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Original Article

Lack of association between the *eNOS* rs1800779 (A/G) polymorphism and the myocardial infarction incidence among the Iraqi Kurdish population

Zhikal O. Khudhur, MSC[a], Goran Othman, PhD[b,c], Galawezh O. Othman, PhD[d], Aziz M. Jafaar, MD[e,f,g], Mahdi Kh. Qadir, MSC[h], Harem Kh. Awla, BSC[i], Sara Sh. Qasim, BSC[j], Shayma M. Hadi, BSC[j], Samiha S. Khan, MBBS[k], Sobia Noreen, PhD[l,*] and Shukur W. Smail, MSC[i,m,**]

[a] *Department of Biology, Faculty of Education, Tishk International University - Erbil, Kurdistan Region, Iraq*
[b] *Department of Medical Laboratory Technology, Erbil Health and Medical Technical College, Erbil Polytechnic University, Erbil, Kurdistan Region, Iraq*
[c] *Department of Medical Laboratory Technology, Al-Qalam University College, Kirkuk, Iraq*
[d] *Department of Biology, College of Education, University of Salahaddin Erbil, KRG-Iraq*
[e] *Department of Endocrinology, Ministry of Health, Kurdistan Regional Government, Erbil-Iraq*
[f] *Department of Endocrinology, School of Medicine, University of Kurdistan Hawler, Erbil, KRG, Iraq*
[g] *Department of Endocrinology, Iraqi Board for Medical Specializations, Erbil, Iraq*
[h] *Department of Physiotherapy, Erbil Health and Medical Technical College, Erbil Polytechnic University-Erbil, Iraq*
[i] *Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Iraq*
[j] *Department of Biochemistry, Private Biolab, Erbil, Kurdistan Region, Iraq*
[k] *Faisalabad Medical University, Faisalabad, Pakistan*
[l] *Department of Pharmaceutical Technology, University of Innsbruck, Innsbruck, Austria*
[m] *Department of Medical Microbiology, College of Science, Cihan University-Erbil, Kurdistan Region, Iraq*

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الملخص

أهداف البحث: ارتبط تعدد الأشكال الجيني لجين سينثاز أكسيد النيتريك البطاني (إينوس) ارتباطا وثيقا بالعديد من أمراض القلب والأوعية الدموية في مجتمعات مختلفة. هدفت الدراسة الحالية إلى التحقق من ارتباط تعدد الأشكال إينوس مع تقدم احتشاء عضل القلب.

طرق البحث: تم تسجيل خمسة وثمانين شخصا صحيحا وثمانين مريضا بمرض احتشاء عضل القلب تم إدخالهم إلى مركز القلب في أربيل في إقليم كردستان العراق في الدراسة. كان جميع المشاركين أكرادا من نفس المجموعة العرقية. تم استخدام "تفاعل البلمرة المتسلسل لنظام الطفرة المقاومة للتضخيم" لتحديد تعدد الأشكال إينوس ومستوى مصل أكسيد النيتريك بواسطة مقياس الطيف الضوئي.

النتائج: كانت الترددات النمط الجيني لأنواع إينوس الثلاثة "أأ" (النوع البري) و"أج" و"ج ج" هي 58.75% و33.75٪ و7.50٪ على التوالي في مرضى احتشاء عضل القلب، وبالنسبة للمجموعة الضابطة كانت 49.41٪ و 43.53٪ و 7.06٪ على التوالي. كما كانت ترددات الأليلات "أ" و "ج" في مجموعة مرضى احتشاء عضل القلب هي 75.6% و 24.4٪ على التوالي، بينما كانت في المجموعة الضابطة 71.2٪ و 28.8٪ على التوالي. كذلك كشفت النتائج عن عدم وجود ارتباط بين توزيع الأنماط الجينية ومستوى أكسيد النيتريك في مصل الدم مع زيادة خطر الإصابة باحتشاء عضل القلب.

الاستنتاجات: خلصت الدراسة إلى عدم وجود ارتباط بين الأنماط الجينية والأليلات لإينوس مع قابلية حدوث احتشاء عضل القلب.

