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Research Article

Association of Lipid Profile, Atherogenic Indices, and LPL Hind-III Gene Polymorphism with Coronary Artery Disease Positive Subjects

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ABSTRACT

Dyslipidemia is renowned as a prominent risk factor for the development of atherosclerosis and associated cardiovascular diseases such as formation of plaques in arteries, atherosclerosis, myocardial infarction, sudden coronary death, stable angina and unstable angina. CAD may be due to dysfunctional mutations in lipoproteins or lipoprotein-related genes. Lipoprotein lipase (LPL) plays an important role in lipid metabolism. Our aim of the present study is to determine the association and prediction of risk cases by using atherogenic indices and *Hind-III* LPL gene polymorphism. Atherogenic indices are a powerful indicator to predict the risk of coronary artery diseases. The atherogenic indices of CAD negative and positive are CRI-I (4.68±0.08; 6.46±0.12), CRI-II (3.03±0.06; 3.99±0.15), TG/HDL-c (3.27±0.19; 7.40±0.62), AIP (0.45±0.02; 0.81±0.03) and AC (3.68±0.08; 5.39±0.13) observed respectively. These results indicate atherogenic indices are may be useful for identifying an individual at higher risk of cardiovascular disease in the clinical practices especially and not markedly deranged or in centers with insufficient resources to predict the CVS risk. In the case of Hind-III LPL gene polymorphism; TT genotype frequency was found to be significantly higher in CAD positive subjects than the controls and CAD negative subjects. More than threefold was increased risk for CAD development under the codominant model. Correspondingly, the T allele frequency of intron 8 T >G polymorphism was elevated in CAD positive subjects (95 % CI; 2.19 (1.28- 3.75) p=0.003) compared to controls. LPL intron 8 T >G gene polymorphism (rs320) results support the above data; T allele (H^+) was associated with various cardiovascular risks such as positively correlated with carotid artery atherosclerosis, higher risk of myocardial infarction and higher plasma triglycerides and lower HDL-cholesterol.

Keywords: Coronary artery disease, Lipoprotein lipase, Atherogenic indices, Gene polymorphism.

INTRODUCTION

Dyslipidemia is renowned as a prominent risk factor for the development of atherosclerosis and another cardiovascular disease (CVD)[1,2]. Dyslipidemia is manifest as elevated levels of lipid profile; TC (total cholesterol), TGs (triglycerides), LDL-c (low-density lipoprotein cholesterol), VLDL-c (very low-density lipoprotein cholesterol), and decreased of high-density lipoprotein cholesterol (HDL-c) in blood. According to world health organization (WHO), cardiovascular disorders are one of the morbidity and mortality accounting for 3 out of every 10 deaths is due to dyslipidemia. These were expected increase to annual death of 23.3 million by 2030[3]. In India, according to National commission on macroeconomics and health (NCMH), there would be around 62 million patients with coronary artery disease (CAD) by 2015 in India and these 23 million would be patients younger than 40 years of[4]. CAD is characterized by a group of diseases that includes the formation of plaques in arteries, atherosclerosis, myocardial infarction, stable angina and unstable angina[5]. A common symptom is chest pain or discomfort which travels into the shoulder, occasionally it may fell like heartburn. Usually, some symptoms occur with exercise or emotional stress. Usually, coronary artery disease is the blockage or narrowing of the coronary arteries, usually caused by atherosclerosis. Atherosclerosis is characterized by thickening, hardening and loss of elasticity of arteries walls due to the accumulation of fatty streaks. It was promoted by abnormal lipoproteins, mainly by TGs, LDL-c and inadequate removals of fats from the macrophages and finally formation of multiple athermanous plaques within the arteries[6]. CAD may be due to dysfunctional mutations in lipoproteins or lipoprotein-related genes (e.g., receptors, catabolic enzymes), multifactorial inheritance and environmental factors (e.g., diet, exercise, tobacco). The common variants of genes involved in lipid metabolism are associated with modest changes in protein function that might be an important risk factor to the population[7]. A lipoprotein lipase (LPL) play an important role in lipid metabolism by hydrolyzing triglycerides (TGs), LPL is the rate-limiting step in the removal of triglyceride-rich lipoproteins, such as chylomicrons (CM) and very low-density lipoproteins (VLDL) from the circulation[8]. Mature LPL composed of

