ORIGINAL ARTICLE

CONTENTS

Association between rs1799983 polymorphism of *eNOS* gene and essential hypertension in Iraqi hypertensive patients

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ABSTRACT

Aim: To investigate allele frequencies of rs1799983 polymorphism *eNOS* genes and to determine association between rs1799983 polymorphism of *eNOS* gene and essential hypertension in Iraqi hypertensive patients.

Materials and Methods: This is an observational cross sectional descriptive single center study. ninety hypertensive patients were recruited by specialist cardiologist and conducted at AL-Diwaniyah teaching hospital and department of pharmacology and therapeutics, college of medicine, university of Al-Qadisiyah, Iraq. DNA samples were genotyped by PCR-tetra-arm method. NO level was measured by using ELISA kit.

Results: Regarding rs1799983 the most frequent allele was G (73%) and the most frequent genotype was GG (55%). Our results indicate lack of substantial link between genotype frequencies of rs1799983 polymorphism and NO level (p=0.88) and thereby there is no statistically significant effect on SBP and DBP (p = 0.051).

Conclusions: our study demonstrated lack of significant association between this polymorphism and essential hypertension in Iraqi hypertensive patients.

KEY WORDS: rs1799983 polymorphism, *eNOS* gene, essential hypertension, Iraqi hypertensive patients

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INTRODUCTION

Due to the fact that the majority of individuals with hypertension (HTN), a prevalent chronic treatable condition, are asymptomatic, it is sometimes known as "the silent killer". Despite advancements, doctors still encounter difficulties in achieving optimal blood pressure (BP) values in all hypertensive patients because of these patients' variable medication responses. BP is the result of peripheral vascular resistance (PVR) and cardiac output (CO) [1]. Therefore, it is believed that elevated CO and/or elevated PVR are the causes of hypertension. Heart rate (HR) and stroke volume together determine CO; the size of the vascular compartment and myocardial contractility both affect stroke volume. Nearly half of the adult population in the US has hypertension, according to the American Heart Association's 2017 recommendations [2, 3]. In Arab nations, it is thought that 30% of adults suffer from hypertension [4]. Follow-up research conducted in Jordan examining the prevalence of hypertension between 1994 and 2009 found that it increased from 29.4% to 32.3% [5]. According to data on the prevalence of hypertension from 2009 to 2017, about one-third of Jordanian adults had the disease [6]. In 2008 the prevalence of HTN in Iraq for both sexes was 29.4 according to the World Health Organization (WHO) Eastern Mediterranean Region health statistics [7]. The prevalence of HTN in Thi-Qar was 26.5% in 2014 [8]. Hypertension is a multifactorial disease, and the causes for these disappointing results are complex, including drug non-adherence, which may be due to treatment costs or adverse effects, and inter-individual genetic variability [3]. 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 and also the connections between certain genes and treatment responses in various ethnic groups [4]. One of important genes is *NOS3* gene, which encode eNOS enzyme that mediate nitric oxide (NO) production. Nitric oxide is a vasodilator substance that produced by endothelium. Endothelial dysfunction may be related to both direct pressure-induced damage and increased oxidative stress in the presence of chronic HTN (oxidative stress is caused by either decreased breakdown or increased generation of reactive oxygen species (ROS)). By producing too many free radicals, such as superoxide anions, which bind to NO, ROS can exacerbate vascular dysfunction by increasing the production of the proinflammatory

oxidant ($ONOO^-$) and reducing nitric oxide bioavailability. Reduced nitric oxide bioavailability is a crucial factor linking oxidative stress to hypertension and endothelial dysfunction [9]. Under normal circumstances, NOS catalyzes the conversion of electrons from NADPH, arginine and oxygen into citrulline and NO. The co-factors tetrahydrobiopterin (BH4), oxygen and NADPH are among those known to be necessary for NOS [2, 3]. After being synthesized, it is transferred from the endothelial cell membrane to vascular smooth muscle cells, where it activates guanylate cyclase and causes it to convert GTP to cGMP, which subsequently leads to calcium removal and cell relaxation [10]. Elevated blood pressure and the development of HTN are brought on by the interruption of NO synthesis via suppression of constitutively expressed eNOS in both animal and human [11]. The *NOS3* or *eNOS* gene encodes the endothelial nitric oxide synthase (eNOS). The *eNOS* gene is found on chromosome 7q36 and is made up of 26 exons and 25 introns that encode for a one hundred-thirty five KDa proteins with one thousand- two hundred three amino acids that span approximately 23 kilobases of the genome (Fig.1). *eNOS* gene is highly polymorphic [12, 13].

