Association Between Paraoxonase 1 (PON 1) And Apolipoprotein E (APO E) Polymorphism with Lipid Profile in Acute Coronary Syndrome Patients

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Doi: 10.23918/eajse.v9i1p340

Abstract: Objective: Acute coronary syndromes (ACS) are categorized by a sudden reduction in blood supply to the heart and include ST-segment elevation myocardial infarction (STEMI), non-STEMI (NSTEMI), and unstable angina. The aim of the present study is to evaluate whether the PON1 and APO E genes single nucleotide polymorphisms (SNPs) are associated with the presence of acute coronary syndrome (ACS) and lipid profiles in a case-control association study in an Iraqi Kurdistan population.

Methods: We genotyped two single nucleotide polymorphisms (SNPs) in APOE and PON 1 genes and compared patients who had an initial ACS with patients who presented with stable exertional angina.

Results: A total of 77 samples, including patients with ACS (N = 61), and those with chest pain syndrome (controls, N = 16) were enrolled. Genotyping for PON1 and APO E genes was performed using the PCR assay.

A series of statistical analyses were performed to investigate the association between PON1 and APO E genes SNPs and the susceptibility to ACS. The results revealed a non-significant association of APOE with ACS risk in which £2 (P = 0.585) which was found in all patients and control except 2 patients and 1 control that carried £3 (P = 0.508). £3 was the risk factor, PON 1 was non significantly associated with ACS in which the A allele (P=0.781) in which mostly found in ACS patients in comparison to the control group and it's a strong risk factor for the disease. Also, T allele (P=0.127) found mostly in ACS patients in comparison to the control group. Logistic regression analyses further revealed an increased risk for ACS in A allele carrier, when compared to those with TT homozygotes (odds ratio: 1.199, 95% CI 0.420-3.756, P = 0.781). Furthermore, there were not any significant differences between lipids profile with both genes (APO E and PON 1).

The results of the present study suggested that the PON1 and APO E may play a role in the pathogenesis of Acute coronary syndrome in Kurdistan /Erbil population and that a decrease in PON1 activity may be a valuable marker for monitoring the development of the atherosclerosis process and the associated cardiovascular complications.

Keywords: PON 1 Gene, APO E Gene, Polymorphism, Lipid Profiles, Acute Coronary Syndrome Patients

1. Introduction

Globally the most common reason for death is cardiovascular diseases (CVDs), estimated to claim 17.9 million deaths annually. Coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions which all belong to CVDs (heart and blood disorder)(Roth et al., 2020).

Received: December 15, 2022
Accepted: February 15, 2023
Hayder, Y., & Shekha, M.S. (2023). Association Between Paraoxonase 1 (PON 1) And Apolipoprotein E (APO E) Polymorphism with Lipid Profile in Acute Coronary Syndrome Patients. *Eurasian Journal of Science and Engineering, 9*(1),340-352.

In the meantime, it is expected that CVDs will be the main cause for the death of more than 23 million people worldwide in 2030 (Amini, Zayeri, & Salehi, 2021). Coronary heart disease is a type of stable or unstable disease that can rapidly proceed to acute coronary syndrome (ACS) in a few years, resulting in myocardial infarction as a result patients' sudden death (Bergmark, Mathenge, Merlini, Lawrence-Wright, & Giugliano, 2022). ACS is described as ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UAP) (Bhatt, Lopes, & Harrington, 2022). ACS entails a range of symptoms associated with acute myocardial ischemia produced by rupturing of coronary artery plaque resulting in thrombosis-induced significant coronary artery stenosis or blockage (Xu et al., 2022).

Oxidative stress is the basic factor of atherosclerosis that leads to buildup of fatty lesions, inflammation, and arterial wall destruction (El Hadri, Smith, Duplus, & El Amri, 2021). A critical factor in the expansion of atherosclerotic plaques could be Low-density lipoprotein (LDL) oxidative changes in the artery wall (Glanz, Bezsonov, Soldatov, & Orekhov, 2022). Oxidized LDL formation increases by oxidative stress, in early studies, LDL was widely recognized as the primary cause of atherosclerosis.(Khatana et al., 2020).

Apolipoprotein E (APOE) is a polymorphic glycoprotein that serves multiple functions in lipid metabolism such as; the formation of chylomicrons, very low-density lipoproteins, and HDL, also cholesterol transportation from peripheral tissues to the liver and these all depend on apo E (Tréguier, Bull-Maurer, & Roingeard, 2022). On chromosome 19, the APOE gene, which codes for alleles 2, 3, and 4, has been related to alterations in lipid profiles and the initiation of coronary artery disease (CAD) (Hou et al., 2020).

