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Original Article

Role of Atherogenic Indices and Association of LPL Intron 6 (C >T) Gene Polymorphism with Dyslipidemia Subtypes in the Prediction of Cardiovascular Risk

Pusapati Madan Ranjit[1,*], Girijasankar Guntuku[2], Ramesh Babu Pothineni[3]

[1]Research Scholar, College of Pharmacy, Jawaharlal Nehru Technological University-Kakinada, Kakinada, Andhra Pradesh, INDIA.
[2]University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, INDIA.
[3]Director and Chief Cardiologist, Cardiac and Multispecialty Hospital, Vijayawada, Andhra Pradesh, INDIA.

ABSTRACT

Background and Objectives: Dyslipidemia is renowned as a prominent risk factor for development of atherosclerosis and other Cardiovascular disease. The Aim study to estimation of lipid profile and predict the cardiovascular risk among the dyslipidemia subtypes and also determine the association of LPL intron 6 C >T (rs285) gene Polymorphism in dyslipidemia subtypes. **Methods:** A total number of 258 subjects were participated in our study, in which 129 members were with elevated lipid profile considered as cases and 129 numbers with normal lipids considered as control subjects. Among the 129 cases, they are further divided into dyslipidemia subtypes. All the sample were further used for analysis of lipid profile and determined the gene Polymorphism by RFLP. **Results:** In our study observed, elevated levels of lipid profile [TC (241.53±6.45), TGs (295.40±28.31), LDL-c (143.69±7.05), VLDL-c (59.08±5.66) and lower levels of HDL-c (39.70± 1.23) and higher values atherogenic indices in combined lipidemia type than the other types and controls. In genotype analysis was not showed significant association of intron 6 C >T gene polymorphisma in both hypercholesterolemia [odd's ratio: 0.77(0.33-1.79); 0.18(0.05-0.62); χ^2 p: 0.025] and hyperlipidemia [odd's ratio: 0.40 (0.14-1.12); 0.82 (0.33-2.03); χ^2 p: 0.177] cases compared with control subjects. But observed, higher risk with CT and CC genotypes in both hypertriglyceridemia and combined dyslipidemia cases. In combined lipidema cases the comparison of lipid profile of genotypes, CC genotypes showed significantly elevated triglycrides (349.5±39.49; $p<0.05$) and VLDL-c (69.91±7.898; $p<0.05$) than CT genotypes. **Conclusion:** Combined lipidemia is one of the major forms of dyslipidemia and atherogenic indices are used as strong predictors to assess the cardiovascular risk in the clinical practices. In case genotype analysis, LPL intron 6 C >T gene Polymorphism may be associated with elevated levels of triglycrdies and VLDL-c.
Key words: Dyslipidemia, Lipid profile, Atherogenic indices, Lipoprotein lipase, Gene polymorphism.

Correspondence
Mr. Pusapati Madan Ranjit
Research Scholar, College of Pharmacy, Jawaharlal Nehru Technological University-Kakinada,(JNTUK) Kakinada, Andhra Pradesh, INDIA.
Ph.no: +91 8106345367
E-mail address: madanranjit@gmail.com

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INTRODUCTION

Dyslipidemia is renowned as a prominent risk factor for development of atherosclerosis and other cardiovascular disease (CVD).[1-3] Dyslipidemia is manifest as elevated levels of one or many lipids such as total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c), triglycerides (TGs) and decreased levels of high density lipoprotein cholesterol (HDL-c) in human blood. World health organization (WHO) estimates the more than half of global cases of ischemic heart disease and more than 4 million deaths per year was due to dyslipidemia In India, there would be around 62 million patients with coronary artery disease by 2015 in India and in which, 23 million would be patients younger than 40 years of age.[4]

An elevated level of lipid profile depends upon various factors like, age, gender,[5] race[6] life style (dietary content),[7] Disease status such as diabetic,[8] thyroid complication[9] and certain medication[10-13] and genetic factors.[14] As per Indian Council of Medical Research-India Diabetes (ICMR–INDIAB) study observed, 29.5% had hypertriglyceridemia (HT), 13.9% had hypercholesterolemia (HC), 11.8% had high LDL-C (HL) levels, 72.3%, had low HDL-C and 79% had abnormalities in one of the lipid parameters in urban and rural India.[15]