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448 amino acids and its gene was located on chromosome 8p22, with 9 exons and 29.6 kb[9,10]. *Hind III* (rs320) polymorphism is the one of the most common polymorphism in LPL gene. It is an 8th intronic base transition of thymine (T) to guanine (G) at position +495, which abolishes the restriction site for the enzyme Hind-III. Several studies have shown that the common T allele (H^+) is significantly associated with high triglycerides (TG) levels and low HDL levels compared to the rare G allele (H^-)[11-18]. Dyslipidemia may come to notice only during routine health check-up of the individuals, there may be no signs and symptoms are associated with it. Sometimes, lipid abnormalities may be diagnosed for the first time after a person suffers from cardiovascular disorders such as myocardial infarction or atherosclerosis or stroke. Hence, the aim of the present study is to determine the association and prediction of risk by using atherogenic indices and *Hind-III* LPL gene polymorphism.

METHODOLOGY

Study design

The present study was carried out at Dr. Ramesh Cardiac and Multispecialty Hospital LTD, Vijayawada, Andhra Pradesh, India. The study subjects were randomly selected; who were a visit to the hospital for their general health check up. The study protocol was approved by the Institutional Ethical Committee and it was conducted during the period from 2012-2014.

Selection of subjects

Selection of the subjects based upon past and present health status of the individual and implementing the certain inclusion and exclusion criteria's. An informed written consent was obtained from all subjects participated in the study.

Inclusion criteria's

Selection of cases and control subjects based upon the plasma lipid abnormality cut off values given by an expert panel of the National cholesterol education program (NCEP)[19]. Based upon certain inclusion criteria such as abnormal levels of lipid profile and other cardiac-related diagnostic tests (ECG, 2D-Echo, TMT and certain clinical symptoms of CAD) cases subjects were selected and they were further segregated into CAD positive (CADP) and CAD negative (CADN) subjects. Those subjects were in normal lipid profile considered as control subjects.

Exclusion criteria's

Exclusion criteria's such as subjects with hepatic disorders, metabolic, renal disease diabetes mellitus and those who were on exogenous hormones supplement or on hormone replacement therapy or use of lipid-lowering drugs were excluded from the study.

Data collection of subjects

Systematic examination of each subject was carried out; it included name, age, and address, type of diet, occupation, physical exercise, present & past medical illness and family history. Anthropometric assessments such as height in meter (m); weight in kilogram (kg); and body mass index (BMI) was calculated by weight in kilograms divided by the square of the height in meter (kg/m^2). A total number of 258 subjects participated in our study, in which 129 members were with elevated levels lipid profile considered as cases and 129 numbers with normal lipid profile considered as control subjects. Among the 129 cases subjects, 84 subjects were as CAD negative; and the rest of the 45 subjects were considered as CAD positive; enrolled with individuals with abnormal clinical, cardiologic and precordial pain and characteristic electrocardiographic changes[20].

Collection of a blood sample and estimation of lipid profile

Fasting blood samples were collected in the morning between 7 a.m. and 8 a.m. by venepuncture of antecubital vein with all aseptic precautions, using a dry disposable syringe under sterile conditions. Fresh serum was used for estimation of TC, TGs, and HDL-c respectively. The tests were carried out in an automated clinical autoanalyzer. Further, low LDL-c, VLDL-c, and Non-HDL-c were calculated by using Friedewald's formula[21]. Further, atherogenic indices like, Castelli's Risk Index-I (CRI-I)=TC/HDL-c, Castelli's Risk Index-II (CRI-II) = LDL-c/HDL-c, Atherogenic Coefficient (AC) = (TC– HDL-c)/HDL-c[22-24], Atherogenic Index of Plasma (AIP) = log (TG/ HDL-c)[25]and TG/HDL-c ratio[26] are calculated from the individuals.

Isolation of genomic DNA

Genomic DNA was extracted from 5ml of fresh whole blood by the rapid non-enzymatic method, where cellular proteins are salted out with saturated sodium chloride solution in the course of dehydration and precipitation. Then the DNA was precipitated with 100% ethanol[27].

