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Evaluation of Myeloperoxidase (MPO) Genetic Polymorphism in Iraqi patients with Acute Myocardial Infarction (AMI)

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Abstract

Acute Myocardial Infarction (AMI) is still most caused by plaque rupture. ST-segment elevation Myocardial Infarction (STEMI) and non-ST-segment elevation Myocardial Infarction (NSTEMI) are two types of AMI that have distinct clinical characteristics. Several studies have found a strong association between Myeloperoxidase (MPO) and a wide range of AMI. MPO – 463 is a frequent MPO single nucleotide polymorphism (SNP) that has a G to A mutation at position 463 bp. Meanwhile, another SNP, MPO-129, is found in the MPO gene promoter. To our knowledge, this is the first study to investigate the association between MPO -463 and MPO-129 with patient survival in an IRAQI population.This study aimed to evaluate two single nucleotide polymorphisms (SNPs) frequency in MPO gene of Iraqi patients with AMI in comparison with control and the association between polymorphisms of these SNPs and the Incidence of AMI in Iraqi patients.The outcomes demonstrated that three different genotypes are found by genotyping the MPO genes (-463) &(-129). They are GG genotype, AG genotype, and AA genotype. The AG genotype of MPO-463 and MPO-129 may be considered as a risk factor in the clinical condition of non-ST-segment elevation Myocardial Infarction (NSTEMI) andST-segment elevation myocardial Infarction (STEMI.

Keywords: Acute Myocardial Infarction (AMI) , ST-segment elevation myocardial Infarction (STEMI) , non-ST-segment elevation Myocardial Infarction (NSTEMI) , Myeloperoxidase (MPO)

1. Introduction

In both the developed and developing worlds, acute myocardial infarction (AMI) is one of the leading causes of mortality. Despite the rapid implementation of approved therapy, it has a significant morbidity rate[1].Acute myocardial infarction (AMI) is described as myocardial necrosis caused by a coronary artery blockage. Chest pain is a common symptom. The electrocardiogram (ECG) and the presence or absence of serological markers are commonly used to make a diagnosis[2]. The majority of the area at risk becomes necrotic if the coronary artery remains obstructed. Reestablishing blood flow to the area at risk via coronary revascularization is the cornerstone of current treatment to prevent myocardial tissue loss[3]. ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) are two types of AMI that have

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distinct clinical characteristics, prognoses, therapeutic options, and time[4].Several studies have found a strong association between Myeloperoxidase (MPO) and a wide range of AMI, with increasing circulating MPO levels being linked to increased AMI risk [5].Myeloperoxidase MPO) is an inflammatory and Oxidative stress marker that rises after a heart attack[6].Numerous studies have suggested that MPO plays a function in the progression of the atherosclerotic process. Mechanistic links exist between MPO and the generation of atherogenic lipoproteins, nitric oxide (NO) consumption, and the development of endothelial dysfunction[7].Many CVD events have been linked to oxidized-LDL (Ox-LDL), also known as an atherogenic lipid. In the presence of H_2O_2, MPO can stimulate undesirable events inside the confines of LDL, resulting in Ox-LDL production[8].The most common outcomes of spontaneous thrombotic arterial blockage are myocardial infarction and stroke, which are the leading causes of death worldwide.[9].MPO is a heme protein that is encoded by a single 11-kb gene on chromosome 17q23.1 that has 11 introns and 12 exons[10]. The G nucleotide in this MPO -463G/A gene polymorphism creates a key binding site for the transcription factor specificity protein 1 (sp1), which can increase MPO gene transcription by up to 25-fold[11]. Due to the modify of an SP1 (specificity protein-1)-binding site, MPO rs2333227(-463) has been linked to considerably reduced transcriptional activity[12]. Further research revealed that the -129 G/A polymorphism, which is found in the promoter region of the MPO gene, eliminates an SP1 binding site and is associated with lower circulating MPO levels[13]. On chromosome 17, the single-nucleotide polymorphisms 463G A and 129G A are found in the promoter region of the MPO gene. Several studies have found various links between these genotypes, MPO plasma levels, and coronary artery disease[14]. MPO is linked to the onset and progression of coronary artery disease. Single-nucleotide polymorphisms in the MPO gene-129 and -463 loci influence MPO activity and expression [15]. MPO463G/A is a frequent MPO single nucleotide polymorphism (SNP) that has a G to A mutation at position 463 bp. Meanwhile, another SNP, MPO129G/A, is found in the MPO gene promoter. Both of these SNPs have been shown to influence the binding of the sp1 and, as a result, MPO protein production[16].Several studies showed that there is a significant association between MPO polymorphism and Acute Myocardial Infarction (AMI).This study investigates the association between AMI risk and MPO polymorphisms.To our knowledge, this is the first study to investigate the association between MPO4-63G/A and -129G/A with patient survival in an IRAQI population.