Apart from the epidamalogical and habitats, the common variants of genes central to lipid metabolism that are associated with modest changes in protein function might be important contributors to risk at a population level.[16] Lipoprotein lipase (LPL) plays an important role in lipid metabolism by hydrolyzing triglycerides in circulating lipoproteins, which constitutes the rate-limiting step in removal of triglyceride-rich lipoproteins, such as chylomicrons (CM) and very low-density lipoproteins (VLDL) from the circulation.[17] Mature LPL is a 448 amino acids and it was located on chromosome 8p22, with 9 exons and 29.6 kb.[18-19] LPL intron 6 *C >T* (rs285 C/T in dbSNP) is the one of the most common polymorphism in LPL gene at position 19957678 of chromosome 8. It was intron '6' base transition of cytosine (C) to thymine (T) transition 1.57 kb from SA-site.[20]

Hence, the aim of the present study is prediction of cardiovascular risk by using estimation of lipid profile and atherogenic indices like, TGs/HDL-c, Atherogenic index of plasma [AIP] and Atherogenic Coefficient [AC]) along with clinically used indices [Castelli's Risk Index-I (CRI-I) and Castelli's Risk Index-I (CRI-II)] among the dyslipidemia types such as HT(elevated levels of triglycerdies), HC(elevated levels of total cholesterol), HL (elevated levesl of LDL-c) and combined lipidemia (CL: Elevated levels of both TC and TGs) cases. And also, to determine the association of LPL intron 6 *C >T* (pvu II) polymorphism with dyslipidemia subtypes.

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 we visit to hospital for their general health checkup. The study protocol was approved by the Institutional Ethical Committee and it was conducted during the period from 2012-2014.

History of participants

Collect the present and past disease, history of the participant, before included in the study. It was based on the any subject was previously suffered with any disease conditions like thyroid, diabetes, hormonal therapy and metabolic dysfunction. The present study was designed based on cohort type study and selection of subjects by implementing the certain inclusion and exclusion criteria's. Based upon levels of lipid profile subjects was divided into cases and control subjects.

Exclusion criteria

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

Inclusion criteria

Based on elevated types of lipids in cases were further classified into hypercholesterolemia (HC: ↑ TC, ↓TG, ↑LDL-c); Hypertriglyceridaemia (HT: ↑TGs, ↓TC, ↑LDL-c); Combined Lipidemia (CL: Both ↑TC, TGs, ↑LDL-c); Hyperlipidemia (HL: Only ↑LDL, ↓TC and TGs) subjects.(↑: Increased; ↓: Decreased).

Data collection and selection of subjects

Complete examination of each subject was carried out; it included name, age and address. An informed written consent was obtained from all the study subjects who participated in our study. Anthropometric parameter like body mass index (BMI) was calculated by weight in kilograms (kg) divided by the square of the height in meter (kg/m^2). Selection of subjects based upon the plasma lipid abnormality cuts off values given by an expert panel of the National Cholesterol Education program (NCEP).[21] A total number of 258 subjects were 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, they are further divided into hypercholesterolemia (n=34), hypertriglyceridemia (n=30); combined lipidemia (n=30) and hyperlipidemia (n=35).

Collection of a blood sample and analysis 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, total cholesterol (TC), triglycerides (TGs) and high density lipoprotein cholesterol (HDL-c) respectively. The tests were carried out in an automated clinical auto analyzer. Further, low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and Non HDL-c were calculated by using Friedewald's formula.[22] 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,[23-25] TG/HDL-c ratio[26] and Atherogenic Index of Plasma (AIP) = log (TG/ HDL-c)[27] are calculated for the individuals.

Genomic DNA isolation and LPL intron 6 (C >T) genotyping(rs285)

Genomic DNA was extracted from 5 ml of fresh whole blood by 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.[28] Primer sequences, polymerase chain reaction (PCR) conditions and restriction enzyme digestions were as follows (oligonucleotides were synthesized by Bio-serve, Gene valley, Hyderabad, Telengana, India). In the region of intron 6, the LPL gene containing C >T (rs285) polymorphism was amplified using the following primers: forward primer: 5'-TAGAGGTTGAGGCACCT-GTGC-3' and Reserse primer was 5'- GTGGGTGAATCACCTGAGG-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 68.2°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 Pvu II restriction enzyme (New England Biolabs.UK) for 16 h at 40°C. The resulting genotypes (Figure 3) products are CC (Homozygote wild: 592bp; 266bp), CT (Heterozygotes: 858 bp; 592bp; 266bp) and TT (Homozygote mutant: 858bp) were electrophresed on 2% agarose gel.