AIM

The main aim of the present research is to investigate allele frequencies of rs1799983 polymorphism eNOS genes and to determine association between rs1799983 polymorphism of eNOS gene and essential hypertension in Iraqi hypertensive patients.

MATERIALS AND METHODS

This is an observational cross sectional descriptive single center study for hypertensive patients of Iraqi nationality, diagnosed according to JNC-8. All candidate patients diagnosed and recruited by specialist caregiving physician/cardiologist. The study was conducted at Al-Diwaniyah teaching hospital and Department of Pharmacology and Therapeutics, Medicine College, Al-Qadisiyah University, Iraq.

SUBJECTS

Ninety adults (37 male and 53 female) aged 20-70 years old were enrolled in this study. Patients with renal or hepatic impairment, pregnancy, heart failure, obesity (BMI ≥ 30) and psychiatric patient were considered as exclusion criteria. The study was approved 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.

BLOOD SAMPLE

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

DNA ISOLATION AND GENOTYPING

Genomic DNA from blood samples was isolated using a DNA extraction kit (Frozen Blood) (Geneaid, USA). The tetra-primer ARMS-PCR technique was performed for genotyping and detecting *eNOS* (rs1799983) gene polymorphism in blood samples. In order to identify the genotype, the ARMS-PCR tetra-primer uses 4 primers in a single PCR. Two non-allele specific primers amplify the area containing the single nucleotide polymorphism at the beginning of the reaction. Therefore they are called outer primers. Two allele-specific primers, called inner primers, which will produce allele-specific fragments, use the outer fragment of the primer as a template in its production [14]. Two allele-specific fragments in an agarose gel can be recognized by their differing diameters by positioning the outer primers at various distances from the polymorphic nucleotide [15]. The online website "PRIMER1: primer design for tetra-primer ARMS-PCR" (http://primer1.soton.ac.uk/primer1.html) was used for design primers (Table 1). BLAST program in NCBI server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to test the primers specificity.

PCR conditions were as following: after denaturation at 95°C for 5 min, 35 cycles were performed (95°C for 1 min, annealing temperature 63°C for 1 min, followed by extension at 72°C for 1 min) and final extension at 72°C for 7 min to amplify the target DNA. DNA were separated by electrophoresis on 1% agarose gel and visualized with ethidium bromide. (Fig.2).

STATISTICAL ANALYSES

Statistical analyses were made by SPSS version 25. For each SNP, allele and genotyping frequencies were calculated. P value <0.05 was considered statistically significant. The data were shown as mean ± SE.

RESULTS

The genotype frequencies of the rs1799983 (G894T) polymorphism among patients with essential hypertension did not significantly differ from those predicted un-