Human paraoxonase 1 (PON1) is an esterase which hydrolyzes oxidized lipids and catalyzes organophosphate paraoxon hydrolysis, which are both important in the development and progression of atherosclerosis (Taler-Verčič, Goličnik, & Bavec, 2020). Vascular disorders that are developed by atherosclerosis PON1 activity is thought to be a unique risk factor for these disorders (Vavlukis, Vavlukis, Krsteva, & Topuzovska, 2022). PON1 is a high-density-lipoprotein-(HDL)-associated esterase which shows that it participates in the HDL antioxidant and anti-atherosclerotic activities (Bounafaa et al., 2015).

The importance of PON1 and APOE in the development of atherosclerosis has been established in recent decades. (Eichner et al., 2002; Mahrooz, Mackness, Bagheri, Ghaffari-Cherati, & Masoumi, 2019). Several studies have recently focused on the links between PON1 and APOE polymorphisms and cardiovascular and cerebrovascular diseases (Broce et al., 2019; Vavlukis et al., 2022). However, the PON1 and APOE polymorphisms and ACS correlations and their association with lipid profiles that have been found were absent in our region. Additionally, there is no data available on the connection between APOE polymorphism and the risk of CAD in Kurdistan. Therefore, this study was carried out to determine the association between PON1 and APOE polymorphism with presence of acute coronary syndrome (ACS)and lipid profiles and applied in our society.

2.Materials and Methods

2.1 Study Selection

A controlled case study was carried out with 61 patients (43 males and 18 females) aged between 52-65 years who were confirmed to possess ACS by coronary angiography findings at Surgical Specialty

Hospital – Erbil Cardiac Center\ Kurdistan\ Iraq. A total of 16 subjects (11 females and 5 males) with age ranged between (51-72 years), age and sex matched healthy volunteers who had visited the cardiac center and had normal angiography were recruited as controls to compare all the parameters with patients. The control group with no known diseases, including other coronary heart diseases, thyroid disease, pulmonary hypertension, heart failure, diabetes, or atrial fibrillation, based on medical history, physical examination, electrocardiography, echocardiography and biochemical measurements. We defined ACS as acute chest pain together with electrocardiogram changes suggesting myocardial ischemia and/or the elevation of cardiac markers (positive troponins, creatinine phosphokinase, or creatine kinase MB fraction above 2 times the upper limit of normal) (Association & zhi, 2007; Members et al., 2012; Thygesen, Alpert, White, & Cardiology, 2007).

Moreover, the data was collected using standardized case report forms. Demographic characteristics, medical history, symptoms at presentation, duration of prehospital delay, biochemical and electrocardiographic findings, treatment practices, and outcomes were collected. The human Ethic committee of the Biology Department- College of Science- University of Salahaddin approved the study protocol in accordance with the principles outlined in the Declaration of Helsinki for human studies (1997). Meanwhile, all subjects provided informed consent.

2.2 Lipid Analysis and Biochemical Markers

The serum separated from the blood was used for the lipid profile analysis which includes Total cholesterol (TC), Triglycerides (TG), HDL, LDL, assayed on Shar Hospital laboratory by performing a Cobas c311 analyzer (Roche Diagnostics System, Mannheim, Germany).

2.3. Molecular Analysis

2.3.1. Genotyping of APOE Gene Polymorphism

The DNA was extracted from blood samples by using Genomic DNA kit (Jena bioscience), depending on manufacturer's instructions. The polymerase chain reaction (PCR) was conducted with the following primers: Cys158/Arg158 (59-ATGCCGATGACCTGCAGAATT-39)/ (59ATGCCGATGACCTGCAGAATC-39), Cys112/Arg112(59CGCGGACATGGAGGACGT-TT-39)/(59-CGCGGACATGGAGGACGTTC-39), and a common reverse primer (59GTTCAGTGATTGTCGCTGGGCA-39). The common primer was paired with Cys158/Arg158 or Cys112/Arg112 and produced an amplicon of 451 and 588 base pairs (bp), respectively. A 218-bp fragment of the LDLR gene was co-amplified to function as an internal positive control, and the PCR primer sequences were 59- GTGGCACCGAGACCAAACTC-39 and 59-AGTGCAAGGAGACCACGGGA-39. The PCR conditions were denaturation at 95uC for 5 min, followed by 35 cycles of 95uC for 30 sec, 63uC for 30 sec and 72uC for 30 sec, and a final extension at 72uC for 5 min. At the end of the PCR cycles, the products were resolved by electrophoresis on 2% agarose gels to validate the amplification of the specific PCR product expected (Wu et al., 2014).

2.3.2 Genotyping of PON 1 Gene Polymorphism

The DNA was extracted from blood samples by using Genomic DNA kit (Jena bioscience), depending on manufacturer's instructions. Tetra-primer amplification refractory mutation system (ARMS) was designed for the detection of T172A (L55M) polymorphism and of -C107T PON1

according to the Ye et al. (2001) procedure. The primers used are shown in Table 1

PCR was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, South Korea) according to the manufacturer recommended protocol. Into a 0.2-mL PCR tube containing the AccuPower PCR PreMix, 1 µL template DNA (~100 ng/µL), 1 µL of each primer (10 µM) and 15 µL DNase-free water were added. The total volume for the PCR was 20 µL.