Statistical Analysis

The collected data were analyzed by using a graph pad prism, version 6. The differences between the groups were determined by performing the one-way analysis of variance (ANOVA), Dunnett Multiple Comparison test, data was expressed as mean ± standard error mean (SEM). Pearson's and regression analysis also preformed. 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. Genotypes analysis performed by calculating the odd'd ratio, chi-square test and Hardy-Weinberg equilibrium calculation.

RESULTS

Table 1 showed the mean±SEM values of age and BMI of the both control and cases. BMI of the control (24.84± 0.39) not showed significant ($p>0.05$) with cases like HC (25.29± 0.65), HT (26.25± 0.83), CL (26.68± 0.77) and HL (26.91± 0.94). Higher lipid profiles (mg/dL) (Table 2 and Figure 1) are observed in all cases than control subjects. Elevated levels of the total cholesterol (225.76± 3.15) and LDL-c (156.00±3.35) in HC subjects, While in HT cases elevated levels of TGs (211.63± 13.25), LDL-c ,VLDL-c(42.32±2.65) and decreased the concentration of HDL-c (33.60± 1.19) observed. Likewise, in HL cases we are observed elevated levels of LDL-c (119.27±1.60). On the other hand, we are observed higher concentration ($p<0.01$) of TC (241.53± 6.45), TGs (295.40± 28.31), LDL-c (143.69±7.05), VLDL-c (59.08±5.66) and lower levels of HDL-c (39.70± 1.23) in combined lipidemia (CL) subjects than control subjects.

Table 3 and Figure 2 showed the comparison of mean±SEM values of atherogenic indices of the control and cases subjects. Among the dyslipidemia types' hypertriglyceridemia (HT) and combined lipidemia cases (CL) showed significantly higher ($p<0.01$) atherogenic indices (CRI-I: 5.56± 0.20; 6.16±0.16, CRI-II: 3.22±0.15; 3.67±0.18, TG/HD-c: 6.69±0.57; 7.57±0.74, AIP: 0.78± 0.03; 0.83± 0.03, AC: 4.55± 0.20; 5.05± 0.17) respectively than other cases and control subjects. Table 4 showed the Pearson's correlation (two tailed) and linear regression analysis of the dyslipidemia types according to their respective elevated lipoprotein. Pearson's correlation analysis of the respective lipoprotein (triglycerides) of the HT and CL types showed significantly correlated with all atherogenic indices, except with CRI-I in HT cases, CRI-I and AC in CL cases. Likewise, linear regression analysis TGs also showed significantly with atherogenic indices of the both HT and CL cases.

Table 5 showed the genotypes distribution of LPL Intron 6 C >T gene polymorphism of control and dyslipidemia sub types of dyslipidemia cases. The distribution of genotypes in control are TT (22.48%), CT(40.31%) and CC (37.20%).The allele frequencies were T(42.63%) and C(57.36%) observed like this. In present our study was not observed significant association of intron 6 C >T gene polymorphism in both hypercholesterolemia [odd's ratio: 0.77(0.33-1.79); 0.18(0.05-0.62); χ^2 p: 0.025] and hyperlipidemia [odd's ratio: 0.40 (0.14-1.12); 0.82 (0.33-2.03); χ^2 p: 0.177] cases compared with control subjects. In case of hyperlipidemia subjects showed significant with allele frequency (χ^2 p:0.002) with control subjects but not observed in hypercholesterolemia subjects (χ^2 p:0.826).In case of hypertriglyceridemia subjects 3 times risk with CT [odd's ratio:3.06 (0.63-4.80)] and 5 times risk with CC [odd's ratio:5.13

