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SINGLE NUCLEOTIDE POLYMORPHISM IN CORONARY ARTERY DISEASE: IS IT GOOD OR BAD?

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Abstract

Introduction: Coronary artery disease (CAD) development and progression were stimulated by environmental or genetic factors. Single Nucleotide Polymorphism (SNP) in apolipoprotein genes involved in lipid and lipoprotein metabolism were primary candidates as susceptibility genes for coronary atherosclerosis.

Aims and Objectives: Distribution and association of APOA1 gene (-75 G>A), and APOC3 gene (3238 C>G) polymorphisms with serum lipid parameters in CAD subjects will provide the link between SNP and serum lipid parameters as well as CAD outcome.

Materials and Methods: One hundred and fifty diagnosed CAD patients and one hundred and fifty healthy controls were included. Serum lipid profile was measured and APOA1 gene (-75 G>A), and APOC3 gene (3238C>G) polymorphism were detected by DNA analysis. Data were analyzed by one-way ANOVA, Chi-square, and Fisher exact tests.

Results: In APOC3 gene (3238 C>G) polymorphism, there was a statistically significant difference in triglycerides between CC (Wild) and CG+GG (Mutant) genotypes ($p<0.001$) in the dominant genetic model and also statistically a significant difference in triglycerides between CC+CG (Wild) and GG (Mutant) genotypes ($p<0.001$) in the recessive genetic model.

Conclusion: Results of the present study could help to understand the association of APOA1 gene and APOC3 gene polymorphism with serum lipid profiles and CAD and further researches were needed to confirm the influence of other contributing factors for the development of CAD.

Keywords: Polymorphism, Apolipoprotein A1, APOA1 gene (-75 G>A), Apolipoprotein C3, APOC3 gene (3238 C>G), Coronary Artery Disease

Introduction

Coronary artery disease (CAD) was becoming among the major public health concerns worldwide. In 2015, it was responsible for 17.9 million deaths worldwide, representing 31% of all global deaths, a number that is expected to grow to more than 23.6 million by 2030 (1). CAD develops when the main artery that supplies the myocardium with blood, oxygen, and nutrients becomes damaged and diseased. This is a gradual decades-long process resulting in slowly progressive narrowing of the coronary artery, by lipid-burdened plaques that eventually lead to symptoms of ischemia, a state where coronary artery narrowing or atherothrombotic occlusion causes the deficiency of oxygen and nutrient supply to the myocardium. CAD develops silently and can be asymptomatic for many years, or throughout an individual's life (2). Coronary artery disease development and progression are stimulated by environmental or genetic factors. Although environmental factors such as tobacco use, diabetes mellitus, and hypertension play an important role in atherosclerosis development, genetic factors represent consequential determinants of atherosclerotic cardiovascular disease risk (3).

The Human Genome Project has guided in new opportunities for studying the genetic non-Mendelian disorders through the production of single nucleotide polymorphism (SNP) maps and genome-wide SNPs. An enormous number of candidate genes, genetic polymorphisms, and susceptibility loci associated with CAD have been

recognized in recent years and, their number is promptly increasing (4). SNPs in apolipoprotein genes involved in lipid and lipoprotein metabolism are primary candidates as susceptibility genes for coronary atherosclerosis.

