AL-QADISIYAH MEDICAL JOURNAL , QAD.MED.J. 21 (1): JANUARY-JUNE 2025
WWW.qmj.qu.edu.iq

REVIEW ARTICLE

The Prevalence of Endothelial Nitric Oxide Gene Polymorphisms in Iraqi Patients with Essential Hypertension

Ghufran K Salman[1], Bassim I Mohammad1[1]

1Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiyah, Iraq

*Corresponding author: E-mail: ghufran.k1994@gmail.com

Abstract:

Background: Due to the polygenic character of hypertension, it is particularly challenging to identify the single nucleotide polymorphisms most likely to be linked with essential hypertension, as well as the connections between certain genes and treatment responses in various ethnic groups. One of the important genes is the eNOS gene, which encodes the eNOS enzyme that mediates NO production. This study involves rs1799983 (G894T) and rs2070744 (T-786C) of the eNOS gene. The objective of this observational cross-sectional descriptive study is to examine frequencies of alleles of eNOS3 genes. Methods: Ninety hypertensive patients were recruited by a specialist cardiologist and conducted at Al-Diwaniyah Teaching Hospital and the Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiyah, Iraq. DNA samples were genotyped by the PCR-tetra-arm method. Results: Regarding rs1799983, the most frequent allele was G (73%), and the most frequent genotype was GG (55%), while for rs2070744, the most frequent allele was T (66%), and the patients had a higher frequency of TT + TC genotypes (43% and 47%, respectively). Conclusion: This study concluded the most common allele for rs1799983 was the G allele (73%). Regarding rs2070744, the most frequent allele was T (66%).

Keywords: *eNOS* gene, polymorphism, Iraqi patients, essential hypertension.

Introduction

Hypertension (HTN) is a common chronic treatable condition with high prevalence. HTN is a risk factor for several medical conditions such as stroke, heart disease, congestive heart failure, kidney disease, and retinal damage [1]. About 95% of adult cases of hypertension are due to essential hypertension (EH). While 5% of cases of chronically increased BP are caused by secondary causes of hypertension [2]. Primary hypertension is the form that affects adults more frequently and tends to develop gradually over time. It has no known etiology. However, some individuals have elevated blood pressure as a result of an underlying condition known as secondary hypertension [3]. A scientific study conducted in 2005 found that in 2000, 26.4% of adults (26.6% of men and 26.1% of women) had hypertension, and it projected that 29.2% would be expected to have this illness by 2025. In 2000, it was found that 972,000,000 adults worldwide had HTN, with 333,000,000 living in economically developed nations and 639,000,000 in those in less developed nations. In 2025, the total number of adults that have HTN was expected to increase to sixty percent, totaling 1.56 billion. [4]. HTN has multiple causes. Variable response to antihypertensive medication may be due to drug non-adherence (which may be due to treatment costs or adverse effects) and inter-individual genetic variability [5]. Due to the polygenic character of HTN, it is particularly challenging to identify the single nucleotide polymorphisms most likely to be linked with essential hypertension (EH) and also the connections between certain genes and treatment responses in various ethnic groups [6]. One of the important genes is the endothelial nitric oxide synthase (NOS3 or eNOS) gene, which encodes the eNOS enzyme that mediates nitric oxide (NO) production. Endothelial cells continuously produce NO in response to flow-induced shear stress, which causes the relaxation of vascular smooth muscle. Under normal circumstances, NOS stimulates the conversion of electrons from NADPH, oxygen, and arginine into citrulline and nitric oxide. The cofactors tetrahydrobiopterin (BH4), oxygen, and NADPH are among those known to be necessary for NOS [7, 8]. (Figure 1)

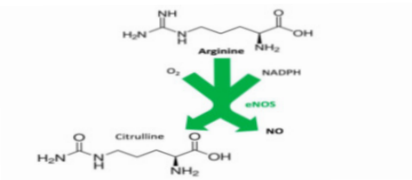

Figure 1: synthesis of nitric oxide (NO)