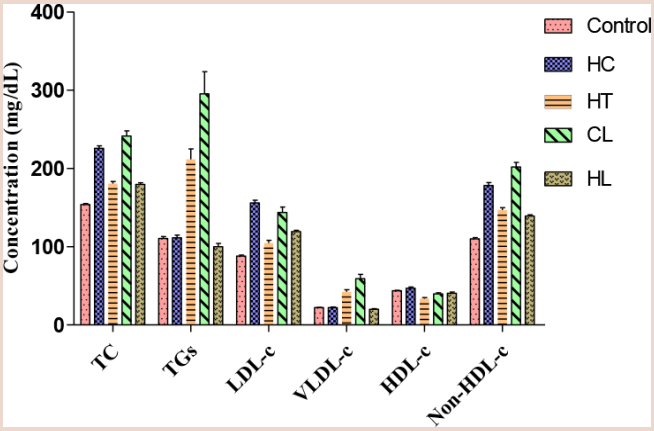

Figure 1: Comparison of lipid profile within types of dyslipidemia cases (DC).

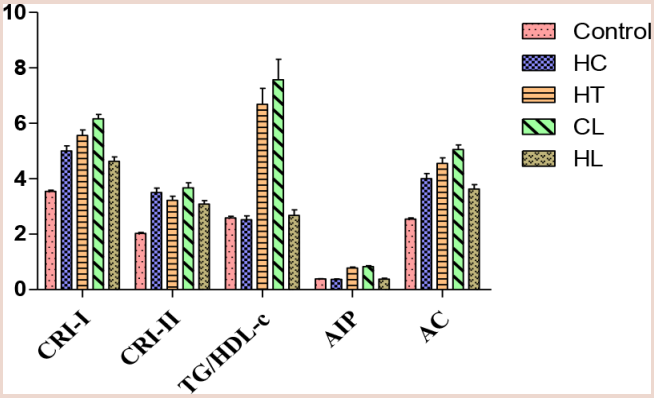

Figure 2: Comparison of atherogenic indices within types of dyslipidemia cases (DC).

Table 1: Showed the comparison of mean±SEM values of the Age, BMI, of the dyslipidemia types by using the Dunnett Multiple Comparisons Test (ANOVA).

Parameter	Controls (n=129)	DC (n=129)			
		HC (n=34)	HT (n=30)	CL (n=30)	HL (n=35)
Age	50.17±1.29	50.47±2.02^{ns}	49.50±2.05^{ns}	54.23± 1.93^{ns}	47.20±2.10^{ns}
BMI	24.84± 0.39	25.29± 0.65^{ns}	26.25± 0.83^{ns}	26.68± 0.77^{ns}	26.91± 0.94^{ns}

Table 2: Showed the comparison of mean±SEM values of the FBG (mg/dL) and lipid profile (mg/dL) of the dyslipidemia types by using the Dunnett Multiple Comparisons Test (ANOVA).

Parameter	Controls (n=129)	DC (n=129)			
		HC (n=34)	HT: (n=30)	CL: (n=30)	HL (n=35)
FBG	87.96±0.47	87.91±0.79^{ns}	87.93±0.88^{ns}	89.46±0.97^{ns}	88.20±0.93^{ns}
TC	153.71± 1.46	225.76± 3.15**	180.50± 2.86**	241.53± 6.45**	179.54± 2.08**
TGs	110.53± 2.32	111.47± 3.44^{ns}	211.63± 13.25**	295.40± 28.31**	99.829± 4.43^{ns}
LDL-c	87.99±1.26	156.00±3.35**	104.59±3.44**	143.69±7.05**	119.27±1.60**
VLDL-c	22.10± 0.46	22.29±0.68^{ns}	42.32±2.65**	59.08±5.66**	19.96±0.88^{ns}
HDL-c	43.61± 0.46	46.82± 1.64^{ns}	33.60± 1.19**	39.70± 1.23**	40.45± 1.57^{ns}