For detection of L55M polymorphism, PCR cycling conditions were as follows: 5 min at

95°C; 30 cycles of 30 s at 95°C, 30 s at 59°C and 40 s at 72°C; 10 min at 72°C (). Each reaction was verified on a 2% agarose gel. Product sizes were: 262 bp for the A allele (allele M), 351 bp for the T allele (allele L), and 571 bp for the two outer primers (Figure 1A). For determination of -107C/T polymorphism, PCR cycling parameters were 5 min at 95°C followed by 25 cycles of 20 s at 95°C, 20 s at 66°C, 15 s at 72°C and 10 min at 72°C -. Product sizes were: 116 bp for the C allele and 174 bp for the T allele, while the product size of the two outer primers was 246 bp (Hashemi et al., 2010).

2.4 Statistical Methods

Data collection and statistical analysis were performed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 9 for windows version 9.3.1 (GraphPad Software, San Diego, CA, USA). Multivariate logistic regression analysis was performed. The odds ratio (OR) and 95% confidence interval (CI) were also estimated. Differences were considered significant as $P< 0.05$.

Qualitative variables were presented as frequencies and percentages. The distributions were assessed for normality using the Kolmogorov-Smirnov test. Additionally, the area under the receiver operating characteristic (ROC) curves generated optimum cut-off values for coronary artery disease risk markers with the severity of ACS. Multivariate logistic regression analysis was performed to eliminate the influence of confounding factors for ACS.

3. Results

3.1 Association of Lipid Profiles in Patients with ACS

In total 77 participants' data were analyzed. The mean age ± SD was approximately similar between the two groups, being 58±10.45 for patients' group and 56.50±11.13 for control group. Lipid parameters of patients and controls and some other baseline characteristics are given in Table 2. These results indicate that there is not significant difference between both patients and control subjects.

3.2 Association Between Some Biomarkers with ACS

Multiple logistic regression analysis was performed. Among the risk factors assessed patients with, family history of premature heart disease showed non-significant association with ACS when compared to control groups [OR 0.652 (95% CI 0.165 to 2.550), p = 0.533]. Furthermore, results also revealed that patients with diabetes have no significant difference. However, depending on the odds ratio it poses a great risk factor for ACS [OR 1.145 (0.239 to 5.106), p =0.859]. On the other hand, gender and hypertension showed a different result and they have a significant difference which illustrates that they are good indicators for acute coronary syndrome but not a risk factor [OR 0.125 (95% CI 0.021 to 0.555), p = 0.011] [OR 0.134 (95% CI 0.020 to 0.636), p = 0.020] respectively]. Thus, the positive family history of hypertension and gender appeared to be the most significant predictors for development of ACS. Nevertheless, high stress and body mass index (BMI)showed

non-significant difference but both are risk factors for the disease, more specifically stress, which is a strong risk factor [OR 1.302 (95% CI 0.180 to 14.01), p = 0.804] [OR 0.8736 (95% CI 0.752 to 0.997), p = 0.056] respectively. Table 3.

3.3 Interpretation of ROC Curves with Respect to Lipid Profiles

The diagnostic accuracy of lipid profiles for ACS was determined by measuring the area under the ROC curve (AUC), 95% confidence interval, sensitivity (SE%), specificity (SP%), positive predictive value (PP%), negative predictive values (NP%), (Table 5). According to ROC given in cholesterol the AUCs was 0.639 with a detectable cutoff value of ≥158.00 keeping acceptable sensitivity (50.82%) and positive and negative predictive values of 0.888 % and 0.291% suggesting that cholesterol appeared to be not an important predictive marker of ruling out ACS in the present study population (Table 5). When considering the triglyceride cut off value of ≥160.00 mg/dl showed a high sensitivity and negative predictive values (Se 81.25 %, NPV 0.305%, AUC = 0.53) compared to other makers suggesting that LDL may have a value in ruling out major vessel disease and luminal narrowing by atheroma (Table 5). However, LDL has shown a moderate but not significant sensitivity and negative predictive values of 36.07 % and 0.251 % respectively (AUC 0.591 .and the last one HDL). Thus, when considering severity of CAD, the HDL cut off value of ≥60.00 mg/dL may be an important predictor in ruling out major vessel disease and luminal narrowing by atheroma, see Table 4.

3.4 Molecular Analysis

3.4.1 Distribution of APOE Genotypes and Alleles Among the Study Subjects

A total of 61 ACS cases and 16 control subjects were evaluated, and the frequencies of the APOE genotypes are shown in Table 5 and Figure 1. The frequencies of the Cysteine /Arginine 158 (£2) in patient group were 59/61 while Cysteine /Arginin 158 -112 (£3) was 2/61. Whereas in control group 15/16 was Cyctine /Arginin 158 (£2) and 1/16 was Cyctine /Arginin 158-112 (£3). According to the odds ratio (OR-1.967), which is a very strong risk factor.