الكلمات المفتاحية: سينثاز أكسيد النيتريك البطاني؛ احتشاء عضل القلب؛ أكسيد النيتريك؛ تعدد الأشكال؛ الأكراد

* Corresponding address: Department of Pharmaceutical Technology, University of Innsbruck, Innsbruck 6020, Austria.
** Corresponding address. Department of Biology, College of Science, Salahaddin University, Erbil, Erbil 44001, Iraq.
E-mails: sobianoreen07@gmail.com (S. Noreen), shukur.smail@su.edu.krd (S.W. Smail)

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Abstract

Objectives: The genetic polymorphisms of the endothelial nitric oxide synthase (*eNOS*) gene are strongly associated with several cardiovascular diseases (CVDs) in various populations. The current study aimed to investigate the association of the *eNOS* rs1800779 (A/G) polymorphism with the progress of myocardial infarction (MI).

Methods: Eighty-five healthy subjects and 80 patients with MI admitted to the Erbil Cardiac Centre in the Kurdistan Region of Iraq were enrolled in the study. All participants were Kurdish from the same ethnic group. The amplification refractory mutation system polymerase chain reaction (ARMS-PCR) was used to determine the rs1800779 (A/G) polymorphism of *eNOS*, and the nitric oxide (NO) serum level was detected by spectrophotometer.

Results: The genotypic frequencies of the *eNOS* rs1800779 AA (wild type), AG, and GG were 58.75%, 33.75%, and 7.50%, respectively, in the MI patients, and 49.41%, 43.53%, and 7.06%, respectively, for the control group. The frequencies of the A and the G alleles were 75.6% and 24.4%, respectively, in the MI group, and 71.2% and 28.8%, respectively, in the control subjects. The results revealed a lack of association of the rs1800779 genotype distribution with the level of NO serum and increased risk of MI.

Conclusion: The study concluded that there is a lack of association between the genotypes and alleles of the rs1800779 *eNOS* and susceptibility to MI in the studied population.

Keywords: Endothelial nitric oxide synthase; Kurdish population; Myocardial infarction; Nitric oxide; Polymorphism

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Introduction

Myocardial infarction (MI) leaves heart tissue with oxygen deficiency, causing oxidative stress and irreversible death of cardiomyocytes.[1] MI may lead to impairment in diastolic and systolic function and make the patient prone to arrhythmias.[2] MI could be responsible for more than 15% of mortality worldwide.[3] In coronary thrombosis, plaque rupture is critical in pathogenesis.[4]

Nitric oxide (NO) is identified as the endothelium-derived relaxing factor produced by multiple isoforms of nitric oxide synthase (NOS).[5,6] NO produced by *eNOS* has vasorelaxant activity and triggers vascular smooth muscle proliferation, platelet aggregation, and cardiovascular system homeostasis.[7,8] The inducible nitric oxide synthase (iNOS) is mainly present in macrophages activated in response to endotoxin and cytokines. MI and other pathologic conditions can activate iNOS.[9]

The controversy over the role of NO and its effect on CVDs has been attributed to the various pathophysiological impacts that rely mainly on the NO levels which are synthesized by NOS, such as the metabolic conditions of the cells, allosteric modulators of the NO, the different experimental models using redox, and the energy state. NO is like a double-edged sword: it has a vasodilatory effect[10] in some conditions, and on the other hand, it acts as a precursor for free radical formation.[11]

The *eNOS* gene is located on Chromosome 7q35-36, which is responsible for converting arginine amino acids into NO and L-citrulline. The *eNOS* polymorphism has been linked to many CVDs and metabolic syndromes, such as inflammatory diseases and hyperlipidemia.[12,13] Several polymorphisms have been detected in the *eNOS* gene, of which three are well established to be associated with MI: the promoter T786C, the exonic G894T, and the intronic 4a/b polymorphism.[14] The other polymorphisms that are strongly associated with CVDs and cholesterol metabolism include the rs743507, rs1800779, and rs1799983 variants. Among the reported polymorphisms of the *eNOS* gene, a significant association of the 4a/b polymorphism in intron 4 of the *eNOS* gene with coronary artery disease (CAD) has been reported.[15,16] So, the 9p21.3 risk locus also increases the risk of CAD.[17]

