Original Article

Biochemistry

Dose-dependent Impacts on the Diagnostic Efficacies of Atherogenic Lipids in Adult Indian Smokers

VEERENDRA KUMAR ARUMALLA, NUTAKKI VANI, J RAMARAO

ABSTRACT

Background: Smoking is a major risk factor for atherosclerosis and coronary heart disease, with a dose-response correlation between the number of cigarettes smoked and the altered lipid profile. In the context of its high prevalence in India, the atherosclerotic risk in smokers is a major concern.

Methodology: A total of 900 healthy male individuals (600 smokers of the age range of 25-35 years and 300 age and sex matched non-smoking controls) were recruited and screened for circulating lipids, total cholesterol/high-density lipoprotein cholesterol ratio(TC/HDL), and the atherogenic index of plasma (AIP = log Triglycerides/HDL).

Results: The TC/HDL ratio, AIP and all the lipids were found to be increased significantly in the study group smokers as compared to their values in the non-smoking controls ($p<0.001$). HDL was found to be significantly decreased ($p<0.001$). The same was true when the group 2 heavy smokers were compared with the healthy, non-smoking controls ($p<0.001$). When comparisons were made between the control group and the group1 light smokers, all the variables were found to be increased, except HDL, but the difference was significant only for the TC/HDL ratio ($p< 0.001$), with a significant decrease in HDL ($p<0.001$). Rather than the lipid profile, the TC/HDL ratio and AIP showed higher efficiency in indicating the atherogenic lipid abnormalities which were associated with smoking.

Conclusions: To conclude, smoking is positively related to lipid abnormalities. The TC/HDL ratio has the best discriminatory power to indicate the atherogenic lipid abnormalities and thereby, the future atherogenic risk which is associated with smoking.

Key Words: Smoking, Atherogenic risk, Lipid profile, Atherogenic index of plasma

INTRODUCTION

Coronary artery disease (CAD) is one of the major health problems which are responsible for the increasing mortality and morbidity in the Indian subcontinent as well as in ethnic Indian communities all over the world [1]. The smoking prevalence is high and it is increasing in many parts of the world, especially in developing countries [2]. There was a dose-response correlation between the CAD morbidity and mortality and the number of cigarettes which are smoked [3].

Dyslipidaemia has been found to be associated with coronary heart disease (CHD). It has been proposed that smoking leads to dyslipidaemia in the form of increased serum total and low-density lipoprotein (LDL) cholesterol levels [4-7], triglycerides (TGLs) [5-7], and decreased high-density lipoprotein (HDL) [5, 6]. Smokers also exhibit dose-dependent elevations of TGLs and total, and LDL cholesterol, as well as decreased levels of HDL cholesterol and apolipoprotein A-I (apoA-I) as compared to the non-smokers [8]. The total cholesterol (TC)/HDL and the LDL/HDL molar ratios have a good predictive value for future cardiovascular events [9]. Dobiasova and Frohlich [10] proposed a term 'Atherogenic Index of Plasma (AIP)', which was defined as log (triglycerides/HDL), that indicated that plasma atherogenecity was also a significant independent predictor of CHD [11]. However, these data are based on studies which were conducted in western counterparts and the data from India are scarce [5-7]. None of those reports dealt with AIP in patients with different smoking status.

Recently, Jindal SK et al reported that the prevalence of smoking was 28.5% in men and 2.1% in women [12] In the context of its high prevalence in India, the atherosclerotic risk in smokers was a major concern in our study, with reference to the different smoking categories. Our objective was primarily to evaluate and compare the serum lipid profile, the TC/HDL ratio and the atherogenic index in smokers with that of the healthy controls and to calculate the efficiency of all the studied parameters as the indicators of atherogenic lipid abnormalities in smokers.