3.4.2 Distribution of PON 1 Genotypes and Alleles Among the Study Subjects

The frequency of PON1 T172A (L55M) polymorphism I n ACS patients and normal subjects is shown in Table 6 and Figure 2. The wild-type genotype (TT) was observed in 22/61 of the patients; whereas 15/61 were heterozygous (AT) and 24/61 were homozygous (AA). In the control group, the frequencies of genotypes were 2/16 for TT, 7/16 for AT and 7/16 for AA. There were significant differences regarding PON1 T172A polymorphisms among RA patients and normal subjects ($P < 0.05$).

3.4.3 Association Between Lipid Profile with The Genes (APOE and PON 1)

In this study, the lipid profile was assessed to determine the degree to which it was correlated with our genes (PON1 and APOE). As a result, there was no discernible difference between the lipid profiles of patients with coronary artery disease and controls with the specific genotypes (APOE [cystine arginine158]) as indicated by the p values (P-0.060.0.499,0.166,0.184) for TC, TG, HDL, and LDL, respectively. As shown in table 6, the lipid profiles and a specific genotype of PON 1 (AA) did not substantially differ from one another for the TC, TG, HDL, and LDL measurements, respectively. The p values for patient and control were (0.359,>0.999,0.476,0.152). see Table 7 and 8.

4. Discussion

The acute coronary syndrome is a significant financial and medical burden. Nearly half of all deaths from coronary heart disease occur because of ACS, hence the morbidity and mortality caused by ACS are significant. There are many factors such as hypertension, age, diabetes, stress and others, which make ACS a major issue. In this study among 61 patients, 39 of them had hypertension and 19 of them had been diagnosed diabetes.

Based on our result there was a significant difference between hypertension and gender. That being said, the overall prevalence of hypertension among ACS patients was higher than that which had been reported in the general population. The relationship between HTN and acute coronary syndromes may be explained by genetic susceptibility, insulin resistance, sympathetic hyperactivity, and vasoactive molecules (such as angiotensin II); also, hypertension is linked to atherosclerosis development (which in turn contributes to progression of myocardial infarction) {Picariello, 2011 #57}. Additionally, diabetes and high stress are another two strong risk factors that can be used as a biomarker for ACS. Our results are agreement with (Norhammar et al., 2004) which revealed that diabetes is a primary causes of coronary artery disease. Despite advances in the management of individuals with unstable coronary syndromes, this disease is still associated with much higher mortality and morbidity in diabetes patients. They showed that early revascularization occurs in patients with unstable coronary syndromes. Diabetic individuals undergoing stable coronary procedures have a higher risk of complications and a worse outcome than nondiabetic ones.

Another method for identifying and monitoring acute coronary syndrome is the lipid profiles. But in this study the lipid profiles (total cholesterol, triglyceride, LDL, HDL) did not showcase significant difference, as mentioned before may be due to the sample size or all samples were collected following catheterization. That is to say, it is possible that patients in this study may have taken medications that had an impact on the results, as opposed to the previous studies. Numerous studies have shown that total cholesterol and LDL cholesterol are important modifiable risk factors for atherosclerotic vascular disease and its clinical symptoms. A vast number of randomized clinical trials have shown that lowering LDL with specific lipid-lowering drugs lowers the risk of first or recurrent heart attack (Sachdeva et al., 2009). We discovered that the prevalence of hypertension among ACS patients was greater in women than in males at every decile of age, and the inverse is observed at older ages (Lin et al., 2013).

Apolipoprotein E (APOE = protein, APOE = gene) is required for plasma lipoprotein metabolism and transportation of lipid in tissues. The genetic polymorphism of the APOE is determined by three common alleles: APOE*2, *3, and *4 (Corbo & Scacchi, 1999).

The majority of cases from patients and controls were APOE £2 (Arginine/Cystine 158,) which is not a risk factor for ACS and does not show any association with lipid profile. APOE-e2 (Arg158RCys) has been associated with reduced levels of TC and LDL cholesterol, which are opposite to the finding (Wu et al., 2014). Whereas, APOE £3(Cystine/Arginine 158-112,) only appeared in three cases, accounting for a very substantial risk factor. There were not any association between this gene and lipid profiles which is mentioned in Wu and his coworkers paper APOE-e3 has a cysteine at residue 112 and an arginine at 158, which are critical for APOE to operate normally (Wu et al., 2014).