SUBMITTED: 26/ JULY /2023 | ACCEPTED: 13/JANUARY/2024 | PUBLISHED: 30/JUNE/2025 |

A decrease in the endothelial L-arginine nitric oxide pathway activity may contribute to or initiate the occurrence of EH by stimulating and maintaining increases in peripheral vascular resistance [9, 10]. Nitric oxide is a relaxing factor derived from endothelium and considered a local blood flow regulator in normal subjects [11, 12, 13].Endothelial dysfunction plays an important role in the pathophysiology of hypertension [14]. Endothelial dysfunction may be related to both direct pressure-induced damage and increased oxidative stress [oxidative stress is caused by either decreased breakdown or increased production of reactive oxygen species (ROS)]. Numerous enzyme systems, such as NADPH oxidase, cyclooxygenase (COX), and xanthine oxidase, as well as decreased superoxide dismutase activity, all contribute to the production of reactive oxygen species. Reactive oxygen species can cause vascular dysfunction due to high production of free radicals (e.g., superoxide anion), which bind to nitric oxide (NO) and reduce NO bioavailability. Reduced NO bioavailability is a crucial factor linking oxidative stress to hypertension and endothelial dysfunction. [15]. Nitric oxide was produced by the endothelial nitric oxide synthase enzyme (eNOS). The NOS3 or eNOS gene encodes the eNOS enzyme. The eNOS gene is located on chromosome 7q36 and is made up of 26 exons and 25 introns that encode for a 135 kDa protein with 1,203 amino acids that spans approximately 23 kilobases of the genome. (Figure 2) [16, 17]. The eNOS gene is highly polymorphic.

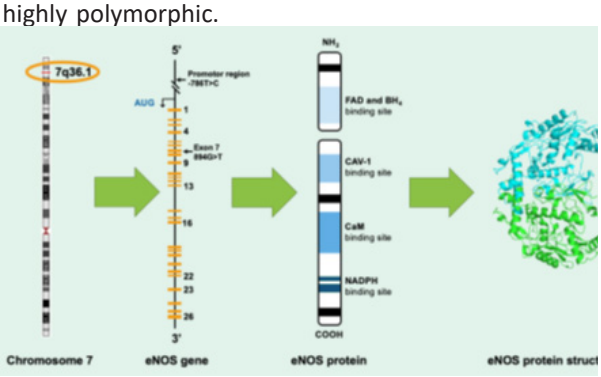

Figure 2: Scheme illustrates location endothelial nitric oxide synthase gens (eNOS) on the chromosome 7.

Aim

To determine the frequency of rs1799983 and 2070744 polymorphisms of eNOS gene.

Materials and Methods

This clinical trial is an observational, cross-sectional, descriptive, single-center study for hypertensive patients of Iraqi nationality who are diagnosed according to JNC 8. Specialist caregiving physicians/cardiologists diagnosed and recruited all candidate patients. The study was conducted at Al-Diwaniyah Teaching Hospital and the Department of Pharmacology and Therapeutics, Medicine College, Al-Qadisiyah University, Iraq.

Subjects

Ninety adults were recruited in this study (37 male and 53 female) aged 20-70 years old. Patients with renal or hepatic impairment, pregnancy, heart failure, obesity (BMI ≥ 30), and psychiatric patients were considered as exclusion criteria. The study was accepted by the Ethics Committee of the Medicine College, University of Al-Qadisiyah, and procedures were explained to all participants, and informed consent was taken from all patients.

Physical measurements

The measurements of blood pressure were taken using a mercury sphygmomanometer. Before taking the measurements, the subjects were advised to sit quietly and rest for 5 min. Blood pressure was calculated as the mean of three subsequent readings. HTN was defined as DBP ≥ 90 mmHg and SBP ≥ 140 mmHg. The weight of the patients included in the study is measured by electronic scale in kg. The height is measured by a height scale in m^2. Depending on these two anthropometric parameters, body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

Blood Sample

Blood samples of 4 ml were collected from the patients that were aspirated from the antecubital vein and divided into two portions: One milliliter (ml) of the patient's whole blood was collected in a tube containing EDTA for DNA extraction and stored until the time of DNA extraction at -20°C. Three milliliters (ml) of the patient's whole blood collected in a gel tube were spun at 5,000 revolutions per minute for five minutes, and the serum was collected to be used in biochemical tests.