METHODOLOGY

Smokers and Controls

600 healthy male smokers who were aged 25-35 years, who were patient attendees, who attended the General Medicine Out-Patients Clinic, Kakatiya Medical College, Warangal, A.P, India, were recruited into the study, along with 300 age, sex and BMI matched healthy controls. The smokers and the control subjects were selected randomly. Informed consent was taken from all the participants. Our study was approved by the institutional ethical committee.

Inclusion Criteria: Smokers with a history of cigarette smoking for more than 5 years and who continued smoking were taken into the study and grouped according to their smoking status into group1 and group2, who were respectively smoking less than and more than 10 cigarettes per day.

Exclusion Criteria: Participants with Diabetes mellitus, hypertension, coronary artery disease, hepatic impairment, renal disease, thyroid disorders and obesity, and who were on β-blockers, lipid lowering drugs and Thiazide diuretics were excluded from the study.

Sampling and analysis

After 12hrs of overnight fasting, 5ml of blood was drawn from all the study participants by venipuncture. The samples were centrifuged at 3000rpm for 15 min for the immediate analysis of TC, TGL, and HDL in serum by specific methods [13]. LDL was calculated by using Friedwald's formula. The TC/HDL ratio and the AIP were calculated. AIP was calculated by taking the log of (TGL/HDL). The efficiency for each of the studied parameter/indices was presented by calculating the ratio of the sum of the true positives and the true negatives to that of the total number of evaluated cases.

Statistical analysis

All the data obtained was presented as mean±SD. Any differences in the parameters between the groups were tested for significance by the ANOVA test. Comparisons were made between the whole smokers vs the controls, the controls vs group1, the controls vs group2 and group1 vs group2. A 'p' value of < 0.05 was considered to be significant statistically. The area beneath the receiver operating characteristic (ROC) curves was used to determine the diagnostic efficacies of all the parameters which were studied in the atherogenic risk prediction among the smokers. 'Diagnostic efficacy' was defined as the ratio of the sum of the true positives and the true negatives to that of the total number of the evaluated cases. All the statistical analyses were performed by using SPSS for Microsoft windows, version 17.0.

RESULTS

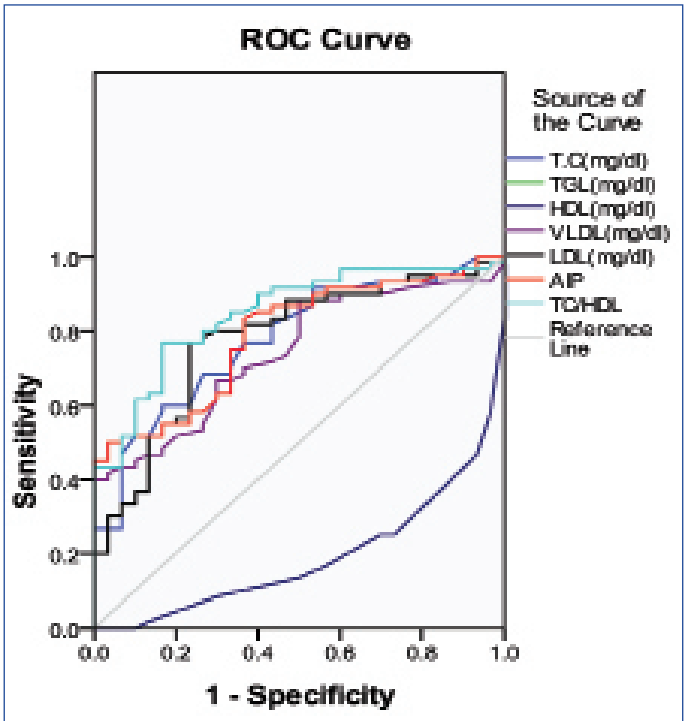
The circulating levels of the lipids, the TC/HDL ratio, and the AIP of the groups are shown in [Table/Fig 1]. The TC/HDL ratio, the AIP and all the lipids except HDL were found to be increased significantly in the study group smokers as compared to their levels in the non-smoking controls ($p<0.001$). HDL was found to be significantly decreased ($p<0.001$). The same was true when the group 2 smokers were compared with the healthy non-smoking controls. When comparisons were made between the control group vs the group 1 smokers, all the variables were found to be increased, except HDL, but the difference was significant only for the TC/HDL ratio ($p< 0.001$), with a significant decrease in HDL ($p<0.001$). Similarly, when the group 1 data was compared with that of the group 2 data, all the parameters which were studied were found to be increased statistically ($p<0.001$), but the HDL concentrations remained unchanged ($p = 0.66$). When the group 1 data was compared with the group 2 data for studying the dose dependent relationships, we found an increase in all the studied parameters, except HDL, which was decreased.