Apolipoprotein A1 is a protein that in humans is encoded by the *APOA1* gene. The *APOA1* gene is positioned on the 11^{th} chromosome, with its definite location being 11q23-q24. The gene comprises 4 exons and 3 introns. ApoA1 is the major protein component of HDL particles in plasma (5). *APOA1* gene (-75 G>A) polymorphism has anti-atherogenic effects which increase the plasma ApoA1 level and HDL cholesterol (HDL-C). These are involved in the clearance of lipid particles from circulation. Therefore, *APOA1* gene (-75 G>A) polymorphism is associated with a decreased risk of coronary artery disease (6). *APOA1* gene product is 433 bp (base pairs). There are 3 restriction sites for *Msp I* endonuclease in the *APOA1* gene at -75 bp, +37 bp, and +83 bp, and, therefore four fragments are produced that are 45 bp, 65 bp, 113 bp, and 209 bp. When there is G to A transition at -75 bp, this restriction site is lost, and it produces a fragment of 179 bp instead of 113 and 66 bp (7). *APOC3* gene (3238 C>G) polymorphism has an atherogenic effect by increasing the plasma ApoC3 and triacylglycerol levels. Apolipoprotein C3 gene was discovered in ApoA1-C3-A4 gene cluster on chromosome 11q23.3. ApoC3 was found to inhibit lipoprotein remnant uptake by the liver (8). Several polymorphisms have been found in the *APOC3* gene at the 5′ promoter region, exon 3, and 3′ untranslated region (3′UTR). *APOC3* gene (3238 C>G) polymorphism is also called *Sst I* polymorphism because *Sst I* restriction enzyme (purified from *Streptomyces Stanford)* can digest its polymerase chain reaction (PCR) product. So, the C allele is also called S1 allele and the G allele is S2 allele (9). After digestion with *Sst I* restriction endonuclease, genotypes are defined as CC, CG, and GG and classified based on the absence or presence of the *Sst I* restriction sites. There is no restriction site in CC genotype (428 bp). Products are digested into three fragments in CG genotype (428 bp, 269 bp, and 159 bp). Products are digested into two fragments in GG genotypes (269 bp and 159 bp) (10). So, the relationship between the *APOC3* gene (3238 C>G) polymorphism and genetic susceptibility to CAD has attracted significant clinical and epidemiological research.

Although many studies reported that *APOA1* and *APOC3* genes in CAD is suggested to be implicated in the risk factor for CAD, some association studies have reported conflicting results. The present study aims to provide the genotype and allele frequencies distribution of these SNPs and the association of polymorphism of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) and serum lipid profiles in the development of atherosclerosis in Myanmar population. The discovery of the genes involved in the development of CAD will provide clinicians and scientists in designing more effective therapies and in identifying high-risk individuals for early intervention.

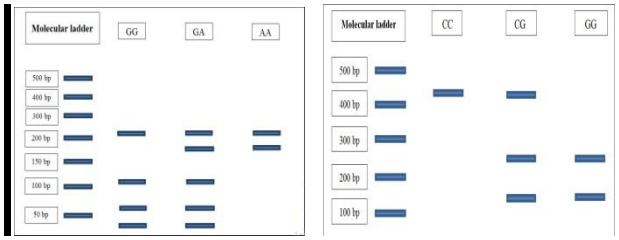
MATERIALS AND METHODS

This cross-sectional comparative study was conducted in the Biochemistry department of a tertiary care hospital and Post-graduate Common Research Laboratory of Medical Academy. The cases were selected randomly from subjects attending the Cardiac department of tertiary care hospital during the period of one and half year, from January 2018 to June 2019. One hundred and fifty CAD diagnosed patients (both sexes) and one hundred and fifty age and sex-matched normal healthy controls were included in this study. CAD was diagnosed by a certified Cardiac physician. Exclusion criteria for subject selection were the presence of heart diseases except for CAD, severe liver disease, severe kidney disease, and severe acute illness. After getting written informed consent from the subjects and taking care of ethical issues, history taking, physical examination, and collecting blood specimen were done.

The six mL of 10 hr overnight fasting venous blood sample was collected. In this total 6 mL of the blood sample, 3 mL was collected in the plain tube for lipid profile and 3 mL was collected in EDTA tube for DNA analysis. Serum was separated from the plain tube by centrifuged with 2000 rpm for 2 minutes within 3 hours and stored at -20°C for estimation of lipid profile in batches. EDTA tube blood was also stored at -20°C for DNA analysis in batches. Total Cholesterol (TC) and High-Density Lipoprotein Cholesterol (HDL-C) were analyzed using cholesterol esterase enzymatic endpoint method, triglycerides (TG) was analyzed using lipoprotein lipase enzymatic endpoint method by Roche Cobas C 111, Switzerland. Low-Density Lipoprotein Cholesterol (LDL-C) was obtained using Friedewald's formula (11).