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

(1.10-3.87)] genotypes was observed with control subjects and no significant (χ^2 p: 0.123) association with genotypes but showed significant association with allele frequencies (χ^2 p: 0.011). In combined lipidemia cases (CL) both CT and CC genotypes showed significant association with both genotype [CT: odd's ratio: 12.92(0.73-27.4); CC: odd's ratio: 23.72(1.37-408.0)] (χ^2 p:0.011) and allele frequencies (χ^2 p:0.0005) with control subjects. Table 6 showed comparison of LPL intron 6 C >T gene polymorphism with in dyslipidemia sub types cases. Here, hyperlipidemia cases considered as control and compared the resto the dyslipidemia subtypes. All the cases showed significant assosication with both genotypes and allele frequiences. In genotypes analysis we were observed higher risk of CT and CC genotypes in combined dyslipidemia cases. In combined lipidema cases (Table 7) the comparison of lipid profile of genotypes, CC genotypes showed significantly elevated triglycrides (349.5 ± 39.49; $p<0.05$) and VLDL-c (69.91 ± 7.898; $p<0.05$) levels than CT genotypes.

DISCUSSION

Dyslipidemia is directly related with showing that with an increase in the BMI in both male and females.[29] However, recent studies are explained that BMI has been widely used as an indicator of total adiposity; its limitations are clearly recognized by its dependence on race.[30] In our study also observed BMI was not shown a significant association with cases

Table 3: Showed the comparison of mean±SEM values of atherogenic indices of the dyslipidemia types by using Dunnett Multiple Comparisons Test (ANOVA).

Parameter	Controls (n=129)	DC (n=129)			
		HC (n=34)	HT: (n=30)	CL: (n=30)	HL (n=35)
CRI-I	3.55±0.04	5.00±0.^{19**}	5.56± 0.^{20**}	6.16±0.^{16**}	4.63±0.^{16**}
CRI-II	2.03±0.03	3.50±0.^{16**}	3.22±0.^{15**}	3.67±0.^{18**}	3.09±0.^{12**}
TGs/HDL-c	2.59±0.06	2.52±0.^{14} ns	6.69±0.^{57**}	7.57±0.^{74**}	2.68±0.^{20}ns
AIP	0.39± 0.01	0.37± 0.^{02}ns	0.78± 0.^{03**}	0.83± 0.^{03**}	0.38± 0.^{03}ns
AC	2.55± 0.04	4.00± 0.^{19**}	4.55± 0.^{20**}	5.05± 0.^{17**}	3.63± 0.^{16**}

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

Table 4: Showed the Pearson's correlation (two-tailed) and linear Regression analysis of the dyslipidemia types.

Dyslipidemia types	Pearson's correlation		Linear regression	
	r	p value	r^2	p value
Hypercholesterolemia				
TC *vs.*CRI-I	0.2655	0.1291^{ns}	0.07049	0.0704^{ns}
TC *vs.*CRI-II	0.2957	0.0896^{ns}	0.08741	0.0896^{ns}
TC *vs.*TG/HDL-c	0.04179	0.8145^{ns}	0.00174	0.8145^{ns}
TC *vs* AIP	0.07478	0.6743^{ns}	0.00559	0.6743^{ns}
TC *vs.*AC	0.2655	0.1291^{ns}	0.07049	0.1291^{ns}
Hypertriglyceridemia				
TGs *vs.*CRI-I	0.3679	0.0454	0.1354	0.0454
TGs *vs.*CRI-II	-0.1917	0.3101^{ns}	0.0367	0.3101^{ns}
TGs *vs.*TG/HDL-c	0.9167	< 0.0001	0.0367	< 0.0001
TGs *vs* AIP	0.8832	< 0.0001	0.7800	< 0.0001
TGs *vs.*AC	0.3688	0.0449	0.1360	0.0449
Combined lipidemia				
TC *vs.*CRI-I	0.4076	0.0254	0.1661	0.0254
TC *vs.*CRI-II	0.2979	0.1098^{ns}	0.0887	0.1098^{ns}
TC *vs.*TG/HDL-c	0.0830	0.6628^{ns}	0.0068	0.0068^{ns}
TC *vs* AIP	0.0813	0.6691^{ns}	0.0066	0.6691^{ns}
TC *vs.*AC	0.0578	0.0578^{ns}	0.0033	0.0033^{ns}
TGs *vs.*CRI-I	0.1446	0.4460^{ns}	0.0209	0.4460^{ns}
TGs *vs.*CRI-II	-0.6359	0.0002	0.4460	0.0002
TGs *vs.*TG/HDL-c	0.9581	< 0.0001	0.9179	< 0.0001
TGs *vs.* AIP	0.9243	< 0.0001	0.8543	< 0.0001
TGs *vs.*AC	0.1750	0.3549^{ns}	0.0306	0.3549^{ns}
Hyperlipidemia				
LDL-c *vs.* CRI-I	0.1814	0.2970^{ns}	0.03291	0.2970ns
LDL-c *vs.* CRI-II	0.2679	0.1197^{ns}	0.07178	0.0717^{ns}
LDL-c *vs.*TG/HDL-c	-0.1134	0.5165^{ns}	0.01287	0.5165^{ns}
LDL-c vs.AIP	-0.1265	0.4688^{ns}	0.01601	0.0160^{ns}
LDL-c *vs.*AC	0.1814	0.1814^{ns}	0.01601	0.2970^{ns}