The data which was obtained according to the ROC curves for all the parameters which were studied among the different groups are shown in [Table/Fig 2] and the ROC curves for the lipids, the TC/HDL and the AIP which were obtained from the total smokers and the controls are depicted in [Table/Fig 1]. The ROC curve analysis for studying the diagnostic efficacies of different parameters in the atherogenic risk estimation, revealed that the area under the curves for the TC/HDL ratio and the AIP were found to be 0.84 and 0.78.

The group 1 and the group 2 smokers as a whole were compared with the non-smoking control group and we observed a significant increase in the serum cholesterol, TGL, LDL and VLDL, followed by a significant decrease in HDL in the smokers, as is evident from our data. This was well in agreement with the findings of Padmavathi P et al [5]. In addition, we also observed a significant increase in the TC/HDL ratio and the AIP in the smokers, when they were considered as a whole, vs the controls. Thus, it was evident from our data, that the TC/HDL ratio had a higher diagnostic efficacy in the atherogenic risk prediction in smokers.

DISCUSSION

The relationship between CHD and smoking was first developed by White *et al* [14], and later by Doll *et al* [15]. The incidence of developing CHD is directly related to the number of cigarettes which are smoked [16]. It has long been established that nicotine has a



[Table/Fig-2]: ROC curve analysis for lipid profile, TC/HDL ratio, and AIP between non-smoking controls and total smokers

Variable	Controls	Total Smokers	Group1	Group2	P value
T. Chol	163.3±21.7	192.7±31.8	175.6±25	209.7±28.9	0.001*, 0.180†, 0.001‡, 0.001§
TGL	100.5±24.3	134.7±41.4	115.9±31.5	153.5±42	0.001*, 0.213†, 0.001‡, 0.001§
HDL	43.0±2.6	39.6±2.6	40.6±2.7	38.7±2.2	0.001*, 0.003†, 0.001‡, 0.66§
LDL	100.2±20.7	126.1±26.8	111.8±22.5	140.3±23.2	0.001*, 0.137†, 0.001‡, 0.001§
VLDL	20.1±4.8	26.9±8.3	23.1±6.3	30.7±8.4	0.001*, 0.213†, 0.001‡, 0.001§
TC/HDL	3.8±0.5	4.9±0.9	4.3±0.7	5.4±0.7	0.001*, 0.011†, 0.001‡, 0.001§
AIP	0.3±0.1	0.51±0.1	0.4±0.1	0.57±0.1	0.001*, 0.028†, 0.001‡, 0.001§

[Table/Fig-1]: Mean±SD and P values (ANOVA) of lipid profile, TC/HDL ratio, and AIP among various groups
* (controls Vs Total-Smokers), † (Controls Vs Group1), ‡ (Control Vs Group2), § (Group1 Vs Group2).