2. Materials and methods

2.1. Study population

The study was performedfrom September 2020 to August 2021.Eighty (80) patients (40 NSTEMI, 40 STEMI)and 40 control offered with chest pain presented to the Coronary Care Unit (CCU) in Ibn Al-Bitar Specialized Center for Cardiac Surgery at Al-Imamian Al-Kadhimiyain Medical City, and Al Yarmuk General Teaching looking for medical help about their newly developed symptoms. Five milliliters of venous blood sample were withdrawn from each patient (12-48) hours (before the sample is taken) presented to the Coronary Care Unit with acute chest pain. The same quantity of blood was drawn from the control group subjects.One milliliter was added to an EDTA tube to be used later for genetic analysis, while blood wastransferred into a gel tube.The tubes were stored at -20 ºC until analysis which was done within 6 weeks after the collection.

2.2. DNA Extraction

The DNA was extracted by using a Quick-DNA™ Blood MiniPrep kit (Catalog No.D3025).The technique for extracting DNA from 10 µl of whole blood, serum, or plasma. Blood can be utilized fresh orstored at -20ºC for future use**.**and can be preserved in EDTA. If the material cannot be handled immediately, it can be "stabilized" for later processing, though blood samples should be treated immediately.

2.3. PCR Analysis

The SNP at – 463 locus, – G463A (rs2333227), was genotyped by PCR/restriction fragment length polymorphism(RFLP) with the following Sequence

of forwarding 5' CGGTATAGGCACACAATGGTGAG - 3', and Reverse 5'-GCAATGGTTCAAGCGATTCTTC - 3 'primers. The cycling conditions were 95°C for 2 min one cycle, followed by 35 cycles at 94°C for 30 s, *62 °C* for 1 min, and 72°C for the 30s. The amplified PCR product (350 bp) was digested with 0.5 µl of restriction Enzyme AciI (BIO LAB/ USA).Where The SNP – G129A (rs34097845), was genotyped by PCR/RFLP with the Sequence of forwarding5'-CCTCCACAGCTCACCTGATAT - 3', and Reverse 5'-CGCTTGAACCATTGCACATCA – 3' primers. The cycling conditions were 94°C for 3 min, followed by 35 cycles at 94°C for 1 min, 52 °C for 1 min, and 72°C for 45 s. The amplified PCR product (278 bp) was digested with 0.5 µl of Restriction Enzyme ApaI (BIO LAB/ USA).

2.4. Agarose gel electrophoresis of DNA

Electrophoresis has been applied to decide DNA pieces after the cycle of extraction or to recognize the consequence of the association of PCR during the presence of the standard DNA to recognize the group size of the result of the Interaction of PCR-RFLP on the Agarose gel.Prepared the Agarose gelaccording to Sambrook[17], a 1 % agarose gel was created by dissolving 1.5 g of agarose in 100 ml of TBE solution.

2.5. Statistical Analysis

The statistical program for social sciences (SPSS) version 23 was used for all statistical analyses.The analysis of variance (ANOVA) test was used to identify the significant difference between the study groups and was used to assess the normality of the distribution of all variables.The Chi-square (χ^2) test was used to do the genetic analysis. Significant P values were less than 0.05, while very significant P values were less than 0.01[18]. In the absence of other evolutionary effects, the Hardy–Weinberg equilibrium or principle asserts that allele and genotype frequencies in a population will remain constant from generation to generation[19].

3. Results

3.1. Physiological Analysis:

Age Distribution: The standard error of mean age (Mean±SE) with Control group (56.15±1.53) year. The standard error of mean age (Mean±SEM) NSTEMI patients' group (55.78±1.26) year, and the standard error of mean age of STEMI (58.80±1.33) year. There was No significant between the three study groups.(See table1)

Gender Distribution: Seventy-two (60%) of the patients and control were male and Forty-eight (40%) were females. The observed frequencies in the male group were higher than expected and there were statistically no significant differences between the frequency of the patient in male and female groups(P=0.901).