For DNA analysis, DNA was isolated by the phenol-chloroform method. DNA was amplified using the following oligonucleotide primers (Thermo Fisher Scientific, USA) by polymerase chain reaction (PCR) for *APOA1* gene; (Forward primer: 5'-AGGGACAGAGCT GATCCTTGAACTCTTAAG-3') (Reverse primer: 5'-TTAGGGGACACCTAGCCCTCAGGAAGAGCA-3') and for *APOC3* gene; (Forward primer: 5'-GGTGACCGATGGCTTCAGTTCCCTG-3') (Reverse primer: 5'-CAGAAGGTGGATAGAGCGCTGGCCT-3').

The *APOA1* gene PCR products were digested with *Msp I* endonuclease (Thermo Fisher Scientific, USA), and *APOC3* gene PCR products were digested with *Sst I* endonuclease (Thermo Fisher Scientific, USA) overnight at 37°C. The digested products were separated by 10% polyacrylamide gel electrophoresis. The lengths of each digested DNA fragment were determined by comparing the migration of a standard DNA marker (Thermo Fisher Scientific, USA). After electrophoresis, the separated bands of DNA were visualized under UV light, and genotypes were determined by 50 bp and 100 bp DNA step ladders.



A B

Figure 1: Electrophoretic pattern of PCR product digested with (A) *Msp I* restriction enzyme for *APOA1* gene and (B) *Sst I* restriction enzyme for *APOC3* gene

One-way ANOVA method was applied to calculate the significance of the difference between the means on a 95% confidence interval of results. Chi-square (χ^2) distribution was used to assess the differences between genotypes and allele frequencies of *APOA1* and *APOC3* genes and also the deviation from Hardy-Weinberg equilibrium (HWE). Allelic and genotypic frequencies were calculated by direct gene counting method and the association of *APOA1* and *APOC3* polymorphisms and disease phenotype were determined by Fisher exact test. These data analysis were done by using SPSS version 20.2 software.

RESULTS

There were 91 (60.7%) male and 59 (39.3%) female in CAD group and 77 (51.3%) male and 73 (48.7%) female in control group, 48 (32%) in ≤50 years age and 102 (68%) in >50 years age in CAD group and 63 (42%) in ≤50 years age and 87 (58%) in >50 years age in control group. In comparison the demographic measures and biochemical parameters of CAD group and control group, all values were statistically significant ($p < 0.05$) except DBP parameter (table 1). In the *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism, there were no statistically significant association between genotypes frequency distribution of polymorphism and CAD (p = 0.61, 0.31) and also no significant difference between allele frequency distribution and CAD (p = 0.66, 0.27) (table 2). In the *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism, there were no statistically significant association between genotypes frequency distribution of polymorphism and serum lipid profile except TG (p<0.001) in *APOC3* gene (3238 C>G) polymorphism (table 3).

According to the dominant genetic model of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism, there were no statistically significant difference in serum lipid profiles between GG (Wild) and GA+AA (Mutant) genotypes of *APOA1* gene (-75 G>A) polymorphism, serum lipid profiles between CC (Wild) and CG+GG (Mutant) genotypes of *APOC3* gene (3238 C>G) polymorphism except TG (p<0.001) (table 4). According to the recessive genetic model of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism, there were no statistically significant difference in serum lipid profiles between GG+GA (Wild) and AA (Mutant) genotypes, serum lipid profiles between CC+CG (Wild) and GG (Mutant) genotypes except TG (p<0.001) (table 5). The genotype frequency distribution *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism in CAD group and control group have not deviated from HWE (table 6).

DISCUSSION

APOA1 gene (-75 G>A) polymorphism

In the present study, the mean values of biochemical parameters such as blood pressure (BP), body mass index (BMI), TC, TG, HDL-C, and LDL-C levels of CAD group were significantly higher than those of control group. The genotype distribution of *APOA1* gene (-75 G>A) polymorphism of the study population was in agreement with HWE (p = 0.33) in CAD group and (p = 0.78) in control group. So, there were no sampling errors and *APOA1* gene (-75 G>A) polymorphism genotyping was valid.

There was no significant association between CAD and genotype frequency distribution (χ^2 = 0.99, p=0.61), as well as allele frequency distribution (χ^2= 0.19, p= 0.66) of the *APOA1* gene (-75 G>A) polymorphism in the present study. This finding was consistent with the findings of some studies in the Chinese (12), in the Australian (13), and in the Indian population (14). CAD is a multifactorial disease, this multifactorial differences could be a consequence of the interplay between several risk factors (both environmental and genetic), which very often provide a variety of results in different ethnicities.