Polymer chain reaction (PCR) and Restriction fragment length polymorphism (RFLP)

Primer sequences, polymerase chain reaction (PCR) conditions and restriction enzyme digestions were as follows (Oligonucleotides were synthesized by Bio-serve, Gene valley, Hyderabad, Telangana). In the region of intron 8, the LPL gene containing *Hind III* polymorphism(rs320) region was amplified using the following primers: forward primer: 5'-GATGCTACCTGGATAATCAAAG-3 and the reverse primer was 5'- CTTCAGCTAGACATTGCTAGTGT-3'. PCR conditions include initial denaturation for 6 min at 95 °C followed by 34 cycles of denaturation at 95 °C for 1.00 min, annealing at 56.4°C for 0.40s and extension at 72.0 for 1.00 min, followed by final extension at 72°C for 1min, followed by final extension at 72°C for 7 min. Amplified PCR products were digested with *Hind III* restriction enzyme (New England Biolabs.UK) for 16h at 40°C.The resulting genotypes (Fig:1) products are CC (Homozygote wild: 213bp; 142bp), TG (Heterozygote's: 355bp; 5213bp; 142bp) and GG (Homozygote mutant: 355bp) were electrophoresed on 2% agarose gel.

Statistical Analysis

The collected data were analyzed by using graph pad prism, version 6. The differences between the groups were determined by performing the one-way analysis of variance (ANOVA), data were expressed as mean ± standard error mean (SEM). The statistical significance was set at the *p*-value of $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ and $^{ns}p>0.05$ is considered as non-significant. For genotype

analysis performed odd's ratio, chi-square test, and Hardy-Weinberg equilibrium.

RESULTS

Table 1 showed the mean ± SEM values of the age and BMI of the dyslipidemia cases and control subjects. No significant difference was observed between the control and cases. Table 2 showed mean ± SEM values of the lipid profile of control, CAD negative and positive subjects. All cases showed a higher concentration of lipid profile than the controls. When compared with in cases group, lipid profile of CAD positive subjects showed significantly higher values of total cholesterol (221.47±42.74; $p<0.001$), triglycerides (251.24±22.25; $p<0.001$), LDL-c (136.69±6.13; $p<0.001$), VLDL-c (50.24±4.45; $p<0.001$) and Non-HDL-c (186.73±5.52; $p<0.001$) and lower levels of HDL-c value (40.36±0.82; $p<0.001$) was observed than CAD negative subjects. Table 3 showed mean±SEM values of atherogenic indices (Figure 4) of dyslipidemia cases and control subjects. The atherogenic indices of CAD negative and positive are CRI-I (4.68±0.08; 6.46±0.12), CRI-II (3.03±0.06; 3.99±0.15), TG/HDL-c (3.27±0.19; 7.40±0.62), AIP (0.45±0.02; 0.81±0.03) and AC (3.68±0.08; 5.39±0.13) observed respectively. Within the comparison of CAD negative and positive subjects, CAD positive subjects showed significantly ($p<0.001$) higher values of indices than the others. Table 4 showed the Pearson's correlation and linear regression analysis of the triglycerides, HDL-c and LDL-c with atherogenic indices. Triglycerides showed significant ($p<0.001$) correlation and regression analysis with VLDL-c and some atherogenic indices. A negative correlation was observed in HDL-c with atherogenic indices, in which CRI-I and AC were correlated significantly. Regression analysis also associated with CRI-I and AC. In the case of LDL-c was significant with CRI-II, TG/HDL-c and AIP in both correlation and regression analysis. Genotype distribution, Allele frequency, Chi-square, Odds ratio and 95% confidence interval (CI) of the LPL Intron 8 T>G in controls, CAD negative and positive cases showed in Table 5. The study follows the Hardy-Weinberg equilibrium. In frequency of TT genotype in Intron 8 T>G polymorphism is high among the CAD-positive (60.00%), followed by CAD negative (48.80%) and controls (36.43%). TT genotype frequency was found to be significantly elevated in CAD positive subjects compared to controls and CAD negative subjects more than threefold increased risk for CAD development under the codominant model. Correspondingly, the T allele frequency of intron 8 T >G polymorphism was elevated in CAD positive subjects (95 % CI; 2.19 (1.28- 3.75) p=0.003) compared to controls. Table 6 comparison between the disease subjects; TT (60.00%; 95 % CI, 1.31(0.40-4.28) genotypes frequency was higher in CAD positive subjects. The T allele frequency polymorphism was elevated in CAD positive subjects (74.44%; 95 % CI; 1.34 (0.75-2.38) compared to controls. The elevated levels of lipid profiles (Table 7) were observed TT genotypes in CAD positive subjects than another genotype with not significant variation.