Cardiac biomarkers in predicting death due to MI were previously noted,[18,19] but the markers for the prevention of MI remain elusive. Therefore, determining functionally important polymorphisms of the *eNOS* gene will determine whether a person is prone to MI, and can explain the diverse prevalence of this severe disease in the Kurdish population. In addition, preliminary data indicate that the Glu-Asp298 polymorphism in exon 7 of the *eNOS* gene is associated with coronary spasms.[20,21] However, the implications of these polymorphisms concerning MI remain to be established. The *eNOS* rs1800779 polymorphism has been considered to be related to migraine disorder[22] and type 2 diabetes mellitus (T2DM).[23]

The present study aimed to identify a putative association of the rs1800779 (A/G) *eNOS* polymorphism with MI among the Kurdish population from Iraq.

Materials and Methods

Study design and participants

The study was carried out between February and May 2021, and the samples were collected at the Coronary Care Unit in the Erbil Cardiac Center Hospital, Iraq. The Scientific and Ethics Committee of Salahaddin University-Erbil approved the proposal and the experimental design (approval number: 715414; approved on January 3, 2021). The enrolled subjects were 165 in total, of which 80 were patients suffering from acute MI registered in the Coronary Care Unit for treatment, and 85 participants were healthy controls (Table 1). Healthy controls without CVDs or any other diseases were included. The presence of any other diseases was excluded from the healthy controls. Several criteria were considered to diagnose MI, such as ECG registrations; the medical history of MI patients; laboratory testing of serum troponin I, highly sensitive cardiac troponin T (hs-cTnT), and creatine kinase-myocardial band (CK-MB); and

echocardiographic results. Before the research began, all participants were asked to read and sign an informed consent form. Cancer patients, diabetics, and patients with CVDs other than MI were excluded from the study (Table 2).

Sample size calculation

The sample size was calculated by using the equation proposed by Hulley et al.[24]:

$N = AB/(E/S)^2$

$A = (1/q_1 + 1/q_0)$

$B = (Z_\alpha + Z_\beta)^2$

N = sample size
Z_α = standard normal deviate for α (the alternative hypothesis is two-sided, $Z_\alpha = 1.96$ when $\alpha = 0.05$) (Appendix 6A on page 73 of Hully et al.[24]).
Z_β = standard normal deviate for β (Z_β = 0. 0.8416 when $\beta = 0.20$, statistical power (1-β) = 80%) (Appendix 6A on page 73 of Hully et al.[24]).
E = standardized effect size (we assume E = 0.70 for this study) (Appendix 6A on page 73 of Hully et al.[24]).
q_1 = proportion of subjects in the MI group (exposed) = 0.10
q_0 = proportion of subjects in the healthy control (unexposed); $1 - q_1 = 0.90$
S = standard deviation of the outcome in the population = 0.65
$A = (1/q_1 + 1/q_0) = 11.1111$
$B = (Z_\alpha + Z_\beta)^2 = 7.8489$
$N = AB/(E/S)^2 = 75.196$

Our minimum sample size was 75, and we enrolled 80 MI patients. Based on this number, 85 healthy controls without diseases were also enrolled in this study.

Collection of samples

Blood samples were taken from participants' peripheral veins using 5 mL syringes directly on admission. From each sample, 2 mL of blood was collected in K2EDTA tubes (5.4 mg) for direct DNA extraction, and 3 mL of blood was placed in gel tubes to assess serum NO levels. The manufacturer's instructions were followed for extracting blood DNA using the spin column technique (Qiagen, Germany). The DNA yield and purity were measured using a Nano-drop TM 1000 spectrophotometer after DNA extraction (Thermo Scientific, USA). The serum was separated by centrifuging the blood-filled gel tubes at 2000 rpm for 10 min and then freezing it in aliquots at −80 °C. These aliquots were afterward utilized to calculate the NO level.