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

Table 5: Genotypes distribution of LPL Intron 6 C >T gene polymorphism of control and Dyslipidemia sub types of Dyslipidemia cases

Geno types N (%)	Control N % Genotypes (n=129)	HL (n=35)		HC(n=34)		HT (n=30)		CL(n=30)	
		N (%) Genotypes	Odd's ratios	N (%) Genotypes	Odd's ratios	N (%) Genotypes	Odd's ratios	N (%) Genotypes	Odd's ratios
TT	29(22.48%)	13(37.14%)	1.00(Ref)	11(32.35%)	1.00(Ref)	2(6.66%)	1.00(Ref)	0(0.00%)	1.00(Ref)
CT	52(40.31%)	18(51.42%)	0.77 (0.33-1.79)	8(23.52%)	0.40 (0.14- 1.12)	11(36.66%)	3.06 (0.63-4.80)	11(36.66%)	12.92 (0.73 -27.4)
CC	48(37.20%)	4(11.42%)	0.18 (0.05- 0.62)	15(44.11%)	0.82 (0.33- 2.03)	17(56.66%)	5.13 (1.10-3.87)	19(63.33%)	23.72 (1.37-408.0)
χ^2 p			0.025		0.177		0.123		0.011
Allele frequency	(n=258)	(n=70)		(n=68)		(n=60)		(n=60)	
T	110(42.63)	44(62.85%)	1.00(Ref)	30(20.4%)	1.00(Ref)	15(25%)	1.00(Ref)	11(18.33%)	1.00(Ref)
C	148(57.36%)	26(37.14%)	0.43 (0.25- 0.75)	38(55.88%)	0.94 (0.54- 1.61)	45(75%)	2.23 (1.18- 4.20)	49(81.66%)	3.31 (1.64- 6.66)
χ^2 p			0.002		0.826		0.011		0.0005
HWE	3.99	0.36		9.29		0.01		1.51	

Where, χ^2 p (Chi-square) $p<0.05$ is significant, HWE (Hardy-Weinberg equilibrium)

Table 6: Comparison of LPL Intron 6 C >T gene polymorphism Dyslipidemia subtypes

Variables		Genotype (%)				Allele (%)			
	N (%)	TTn (%)	CT n (%)	CCn(%)	χ^2 p	N (%)	Tn(%)	Cn(%)	χ^2 p
DC	129					258			
HL:	35(27.13%)	13(37.14%)	18(51.42%)	4(11.42%)		70(27.13%)	44(62.85%)	26(37.14%)	
HC:	34(26.35%)	11(32.35%)	8(23.52%)	15(44.11%)		68(26.35%)	30(20.4%)	38(55.88%)	
OR(95%CI)		1.00(Ref)	0.52 (0.16- 1.67)	4.43(1.13-17.35)	0.014		1.00(Ref)	2.14(1.08 - 4.23)	0.027
HT:	30(23.25%)	2(6.66%)	11(36.66%)	17(56.66%)		60(23.25%)	15(25%)	45(75%)	
OR(95%CI)		1.00(Ref)	3.97(0.74-21.05)	27.63(4.36-174.8)	0.001		1.00(Ref)	5.07(2.37- 10.85)	0.001
CL:	30(23.25%)	--	11(36.66%)	19(63.33%)		60(23.25%)	11(18.33%)	49(81.66%)	
OR(95%CI)		1.00(Ref)	--	--	0.001		1.00(Ref)	7.53(3.33- 17.02)	0.001

Where, OR: odd's ratio, χ^2 p: Chi-square $p<0.05$ is significant.