Smoking: The number of smokers in the control group was 13(20.00%). The number of smokers in the NSTEMI group was 25 (38.46%) which was significantly higher than that of the control (p =0.006). The number of smokers in the STEMI group 27 (41.53%) was significantly higher than that of the control (P=0.001). there were statistically no significant differences between the smoker of the patient groups (0.642)

Hypertension*:* the number of hypertensive in the control group were 6 (12.76%). While in the STEMI group the number of hypertensives 19(40.42%) were significantly higher than that of the control (P=0.002). The number of patients with hypertension in the STEMI group were22(88%) significantly higher than that of the control (P=0.001).

Diabetes: the number of diabetics in the control group was 5 (11.62 %). The number of diabetics in the NSETMI group 18 (41.86 %) was significantly higher than that of the control (P=0.002). The number of diabetics in the STEMI group 20(46.51%) were significantly higher than that of the control (P=0.001).

***BMI*:** Eighteen (22.22%) of the Control group there had a BMI > 25 (*i.e.;* obese).Thirty (38.27%) of the NSETMI group had a BMI > 25 (Obese).While Thirty-two (39.50) of the STEMI group had a BMI >25 (Obese). The Chi-square test reveals a higher significant difference between patients (NSTEMI, STEMI) and the control group (p=0.001).

Table 1 The demographic and clinical characteristics of NSTEMI, STEMI and controls

demographic	control	NSTEMI	p-value	STEMI	p-value
mean age(year)	56.15	55.78	0.848	58.8	0.178
male (n)	23	25	0.653	24	0.822
female (n)	17	15		16	
Smoker n(%)	13(20.00)	25(38.46)	**0.006**	27(41.53)	**0.001**
Non-Smoker n(%)	27(49.09)	15(27.27)		13(23.63)	
Hypertensive n (%)	6 (12. 76)	19(40.42)	**0.002**	22(46.80)	**0.001**
Non-Hypertensive n(%)	34(46.57)	21(28.76)		18(24.65)	
Diabetic n(%)	5(11.62)	18(41.86)	**0.002**	20(48)	**0.001**
Non-Diabetic n(%)	35(45.45)	22(28.87)		20(25.97)	
Non-Obese n(%)	22(58.41)	9(23.07)	**0.001**	8(20)	**0.001**
Obese n(%)	18(22.22)	31(39.50)		38(39.50)	

(P=0.028,OR=2.140) in the NSTEMI groupand (P=0.008,OR=2.497)in the STEMI group.

3.2. Molecular Analysis :

Single Nucleotide Polymorphism(SNP) -463 of MPO gene.

Table 2shows the genotype& allele distribution ofSNP-463 MPO gene polymorphismsin control and patients with NSTEMI and STEMI, the genotyping reveals that there are three different genotypes:genotype (GG), genotype AG, and genotype (AA). The genotype (AG) shows the highest percentage of 62.5% genotype in the STEMI group compared to 60.0% in the NSTEMI group and the lowest percentage of 27.5% in the control group. The MPO-463 Aallele was identified in 23.8%of thecontrol, 40.0%of the NSTEMI group,and 43.8% of the STEM group.Comparing the genotype frequency distributions of GG and AG genotype revealed that with AG genotypehad a significantly higher risk of developing the NSTEMI group (P=0.003,OR=4.545)and theSTEMI(P=0.001,OR=5.682). Meanwhile, no significant differences were observed regarding disease risk of the AA genotype compared with the GGgenotype (P=0.353,OR=2.083) in the NSTEMI group and (P=0.138, OR=3.125) in the STEMI group frequencies. Comparing the A and Gallele distributions indicated that -463Aconferred a significant risk of developing AMI