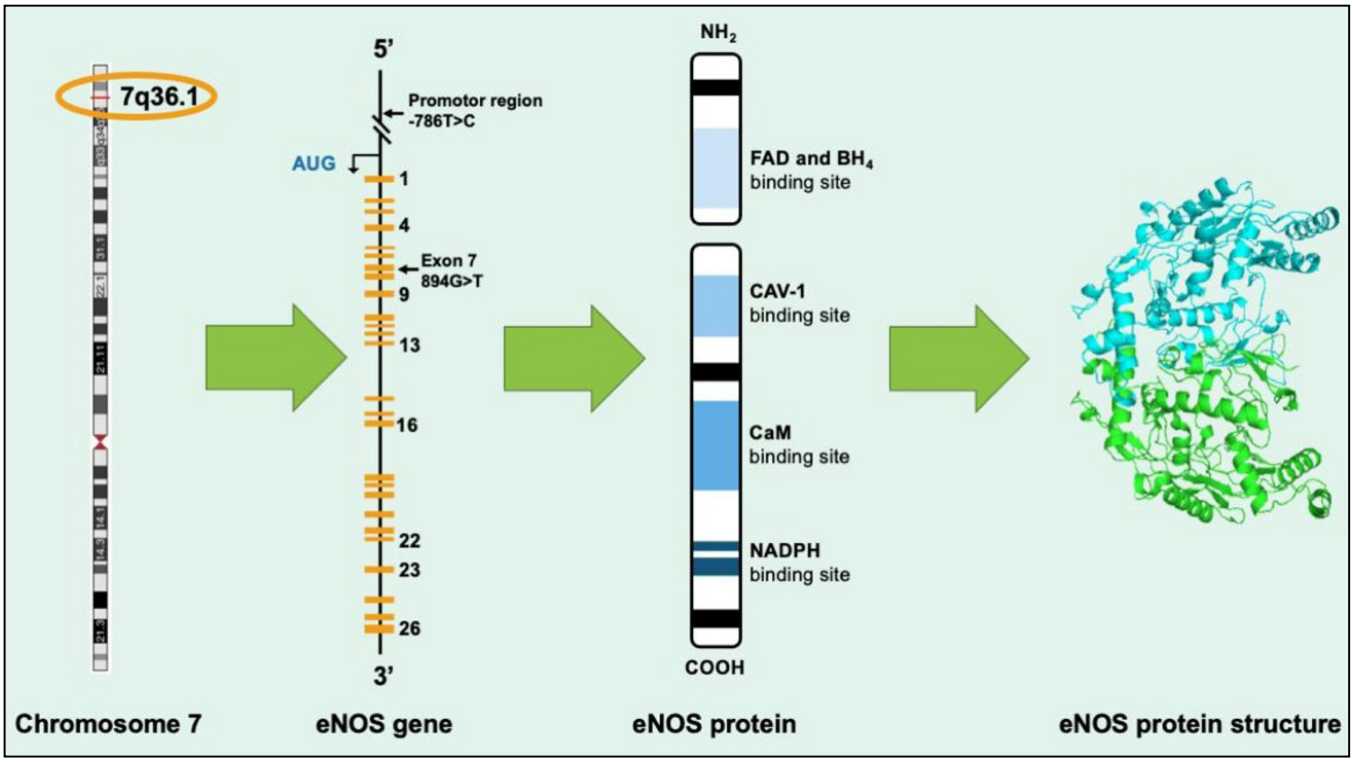

Fig. 1. Location of the endothelial nitric oxide synthase gene (eNOS) on chromosome 7.

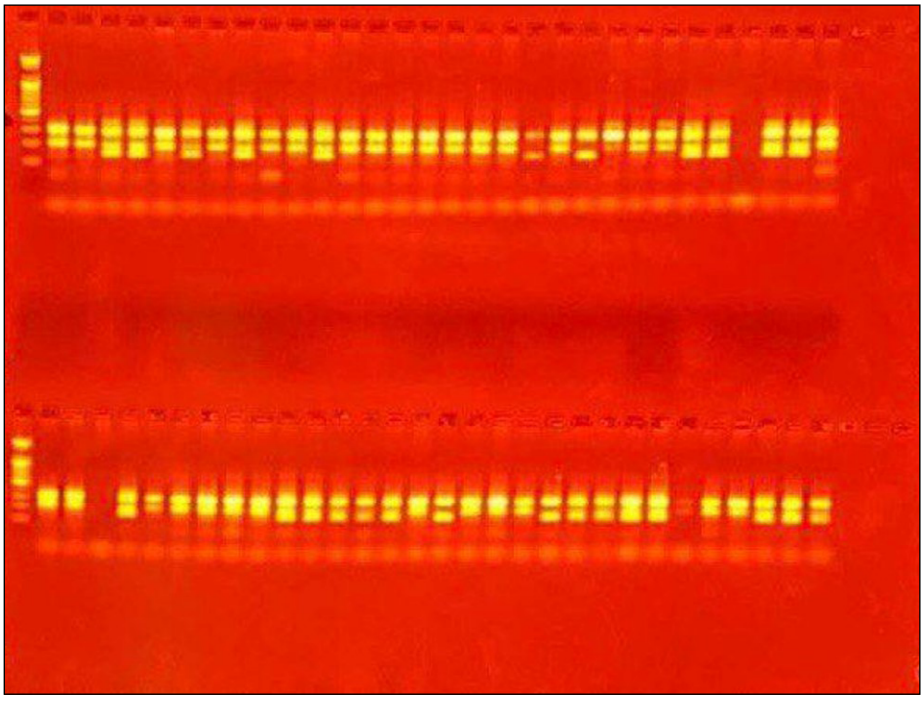

Fig. 2. Agarose gel electrophoresis image that show the PCR product analysis of eNOS (rs1799983) gene from some blood sample patients.