DISCUSSION

In our study observed, higher value BMI observed in cases than control subjects but not showed significantly ($p>0.05$) different. BMI has been widely used as an indicator of total adiposity; its limitations are clearly recognized by its dependence on race[28]. Prior epidemiologic studies have shown that increasing body mass index (BMI) is associated with higher total cholesterol and low-density lipoprotein cholesterol (LDL). However, these studies were limited by under-representation of obese subjects[29]. Plasma lipid profile of the cases showed higher values than control subjects. Elevated serum triglycerides are one of the important risk factors for developments of atherosclerosis, possible mechanisms for an explanation; elevated triglycerides might promote and responsible for the generation of small dense LDL-c. Further, these small LDL-c particles are high susceptible to oxidation than larger lipoproteins[30] because they contain a greater proportion of polyunsaturated fatty acids and the surface apolipoprotein B (Apo B) is exposed to oxidizing agents[31]. This modified LDL-c was responsible for the development of atherosclerosis explained by several mechanisms such as oxidized LDL-c particles no longer recognized by LDL receptor[32] and promote cell death at higher levels of oxidized LDL-c and also increases the expression of matrix metalloproteinase's, which play a key role in plaque instability and rupture[33]. Due to these effects, endothelial function was partly impaired[34,35], as a result, changes in the expression of nitric oxide syntheses enzymes and stimulation of pro- inflammatory condition by the encouragement of the synthesis of a variety of cytokines and growth factors[36-38]. All of these changes contribute to the development of atherosclerosis. HDL-c plays a part role in reverse cholesterol transport and also protects LDL from oxidation[39,40] and its ability of paraoxinase 1 (PON1) to protect LDL-c from oxidation[41]. A number of research studies indicate low levels of HDL-c are known to be an independent and powerful predictor of atherosclerosis[42]. Elevated levels of VLDL-c and Non-HDL-c are also important predictable markers for the development of CAD. Previous studies are also explained that plasma VLDL-c levels correlate with increased density and decreased the size of LDL-c particles[43,44] . In our study observed elevated levels of TC, TGs, LDL-c, VLDL-c, Non-HDL-c and reduced levels of HDL-c in CAD positive than CAD negative subjects. Due to higher abnormal of levels of lipid profile in CAD positive subjects may be responsible for early development of cardiovascular disorders. CAD negative subject is also observed abnormal lipid profile that indicates the further development of coronary vascular disorders during their life period. Assessment of the relative proportions of cholesterol in these two fractions like pro-atherogenic lipoproteins and anti-atherogenic HDL-c can be valuable than the individual lipid measurements. One of the methods is to compare levels of HDL-c and non-HDL cholesterol. Another method is the use of atherogenic indices. These are a powerful indicator for assessment of the risk for

Table 1: Mean ± SEM values of Age and BMI of the coronary artery disease negative (CADN) and Coronary artery disease positive (CADP) of dyslipidemia cases.

Parameter	Control (n=129)	Dyslipidemia Cases (n=129)		*p Value*	
		CADN (n=84)	CADP (n=45)	CADN *vs.*	CADP
Age (Years)	50.17± 1.29	48.92±1.34	52.66±1.51	>0.05	
BMI	24.84±0.39	26.12±0.52	26.57±0.64	>0.05	

Where, $^{*}p<0.05$;$^{**}p<0.01$;$^{***}p<0.001$considered significant and $^{ns}>0.05$ non significant.

Table 2: Comparison of Mean ± SEM values of FBG and lipid profile of the Coronary artery disease negative (CADN) and Coronary artery disease positive (CADP) of dyslipidemia cases.

