers and non-smokers²¹. Contrary report to this has also been documented by Nesje LA et al²² who found no significant difference between smokers and non-smokers concerning triglycerides and total cholesterol.

Dyslipidemia is a well-established risk factor for the development of coronary artery disease²³. Our study demonstrated presence of dyslipidemia in chronic smokers. Increased levels of triglyceride, total cholesterol, LDL-C and lower HDL-C in smokers points to coronary artery disease risk.

The biological mechanism linking smoking and atherogenesis, the process leading to cardiovascular diseases, is complex and not fully understood. The current opinion is that atherosclerosis is an immune/ inflammatory response of the intima to endothelial injury^{24,25}. It is also well known that the injury is mainly initiated by lipid accumulation.^{25,26} Native plasma lipids, in particular native LDLs, can freely enter the intima and are taken up by vascular cells via LDL receptor-mediated endocytosis. Nevertheless, they do not primarily initiate an inflammatory response, they are not phagocytosed by

monocytes, and they do not initiate atherosclerotic alterations^{26,27}. Oxidation or other modifications of LDL, however, substantially alter its role: oxidized or modified lipids are chemotactic for monocytes, induce migration, initiate inflammatory responses, alter the endothelium, induce differentiation of monocytes into macrophages, and are avidly taken up by macrophages via scavenger receptors^{26,28,29}.

Modified LDL is recognized by scavenger receptors distinct from the 'classical' LDL receptors present on all mammalian cells³⁰. Scavenger receptors bind and internalize modified low-density lipoprotein (LDL). Because the expression of scavenger receptors is not down-regulated by cholesterol, macrophages expressing scavenger receptors can internalize substantial quantities of cholesteryl ester from oxidized LDL leading to foam cell formation³¹.

Intravascular lipids are almost completely derived from plasma as a result of unphysiologically high plasma lipoprotein concentrations. It is important to note that the individual plasma lipid profile is the result of interactions between genetic and environmental factors²⁶.

In summary, atherosclerosis results from multiple complex interactions among various factors injuring the endothelium followed by healing or repair processes of the arterial wall and occurs in a hyperlipidaemic and dyslipoproteinaemia environment.

Endothelial dysfunction is mainly caused by diminished production or availability of NO³². It has been demonstrated that the serum concentration of nitrate and nitrite, metabolic end-products of NO, is significantly decreased in smokers relative to that in nonsmokers³³. In cigarette smokers, low-density lipoprotein (LDL) is more prone to oxidation due to higher level of ROS and reactive nitrogen species³⁴. Oxidatively modified LDL limits the bioactivity of

endothelium-derived NO; and, in turn, the loss of NO bioactivity is associated with increased inflammatory cell entry into the arterial wall³⁵. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Antioxidant mechanism of vitamin C include an inhibition of smoke- induced leukocyte adhesion to vascular endothelium, and protection of LDL against atherogenic modification¹⁵.

It was seen that mean value of urinary vitamin C was higher in smokers as compared to nonsmokers. The mean value of urinary vitamin C was higher in smokers who smoked higher number of cigarettes/day as compared to smokers who smoked lesser number of cigarettes/day. The two group of subjects were comparable regarding the variable known to affect vit C status. Thus it seems reasonable to speculate that the difference in fasting urinary vitamin C levels we observed between the two groups were secondary to cigarette smoking and theoretically lower plasma vitamin C level in smokers could be caused by increased urinary excretion of vitamin C in smokers.

Wei wei et al¹³ using NHANES III data reported that serum vitamin C was inversely associated with serum cotinine even after diet adjustment, this indicates that declines in serum level of vitamin C in smokers is a metabolic effect of smoking.

The lowered serum vitamin C levels in smokers could be due to either impaired vitamin C absorption or increased turnover. On the basis of studies measuring urinary vitamin C excretion in conjunction with the administration of known vitamin C intakes, Pelletier suggested the presence of impaired bioavailability but normal turnover of vitamin C in smokers^{36,37}. On the other hand, Kallner, et al,³⁸ measured vitamin C kinetics using radio-labeled ascorbic acid and demonstrated increased turnover in smokers but only small differences in

absorption when compared to non-smokers. Others have reported that smoking acutely increases urinary excretion of vitamin C,³⁹ also suggesting an accelerated metabolism in smokers. Our study is consistent with this experimental study. So smokers not taking nutritional supplements, may be at substantial risk.

As statistical analysis were dependent on the accuracy of self reported smoking habits and age this could limit significance of the result. The result of our study may not be generalizable at this stage to the entire population because of low sample size and local geographical representation.

Because the health benefits of smoking cessation occur faster for cardiovascular than other diseases, policies that prevent and reduce smoking will have immediate and large benefits for reducing cardiovascular mortality⁴⁰.

CONCLUSION

To conclude smoking causes alteration in lipid profile. Amount and duration of smoking also influences dyslipidaemia. Increased amount and duration of smoking causes more of dyslipidaemia. Smokers excrete more ascorbic acid in urine as compared to non-smokers. Low plasma ascorbic acid in smokers might be due to increased excretion of ascorbic acid in urine. There is relationship between tobacco use and atherosclerosis but mechanism is poorly understood. The rapid reduction in risks of cardiac events after cessation of smoking implies that tobacco use promote thrombosis & contribute in formation of atherosclerotic plaque. Anti-smoking advise should be an important part of public health system.

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