Single Nucleotide Polymorphism (SNP) -129of MPO gene

Table 3shows three different genotypes are found by genotyping the -129 MPO gene and they are; GG genotype, AG genotype, and AA genotype. The highest percentage of the GG genotype is found in thecontrol group 65.0%compared to 42.5%in the NSTEMI and 42.5% in STEMI. While the highest percentage of the AG is found in the NSTEMI group 50.0%compared to 47.5%in STEMI and 25.0%in the control group. Whereas the genotype (AA) has a percentage of 7.5 % in the NSTEMI group compared to 10.0% in STEMI & Control.The MPO-129 Aallele was identified in 22.5%of the control, 32.5%of the NSTEMI group, and33.8%of the STEM group.Comparing the genotype frequency distributions of GG and AG genotype showedthat with AG genotypehad a significantly higher risk of developing the NSTEMI group (P=0.024,OR=3.059) and the STEMI group (P=0.032,OR=2.906).Meanwhile, no significant differences were observed regarding disease risk of the AA genotype and A allele compared with the GGgenotype (P=0.868,OR=1.147) in the NSTEMI group and (P=0.582, OR=1.529) in the STEMI group and G allele (p=0.07, OR=0.546) in the NSTEMI group and (p=0.140, OR =1.749) in the STEMI group frequencies,respectively.

Haplotype Analysis between SNP1(-463) & SNP2 (-129)

We performed haplotype analysis by considering SNP 1 (–463) and SNP 2 (–129)in the NSTEMI

group and the control groups for which we did NOT find a significant association of alleles of SNP 1 and Alleles of SNP 2 (Haplotypes) that showed nosignificant effect with the disease.
We also performed haplotype analysis by SNP 1(–463) and SNP 2 (–129)in STEMI and control groups for which we did find a significant association of alleleA ofSNP 1and Allele G of SNP 2 that showed

significant risk effect with disease (p = 0.033, OR=2.906)and we did find a significant association of allele A of SNP 1 and Allele A of SNP 2 that showed significant risk effect with disease (p=0.026, OR=2.941). whereas on the other hand, we did not findany significant association of ether allele G in SNP 1 and allele A in SNP 2. See table 4.

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Table 2: Distribution of genotypes &alleles of SNP-463 MPO gene in the Control,NSTEMI, and STEMI groups

Genotype -463		Control	NSTEMI	p-value	Odds ratio 95%CI	STEMI	p-value	Odds ratio 95%CI
GG	n	25	12	ref		10	ref	
	%	62.5%	30.0%			25.0%		
AG	n	11	24	**0.003***	**4.545**	25	**0.001***	**5.682**
	%	27.5%	60.0%		**1.686-12.251**	62.5%		**2.049-15.759**
AA	n	4	4	0.353	2.083	5	0.138	3.125
	%	10.0%	10.0%		0.443-9.790	12.5%		0.693-14.082
AlleleG		61	48	ref		45	ref	
	%	76.3%	60.0%			56.3%		
AlleleA		19	32	**0.028***	**2.140**	35	**0.008***	**2.497**
	%	23.8%	40.0%		**1.082-4.233**	43.8%		**1.257-4.921**
	n	80	80			80		
	%	100.0%	100.0%			100.0%		

STEMI: ST-segment elevationMyocardial Infarction
NSTEMI: non-ST-segment elevationmyocardial infarction
-463: Single Nucleotide Polymorphism(SNP)of MPO gene

Table 3: Distribution of genotypes &alleles of SNP-129 MPO gene in the Control,NSTEMIAND STEMI groups

Genotypes -129		**Control**	**NSTEMI**	**p-value**	**Odds ratio 95%CI**	**STEMI**	**p-value**	**Odds ratio 95%CI**
GG	n	26	17	ref		17	ref	
	%	65.0%	42.5%			42.5%		
AG	n	10	20	**0.024***	**3.059**	19	**0.032***	**2.906**
	%	25.0%	50.0%		**1.015-8.107**	47.5%		**1.091-7.741**
AA	n	4	3	0.868	1.147	4	0.582	1.529
	%	10.0%	7.5%		0.228-5.779	10.0%		0.336-6.956
Allele G		62	54	ref		53	ref	
	%	77.5%	67.5%			66.3%		
Allele A		18	26	0.07	0.546	27	0.140	1.749
	%	22.5%	32.5%		0.95-3.531	33.8%		0.822-3.670

STEMI: ST-segment elevationMyocardial Infarction
NSTEMI: non-ST-segment elevationmyocardial infarction
-129: Single Nucleotide Polymorphism(SNP)of MPO gene

Table 4Haplotype association of myeloperoxidase (MPO) –463 and–129 genotype and allelic frequencies in the NSTEMI,STEMI,and control groups