The mean values of TC, TG, HDL-C, and LDL-C levels showed no significant differences between genotypes (GG, GA, and AA) of *APOA1* gene (-75 G>A) polymorphism in the present study. This finding disagreed with the previous study of the Chinese population showed that there was a significant difference in serum lipid profiles between genotypes of *APOA1* gene (-75 G>A) polymorphism (15). According to the results of the dominant genetic model and recessive genetic model, there was no significant difference in serum lipid profiles between wild and mutant genotypes of *APOA1* gene polymorphism. The regulation of *APOA1* gene expression is at the transcription level and is influenced by various factors like hormone, diet, developmental, physiological, and various environmental stimuli. The alteration in hormonal or metabolic status can affect the expression of *APOA1* gene (16).

APOC3 gene (3238 C>G) polymorphism

Genotype frequency distribution of the study population was in agreement with HWE (p=0.94) in CAD group and (p=0.99) in control group. So, there were no sampling errors, and *APOC3* gene (3238 C>G) polymorphism genotyping was valid.

In the present study, there was no significant association between CAD and genotype frequency distribution (χ^2=2.32, p=0.31) as well as allele frequency distribution (χ^2=1.20, p=0.27) of the *APOC3* gene (3238 C>G) polymorphism. This finding was consistent with the findings of some studies in the Austrian (17), in the Japanese (18), in the French (19), in the Italian (20), and in the Indian population (21). However, some studies carried out on the Italian (22), the Indian (23), the Egyptian (24), and the Chinese population (25) (26) showed that the

significant association between *APOC3* gene (3238 C>G) polymorphism and CAD.

This inconsistency may be the differences in ethnic groups. This difference is reflected in the CAD morbidity and mortality rates among western populations which are much higher than that found in eastern populations (18). The present study revealed that there was a significant difference in mean serum TG concentrations of the CC, CG, and GG genotypes of *APOC3* gene (3238 C>G) polymorphism (p<0.001). Mean serum TC, HDL-C and LDL-C concentrations of the study population were not a significant difference between genotypes of these polymorphisms. This finding of the present study agreed with the reporting of some studies in the Italian (22), in the Egyptian (24), in the Indian (14), and in the Chinese population (26).

Regulation of *APOC3* gene expression is influenced by various factors like hormone, diet, developmental, physiological, and various environmental stimuli (25). The human *APOC3* gene expression is controlled by positive and negative elements that are spread throughout the APOA1-C3-A4 gene cluster on the long arm of chromosome 11. The several restriction fragment length polymorphisms in and around the *APOC3* gene have been linked with hypertriglyceridemia and CAD in several different populations. The association of *APOC3* gene (3238 C>G) polymorphism with CAD may arise from the fact that it influences the expression of the *APOC3* gene or that it is in linkage disequilibrium with the mutation in the A1 or C3 genes or an adjacent gene predisposes to CAD (27).

CONCLUSION

The finding of the present study revealed that *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism were not concerned with the risk of CAD. The high TG concentration can cause CAD in people who possess GG genotype of *APOC3* gene (3238 C>G) polymorphism in the study population. The results of the present study could help to understand the association of *APOA1* gene and *APOC3* gene polymorphism with serum lipid profiles and CAD and further researches were needed to confirm the influence of other contributing factors for the development of CAD.

Table 1: Demographic measures and Biochemical values

Characteristics	CAD (n = 150)		Control (n = 150)		Statistics	
	Mean	SD	Mean	SD	*t*	*p*-value
Age (years)						
≤50	48 (32%)		63 (42%)			
>50	102 (68%)		87 (58%)			
Sex						
Male	91 (60.7%)		77 (51.3%)			
Female	59 (39.3%)		73 (48.7%)			
SBP (mmHg)	125.57	17.29	118.23	17.48	3.65	<0.001
DBP (mmHg)	79.93	9.65	77.00	9.17	2.69	0.007
BMI (kg/m^2)	25.62	2.03	24.71	2.04	3.87	<0.001
TC (mg/dl)	210.66	48.68	181.84	35.62	6.35	<0.001
TG (mg/dl)	187.08	69.33	151.48	42.63	5.36	<0.001
HDL-C (mg/dl)	41.91	8.94	46.93	20.03	-4.28	<0.001
LDL-C (mg/dl)	117.64	42.59	105.25	41.57	1.15	<0.001