der conditions Hardy- Weinberg equilibrium (P>0.05). The genotype frequencies (GG, GT, TT) were 48 (55%), 31 (36%) and 3 (9%), respectively, with the most common allele being G - 127 (73%). The mean ± SE plasma level of NO in homozygous GG carriers was 72.3±2.9 µmol/L. In homozygous TT carriers, the plasma level was 74.6±9.4 µmol/L. On the other hand, plasma level of NO in heterozygous GT was 74.7±3.9 µmol/L. There was no statistically significant association between genotype frequencies and NO level (p = 0.88) (Table 2).

As shown in Table 3, the mean ± SE systolic blood pressure in homozygous GG, heterozygous GT, and homozygous TT carrier patients was 149 ± 14.5 mmHg, 152 ± 15.4 mmHg, 150 ± 15.9 mmHg respectively. On other hand, the mean ± SE diastolic blood pressure in homozygous GG, heterozygous GT and homozygous TT carrier patients was 87.9 ± 6.6 mmHg, 91.6 ± 6.7 mmHg, 87.8 ± 6.8 mmHg respectively. There was no statistically

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		

Table 2. Mean ± SE values of NO level and genotyping frequency in Iraqi patients with essential hypertension eNOS rs1799983 (G894T) polymorphism

Genotype rs1799983 (G894T)	Numbers	Mean NO level (µmol/L)	S.E	P value
GG	48	72.3	2.9	0.88
GT	31	74.7	3.9	
TT	8	74.6	9.4	

Table 3. Effect of eNOS rs1799983 (G894T) polymorphism on systolic and diastolic blood pressure in Iraqi hypertensive patients

Genotype rs1799983	Systolic BP mean (mmHg)	S.E	P value	Diastolic BP mean (mmHg)	S.E	P value
GG	149	2.1	0.6	87.9	0.9	0.051
GT	152	2.7		91.6	1.2	
TT	150	5.6		87.8	2.4	

significant effect of eNOS rs1799983 on systolic and diastolic blood pressure.

DISCUSSION

Due to the polygenic character of HTN, it is particularly challenging to identify the single nucleotide polymorphisms most likely to be associated with this condition as well as the connections between certain genes and treatment responses in various ethnic groups [4]. Notably, of the 40% of patients undergoing treatment, almost 65% do not achieve the target <140/90 mmHg [16]. In our cross sectional study, we investigate the association between *eNOS* rs1799983 polymorphisms and EH in Iraqi hypertensive patients. Regarding rs1799983 the most frequent allele was G (73%) while the most frequent genotype was GG (55%) ($p = 0.7$). Our study was demonstrated lack of association between rs1799983 and essential hypertension ($p > 0.05$). Many studies have looked into the relationship between the EH and rs1799983 variant. However, the findings have been inconclusive and controversial. Some studies have found a greater T allele frequency in those with hypertension, and T allele has been linked to resistance to conventional treatment [15, 17]. 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 G allele and the outcome, all-cause death [18, 19]. 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. On the other hand, other studies reveal a lack of evidence of an association between this polymorphism and essential hypertension in Australians [20] and Japanese [21]. Gamil et al. showed absence of linkage between rs1799983 and essential hypertension among Sudanese people [22].

CONCLUSIONS

Our study is the first in Iraq to investigate the linkage between *eNOS* rs1799983 polymorphism and essential hypertension in Iraqi hypertensive patients. This study concluded the most common allele for rs1799983 was G allele (73%) while the most frequent genotype was GG, frequency of other genotype GT and TT were 36% and 9% respectively. In this study we demonstrate the lack of significant association between two this polymorphism and essential hypertension.

RECOMMENDATIONS

To corroborate this association, additional research with bigger sample numbers and family-based analyses are needed. Future research should concentrate on the interactions between gene-environment and gene-gene, as well as haplotype patterns.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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