Variables	Best-Cutoff value	Sensitivity	Specificity	Diagnostic efficacy	AUC	95% CI
T.Chol	182.0[a] 153.5 [b] 182.0[c] 174.0 [d]	60.0[a] 83.3[b] 80.0[c] 93.3[d]	83.3[a] 46.6[b] 83.3[c] 53.3[d]	66.6[a] 65.0[b] 81.6[c] 73.3[d]	0.77[a] 0.65[b] 0.90[c] 0.80[d]	0.68-0.87[a], 0.51-0.79[b] 0.83-0.97[c], 0.69-0.90[d]
TGL	151.0[a] 93.0[b] 151.0[c] 125.5[d]	40.0[a] 86.6[b] 63.3[c] 83.3[d]	100.0[a] 50.0[b] 100.0[c] 80.0[d]	60.0[a] 68.3[b] 81.6[c] 81.6[d]	0.74[a] 0.63[b] 0.85[c] 0.76[d]	0.64-0.84[a], 0.48-0.77[b] 0.74-0.96[c], 0.63-0.90[d]
HDL	40.5[a] 40.0[b] 39.5[c] 39.5[d]	73.3[a] 56.6[b] 73.3[c] 73.3[d]	75.0[a] 73.3[b] 93.3[c] 66.6[d]	74.4[a] 65.0[b] 83.3[c] 70.0[d]	0.81[a] 0.72[b] 0.89[c] 0.72[d]	0.72-0.89[a], 0.60-0.85[b] 0.81-0.98[c], 0.58-0.85[d]
LDL	108.6[a] 106.5[b] 113.2[c] 113.2[d]	76.7[a] 60.0[b] 93.3[c] 93.3[d]	76.7[a] 73.3[b] 76.6[c] 60.0[d]	76.6[a] 66.6[b] 85.0[c] 76.6[d]	0.77[a] 0.65[b] 0.89[c] 0.80[d]	o.67-0.87[a], 0.51-0.79[b] 0.81-0.97[c], 0.70-0.91[d]
VLDL	30.2[a] 18.6[b] 30.2[c] 25.1[d]	40.0[a] 86.6[b] 63.3[c] 83.3[d]	100.0[a] 50.0[b] 100.0[c] 80.0[d]	60.0[a] 68.3[b] 81.6[c] 81.6[d]	0.74[a] 0.63[b] 0.85[c] 0.76[d]	0.64-0.84[a], 0.48-0.77[b] 0.74-0.96[c], 0.63-0.90[d]
TC/HDL	4.1[a] 4.1[b] 4.2[c] 4.3[d]	76.7[a] 56.6[b] 96.6[c] 96.6[d]	83.3a 83.3b 83.3c 63.3d	78.8[a] 70.0[b] 90.0[c] 80.0[d]	0.84[a] 0.73[b] 0.95[c] 0.84[d]	0.76-0.92[a], 0.60-0.86[b] 0.91-1.0[c], 0.74-0.93[d]
AIP	0.5[a] 0.3[b] 0.5[c] 0.5[d]	50.0[a] 76.6[b] 80.0[c] 83.3[d]	96.7a 63.3b 96.6c 80.0d	65.5[a] 70.0[b] 88.3[c] 81.6[d]	0.78[a] 0.68[b] 0.89[c] 0.78[d]	0.69-0.88[a], 0.54-0.82[b] 0.80-0.98[c], 0.66-0.91[d]

[Table/Fig-3]: Best-cutoff, sensitivity, specificity, diagnostic efficacy, AUC and 95% CI values for lipid profile, TC/HDL ratio, and AIP obtained by ROC Analysis among various groups.
[a](controls Vs Total-Smokers), [b](Controls Vs Group1), [c](Control Vs Group2), [d](Group1 Vs Group2)

considerable influence on the increase in the lipid levels in blood [17]. The increased total lipids are considered to be an important contributory factor for the development of atherosclerosis [18]. Cigarette smoking has been found to increase the concentrations of TGL and to lower the concentration of HDL cholesterol [5, 6]. The current study showed significantly higher levels of total lipids in smokers as compared to that in the controls. These changes were found to contribute towards the atherogenic potential of cigarette smoking.