Table 2: *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism in the study population

Variables	CAD (n = 150)	Control (n = 150)	Statistic	
			χ^2	*p*-value
***APOA1* gene (-75 G>A) polymorphism**				
Genotypes				
GG	96 (64%)	104 (69.33%)		
GA	53 (35.33%)	44 (29.33%)	0.99	0.61
AA	1 (0.67%)	2 (1.33%)		
Alleles				
G	245 (81.67%)	252 (84%)	0.19	0.66
A	55 (18.33%)	48 (16%)		
***APOC3* gene (3238 C>G) polymorphism**				
Genotypes				
CC	71 (47.33%)	85 (56.67%)	2.32	0.31
CG	62 (41.33%)	55 (36.67%)		
GG	17 (11.33%)	10 (6.67%)		
Alleles				
C	204 (68%)	225 (75%)	1.20	0.27
G	96 (32%)	75 (25%)		

Table 3: Different genotypes of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism and serum lipid profile in the study population

Variables	GG (Wild) (n = 200)	GA (Heterozygous) (n = 97)	AA (Mutant) (n = 3)	ANOVA	
				(F)	*p*-value
***APOA1* gene (-75 G>A) polymorphism**					
TC (mg/dl)	204.02 ± 41.69	204.93 ± 44.83	190.44 ± 16.05	0.17	0.84
TG (mg/dl)	171.97 ± 65.37	165.44 ± 47.80	113.63 ± 7.41	1.69	0.19
HDL-C (mg/dl)	43.95 ± 16.10	41.36 ± 14.32	50.48 ± 23.15	1.23	0.29
LDL-C (mg/dl)	118.65 ± 42.60	122.52 ± 43.86	109.45 ± 33.82	0.35	0.70
***APOC3* gene (3238 C>G) polymorphism**					
Variables	**CC (Wild) (n = 156)**	**CG (Heterozygous) (n = 117)**	**GG (Mutant) (n =27)**	**(F)**	***p*-value**
TC (mg/dl)	201.15 ± 42.48	207.74 ± 41.71	206.24 ± 42.5	0.84	0.43
TG (mg/dl)	154.8 ± 56.09	175.69 ± 51.2	225.07 ± 80.55	18.79	<0.001
HDL-C (mg/dl)	41.92 ± 15.42	45.73 ± 16.43	39.31 ± 11.4	2.93	0.06
LDL-C (mg/dl)	120.54 ± 43.19	120.21 ± 42.51	113.82 ± 43.71	0.29	0.75

Table 4: Different genotypes of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism and serum lipid profile in the study population (Dominant genetic model)

Variables	GG (Wild) (n = 200)	GA+AA (Mutant) (n = 100)	Statistics	
			t	*p*-value
***APOA1* gene (-75 G>A) polymorphism**				
TC (mg/dl)	204.02 ± 41.69	204.50 ± 44.28	-0.09	0.93
TG (mg/dl)	171.97 ± 65.37	163.89 ± 47.92	1.09	0.27
HDL-C (mg/dl)	43.95 ± 16.11	41.63 ± 14.57	1.21	0.23
LDL-C (mg/dl)	118.65 ± 42.61	122.12 ± 43.52	-0.66	0.51
***APOC3* gene (3238 C>G) polymorphism**				
Variables	**CC (Wild) (n = 156)**	**CG+GG (Mutant) (n = 144)**	***t***	***p*-value**
TC (mg/dl)	201.15 ± 42.48	207.46 ± 42.42	-1.29	0.19
TG (mg/dl)	154.8 ± 56.09	184.95 ± 60.67	-4.47	<0.001
HDL-C (mg/dl)	41.92 ± 15.42	44.53 ± 15.78	-1.45	0.15
LDL-C (mg/dl)	120.54 ± 43.19	119.02 ± 42.65	0.31	0.76