Measurement of serum nitric oxide (NO)

The Griess reaction was first described in 1879 by Peter Griess. Because of its simplicity, it has been used extensively in the analysis of numerous biological samples, including serum, urine, CSF, saliva, and cell culture media. In this method, nitrite is first treated with a diazotizing reagent, e.g., sulfanilamide, in acidic media to form a transient diazonium salt. To form a stable azo compound, this intermediate is then allowed to react with a coupling reagent, N-naphthyl-ethylenediamine (NNED). The intense purple color of the product allows for nitrite assays with high sensitivity and can be used to measure nitrite concentrations as low as 0.5 mM. The absorbance of this adduct at 540 nm is linearly proportional to the nitrite concentration in the sample.[25] In our study, the samples were deproteinized by mixing 0.5 mL of serum, 10 μL of sodium hydroxide, and 300 μL of 0.15 M zinc sulfate, and then vortexing each sample for 1 min. The samples were then kept on ice for 15 min, after which they were centrifuged for 10 min at 3000 rpm. Copper-coated cadmium granules were used to convert NO_3 to NO_2. Cadmium granules were stored in 0.1 M sulfuric acid and washed by swirling them with distilled water. A solution of copper sulfate (15 mL, mM/L in 0.2 mole/L glycine buffer, pH 9.7) was used to coat the granules by mixing them for 2 min. Cadmium granules were drained and dried over tissue paper, which was used within 5 min. Then, 0.5 mL of distilled water, standard solutions, and deproteinized samples were added, followed by 0.5 mL of glycine buffer (0.2 mole/L, pH 9.7). Two grams of copper-coated cadmium granules were added to the deproteinized samples; the tubes were shaken for 30 min in an electric shaker and 0.5 mL of the samples (distilled water, standard solutions, and deproteinized samples) was transferred to an appropriately labeled tube, followed by adding 0.5 mL of freshly prepared color reagent (Griess reagent). The Griess reagent was prepared by mixing an equal volume of 0.1% NNED (1 mg NNED/mL distilled water) and 1% sulfanilic acid in 5% phosphoric acid (10 mg sulfanilic acid/mL phosphoric acid). The tubes were placed in a water bath at 25 °C for 15 min to let the color develop. Then absorbance was measured at 543 nm using a 1 mL cuvette filled with distilled water as a blank. Sodium nitrite was used for preparing the standard curve by plotting absorbance versus concentrations. Five standard concentrations of sodium nitrite were used with each assay (2.5, 5, 10, 25, and 50 μmol/L) to generate a new equation for each set of samples assayed. A standard curve was plotted using an optical density value for each standard value versus the concentration of the standards. Then the concentration of each unknown was determined by interpolating from the standard curve.

Measurement of serum cardiac markers

The serum samples were used for measuring hs-cTnT and CK-MB. They depend on the immune-assay principle, and they were measured according to the manufacturer's instructions; Elecsys (Roche Diagnostics, Mannheim, Germany). After measuring the calibrator and quality control, serum samples were placed in the sample area, and 20 μL of serum sample was drawn to run the test. The results were obtained 5 min after the starting time. The analyzer automatically calculates the analyte concentration of each sample. The reference values for hs-cTnT and CK-MB are 0–0.014 ng/mL and up to 6.22 ng/mL, respectively.

Screening of rs1800779 (A/G) polymorphism in the eNOS gene

For genotyping the *eNOS* rs1800779 polymorphism, the ARMS-PCR test was used. The primers were taken from previously published literature[23] (Table 3). First, 2 μL of genomic-extracted DNA, 10 μL of Taq DNA Polymerase 2× Master Mix RED (Ampliqon, Danish), 1 μL of each forward (inner and outer) and reverse (inner and outer) primer, and 4 μL of nuclease-free water were used for a total of 20 μL. Then, the amplification process was started by using a Mini-app thermal cycler (Applied Biosystem, USA) in which the conditions were set, including initial denaturation at 95 °C (5 min), then 35 cycles at 95 °C (60 s) for denaturation, followed by annealing at 62 °C (45 s), and an extension step at 72 °C (60 s). The final extension was set at 72 °C (1 min). The third step, a 5 min extension phase that extends all PCR fragments, was performed at 72 °C. After PCR amplification, the PCR amplicons were separated on 2% agarose gel, stained with ethidium bromide, and visualized with a Bio-Rad UV-trans-illuminator. The amplification of the *eNOS* rs1800779 (A/G) variant resulted in the generation of three genotypes with the expected product sizes: the 208 bp band represented the wild homozygote genotype AA, the 256 bp band represented the mutant homozygote GG, and the heterozygote AG genotype was represented by 405 bp band in the agarose gel.