PON1, a high-density lipoprotein-associated esterase, is suspected to play a vital role in a variety of human diseases, including atherosclerosis and type 2 diabetes. When PON1 activity is low, there is an

increased risk of major cardiovascular events (Shunmoogam, Naidoo, & Chilton, 2018). In PON 1, the homozygote allele (AA), is a significant risk factor for ACS and is present in the majority of patients and controls (OR=1.199). While the wild type allele (TT), which is more commonly found in patients than in controls, is not a risk factor. With that being said, heterozygote (AT) is a very substantial risk factor which is observed in patients and controls with a high ratio.

Lipid profiles and PON1 do not significantly differ from one another. This might be because it is difficult to recruit individuals for the study, or because of regulation that applies before catheterization, or because the blood was drawn improperly, since hemolysis blood produces inaccurate results.

5. Conclusions

This study found that hypertension, diabetes and high stress have a very strong effects on patients with ACS. APOE £2 (Arginine/Cystine 158,) which is not a risk factor for ACS, while APOE £3(Cystine/Arginine 158-112) accounting for a very substantial risk factor. and PON 1, the homozygote allele (AA), is a significant risk factor for ACS. While the wild type allele (TT), is not a risk factor. With that being said, heterozygote (AT) is a very substantial risk factor for ACS patients.

6. Acknowledgements

I'd like to thank Ms. Lina and Eman have been incredible mentors, and I'm also grateful to them, parents, husband. Friends, lab mates, colleagues, and research team Mr. Govand, Abddulla, Omer, Razwan, Harem are all appreciated for the times we had working and socializing together. I'd also like to thank everyone who has been there for me emotionally and intellectually as I've worked on my coursework. I'd like to express my gratitude to everyone in the Surgical Specialty Hospital – Erbil Cardiac Center\ Kurdistan\ Iraq.

7. Conflict of interest

I hereby likewise declare that I fully understand that in case any information that may cause me to be in a position that shall be in conflict of interest with regard to my duties and responsibilities arises after this declaration, I shall thereafter inform the Committee.

Table 1: Primers used for determination of -107C/T and PON1 L55M gene polymorphism.

primers	-107C/T	L55M
Forward outer	5'-GCCAGTCCCATCCCCAAGAGGGTGAGCG-3'	5'-GGCTTTTGTACGTTTTGTG-3'
Reverse outer	5'-GAAAGTGCTGAGCTCCTGCGGTGGGGGC-3'	5'-CCGAAGAACACAAATATGCA-3'
Forward inner	5'-AGCGCCGATTGGCCCGCCACG-3'	5'-CAGAAACTGGCTCTGAAGTCA-3'

Reverse inner	5’-CTGCCGACCCGGCGGGGAGGTGT-3’	5’-TCCATTAGGCAGTATCTCGAA-3’

Table 2: Basic characteristics of the population.

Characteristics	Control (n=16) mean±SE	ACS (n=61) mean±SE	P value
Age	59.94 ±11.13	58.39±1045	0.6053
Male/Female	5/11 (M %31.25 – F %68.75)	43/18 (M %70.5 – F %29.5)	
Marital states	16 (%100)	61 (%100)	>0.9999
Smoking	6 (%37.5)	37 (%60.65)	0.1562
Drinking	1 (%6.25)	6 (%9.836)	>0.9999
Diabetes	5 (%31.25)	19 (%31.147)	>0.9999
Hypertension	7 (43.75)	37 (%60.65)	0.2643
TC	160.2±48.53	171.6±49.22	0.3169
TG	141.1±76.05	149.5±92.85	0.6701
HDL	38.44±10.08	37.54±20.58	0.1488
LDL	83.81±38.74	94.77±38.16	0.2679

Table 3: Impact of covariates on risk of coronary artery disease: multiple logistic regression analysis.

Variable	Odds Ratio	95 % confidence interval	P value	P value summery
Gender	0.1254	0.0201 to 0.556	0.011	*
Hypertension	0.1343	0.020 to 0.637	0.020	*
Diabetes	1.145	0.240 to 5.106	0.859	Ns
Cardiovascular disease	0.6517	0.165 to 2.550	0.533	Ns
High stress	1.302	0.180 to 14.01	0.804	Ns
BMI	o.8736	0.752 to 0.997	0.056	Ns

Table 4: Receiver operating characteristic curves generated optimum cut-off values for coronary artery disease risk markers with the severity of ACS

Biomarker	Cutoff value	Sensitivity %	Specificity %	PPV %	NPV %	AUC 95% CI	P value	DOR
Cholesterol	≥158.00	50.82	75.00	0.888	0.291	0.639 (0.4840-0.795)	0.088	3.243
Triglyceride	≥160.00	81.25	40.98	0.8753	0.3049	0.535 (0.383-0.687)	0.665	3.079
HDL	≥60.00	93.44	0.000	1.000	0.251	0.618 (0.474-0.762)	0.147	
LDL	≥104.00	36.07	81.25	0.874	0.251	0.591 (0.429 - 0.754)	0.257	2.336

Table 5: Genotype distributions of Apo E polymorphisms and their association with coronary artery disease.