Haplotypes		**Control**	**NSTEMI**	**p-value**	**Odds ratio 95% C.I**	**STEMI**	**p-value**	**Odds ratio 95% C.I**
GG	n	50	37	ref		34	ref	
	%	62.5%	46.3%			42.5%		
AG	n	11	17	0.097	2.088	19	**0.033***	**2.540**
	%	13.8%	21.3%		0.875-4.982	23.8%		**1.074-6.008**
AA	n	8	15	0.057	2.534	16	**0.026***	**2.941**
	%	10.0%	18.8%		0.973-6.601	20.0%		**1.133-7.635**
GA	n	11	11	0.529	1.351	11	0.423	1.471
	%	13.8%	13.8%		0.529-3.451	13.8%		0.573-3.774

4. Discussion

Several investigations in recent years have demonstrated that elevated MPO levels are independently linked to an increased risk of AMI. Both polymorphic versions of the MPO gene, MPO –463 and MPO –129, are positioned upstream of the MPO gene's translation initiation codon.

The present case-control study investigated the possible association and clinical significance of MPO gene rs2333227(-463) and MPO gene rs34097845 (-128) inIraqi patients with AMI.The possible associations were investigated between NSTEMI, SEMI, clinical feature (age, gender), and susceptibility polymorphisms to AMI, such as MPO gene in 120 Iraqi subjects, and their result was discussed next pages.

4.1. Clinical assessment

a) Age: The mean age of AMI patients of our study was 55.78years old of NSTEMI group were 58.80 years of STEMI group as showed intable 1, and range from (40-70) years and that agree with a previous study [20], which was stated that in Norway, the risk of AMI in adults < 45 years old was low, but nearly one out of every ten patients with AMI < 45 years old died or had a new cardiovascular incident during follow-up. Efforts to improve risk factor control in these individuals should be stepped up. and with [21] who stated that The most significant risk factor for stroke is advanced age; around 75% of strokes occur in adults aged 65 and up.

b)Gender: Although the frequency of the male patient group is higher than that of the female in our study (see Table1) there were no statistically significant differences between them (p=0.901), this means that the disease could affect both genders but with a propensity to male. This study agrees with a previous study was done by [22] Female AMI patients had a greater readmission rate than male AMI patients after a 6-month follow-up, but there was no difference in death between male and female AMI patients. Although young female AMI patients aged 60–65 years had higher short- and long-term mortality than males, these findings support that there was no significant difference in mortality between old male and old female patients.

4.2. Risk Factors

a) Smoking: twenty-five (38.46%) of NSTEMI group, twenty-seven (41.53%) of STEMI group were smokersand thirteen (20.00%) of control were smokers, there is a highly significant difference between NSTEMI and control group (p=0.006) also there is a highly significant difference between STEMI and control group (p=0.001) (see Table 1). Our results may indicate an association between smoking and AMI and that agrees with [23]who showed that Smoking has been shown to precipitate atherosclerosis and cause rapid AMI through a variety of mechanisms, including (a) increasing serum low-density lipoprotein-cholesterol (LDL-C) and triglyceride concentrations while lowering serum high-density lipoprotein-cholesterol (HDL-C); (b) stimulating the free radical to oxidize LDL-C molecules, causing the oxidized LDL-C molecules to accumulate within the arteries.

b) Hypertension: nineteen of the NSTEMI group (40.42%) were hypertensive, and six of the control (12. 76%) were hypertensive and the number of NSTMI hypertensive group was significantly higher with control (P=0.002). Similarly, the number of patients with hypertension in the STEMI group was 22(46.80%) significantly higher than that of the control (P=0.001).This study agrees with a previous study done by [24] who showed that Hypertension was found to be prevalent in 57.2 % of acute myocardial infarction (AMI) patients in South Korea, and diabetes was shown to be prevalent in 32.3 %. Treatment for high blood pressure lowers the risk of AMI in the general population.