In conformity with previous reports, we found a significant increase in the TC [4-7, 19], TGL [5-7, 20, 21], LDL, and VLDL [5, 6, 19, 21] concentrations, followed by a significant decrease in the HDL [5, 6, 19, 21] levels. Smoking causes an increased activity of HMG-CoA reductase [22] and decreased lipoprotein lipase [6] activities, resulting in elevated levels of TC and TGL, LDL, and VLDL. In addition, nicotine stimulates the sympathetic adrenal system, which leads to increased lipolysis. According to McCall et al [23], the reduced HDL level in smokers is due to its increased catabolism and the inactivation of the lecithin-cholesterol acyl transferse (LCAT) system. In the present study, we also measured plasma atherogenecity by using the AIP and the TC/HDL ratio. Both were found to be significantly high in smokers. An increased TC/HDL ratio has been previously reported [4, 24]. To the best of our knowledge, there are no reports from India concerning the AIP in smokers till date.

From the above information, we propose here, that rather than the lipid profile, the TC/HDL ratio and AIP have higher diagnostic efficacies in atherogenic risk prediction in smokers under different smoking doses. The diagnostic efficacy of TC/HDL, when the non-smoking controls were compared with the total, group2, and group1 smokers, was found to be 78.8, 90.0, and 70.0. The higher diagnostic efficacy of TC/HDL in the group 2 smokers confirmed the dose-dependent response of the atherogenic indices to smoking. However, TGL showed the same diagnostic efficacy as that of AIP in the group 1 vs group 2 analysis. It was suggested that the triglyceride levels was the most important factor which led to CHD [25]. Overall, it is clear that our findings altogether suggested that the TC/HDL ratio and AIP had better diagnostic efficacies in predicting the atherogenic risk and its dose-dependent response in smokers. A dose dependent relationship was studied by several authors [4,6,24]. The change in lipids among the smoking groups may be due to the changes in the smoking dose and the greater the number of cigarettes which were smoked, the higher was the atherogenic risk.

To conclude, smoking is positively related to lipid abnormalities and the TC/HDL ratio has the best discriminatory power to indicate the atherogenic lipid abnormalities which are associated with smoking. Moreover, AIP has the highest diagnostic efficacy in differentiating the atherogenic risk which is associated with heavy smokers from other study groups.

REFERENCES

[1] Enas EA, Yusuf S, Mehta JL. Prevalence of coronary artery disease in Asian Indians. *Am J Cardiol* 1992; 70: 945-9.
[2] Frieden TR, Bloomberg MR. How to prevent 100 million deaths from tobacco. *Lancet* 2007; 369: 1758–61.
[3] Kannel WB. Update on the role of cigarette smoking in coronary artery disease. *Am Heart J* 1981; 101: 319–28.
[4] Venkatesan A, hemalatha A, Bobby Z, Selvaraj N, Sathiyapriya V. Effects of smoking on the lipid profile and the lipid peroxidation in normal subjects. *Indian J Physiol Pharmacol* 2006; 50(3): 273–78.
[5] Padmavathi P, Reddy VD, Varadacharyulu N. Influence of chronic cigarette smoking on the serum biochemical profile in male human volunteers. *Journal of Health Science* 2009; 52(2): 265-70.