Statistical analysis

GraphPad Prism 6 and SPSS programs were used for the statistical analysis of the results. Parametric tests were applied, and all data passed the standard points of the normality tests (according to D'Agostino, Kolmogorov–Smirnov, and Shapiro–Wilk). Continuous variables were compared using a t-test, while categorical variables, such as the *eNOS* genotype and allele frequency, were analyzed using a chi-squared (χ^2) test. Both the 95% confidence interval (CI) and odds ratio (OR) of the genotypes and alleles were calculated to estimate the association of the rs1800779 (A > G) single nucleotide polymorphism (SNP) of the *eNOS* gene with MI patients. Binary logistic regression and one-way ANOVA were applied to find the correlation of the *eNOS* genotype with serum NO. Moreover, a P-value of less than 5% ($P < 0.05$) was considered statistically significant.

Results

Demographic and clinical characteristics of the subjects

The characteristics of the MI patients and the healthy controls are presented in Table 1. The MI patients and the controls showed no significant differences in age or gender. The MI patients showed significantly higher NO levels than the control group, with mean values of 25.95 ± 1.390 and 17.10 ± 0.877, respectively (P = 0.0001) (Figure 1). The mean CK-MB value of the MI group (21.51 ± 5.622) was significantly higher than the mean CK-MB value of the control group (2.777 ± 0.195) (P = 0.001). Moreover, the hs-TnT marker showed a significantly higher mean value in the MI group (0.658 ± 0.180) than in the control group (0.121 ± 0.021) (P = 0.0001).

Table 1: Demographics, clinical characteristics, and risk factors of the MI patients and control subjects.

Variable	Controls mean ± SEM	MI patients mean ± SEM	P-value
N	85	80	
Age	57.03 ± 1.068	60.24 ± 1.223	0.0512*
Sex (males/females)	37/49	32/48	0.693**
Smoking		15 (18.75%)	
Alcohol intake		5 (6.25%)	
Family history		20 (25%)	
Serum NO (μmol/L)	17.10 ± 0.877	25.95 ± 1.390	<0.0001*
CK-MB (ng/mL)	2.777 ± 0.195	21.51 ± 5.622	0.001*
hs-TnT (ng/mL)	0.121 ± 0.021	0.658 ± 0.180	0.005*

CK-MB: creatine kinase-myocardial band, hs-TnT: high-sensitivity cardiac troponin T, MI: myocardial infarction, NO: nitric oxide, N: number of participants. *Comparisons between groups were done via independent t-tests. **The association between males and females was done using a chi-squared (χ^2) test. Data are presented as the mean ± standard error of the mean (SEM). Probability (P)-values less than 0.05 were considered significant.

Genotype and allele comparisons in the MI patients and controls

The distribution of the genetic and allelic frequencies of *eNOS* rs1800779 (A/G) were analyzed, and the results are given in Table 4. In the co-dominant model, the AA genotype frequency in the patients with MI was higher (58.75%) compared to the control subjects (49.41%), and the AG genotype in the control group showed a higher frequency

Table 2: Inclusion and exclusion criteria used in this study.

Inclusion criteria	Exclusion criteria
Age from 30 to 70 years	Age <30 or >70 years
Patients have only MI	Presence of other comorbidities besides MI, such as other CVDs, diabetes, ESRD, etc.
Able to participate in the informed consent process	Patients who requested to withdraw from the study
Males and non-pregnant females	Pregnant females
Chest pain is supported by biochemical laboratory changes, ECG changes, or findings on imaging modalities able to detect myocardial injury and necrosis (24).	

CVDs: cardiovascular diseases, ECG: electrocardiograph, ESRD: end-stage renal disease, MI: myocardial infarction.

Table 3: Sequence of primers used for genotyping *eNOS* in this study (26).