Parameter (mg/dl)	Control (n=129)	Dyslipidemia Cases (n=129)		*p Value*	
		CADN (n=84)	CADP (n=45)	CADN *vs.*	CADP
TC	153.71±1.46	198.01±2.88***	221.47±42.74***	<0.001	
TGs	110.53±2.32	133.20±6.64^{ns}	251.24±22.25***	<0.001	
LDL-c	87.99±1.26	128.22±2.53***	136.69±6.13***	>0.05	
VLDL-c	22.10±0.46	26.64±1.33^{ns}	50.24±4.45***	<0.001	
HDL-c	43.61±0.46	43.38±0.96^{ns}	34.73±1.15***	<0.001	
Non HDL-c	110.10±1.38	154.63±2.53***	186.73±5.52***	<0.001	

Where,$^{*}p<0.05$;$^{**}p<0.01$;$^{***}p<0.001$considered significant and $^{ns}>0.05$ non-significant.

Table 3: Comparison of Mean ± SEM values of atherogenic indices of the Coronary artery disease negative (CADN) and Coronary artery disease positive (CADP) of dyslipidemia cases.

Parameter	Control (n=129)	Dyslipidemia Cases (n=129)		*p Value*	
		CADN (n=84)	CADP (n=45)	CADN *vs.*	CADP
CRI-I	3.55±0.04	4.68±0.08***	6.46±0.12***	<0.001	
CRI-II	2.03±0.03	3.03±0.06***	3.99±0.15***	<0.001	
TGs/HDL-c	2.59±0.06	3.27±0.19^{ns}	7.40±0.62***	<0.001	
AIP	0.39±0.01	0.45±0.02*	0.81±0.03***	<0.001	
AC	2.55±0.04	3.68±0.08***	5.39±0.13***	<0.001	

Where,$^{*}p<0.05$;$^{**}p<0.01$;$^{***}p<0.001$considered significant and $^{ns}>0.05$ non-significant.

Table 4: Pearson's correlation and linear regression analysis of HDL-c with atherogenic indices.

Parameters	Pearson's correlation		Linear regression	
	r value	p value	r^2	P value
HDL-c				
CRI-I	-0.4938	0.0006*	0.2439	0.0006*
CRI-II	-0.2518	0.0953^{ns}	0.0633	0.0953^{ns}
TG/HDL-c	-0.1834	0.2279^{ns}	0.0336	0.2279^{ns}
AIP	-0.2475	0.1012^{ns}	0.0612	0.1012^{ns}
AC	-0.5737	< 0.0001***	0.3292	< 0.0001***

Where, $^{*}p<0.05$;$^{**}p<0.01$;$^{***}p<0.001$significant (Two tailed).

coronary artery diseases. The higher values, higher the risk of developing cardiovascular diseases and *vice versa*[45]. Atherogenic ratios like Castelli's Risk Index-I (CRI-I), Castelli's Risk Index-II (CRI-II), the Atherogenic coefficient (AC), TG/HDL-c ratio, and Atherogenic index of plasma (AIP) are calculated. All these indices are especially useful in predicting the cardiovascular risk and confirmed by a number of other studies. Now, we are applying these indices for predicting the cardiovascular risk in dyslipidemic subjects. The average ratio of total cholesterol to HDL-c (CRI-I) of healthy individuals is about 3.5 or lower[22,46] and in the case of LDL-c/HDL-c ratio (CRI-II) is 3 or lower[47,48]. Another research study explained the association of TC/HDL-c with coronary plaques formation[49]. **(**In PROCAM study observed, subjects with LDL-c/HDL-c (CRI-II) >5 had six times higher rate of coronary events[50]. In our study observed higher values of CRI-I and CRI-II in both CAD negative and positive subjects than the control subjects and also observed higher values both CRI-I and CRI-II in CAD positive subjects than CAD negative subjects. The cad-positive group confirmed by electrocardiographic changes and other clinical characteristics, so this above results indicates and supports this index may be very useful in prediction for coronary artery disorders. Protasio et al. explained that ratio of triglycerides to HDL-c was found to be a powerful independent indicator of extensive coronary disease[26]. In our study observed higher values of

Table 5: Genotype distribution of LPL Intron 8 T > G gene polymorphism controls and dyslipidemia cases.