Table 7: Comparison of lipid profile of the genotypes in combined lipidemia cases by using Unpaired 't' test

Lipid profile	Combined lipidemia			p-value
	TT(n=0)	CT(n=11)	CC(n=19)	
TC	--	233.2 ± 6.638	246.4 ± 9.390	>0.05
TG	--	201.9 ± 10.52	349.5 ± 39.49	<0.05
LDL-c	--	153.9 ± 6.332	137.8 ± 10.42	>0.05
VLDL-c	--	40.38 ± 2.103	69.91 ± 7.898	<0.05
HDL-c	--	38.91 ± 1.569	40.16 ± 1.743	>0.05
Non-HDL-c	--	194.3 ± 6.048	206.2 ± 8.629	>0.05

Where, $p<0.05$ is significant.

subjects. These results indicated that our results correlated with previous studies.

The prediction of cardiovascular risk is based upon determining the comparison concentration of pro-atherogenic lipid profile (TC, TGs, LDL-c and VLDL-c) and anti-atherogenic lipid HDL-c.[21] In our study, we are observing elevated of TGs, LDL-c VLDL-c and lower HDL-c levels in combined lipidemia cases than other types. These clearly indicate that combined lipidemia more risk than other cases and support the other studies.

Elevated serum triglycerides are one of the important risk factors for developments of atherosclerosis, possible mechanisms for explaining; elevated triglycerides might promote and responsible for the generation

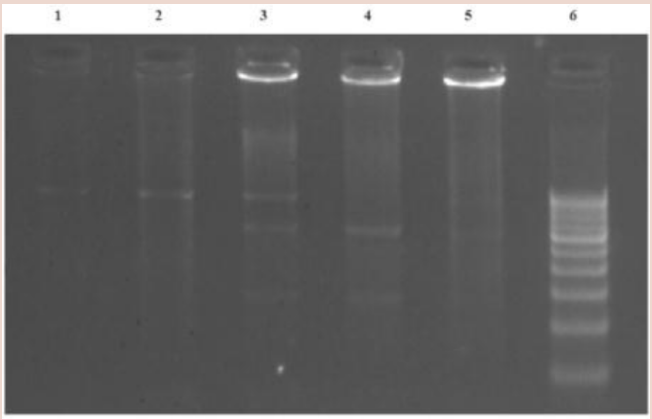

Figure 1: Showed genotypes after digestion of Pvu II enzyme.Lane 6 [Ladder], Lane 4 [Homozygote (wild): C/C: 592bp; 266bp], Lane 3 and 5 [Heterozygotes: C/T: 858 bp; 592bp; 266bp], Lane 1 and 2 [Homozygote (mutant): T/T: 858bp].

of small dense LDL-c. Further, these small LDL-c particles are highly more susceptible to oxidation than larger lipoproteins.[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 synthesis 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.

In clinical practice, assessment of atherosclerosis risk of the individuals by performing an angiogram or any other suitable methods. Other methods to predict the CVD risk by calculating the lipid ratio mentioned above. All these atherogenic indices are shown higher in dyslipidemia types than control subjects. Among the dyslipidemia types HT and CL types higher values than others.

Castelli's Risk Index-I (CRI-I) and Castelli's Risk Index-II (CRI-II) are generally used in clinical practice. The average ratio of total cholesterol (TC) to HDL-c (CRI-I) of healthy individuals is about 3.5 or lower[23,39] and in case of LDL-c/HDL-c ratio (CRI-II) is 3 or lower.[39,40] Another research study explained association of TC/HDL-c with coronary plaque formation.[41] In the PROCAM study observed, subjects with LDL-c/HDL-c (CRI-II) >5 had a six times higher rate of coronary events.[42]

Protasio *et al*. explained that the ratio of triglycerides (TGs) to HDL-c was found to be a powerful independent indicator of extensive coronary disease.[26] 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.[43] 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.[44]