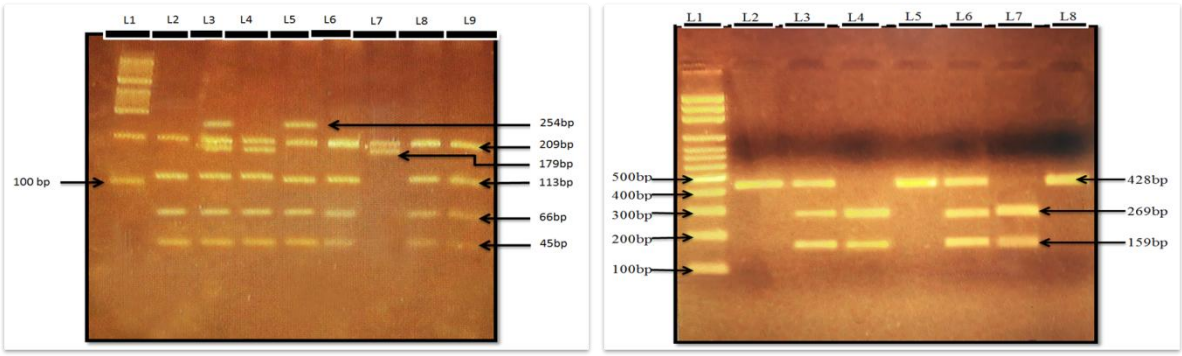
Table 5: Different genotypes of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism and serum lipid profile in the study population (Recessive genetic model)

Variables	GG+GA (Wild) (n = 297)	AA (Mutant) (n = 3)	Statistics	
			t	*p*-value
***APOA1* gene (-75 G>A) polymorphism**				
TC (mg/dl)	204.32 ± 42.67	190.44 ± 16.05	0.56	0.57
TG (mg/dl)	169.84 ± 60.19	113.63 ± 7.42	1.61	0.11
HDL-C (mg/dl)	43.10 ± 15.57	50.48 ± 23.16	-0.81	0.42
LDL-C (mg/dl)	119.91 ± 42.99	109.45 ± 33.82	0.42	0.67
***APOC3* gene (3238 C>G) polymorphism**				
Variables	**CC+CG (Wild) (n = 273)**	**GG (Mutant) (n = 27)**	***t***	***p*-value**
TC (mg/dl)	203.97 ± 42.20	206.24 ± 46.19	-0.26	0.79
TG (mg/dl)	163.76 ± 54.93	225.07 ± 80.55	-5.27	<0.001
HDL-C (mg/dl)	43.56 ± 15.95	39.91 ± 11.41	1.35	0.18
LDL-C (mg/dl)	120.40 ± 42.82	113.82 ± 43.71	0.76	0.45

Table 6: Hardy-Weinberg equilibrium of genotype frequency distribution of *APOA1* gene (-75 G>A) and *APOC3* gene (3238 C>G) polymorphism in the study population

Polymorphism	Expected	Observed	HWE	
			χ^2	*p*-value
***APOA1* gene (-75 G>A) polymorphism**				
CAD group (n = 150)				
Genotypes				
GG	100.8 (67.2%)	96 (64%)	2.21	0.33
GA	44.28 (29.92%)	53 (35.33%)		
AA	4.86 (3.24%)	1 (0.67%)		
Control group (n = 150)				
Genotypes				
GG	105.84 (70.56%)	104 (69.33%)	0.51	0.78
GA	40.32 (26.88%)	44 (29.33%)		
GG	3.84 (2.56%)	2 (1.33%)		

***APOC3* gene (3238 C>G) polymorphism**				
CAD group (n = 150)				
Genotypes CC CG GG	 69.36 (46.24%) 65.28 (43.52%) 15.36 (10.24%)	 71 (47.33%) 62 (41.33%) 17 (11.33%)	0.12	0.94
Control group (n = 150)				
Genotypes CC CG GG	 69.36 (46.24%) 65.28 (43.52%) 15.36 (10.24%)	 71 (47.33%) 62 (41.33%) 17 (11.33%)	0.12	0.94



(A) **(B)**

Figure 2: Electrophoresis patterns of Genotyping result for (A) *APOA1* gene (-75 G>A) polymorphism and (B) *APOC3* gene (3238 C>G) polymorphism

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