	Control (n=16)	ACS (n=61)	OR (95 % Cl)	P value
APOE (£2)	15	59	0.509 (0.057 to 7.821)	585
APO E (£3)	1	2	1.967 (0.128 to 17.60)	0.508

Table 6: Genotype distributions of PON1 polymorphisms and their association with coronary artery disease.

Genotype	ACS patients		Control		Odds Ratio	(95% CI)	P value
	No.	%	No	%			
AA	24		7		1.199	(0.420-3.756)	0.781
AT	15		7		2.385	(0.789 - 7.088)	0.212
TT	22		2	`	0.253	(0.054 - 1.186)	0.127

Table 7: Association between lipid profile with the (APOE)

	Cystine/arginine 158			Cystine/arginine 158-112		
	Control mean±SD	ACS mean±SD	P Value	Control mean±SD	ACS mean±SD	P Value
TC	144.7±38.46	170.8±49.15	0.0602	118.5±167.6	196.0±63.64	0.6667
TG	130.8±66.27	148.9±94.38	0.4988	147.5±208.6	166.0±8.485	>0.9999
HDL	38.40±10.43	37.44±20.90	0.1663)	19.50±27.58	40.50±7.778	0.6667
LDL	80.93±38.28	94.19±37.92	0.1839	63.50±89.80	112.0±57.98	0.6667

Table 8: Association between lipid profile with the (PON 1)

	AA			TT			AT		
	Control mean±SD	ACS mean±SD	P Value	Control mean±SD	ACS mean±SD	P Value	Control mean±SD	ACS mean±SD	P Value
TC	147.4±34.16	171.2±42.56	0.3588	117.5±3.536	162.1±48.46	0.0870	162.9±55.33	184.3±60.34	0.4167
TG	159.3±82.74	150.8±65.82	>0.9999	82.50±31.82	133.9±83.76	0.3551	139.6±76.88	175.3±133.9	0.6796
HDL	33.43±5.884	32.29±5.989	0.4763	38.00±11.31	37.86±27.39	0.5725	43.57±11.75	43.67±21.85	0.5455
LDL	74.43±23.95	95.79±35.96	0.1523	104.5±81.32	90.09±37.18	0.8768	87.29±42.61	98.13±45.58	0.5932

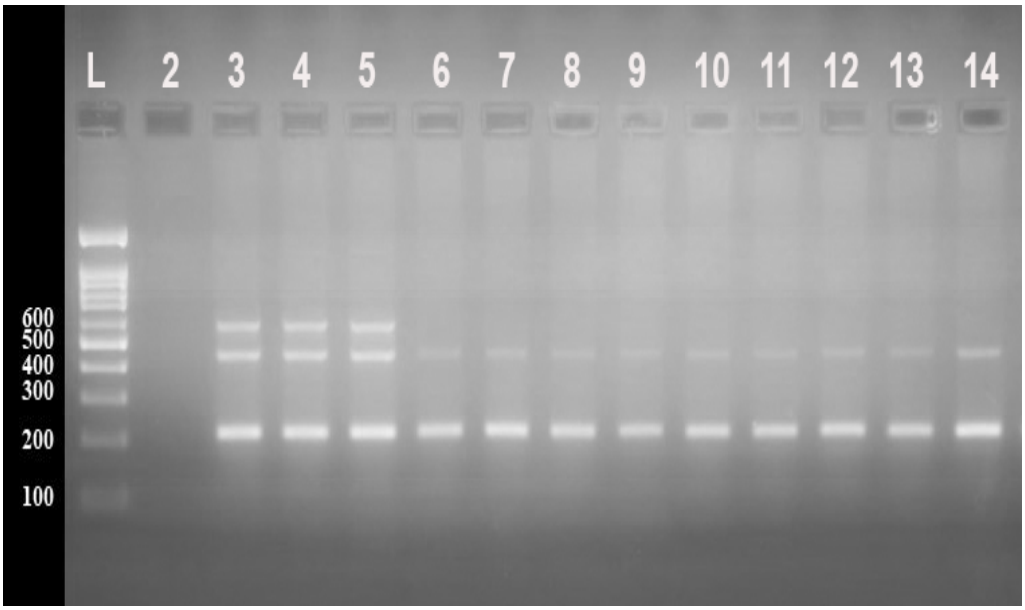

Figure 1: Agarose gel electrophoresed showing APOE gene PCR products: Lane 1: DNA Ladder of 100pb. Lane 2: Negative control. The 218bp is an internal control, Lane 3-5: Amplified the Cyst112/Arg158 type (218/588) bp. Lane 6-14: Cyst112 target amplification (112bp).