C) Diabetes Mellitus (DM): eighteen ofthe NSTMI group (41.86 %) were diabetic and Five of the control group (11.62 %) were diabetic and the frequency of the NSTEMI diabetic group was higher than that in the control group (P=0.002). Twenty of the STEMI group (46.51%) were diabetic, and the number of STEMI diabetic groups was significantly higher than that in the control group (P=0.001). Our results also agreed with a study done by [25] who considered Patients with AMI and diabetes mellitus (DM) are more likely to develop cardiogenic shock (CS), and there is evidence that the frequency of CS after AMI

is rising in the general population. And with [26] who showed that Diabetes mellitus (DM) is expected to impact up to 642 million people worldwide by the year 2040, according to estimates. In patients with DM, the relative risk ratio for acute myocardial infarction (MI) has been reported to range between 1.48 and 3.07.

d) *BMI*: A BMI of >25 (Obese) was found in 32 (39.50 %) of the STEMI group. Thirty-one people in the NSETMI group (38.27%) had a BMI of more than 25. (Obese) While 22.22 % of the Control group had a BMI of 25 or higher (obese). a greater significant difference (p=0.001) between the patients (NSTEMI, STEMI) and the control group. for more information. Our results agreed with [27] who showed that There was a high frequency of abdominal obesity in AMI patients, with independent of other risk variables, substantial relationships between higher waist circumference (WC) and recurring atherosclerotic cardiovascular disease (ASCVD). After a first MI, WC measures can be used in the clinical context to identify patients who are at a higher risk of recurrent ASCVD.

4.3. The role of MPO -463 polymorphism in developing AMI

Concerning the mutational analysis, all groups of a sample Iraqi subjects (80 patients and 40 control) are genotyped for the MPOrs 2333227 SNP, this analysis reveals that We found higher frequencies of both the AG genotype (60.0%) in the NSTEMI group where the AG genotype (62.5%)in the STEMI group compared to its frequency (27.5% AG genotype) in the control group see (Table 2) and that indicates the AG genotype is a risk factor in IRAQI patients (P=0.003, OR=4.545) and (P=0.001, OR=5.682) of the NSTEMI group & the STEMI group respectively. Meanwhile, no significant differences were observed regarding disease risk of the AA genotype compared with the GGgenotype these findings are consistent with many previous studies mentioned below.

A meta-analysis discovered that the myeloperoxidase rs2333227 polymorphism can reduce the risk of coronary heart disease in Asians but not in Caucasians. They also discovered that the A allele is strongly associated with a lower risk of CHD in Asians but not in Caucasians. This could be due to genetic variations and gene-environment interactions[12].

A meta-analysis of published data showed The GG genotype was related to elevated CAD risk in Asians but not in Caucasians, according to the 463G/A polymorphism. In the case of the 129G/A polymorphism, no link was identified between the GG genotype and the risk of CAD in Asians or Caucasians [28].

Other studies demonstrated that MPO 463 A allele frequency in patients and controls varied from 8 to 47 % and 16 to 56 % in different ethnic communities. MPO 463 A frequency in control patients was observed to be 26.7 percent in French Canadian, 22.4 percent in Swedish, and 26.36 percent in Turkish populations[16]. In the present study, it was identified in 40 percent of the NSTEMI group, 43 percent of the STEMI group, and 23.8 percent of controls.

Also, a study done by [10]showedthat The findings are consistent with research in the French Canadian population, which found that recessive allele "A" was statistically linked to a lower risk of coronary artery disease. The incidence of early CAD was dramatically reduced in people with the – 463 AA genotype, and comparable findings were found in our investigation.

4.4. The role of MPO -129 polymorphism in developing AMI

Regarding the mutational analysis, all groups of a sample Iraqi subjects (80 patients and 40 control) are genotyped for the MPO rs34097845 SNP, this analysis reveals that We found, higher frequency AG genotype in both the NSTEMI group (50.0%) and STEMI group (47.5%) compared to control (25.0%) and that indicates the AG genotype is a risk factor of the NSTEMI & STEMI in IRAQI patens. In contrast, lower frequencies of both the AA genotype (7.5%) and the A allele (32.5%) in the NSTEMI group where the AA genotype (10.0%)and the A allele (33.8%) in the STEMI group compared to its frequency (10.0% AA genotype) (22.5% A allele) in the control group. no significant differences were observed see (Table 3).these findings are consistent with previous studies mentioned below.

A previous study showed that two frequent SNPs in the MPO gene promoter, -463G/A and -129G/A, have been discovered to influence the binding of the transcriptional factor specificity protein1 (SP1), hence affecting MPO expression. The AG-genotypes

at locations -463 and -129 werefound to raise the risk of coronary artery disease by 1.53 and 1.94 fold, respectively [29].

Another study showed that Individuals with the GA genotype had ~2-fold higher risk of having CAD than those with the GG genotype (OR=1.94), according to the A allele and GA genotype frequency distributions [16].