Primer description	Primer sequence 5′−3′	Product size (bp)
Forward inner primer (A allele)	TAGTGGCCTTTCTCCAGCCCCTCAGAGGA	208 bp
Reverse inner primer (G allele)	GAGTGCATGCTGGGGTTTGTAGTTCTGGGC	256 bp
Forward outer primer	GCCCACCCCAACCTTATCCTCCACTGCT	405 bp
Reverse outer primer	GCCGCAGGTCAGCAGAGAGACTAGGGCT	

eNOS: endothelial nitric oxide synthase.

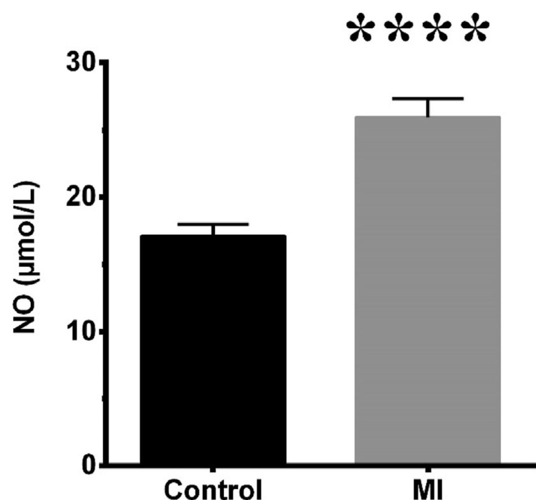

Figure 1: Serum NO levels in control and MI patients. Student's t-test was used to determine differences in NO levels between control and MI patients. MI: myocardial infarction, NO: nitric oxide. Probability (P)-values less than 0.05 were considered significant.

(43.53%) compared to the MI group (33.75%). However, the differences were statistically non-significant (P = 0.194).

The GG genotype in both groups was not different (P = 0.854). In the dominant model, the AG + GG vs. AA showed higher frequency in the control subjects (50.59%) compared to the MI patients (41.25%). However, the difference was insignificant (P = 0.229). Moreover, the GG vs. AA + AG in the recessive model showed an insignificant P-value (P = 0.913), with frequencies of 7.50% and 7.06% in the MI patients and the control subjects, respectively. The frequencies of the A and G alleles among MI cases and control subjects displayed a lack of association with increased risk of MI.

The A allele in the MI patients was more frequent (75.6%) compared to the healthy controls (71.2%), and the G allele was more frequent in the controls (28.8%) when matched with the patients (24.4%). However, the difference between the two groups was statistically non-significant regarding the allele frequencies (P = 0.361).

Serum NO level in the eNOS rs1800779 (A/G) genotypes

The mean serum NO levels in the AA, AG, and GG genotypes were 17.10 ± 0.877, 28.32 ± 1.949, and 21.51 ± 5.918, respectively (Figure 2 & Table 5). The results showed no statistically significant difference in NO levels among different genotypes of the *eNOS* rs1800779 variants among the MI patients.

Logistic regression analysis of serum NO levels and eNOS rs1800779 (A/G) genotypes

Binary logistic regression determined the non-significant association of *eNOS* rs1800779 (A/G) genotypes with the

Table 4: Genotypic and allelic frequencies of the *eNOS* rs1800779 (A/G) polymorphism in the MI patients and the control subjects.

rs1800779 (A/G) SNP	MI (n = 80)		Control (n = 85)		OR	95% CI	P-value
	No.	%	No.	%			
Genotype frequencies							
Codominant							
AA	47	58.75	42	49.41	1.0	–	–
AG	27	33.75	37	43.53	1.534	0.802 to 2.931	0.194
GG	6	7.50	6	7.06	1.119	0.335 to 3.738	0.854
Dominant							
AA	47	58.75	42	49.41	1.0	–	–
AG + GG	33	41.25	43	50.59	1.458	0.787 to 2.699	0.229
Recessive							
AA + AG	74	92.5	79	92.94			
GG	6	7.50	6	7.06	0.9367	0.289 to 3.035	0.913
Alleles							
A	121	75.6	121	71.2	1.256	0.7694 to 2.052	0.361
G	39	24.4	49	28.8			

The association among variables was done using a chi-squared (χ^2) test. CI: confidence interval, OR: odds ratio, MI: myocardial infarction, SNP: single nucleotide polymorphism. Probability (P)-values less than 0.05 were considered significant.