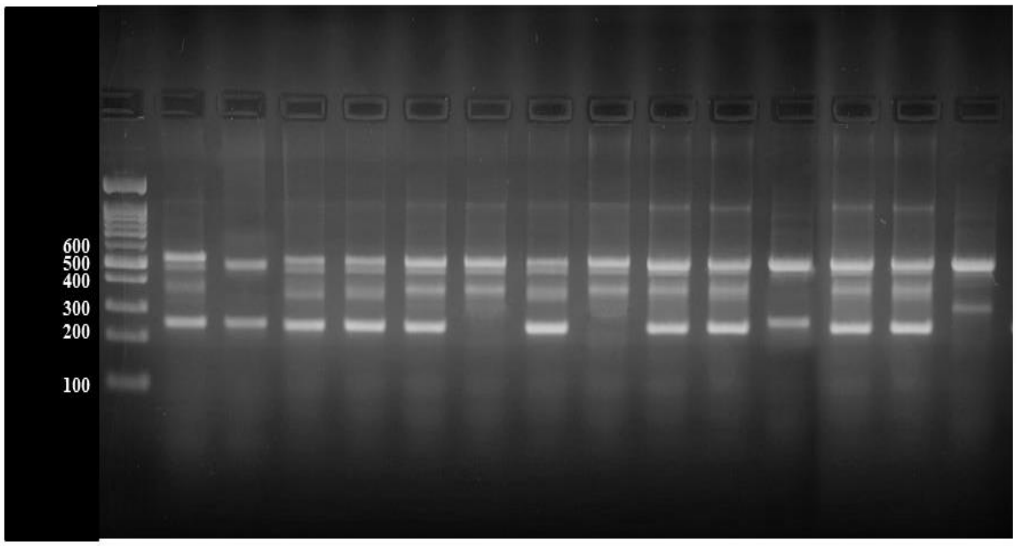

Figure 2: PON1 (L55M) genotyping. PCR products were electrophoresed on agarose gel. Lane 1: Ladder of 100bp. The 571bp amplified fragment as an internal positive control. Lane:2,4,5,6,8,10,11,13,14 belong amplification of Allele A and Allele T (262/351)bp, Lane 3,12:Amplified Allele A (262)bp and Lane 7,9 belong to target amplification of Allele T (351)bp.

References

Amini, M., Zayeri, F., & Salehi, M. (2021). Trend analysis of cardiovascular disease mortality, incidence, and mortality-to-incidence ratio: results from global burden of disease study 2017. *BMC Public Health, 21*(1), 401. doi:10.1186/s12889-021-10429-0

Association, C. S. o. C. o. C. M., & zhi, E. B. o. C. J. o. C. J. Z. x. x. g. b. z. (2007). Guideline for diagnosis and treatment of patients with unstable angina and non-ST-segment elevation myocardial infarction. *35*(4), 295-304.

Bergmark, B. A., Mathenge, N., Merlini, P. A., Lawrence-Wright, M. B., & Giugliano, R. P. (2022). Acute coronary syndromes. *Lancet, 399*(10332), 1347-1358. doi:10.1016/s0140-6736(21)02391-6

Bhatt, D. L., Lopes, R. D., & Harrington, R. A. (2022). Diagnosis and Treatment of Acute Coronary Syndromes: A Review. *Jama, 327*(7), 662-675. doi:10.1001/jama.2022.0358

Bounafaa, A., Berrougui, H., Ghalim, N., Nasser, B., Bagri, A., Moujahid, A., . . . Khalil, A. (2015). Association between Paraoxonase 1 (PON1) Polymorphisms and the Risk of Acute Coronary Syndrome in a North African Population. *PLOS ONE, 10*(8), e0133719. doi: 10.1371/journal.pone.0133719

Broce, I. J., Tan, C. H., Fan, C. C., Jansen, I., Savage, J. E., Witoelar, A., . . . Desikan, R. S. (2019). Dissecting the genetic relationship between cardiovascular risk factors and Alzheimer's disease. *Acta Neuropathol, 137*(2), 209-226. doi:10.1007/s00401-018-1928-6

Corbo, R. M., & Scacchi, R. (1999). Apolipoprotein E (APOE) allele distribution in the world. Is APOE* 4 a 'thrifty'allele? *Annals of human genetics, 63*(4), 301-310.

Eichner, J. E., Dunn, S. T., Perveen, G., Thompson, D. M., Stewart, K. E., & Stroehla, B. C. (2002). Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *Am J Epidemiol, 155*(6), 487-495. doi:10.1093/aje/155.6.487

El Hadri, K., Smith, R., Duplus, E., & El Amri, C. (2021). Inflammation, Oxidative Stress, Senescence in Atherosclerosis: Thioredoxine-1 as an Emerging Therapeutic Target. *Int J Mol Sci, 23*(1). doi:10.3390/ijms23010077

Glanz, V., Bezsonov, E. E., Soldatov, V., & Orekhov, A. N. (2022). Thirty-Five-Year History of Desialylated Lipoproteins Discovered by Vladimir Tertov. *Biomedicines, 10*(5). doi:10.3390/biomedicines10051174

Hashemi, M., Moazeni-Roodi, A., Fazaeli, A., Sandoughi, M., Taheri, M., Bardestani, G., . . . Ghavami, S. (2010). The L 55 M polymorphism of paraoxonase-1 is a risk factor for rheumatoid arthritis. *Genetics and Molecular Research, 9*(3), 1735-1741.