Another study reported that The -129G/A polymorphism is another polymorphism found in the MPO gene's promoter region. Individuals with the -129A allele had lower MPO activity in their neutrophils [30].

A meta-analysis presented that In the case of the 129G/A polymorphism, no link was identified between the GG genotype and the risk of CAD in Asians or Caucasians [28].

5. Conclusion

Iraqi people with the NSTEMI group and the STEMI group are mostly having AG, AA, and GG genotypes of the (rs2333227) & (rs34097845) MPO genes polymorphisms

Iraqi people having AG genotype of the (rs2333227) &(rs34097845) MPO genes are susceptible to developing NSTEMI & STEMI. that indicate the AG genotype may be considered as a risk factor in the clinical condition of NSTEMI & STEMI

6. Conflict of Interests

There are no conflicts of interest stated by the authors regarding the publication of this paper.

7. References

[1] J. Clarke.John-Ross, R. Kennedy, F. Duarte Lau, G. I. Lancaster, and S. W. Zarich, "Invasive Evaluation of the Microvasculature in Acute Myocardial Infarction: Coronary Flow Reserve versus the Index of Microcirculatory Resistance," *J. Clin. Med.*, vol. 9, no. 1, p. 86, 2019.

[2] A. Cranz, "Broken Heart Strings - Psychological Stress in Cardiac Patients after Chordae Tendineae Rupture," *thesis*, p. p.24, 2019.

[3] M. J. M. Silvis *et al.*, "Immunomodulation of the NLRP3 Inflammasome in Atherosclerosis, Coronary Artery Disease, and Acute Myocardial Infarction," *J. Cardiovasc. Transl. Res.*, vol. 14, no. 1, pp. 23–34, 2021.

[4] M. Chiesa *et al.*, "Whole blood transcriptome profile at hospital admission discriminates between patients with ST-segment elevation and non-ST-segment elevation acute myocardial infarction," *Sci. Rep.*, vol. 10, no. 1, pp. 1–14, 2020.

[5] C. J. A. Ramachandra, K. P. M. M. Ja, J. Chua, S. Cong, W. Shim, and D. J. Hausenloy, "Myeloperoxidase As a Multifaceted Target for Cardiovascular Protection," *Antioxidants Redox Signal.*, vol. 32, no. 15, pp. 1135–1149, 2020.

[6] R. Hasan, D. Lindarto, G. A. Siregar, and Z. Mukhtar, "The effect of bay leaf extract syzygium polyanthum (Wight) walp. on C-reactive protein (CRP) and myeloperoxidase (MPO) level in the heart of rat model of myocardial infarction," *Med. Glas.*, vol. 17, no. 1, pp. 41–45, 2020.

[7] S. J. Nicholls and S. L. Hazen, "Myeloperoxidase and cardiovascular disease," *Arterioscler. Thromb. Vasc. Biol.*, vol. 25, no. 6, pp. 1102–1111, 2005.

[8] J. Premkumar, P. Sampath, R. Sanjay, A. Chandrakala, and D. Rajagopal, "Synthetic Guaiacol Derivatives as Promising Myeloperoxidase Inhibitors Targeting Atherosclerotic Cardiovascular Disease," *ChemMedChem*, vol. 15, no. 13, pp. 1187–1199, 2020,

[9] D. Wolf and K. Ley, "Immunity and Inflammation in Atherosclerosis," pp. 315–327, 2019.

[10] S. Maddhuri *et al.*, "Analysis of plasma myeloperoxidase levels and functional gene –463G>A and –129G>A polymorphisms with early onset of coronary artery disease in South Indian population," *Folia Cardiol.*, vol. 11, no. 4, pp. 272–278, 2016.

[11] Y. Y. Li *et al.*, "PRISMA-combined Myeloperoxidase -463G/A gene polymorphism and coronary artery disease: A meta-analysis of 4744 subjects," *Med. (United States)*, vol. 96, no. 12, 2017, .

[12] Y. Q. Zhang, Y. F. Jiang, M. Chen, N. N. Zhang, and Y. F. Zhou, "Association between myeloperoxidase rs2333227 polymorphism and susceptibility to coronary heart disease,"

Arch. Med. Sci., vol. 16, no. 5, pp. 1231–1238, 2020.