Name of Gene (LPL Intron 8 T>G)	Control n=129(%)	Dyslipidemia cases (n=129)					
		CAD Negative (n=84)			CAD Positive (n=45)		
		Genotypes	OR (95% CI)	χ2 (p)	Genotypes	OR (95% CI)	χ2 (p)
Codominant model							
GG	29(22.48%)	10 (11.90%)	1.00(Ref)	χ^2-5.03 (0.08)	5 (11.11%)	1.00(Ref)	χ^2-6.49 (0.03)
TG	53(41.08%)	33 (39.28%)	1.80 (0.77- 4.18)		13 (28.88%)	1.42 (0.46- 4.38)	
TT	47(36.43%)	41 (48.80%)	2.52 (1.10 - 5.81)		27 (60.00%)	3.33 (1.15- 9.62)	
Dominant model							
GG	29(22.48%)	10 (11.90%)	1.00(Ref)	χ^2-8.19 (0.004)	5 (11.11%)	1.00(Ref)	χ^2- 4.70 (0.03)
TG+TT	70(54.26%)	74 (88.09%)	3.06 (1.39 - 6.75)		40 (88.88%)	3.31 (1.18- 9.24)	
Recessive model							
GG+TG	82(63.56%)	43 (51.19%)	1.00(Ref)	χ^2-3.21 (0.07)	18 (40.00%)	1.00(Ref)	χ^2-7.58 (0.005)
TT	47(36.43%)	41 (48.80%)	1.66 (0.95- 2.90)		27 (60.00%)	2.61 (1.30- 5.24)	
Overdominant model							
GG+TT	76(58.91%)	51 (60.71%)	1.00(Ref)	χ^2-0.06 (0.79)	32 (71.11%)	1.00(Ref)	χ^2-2.10 (0.14)
TG	53(41.08%)	33 (39.28%)	0.92 (0.52- 1.62)		13 (28.88%)	0.58 (0.27- 1.21)	
Allele							
G	111(43.02%)	53 (13.69%)	1.00(Ref)	χ^2-5.65 (0.01)	23 (25.55%)	1.00(Ref)	χ^2-8.59 (0.003)
T	147(56.97%)	115 (68.45%)	1.63 (1.08- 2.46)		67 (74.44%)	2.19 (1.28- 3.75)	
HWE (p)	3.38	0.69			2.61		

Where, OR (Odd's ratio), χ2 p (Chi square); p<0.05 is significant; p>0.05 is non-significant; HWE (p) (Hardy-Weinberg equilibrium).

Table 6: LPL Intron 8 T >G gene polymorphism with characteristic CAD positive and negative cases of dyslipidemia cases.

Variables	Genotype (%)					Allele (%)			
	N (%)	GGn (%)	TG n (%)	TTn(%)	χ2 (p)	N (%)	Gn(%)	Tn(%)	χ2 (p)
CAD	129					258			
Negative	84(65.11%)	10(11.90%)	33(39.28%)	41(48.80%)	χ^{2-}	168(65.11%)	53(13.69%)	115(68.45%)	χ^2
Positive	45(34.88%)	5(11.11%)	13(28.88%)	27(60.00%)	1.14 (0.56)	90(34.88%)	23(25.55%)	67(74.44%)	1.01 (0.31)
OR (95%CI)		1.00(Ref)	0.78 (0.22- 2.75)	1.31 (0.40 - 4.28)			1.00(Ref)	1.34 (0.75- 2.38)	

Where, OR (Odd's ratio), χ2 p (Chi-square); p<0.05 is significant; p>0.05 is non-significant.