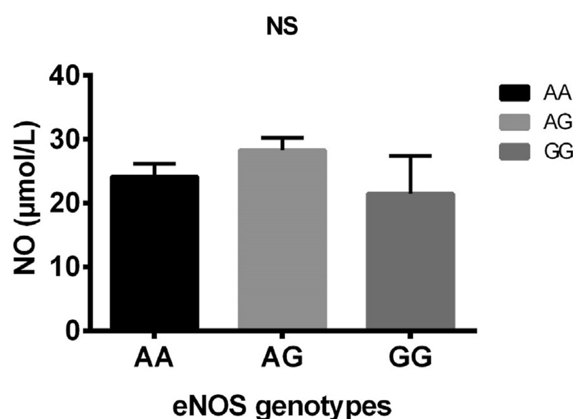

Figure 2: Comparison of serum NO levels in the *eNOS* genotypes. One-way ANOVA was used to determine the differences in NO levels among *eNOS* genotypes. *eNOS*: endothelial nitric oxide synthase, NO: nitric oxide. Probability (P)-values less than 0.05 were considered significant.

Table 5: Serum NO level compared in different genotypes.

rs1800779 (A/G) SNP	NO (mean ± SEM) (μmol/L)	P-value
AA	24.22 ± 1.987	0.300
AG	28.32 ± 1.949	
GG	21.51 ± 5.918	

NO: nitric oxide, SNP: single nucleotide polymorphism. Comparisons between groups were done via one-way ANOVA. Data are presented as mean ± standard error of the mean (SEM). Probability (P)-values less than 0.05 were considered significant.

Table 6: Binary logistic regression analysis to associate serum NO with the AA and non-AA genotypes of the rs1800779 SNP.

Variable	B	SEM	OR	CI	P-value
NO (μmol/L)	0.017	0.018	1.017	0.982−1.053	0.349

Binary logistic regression was done by SPSS 27. The MI patients were divided into those with AA genotypes and those without considered dependent variables (dichotomous variables). B: regression coefficient, CI: confidence interval, OR: odds ratio, SEM: standard error of the mean. A probability (P)-value of less than 0.05 was considered significant.

NO levels of the MI patients (OR = 1.017, 95% CI = 0.982−1.053, P = 0.349) (Table 6).

Discussion

The most common form of coronary artery disease is MI, considered one of the world's major health problems. The endothelial cells of blood vessels are responsible for NO production in the reaction of *eNOS* converting L-arginine to NO. The latter has an essential role in maintaining blood vascular function. Numerous *eNOS* polymorphisms have been studied regarding their relationship with MI. For the first time, the current study aimed to investigate the association between the *eNOS* rs1800779 (A/G) polymorphism and the development of myocardial infarction among the Kurdish population from Iraq.

Our study showed a noticeable elevation in NO levels in MI patients. There are many reasons for this. First, the activity of iNOS is overexpressed in MI patients, leading to an increase in the levels of ONOO− and NO. The hyperactivity of iNOS and NO results in myocardial dysfunction and an increased mortality rate in MI.[9] It has been documented that NO levels in the serum are elevated in many diseases, including heart failure, diabetes mellitus, sepsis, and liver cirrhosis—as well as in MI patients. However, the normal range and the normal concentration that separate the pathological function (free radical function) from the physiological function in patients and healthy controls remain elusive.[26]

In the current study, the mean NO level in the healthy controls was 17.10 ± 0.877 μM. This is not in line with the mean NO level in the study conducted on the Turkish population, which reached 32.6 μM.[27] On the other hand, the mean serum NO concentration in MI patients in our study was 25.95 μM, which was noticeably higher than that of the control subjects. Nevertheless, this result was close to the mean NO concentration in a previous study among Iranian controls.[26]

On the other hand, mean NO concentrations in different healthy populations vary: 55 μM among Japanese,[28] 53.11 μM among Koreans,[29] and 8.8 μM among African-Americans.[30] The difference in NO levels in different populations may be due to the type of foods they eat and environmental pollution.[31,32] Increased NO levels have many pathological consequences; there is evidence regarding the association of NO with oxidative stress. NO plays a detrimental role in inducing DNA fragmentation and mitochondrial dysfunction via the destruction of respiratory chain portions through the production of a highly cytotoxic nitrogen species (peroxynitrites) in reaction with superoxide.[33]

De Sánchez et al. (2012) experimented on isoproterenol-induced myocardial infarction in animal models. They reported that NO had induced MI by two mechanisms: firstly, it impacted systolic and diastolic blood pressures[34]; and secondly, the NO as a free radical stimulated damage to mitochondria, caused energy instability of the cell, and increased the production of NO_2 and NO_3.[35] They also found that the isoproterenol triggered MI mainly by forming NO intermediates.