Atherogenic Index of Plasma (AIP) shown an inverse relationship that exist between TG and HDL-c and that the ratio of TG of HDL-c is a strong predictor of infarction and it was used by some practitioners as a significant predictor of atherosclerosis.[43] Other researchers suggested that, AIP is a highly sensitive marker of 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.[45]

Atherogenic coefficient (AC) is a measure of cholesterol in LDL-c, VLDL-c lipoprotein fractions with respect to good cholesterol or HDL-c. It reflects the atherogenic potential of the entire spectrum of lipoprotein fractions. The higher values, higher the risk of developing cardiovascular diseases and *vice versa*.[46] Pearson's correlation (two tailed) and linear regression analysis also explained the importance of triglycerides with atherogenic indices. All these effects finally observed the development of atherosclerosis and its associated disorders.

Our genotypes results agreed with previous reports, LPL Intron 6 C >T gene polymorphism Callele has been reported to be associated with variation/higher levels in plasma triglyceride levels in (C/C) than the (T/T) of the Intron 6 C >T -polymorphism in a Japanese population.[47] Similar results observed in other studies[48-50] and Seung-Ho *et al*. reported that, *Pvu II* (Intron 6 C >T) site was associated with elevated triglyceride levels in the combined hyperlipidemic group of the in Koreans,[51] another Chinese population study observed that CC genotype in the lipoprotein lipase gene is associated with susceptibility to Hypertriglyceridemia.[52] A recent Macedonian population, LPL Intron 6 C >T polymorphism in the lipoprotein lipase gene with the coronary artery disease reported that no significant differences between the prevalence of the LPL- Intron 6 C >T genotypes in both coronary artery disease patients and control subjects. However, the homozygous genotype (CC) was more prevalent in the CAD group (22.9%) than the control group (15.6%).[53] But, some research reportes are showed no significant effect of intron 6 C >T polymorphism on plasma triglycerides levels in biethnic population (Hispanics and non-Hispanic Whites)[54] and in ECTIM study also observed, LPL Intron 6 C >T polymorphism not showed significant effect on fasting lipid parameters and severity of coronary lesions.[55] Similarly, TT, CC and CC genotypes did not correlate significantly with plasma levels of TC, TG, HDL-c and HDL-c, Hence not showing association with CAD in Saudi population.[56]

CONCLUSION

BMI association with lipid profile based upon the age, gender and race, so our results support the earlier studies. Among the dyslipidemia subtypes hypertriglyceridemia (HT) and combined lipidemia (CL) cases showed, a high lipid profile and higher values of atherogenic indices. These results indicated that, both the cases of HT and CL type of dyslipidemia are riskier than other dyslipidemia types. Elevated triglycerides are known, responsible for elevated levels of VLDL-c and lipoproteins. In our study also observed, in both CL and HT cases, higher concentrations of VLDL-c than other types. In hypertriglyceridemia patients have VLDL-c, rich small LDL-c, large LDL-c particles and low levels of HDL-c. Further, these small LDL-c particles are highly susceptible to oxidation and responsible for the progression /development of atherogenic plaques.[31] Atherogenic indices are shown significantly with hypertriglyceridemia (HT) and combined lipidemia (CL) during our study. So our conclusion, these indices may be useful to predict cardiovascular risk in the clinical practices, especially when in centre with not markedly deranged or insufficient resources.

The our results indicated that CC and CT genotypes associated with significantly elevated levels of lipid profile in combined lipidemia. This Polymorphism may be due to the region containing *Pvu II* site resemble the splicing site in its homology to the accord sequence required for 3'-splicing and the creation of the lariat structure, suggesting that C497T (CAG CTG→TAG CTG) change may interfere with splicing of mRNA and abolishes the restriction site for the enzyme for *Pvu II*. Previously, Fisher *et al*. and Shurui Li *et al*. reported that Pvu *II* polymorphism by RFLP.[57-58] Our conclusion is LPL intron 6 C >T gene Polymorphism may

be associated with elevated levels of lipid profile especially in triglycrdies and VLDL-c.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

BMI: Body mass index; **SNP:** Single nucleotide polymorphism; **DNA:** deoxyribonucleic acid; **dbSNP:** Single Nucleotide Polymorphism Database; **C> T:** Cytosine > Thymine.

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