Analytical techniques

Total cholesterol, HDL, LDL, VLDL, triglyceride, serum urea, creatinine, uric acid, and random blood glucose were measured by enzyme assay.

DNA isolation and genotyping

From blood samples, genomic DNA was isolated by a DNA isolation kit (Frozen Blood) from Geneaid (USA). The Tetra-primer ARMS-PCR technique was performed for genotyping and detecting eNOS (rs1799983) and (rs2070744) gene polymorphisms in blood samples. To identify the genotype, the tetra-primer ARMS-PCR uses 4 primers in a single PCR, which include two allele-specific primers (inner primers) and two non-allele-specific primers (outer primers). At the start of the reaction, outer primers amplify the area containing the single nucleotide polymorphism. When a non-allele-specific primer fragment is produced, it acts as a template for the inner primers, which will form allele-specific fragments. [18]. When putting non-allele-specific primers at various distances from polymorphic nucleotides, the two allele-specific fragments may be observed in an agarose gel by their various sizes. [19]. The online website Primer 1 was used for design primers (Table 1). The BLAST program in the NCBI server was used to test the primers' specificity.

Table 1: The PCR primers with their sequence, amplicon size and annealing temp.

Primer	Sequence	Amplicon	Annealing
eNOS rs1799983	Inner forward CTGCTGCAGGCCCCAGATAAT	T-allele 147 bp.	63
	Inner reverse ACCCTGGAGATGAAGGCAGGA	G-allele 195 bp.	
	Outer forward GCAGAAGGAAGAGTTCTGGGAGC	Two outer primers 298 bp.	
	Outer reverse CCTTCTTGAGAGGCTCAGGGATG		
eNOS 2070744	Inner forward GGCATCAAGCTCTTCCCTGTCC	C- allele 154 bp.	63
	Inner reverse GGCTGAGGCAGGGTCAGACA	T-allele 103 bp.	
	Outer forward CTGGTGTACCCCACCTGCATTCT	Two outer primers 215 bp.	
	Outer reverse GACTCAAGTGGGGGACACAAAAGAG		

The PCR condition was as follows: after denaturation at 95°C for five minutes, 35 cycles were performed (95°C for one minute, annealing temperature 63°C for one minute, followed by extension at 72°C for one minute), and final extension at 72°C for seven minutes to amplify the target DNA. DNA was separated by electrophoresis on 1% agarose gel and visualized with ethidium bromide. (Figures 3 and 4

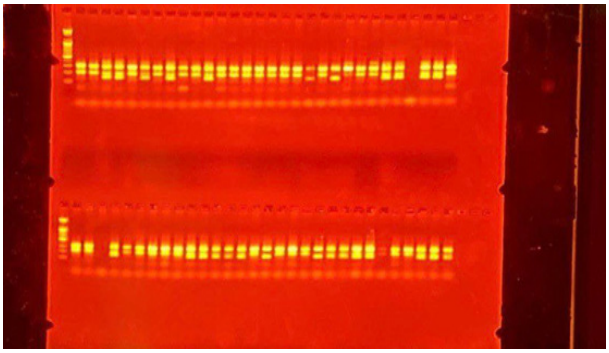

Figure 3: Agarose gel electrophoresis image that show the PCR product analysis of eNOS (rs1799983) gene from some blood .sample patients