[13] B. S. Van Der Veen, M. P. J. De Winther, and P. Heeringa, "Myeloperoxidase: Molecular mechanisms of action and their relevance to human health and disease," *Antioxidants Redox Signal.*, vol. 11, no. 11, pp. 2899–2937, 2009.

[14] K. K. Berg, H. O. Madsen, P. Garred, R. Wiseth, S. Gunnes, and V. Videm, "The additive contribution from inflammatory genetic markers on the severity of cardiovascular disease," *Scand. J. Immunol.*, vol. 69, no. 1, pp. 36–42, 2009.

[15] Z. Zhao, K. Shi, B. Zhu, and X. Xiao, "Association of MPO gene polymorphism with coronary artery injury and therapeutic effect of IVIG combined with heparin in children with KD," vol. 25, no. 7, pp. 38–42, 2019.

[16] S. Arslan, Ö. Berkan, B. Bayyurt, and O. Beton, "artery disease risk and patient survival in a Turkish population," pp. 547–552, 2017.

[17] J. Sambrook, "Joe Sambrook - Molecular Cloning_ A Laboratory Manual-Cold Spring Harbor Laboratory Press (2001).pdf." 1989.

[18] G. Norman, "Likert scales, levels of measurement and the laws of statistics," *Adv. Heal. Sci. Educ.*, vol. 15, no. 5, pp. 625–632, 2010.

[19] J. Masel, "Rethinking Hardy-Weinberg and genetic drift in undergraduate biology," *BioEssays*, vol. 34, no. 8, pp. 701–710, 2012.

[20] J. Jortveit, A. H. Pripp, J. Langrgen, and S. Halvorsen, "Incidence, risk factors and outcome of young patients with myocardial infarction," *Heart*, vol. 106, no. 18, pp. 1420–1426, 2020.

[21] C. Wang *et al.*, "DNA methylation-based biomarkers of age acceleration and all-cause death, myocardial infarction, stroke, and cancer in two cohorts: The NAS, and KORA F4," *EBioMedicine*, vol. 63, p. 103151, 2021.

[22] P. Wang, J. Yao, Y. Xie, and M. Luo, "Gender-Specific Predictive Markers of Poor Prognosis for Patients with Acute Myocardial Infarction During a 6-Month Follow-up," *J. Cardiovasc. Transl. Res.*, vol. 13, no. 1, pp. 27–38, 2020,

[23] S. O. Amen *et al.*, "Prevalence of the Most Frequent Risk Factors in Iraqi Patients with Acute Myocardial Infarction," pp. 6–18, 2020.

[24] Y. H. Kim *et al.*, "Which is the worst risk factor for the long-term clinical outcome? Comparison of long-term clinical outcomes between antecedent hypertension and diabetes mellitus in South Korean acute myocardial infarction patients after stent implantation," *J. Diabetes*, vol. 12, no. 2, pp. 119–133, 2020.

[25] M. Thoegersen *et al.*, "The association of diabetes and admission blood glucose with 30-day mortality in patients with acute myocardial infarction complicated by cardiogenic shock," 2020.

[26] T. A. G. Ed, P. T. A. D. Avarpasand, A. L. I. H. Osseinsabet, F. A. O. Midi, S. A. M. Ehrabi, and T. A. G. Ed, "Original Contribution INTERACTION EFFECT OF DIABETES AND ACUTE MYOCARDIAL INFARCTION ON THE LEFT ATRIAL FUNCTION AS EVALUATED BY 2-D SPECKLE-TRACKING," vol. 46, no. 6, pp. 1490–1503, 2020.

[27] H. Mohammadi *et al.*, "Abdominal obesity and the risk of recurrent atherosclerotic cardiovascular disease after myocardial infarction," *Eur. J. Prev. Cardiol.*, 2020.

[28] Y. Wang, X. Y. Chen, K. Wang, S. Li, and X. Y. Zhang, "Myeloperoxidase polymorphism and coronary artery disease risk," *Med. (United States)*, vol. 96, no. 27, 2017.

[29] M. Dikshit and N. K. Ganguly, *Modulation of Oxidative Stress in Heart Disease*. 2019.

[30] G. Ndrepepa, "Myeloperoxidase – A bridge linking inflammation and oxidative stress with cardiovascular disease," *Clin. Chim. Acta*, vol. 493, no. February, pp. 36–51, 2019.