Hou, J., Deng, Q., Guo, X., Deng, X., Zhong, W., & Zhong, Z. (2020). Association between apolipoprotein E gene polymorphism and the risk of coronary artery disease in Hakka postmenopausal women in southern China. *Lipids Health Dis, 19*(1), 139. doi:10.1186/s12944-020-01323-6

Khatana, C., Saini, N. K., Chakrabarti, S., Saini, V., Sharma, A., Saini, R. V., & Saini, A. K. (2020). Mechanistic Insights into the Oxidized Low-Density Lipoprotein-Induced Atherosclerosis. *Oxid Med Cell Longev, 2020*, 5245308. doi:10.1155/2020/5245308

Lin, Y., Pan, W., Ning, S., Song, X., Jin, Z., & Lv, S. (2013). Prevalence and management of hypertension in patients with acute coronary syndrome vary with gender: Observations from the Chinese registry of acute coronary events (CRACE). *Molecular Medicine Reports, 8*(1), 173-177.

Mahrooz, A., Mackness, M., Bagheri, A., Ghaffari-Cherati, M., & Masoumi, P. (2019). The epigenetic regulation of paraoxonase 1 (PON1) as an important enzyme in HDL function: The missing link between environmental and genetic regulation. *Clinical Biochemistry, 73*, 1-10. doi:https://doi.org/10.1016/j.clinbiochem.2019.07.010

Members, A. T. F., Steg, P. G., James, S. K., Atar, D., Badano, L. P., Lundqvist, C. B., . . . Ducrocq, G. J. E. h. j. (2012). ESC Guidelines for the management of acute myocardial infarction in

patients presenting with ST-segment elevation: The Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC). *33*(20), 2569-2619.

Norhammar, A., Malmberg, K., Diderholm, E., Lagerqvist, B., Lindahl, B., Rydén, L., & Wallentin, L. (2004). Diabetes mellitus: the major risk factor in unstable coronary artery disease even after consideration of the extent of coronary artery disease and benefits of revascularization. *J Am Coll Cardiol, 43*(4), 585-591.

Roth, G. A., Mensah, G. A., Johnson, C. O., Addolorato, G., Ammirati, E., Baddour, L. M., . . . Fuster, V. (2020). Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. *J Am Coll Cardiol, 76*(25), 2982-3021. doi:10.1016/j.jacc.2020.11.010

Sachdeva, A., Cannon, C. P., Deedwania, P. C., LaBresh, K. A., Smith Jr, S. C., Dai, D., . . . Fonarow, G. C. (2009). Lipid levels in patients hospitalized with coronary artery disease: an analysis of 136,905 hospitalizations in Get With The Guidelines. *American heart journal, 157*(1), 111-117. e112.

Shunmoogam, N., Naidoo, P., & Chilton, R. (2018). Paraoxonase (PON)-1: a brief overview on genetics, structure, polymorphisms and clinical relevance. *Vascular health and risk management, 14*, 137.

Taler-Verčič, A., Goličnik, M., & Bavec, A. (2020). The Structure and Function of Paraoxonase-1 and Its Comparison to Paraoxonase-2 and -3. *Molecules, 25*(24). doi:10.3390/molecules25245980

Tréguier, Y., Bull-Maurer, A., & Roingeard, P. (2022). Apolipoprotein E, a Crucial Cellular Protein in the Lifecycle of Hepatitis Viruses. *Int J Mol Sci, 23*(7). doi:10.3390/ijms23073676

Vavlukis, M., Vavlukis, A., Krsteva, K., & Topuzovska, S. (2022). Paraoxonase 1 gene polymorphisms in lipid oxidation and atherosclerosis development. *Front Genet, 13*, 966413. doi:10.3389/fgene.2022.966413

Wu, J., Qiu, L., Guo, X.-z., Xu, T., Cheng, X.-q., Zhang, L., . . . Ni, L. (2014). Apolipoprotein E gene polymorphisms are associated with primary hyperuricemia in a Chinese population. *PLoS One, 9*(10), e110864.

Xu, C., Yu, B., Zhao, X., Lin, X., Tang, X., Liu, Z., . . . Li, L. (2022). Valosin Containing Protein as a Specific Biomarker for Predicting the Development of Acute Coronary Syndrome and Its Complication. *Front Cardiovasc Med, 9*, 803532. doi:10.3389/fcvm.2022.803532