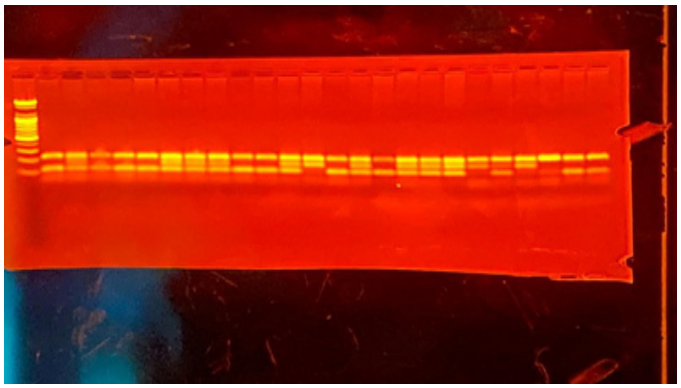

Figure 4: Agarose gel electrophoresis image that show the PCR product analysis of eNOS (rs2070744) gene from some blood .patients

Statistical analyses

Statistical analyses were performed by SPSS version 25. For each SNP, allelic and genotyping frequencies were calculated. A p-value < 0.05 was considered statistically significant. We .presented the data in terms of mean and SD

Results

Demographic data

This study included 90 Iraqi hypertensive patients; 53 (58.9%) were females and 37 (41.1%) were males (Figure 5), with the mean ± SD age (years) of the study group being 53.2 ± 13.8 and .the mean ± SD BMI being 29 ± 5 kg/m²

(Body mass index (BMI

n = 14) of the enrolled patients were considered normal) 15.6% weight, 42.2% (n = 38) were considered overweight, and 42.2% (n = 38) were considered obese. 26 of the obese patients fall into class I obesity, 11 fall into class II, and only one falls into class III. Figure 6

Renal function

The mean ± SD blood urea and serum creatinine of recruited hypertensive patients were 36.9 ± 9.3 mg/dL and 0.9 ± 0.2 mg/dL, respectively. Table 2

Metabolic profile

Among the study patients, the mean ± SD of total serum cholesterol, triglyceride, atherogenic index, and uric acid was 195.8 ± 44.8 mg/dL, 223.7 ± 105.7 mg/dL, 0.69 ± 0.3, and 5.2 ± 1.5 mg/dL, respectively. Table 3

Blood pressure

All patients who participated in this study were hypertensive, so the mean ± SD of systolic blood pressure was 150 ± 14.9 mmHg, while the mean ± SD of diastolic blood pressure was 89.3 ± 6.9 mmHg. Figure 7

Figure 5: Pie chart illustrates the sex distribution of participant .(hypertensive patients in the study (n = 90

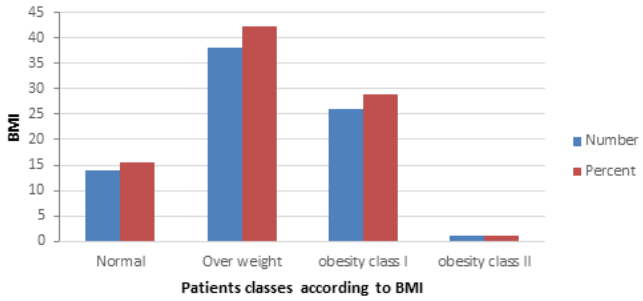

Figure 6: Shows BMI distribution of the enrolled hypertensive .(population (n = 90

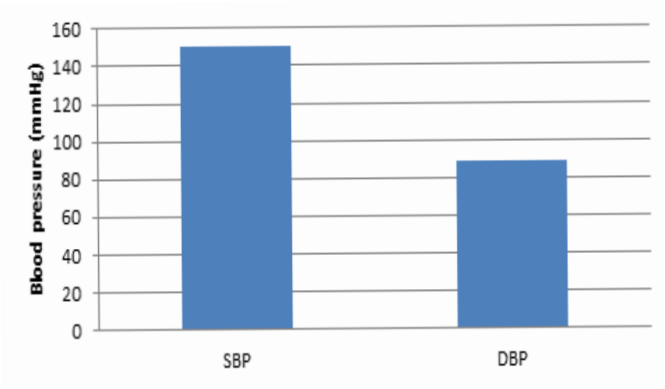

Figure 3.3: Shows the mean systolic blood pressure (DBP) and diastolic blood pressure (DBP) of recruited hypertensive patients (n = 90).