TG/HDL-c ratio in both CAD negative and positive subjects than the control subjects and also observed higher value was observed in CAD positive subjects than negative subjects, this may due to higher levels of triglycerides and lower levels of HDL-c in CAD positive subjects. Initially, TG/HDL-c ratio proposed by Gaziano et al is an atherogenic index that has proven to be a highly significant independent predictor of myocardial infarction, even stronger than TC/HDL-c and LDL-c/HDL-c[51]. Angela Bacelar et al. reported that this ratio is possible to approximately determine the presence and extent of coronary artery disease (CAD) by non-invasive methods[52]. The above results indicate and support this ratio is a very useful predictor for assessment of cardiovascular disorders

Table 7: Comparison of lipid profile of the CADP cases of the DC subjects with their LPL Intron 8 T >G gene genotypes.

Lipid profile	CADP(n=45)			
	GG (n=5)	TG(n=13)	TT(n=27)	TG+TT(n=40)
TC	228.8± 20.27	215.1± 13.60 ^{ns}	223.2± 7.77 ^{ns}	220.6± 6.78 ^{ns}
TGs	216.8± 22.71	257.2± 40.67^{ns}	254.7± 31.70 ^{ns}	255.6± 24.85 ^{ns}
LDL-c	149.0± 11.85	129.9± 13.65 ^{ns}	137.7± 7.67 ^{ns}	135.2± 6.74 ^{ns}
VLDL-c	43.36± 4.54	51.45± 8.13 ^{ns}	50.95± 6.34 ^{ns}	51.11± 4.97 ^{ns}
HDL-c	36.40± 6.80	33.69± 1.97 ^{ns}	34.93± 1.23 ^{ns}	34.53± 1.04 ^{ns}
Non-HDL-c	192.4± 13.65	181.4± 11.94 ^{ns}	188.3± 6.94 ^{ns}	186.0± 6.02 ^{ns}

Where,*p<0.05;**p<0.01;***p<0.001considered significant and ^{ns}>0.05 non significant.

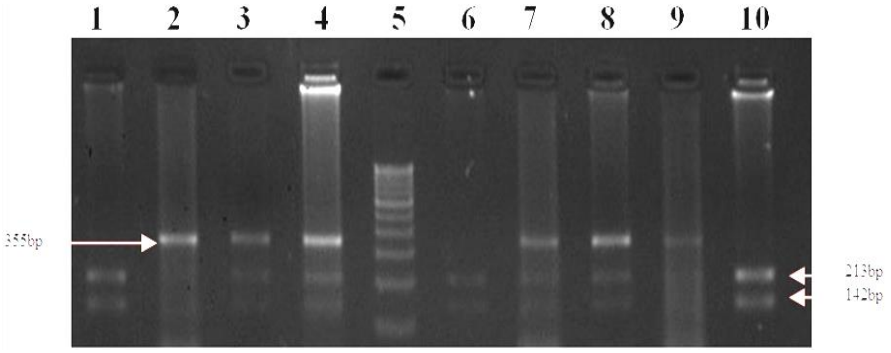

Figure 1: LPL intron 8 T >G gene polymorphism. Lane: 5 [Ladders], Lane: 1, 6 & 10 [Homozygote (wild): T/T - 213bp; 142bp], Lane: 3, 4, 7 & 8 [Heterozygote: T/G-355bp; 213bp; 142bp], Lane: 2 & 9 [Homozygote (mutant): G/G- 355bp].