Significantly higher mean values of the CK-MB band and the hs-TnT markers were recorded in the MI patients in our study. The emergency rooms in Erbil province hospitals use the CK-MB along with the hs-TnT marker to diagnose acute MI, despite the superiority and sensitivity of the hs-TnT marker.[36] CK-MB and hs-TnT are released from the myocardium during damage. As a result, their levels become elevated in MI patients compared to healthy individuals.[37]

The present study showed no significant association between the MI and control groups in rs1800779 *eNOS* genotypes and alleles. Individuals carrying the GG genotype or AG genotype did not have an association with increased risk and susceptibility to developing MI. The G allele was neither a risk factor nor a protective factor for MI. This result is not consistent with the finding of Zhang et al.,[38] who reported

that the rs1800779 SNP in *eNOS* was related to CVD. There are conflicting results regarding the association of the *eNOS* polymorphism in different sites with CVD in other populations.[39–41] Serum NO levels were compared concerning rs1800779 (A/G) genotypes in the MI patients. Despite increasing NO levels, the results showed that the serum NO level was not significantly related to the rs1800779 genotypes (AA, AG, or GG). Moon et al.[29] also documented a lack of association between the *eNOS* polymorphism and NO levels in MI patients in the Korean population.

Elevated NO levels may result from other SNPs of the *eNOS* or other NOS isoforms such as the iNOS enzyme. The latter is the primary marker for MI and is activated during inflammation. In our study, binary logistic regression uncovered that the *eNOS* polymorphism is unrelated to NO production. Therefore, it is not associated with MI. Therefore, this polymorphism cannot be regarded as a risk or protective factor. The rs1800779 SNP generates metabolic syndrome (MetS) through cholesterol and LDL metabolism. As all MetS patients have a higher risk of CVD, the condition is multifactorial. Therefore, the minor allele carriers of the NOS variants might have a higher risk of CVD.[42] According to previous studies on *eNOS* polymorphisms and cholesterol, the association between *eNOS* polymorphisms with cholesterol[43] and oxidized low-density lipoprotein (oxLDL) has been evaluated.[12] Moreover, the OxLDL is considered a risk factor for MI.

There are a few limitations to this study. First, a small sample size is one of the limitations that might increase the standard error of variables. Second, blood was only drawn from patients at the time of admission. The results would have been more meaningful if blood had been retaken at regular intervals and the biomarkers reassessed. The time elapsed between the onset of the attack and the taking of the sample in the emergency department was not considered, since this information was not available for most MI patients. Finally, only ARMS-PCR was used to find SNPs. Sanger sequence analysis is a better way to confirm them.

Conclusion

From the findings of our study, it can be concluded that there is a lack of association between the *eNOS* rs1800779 polymorphism and MI incidence among the Iraqi Kurdish population. Moreover, the studied SNP showed an insignificant association with serum NO levels in the studied population. Further studies with a larger sample size of other *eNOS* polymorphisms are encouraged to understand possible MI risk factors for the Kurdish population.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

This study was approved by the Scientific and Ethics Committee of the University of Salahaddin-Erbil, KRG, Iraq (approval number: 715414) on January 3, 2021. Informed consent was obtained from the participants after the research objectives and aims, voluntary participation, right to autonomy and confidentiality, and the right to withdraw from participation in the study were explained to them.

Authors contributions

Concepts and design by SN, AMJ, and MKQ; experiments performed by ZOK, GOO, and HKA; data analyzed by SWS; tables and figures prepared by MMH, SP, MD, and TS; manuscript written by SWS, MFR, and SN. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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