Table 2: Illustrating values of blood urea and serum creatinine of recruited hypertensive patients (n = 90).

	Minimum	Maximum	Mean	Standard Deviation (SD)
Blood urea (mg/dL)	16.8	60	36.9	9.3
Creatinine (mg/dL)	0.45	1.4	0.9	0.2

Table 3: Illustrating the metabolic profiles characteristics of recruited hypertensive patients enrolled in the study.

	Minimum	Maximum	Mean	Standard Deviation (SD)
Cholesterol	105.0	357.0	195.9	44.8
Triglyceride	91.0	722.0	223.7	105.7
Atherogenic index	0.3	2.7	0.7	0.3
Uric acid	2.9	12.5	5.2	1.5

Genotyping the frequency of eNOS gene polymorphisms.

Genotype frequencies of the rs1799983 (G894T) and rs2070744 (T786C) polymorphisms among patients with essential hypertension did not significantly differ from those expected under conditions of Hardy-Weinberg equilibrium (P>0.05). The frequencies of the GG, GT, and TT genotypes were 48 (55%), 31 (36%), and 3 (9%), respectively. The most frequent allele was G, with a frequency of 127 (73%).

Regarding rs2070744 (T-786C), the frequencies of the (TT, TC, CC) genotypes were 37 (43%), 41 (47%), and 9 (10%), respectively. The allele with the highest frequency is T, accounting for 115, 66%.

Discussion

In our cross-sectional study, the study investigates the prevalence of eNOS rs1799983 and rs2070744 polymorphisms in Iraqi patients with EH. Regarding rs1799983, the most frequent allele was G (73%), while the most frequent genotype was GG (55%). The T allele has a minor frequency (27%), and the TT genotype was 9% (p = 0.7). Many studies have looked into the relationship between the EH and rs1799983 variant. Although the findings have been inconclusive and controversial. Other studies have found a greater frequency of the T allele in those with hypertension. [11, 9] In contrast, studies conducted on Caucasian groups found a higher frequency of the G allele in the hypertensive group, as well as a relationship between the G allele and the outcome, all-cause death [20,12]. These differences could be a sign that another single nucleotide polymorphism or mutation is connected to one of the two alleles, or they could be a sign that the connections that have been discovered are the result of random errors. Other studies reveal a loss of evidence for a relationship between this polymorphism and essential hypertension in Australian people [21] and Japanese people [22]. Gamil et al. showed an absence of linkage between rs1799983 and essential hypertension among Sudanese people [23]. Regarding rs2070744, the most frequent allele was T (66%). The patients had a higher frequency of TT + TC genotypes, 43% and 47%, respectively. While C allele frequency was 34% and CC genotype frequency was 10% (p = 0.95). Gamil et al. showed the frequency of CC + TC genotypes was higher in comparison with the TT genotype (p = 0.04) among Sudanese hypertensive patients. This study established a link between rs2070744 polymorphism and essential hypertension in the eNOS gene promoter among Sudanese patients [23]. Another study performed among the Canadian population revealed that the CC genotype is linked to higher SBP in a healthy cohort and that people who carry the C allele had a 2.16 (95% CI, 1.3-3.7) relative risk of developing hypertension compared to people who do not [24]. Research in a Japanese population, however, found no correlation between essential hypertension and this variation [25].

Conclusion

Our study is the first in Iraq to investigate the prevalence of (allele and genotype frequency) eNOS rs1799983 and rs2070744 polymorphisms in Iraqi patients with (EH). This study concluded the most common allele for rs1799983 was the G allele (73%), while the most frequent genotype was GG; the frequencies of the other genotypes, GT and TT, were 36% and 9%, respectively. Regarding rs2070744, the most frequent allele was T (66%), while the most frequent genotype was TC (47%); the frequencies of the other genotypes, TT and CC, were 43% and 10%, respectively.

Recommendations

Additional research with bigger sample numbers and family-based analyses is needed.

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