in humans. Atherogenic Index of Plasma (AIP) shown an inverse relationship that exist between TG and HDL-c and that the ratio of TG to HDL-c is a strong predictor of infarction and it was used by some practitioners as a significant predictor of atherosclerosis[51]. Other researchers suggested that AIP is a highly sensitive marker of the difference of lipoprotein in patients. AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high cardiovascular risk[53]. In our study observed high values of AIP in both CAD negative and positive subjects than control subjects and also observed higher value was observed in CAD positive subjects than negative subjects. These CAD positive subjects already confirmed by electrocardiographic changes and other clinical characteristics, so this above result indicates and supports this index may be very useful in prediction for CAD. Atherogenic coefficient (AC) is a measure of cholesterol in LDL-c, VLDL-c lipoprotein fractions with respect to good cholesterol or HDL-c. Both Pearson's correlation and linear regression of HDL-c showed significantly with atherogenic indices in our study. Higher levels triglycerides, LDL-c and lower levels of HDL-c have known risk factor development of CAD. Our results are also showed that, higher values of atherogenic indices of both CAD negative & positive subjects than controls. Particularly, higher values are observed in CAD positive subjects than negative subjects. These values are reflected the atherogenic potential of the entire spectrum of lipoprotein fractions. In the case of LPL intron 8 T >G gene polymorphism; several reports are suggested that T (H^+) allele is associated with hypertriglyceridemia[54-56]. The higher frequency of T (H^+allele) was found in white patients with severe coronary atherosclerosis than healthy control and suggested that LPL intron 8 T >G gene polymorphism (rs320) influence atherosclerotic disease[57]. Likewise, Chen et al also reported that this polymorphism was positively correlated with carotid artery atherosclerosis in white male subjects[58,59]. In an another study observe, H^+H^+ (T/T) genotype has a higher risk of myocardial infarction (MI) in patients over 90 years old, while H^-allele (G) carriers are protected against MI[60]. Malygina et al reported that TT (H^+H^+) of LPL gene is one of the markers of predisposition to myocardial infarction (MI), while allele H^- (G allele) is one of the resistance marks in Russian elderly patients with stable effort angina (SEA)[61]. Ma YQ et al., conclude that the T allele of the LPL gene intron 8 T >G polymorphism is associated with higher plasma triglyceride and lower HDL-cholesterol levels in Chinese patients with early-onset diabetes[62]. Zhang et al observed that, the plasma triglycerides (TGs) level of H^+H^+ (T/T) genotype was significantly higher than that of H^+H^- (T/G) and H^-H^- (G/G) genotypes (P<0.05 and P<0.01); the plasma TC level and TG/HDL-C ratio were higher than those of H^+H^- and H^-H^-genotypes (P<0.05) in Chinese type IIb hyperlipoproteinemia subjects[63]. H^-allele was associated with lower levels of triglycerides and higher HDL-c in a southern Brazilian population of European descent and also the H^- haplotypes was associated with a significant protective effect against in coronary artery disorders in male subjects[64]. Similar results, like LPL H^+ H^+ genotype, was a risk factor for myocardial infarction in Brazil and Russian population[65] and significantly associated with myocardial infarction (MI) patients in South Indian population[66], but these polymorphic studies are very limited in India. In another study observed H^- (G) allele was not associated with blood lipids or cardiovascular events[67] and also a recent study conducted by Marcia et al reported H^+/H^+ (T/T) genotype

and the H^+ (T) allele were associated with elevated VLDL-c and triglycerides levels ($P < 0.05$) and reduced HDL-C levels ($P < 0.05$)[68]. Likewise, $H^- H^-$ (G/G), $H^+ H^-$ (T/G) and H^+H^+ (T/T) genotypes of intron 8 T >*G* LPL gene no significant differences in the serum levels of TC, TG, HDL-c and LDL-c in Saudi Population[69] similarly a study between the control and coronary artery disease in Shiraz City observed, this polymorphism doesn't have any significant association with CAD[70]. In our study observed the higher frequency of TT (H^+H^+) genotypes in CAD positive than the negative and control subjects.

CONCLUSION

Our conclusion is, BMI mainly used as an indicator of total adiposity but not an important predictor for assessment of cardiovascular risk. An elevated level of triglycerides, LDL-c, VLDL-c, Non-HDL-c and reduced HDL-c are an important indicator for assessment of cardiovascular disorders risk development. We are concluded and support the earlier studies; these atherogenic indices are a powerful indicator to predict the risk of coronary artery diseases. CAD positive subjects showed higher values of atherogenic indices than CAD negative subjects. These results indicate atherogenic indices are may be useful for identifying an individual at higher risk of cardiovascular disease in the clinical practices especially and not markedly deranged or in centers with insufficient resources to predict the CVS risk. The higher values, higher the risk of developing cardiovascular diseases and *vice versa*. In the case of LPL intron 8 T >G gene polymorphism (rs320) results supports the above data; T allele (H^+) was associated with various cardiovascular risks such as positively correlated with carotid artery atherosclerosis, higher risk of myocardial infarction and higher plasma triglycerides and lower HDL-cholesterol. LPL intron 8 T >G gene polymorphism (rs320) might use as markers for predicting the cardiovascular risk.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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