[6] Neki NS. The lipid profile in chronic smokers – A clinical study. *JIACM* 2002; 3(1): 51-4.
[7] Sharma SB, Dwivedi S, Prabhu KM, Singh G, Kumar N, Lal MK. Coronary risk variables in young asymptomatic smokers. *Indian J Med Res* 2005; 122: 205-10.
[8] Freeman DJ, Griffin BA, Murray E, Lindsay GM, Gaffney D, Packard CJ, et al. Smoking increases the plasma lipoproteins in man: effects of low density lipoprotein cholesterol levels and the high density lipoprotein subfraction distribution. *Eur J Clin Invest* 1993; 23: 630–40.
[9] Grover SA, Levington C, Paquet S. Identifying adults who are at a low risk for significant hyperlipidemia: a validated clinical index. *J Clin Epidemiol* 1999; 52: 49–55.
[10] Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). *Clin Biochem* 2001; 34: 583–8.
[11] Gaziano JM, Hennekens CH, O'Donnell CJ. Fasting triglycerides, high-density lipoprotein and the risk of myocardial infarction. *Circulation* 1997; 96: 2520–5.
[12] Jindal SK, Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D et al. Tobacco smoking in India: Prevalence, quit-rates and respiratory morbidity. *Indian J Chest Dis Allied Sci* 2006; 48: 37-42.
[13] Nader R, Paul B, John A. Lipids Lipoproteins and ApoLipoproteins, In *Tietz Textbook of Clinical Chemistry*, 3rd ed, 1994, Burtis C.A. and Ashwood E.R, Eds.p 809-52. Philadelphia: W.B.Saunders.
[14] Framingham Study: Sorlie P, Gorden T. Body building and mortality. *JAMA* 1980; 243.
[15] Doll R, Hill AB. Mortality in relation to smoking. *Br Med J* 1964; ii: 399-440.
[16] Friedman GD, Dale LG. Mortality in middle-aged smokers and non-smokers. *NEJM* 1979;: 300-213.
[17] Gorden T, Castelli WP. Diabetes, blood lipids and the role of obesity in the coronary heart disease risk for women. The Framingham Study. *Ann Intern Med* 1977; 83: 393-97.
[18] Dawber TR, Kannel WB, Gorden T. Some factors which are associated with the development of coronary heart disease. *Am J Publ Health* 1959; 49: 1349-56.
[19] Akbari MZA, Bhatti MS, Shakoor M. Lipid profile in smoking. *JAMC* 2000; 12(3): 19-21.
[20] Afrin L, Sultana R, Ferdousi S, Ahmed A. Evaluation of serum triglycerides and the total cholesterol status in adolescent smokers. *J Bangladesh Soc Physiol* 2006; 1: 14-18.
[21] Freeman DJ, Griffin BA, Murray E. Smoking and plasma lipoproteins in man: effects on the low density lipoprotein cholesterol levels and the high density lipoprotein subfraction distribution. *European Journal of Clinical investigation* 2008; 23(10): 630-40.
[22] Latha MS, Vijayammal PL, Kurup PA. Effect of exposure of rats to cigarette smoke on the metabolism of lipids. *Atherosclerosis* 1988; 70: 225-31.
[23] McCall MR, Van Den Berg JJM, Frans A. Modification of the LCAT activity and the HDL structure: New links between cigarette smoke and coronary heart disease risk. *Arterioscler Thromb* 1994; 14: 248-253.
[24] Kaori Teshima HI. Cigarette smoking, Blood pressure and serum lipids in Japanese men who were aged 20-39 years. *J Physiol Anthropol* 2001; 20(1): 43-45.
[25] Varley H, Gowenlock HA, Bell M. (Editors) 1984. *Practical Clinical Biochemistry*. Vol.1, 5th ed. William heinemann Medical Books Ltd. London. P677.

AUTHOR(S):
1. Dr. Veerendra Kumar Arumalla
2. Dr. Nutakki Vani
3. Dr. J Ramarao

NAME OF DEPARTMENT(S)/INSTITUTION(S) TO WHICH THE WORK IS ATTRIBUTED:
Department of Biochemistry,
Shri Sathya Sai Medical College and Research Institute,
Ammapettai, 603108.
Kancheepuram (Dist.)
Tamil Nadu, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Veerendra Kumar Arumalla MD
Department of Biochemistry,
Shri Sathya Sai Medical College and Research Institute,
Ammapettai, 603108.
Kancheepuram (Dist.), Tamil Nadu, India.
Phone: +919962313887
E-mail: drveerendraarumalla@gmail.com

DECLARATION ON COMPETING INTERESTS:
No competing Interests.

Date of Submission: **Aug 18, 2011**
Date of peer review: **Oct 15, 2011**
Date of acceptance: **Oct 17, 2011**
Date of Publishing: **Nov 30, 2011**