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ENDOTHELIAL NITRIC OXIDE SYNTHASE POLYMORPHISM AND ISCHEMIC HEART DISEASES IN COHORT OF EGYPTIAN PATIENTS

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ABSTRACT

The aim of the present work was to study the relations between two types 'of genetic polymorphism of eNOS Glu298Asp and T786C and the presence of coronary artery diseases in cohort of Egyptian patients.The study included 76 patients who were diagnosed to have coronary artery diseases from Mansoura University hospitals and 40 healthy control subjects. Blood samples were obtained for complete lipid profile study and molecular study of genetic polymorphism of nitric oxide synthase *Glu298Asp and T786C.*The presence of polymorphism of G-Asp was detected in patients in significantly higher rates than control (39.5% &15% respectively, P=0.006). Also, polymorphism T-C was significantly detected in patients compared to control (32.8% & 10% respectively, P=0.03). The study highlights that Glu298Asp and T786C genetic polymorphism of endothelial nitric oxide synthase is a common finding among patients with ischemic heart disease. The presence of polymorphism is associated with dyslipidemia. Further studies are required to establish this finding.

KEYWORDS: **Endothelial nitric oxide synthase, Polymorphism, Ischemic heart diseases**

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INTRODUCTION

Vascular Endothelial produces significant substance with biological activity among which is nitric oxide (NO). Nitric oxide synthase (eNOS) synthesize NO from amino acid L-rginine. [1]NO has a major function in preventing many cardiovascular diseases like hypertension and atherosclerosis by its relaxation action on smooth muscles in blood vessels and reducing adhesion of leucocytes and platelets to the endothelium and reduce the oxidation of low density lipoprotein associated with atherosclerosis. [2]Alteration in the activity of eNO has been associated with atherosclerosis in some studies. [3, 4]There are variants of eNOS associated with genetic polymorphisms and these variants may be implicated for increase susceptibility to atherosclerosis by affecting NO levels produced by endothelium.[5] One common polymorphism associated with theoretical risks for coronary artery disease is being described with genetic modification in the exon 7 leading to substitution of guanine by thiamine in the position 894 that results in the change of amino acids sequences of nitric oxide from glutamic acid to aspartic acid at location of 298 in eNOS protein. [5-8] Other type of polymorphism that can be associated with increased risk of atherosclerosis is that polymorphism in the 5′-flanking region of the eNOS gene that changes nucleotide thiamine at position 786 to cytosine affecting eNOS expression.[9]The data about the relation between cardiac atherosclerosis diseases and genetic polymorphism of eNOS are contradictory among various ethnic groups. [10, 11]Therefore the aim of the present work was to evaluate the relation between two types of genetic polymorphism, eNOS Glu298Asp and T786C, in coronary artery disease in Egyptian patients, in order to assess the utility of those molecular markers as early accurate diagnostic markers of the disease, as well as identification the category of people prone to the disease.

MATERIAL AND METHODS

The study included 76 patients who were diagnosed to have acute myocardial infarction inpatients in Mansoura University hospitals from January 2015 till August 2015. Definite acute myocardial infarction is diagnosed in the presence of unequivocal ECG changes and/or unequivocal enzyme changes; the history may be typical or atypical. Exclusion criteria were any patients who have any ischemic heart diseases without evident criteria of acute myocardial infarction like patients with angina pectoris, unstable angina and spontaneous angina. Also, patients with ischemic pain due to non-coronary stenosis like aortic stenosis were excluded. informed consent was obtained from each participant for blood samples according to Mansoura Faculty of medicine ethical committee. The study also included 40 healthy control subjects. Data was obtained from each participant about history of Diabetes mellitus and hypertension. After 12 hours fasting, ten milliliter blood was obtained from each subject. Each Blood sample was divided to two tubes; one for laboratory complete lipid profile study including high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol and triglycerides (TG). The other tube was heparinized for peripheral mononuclear cells separation and kept frozen at -70ºC for genetic analysis for genotypic study of nitric oxide synthase gene polymorphism. Inpatients have informed before blood samples taken.

DNA Extraction

DNA extraction was performed from buffy coat with a Qiagen DNA extraction kit (Qiagen Inc., Valencia, CA).

Polymerase Chain Reaction (PCR) for Detection of polymorphism of Glu298Asp

PCR study was performed on isolated buffy coat. The sequences of the primers used were 5′-CATGAGGCTCAGCCCCAGAAC-3′ and 5′-AGTCAATCCCTTTGGTGCTCAC-3.′PCR was performed in a total reaction of 25 µl containing 100 ng of template DNA, 1.0 µM of each primer, and 12.5 µl of PCR Master Mix (Qiagen Inc., Valencia, CA). The protocol for PCR was 94 °C for 4 min for denaturation followed by 35 cycles at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min. Finally, extension was conducted at 72 °C for 5 min. Distilled water was included as a negative control sample in each run to check for contamination [12].PCR amplification was followed by application of MboI restriction enzyme for digestion of PCR product for 16 h at 37 °C. In the presence of polymorphism this results in cleavage of PCR product into two fragments of 119bp and 87 bp. Electrophoresis was performed on 1.5% gel stained with ethidium bromide

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PCR for Detection of T786C polymorphism

This polymorphism of the eNOS was determined by the use of the following primers 5′-ATGCTCCCACCAGGGCATCA-3′ and 5′-GTCCTTGAGTCTGACATTAGGG-3′. PCR amplification was followed by application of NgOAIV restriction enzyme for 16 h at 37 °C. In the presence of polymorphism this results in cleavage of PCR product into two fragments of 203 and 33 bp. Electrophoresis was performed on 1.5% gel stained with ethidium bromide.

RESULTS

The demographic and laboratory data of the studied subjects were summarized in table 1. The mean age± SD of patients was 59.9± 2.1 and they were significantly older than control subjects. The patients were mainly male (78.9%). The risk factors associated with chronic heart disease (CHD) were mainly systolic hypertension (98.7%) followed by smoking (65.7%) and diabetes mellitus (DM) (35.5%). Lipid profile had significantly higher values for cholesterol (377.4± 135.8), TG (251.8± 98.6) and LDL (367.2± 134.5) compared to control subjects (P=0.0001). Meanwhile, HDL was significantly lower in patients (26.2± 11.6) than control (P=0.0001).The presence of polymorphism of G-Asp was detected in patients in significantly higher rates than control (39.5%& 15%, respectively, P=0.006). Also, polymorphism T-C was significantly detected in patients compared to control (32.8%& 10% respectively, P=0.03), table 2.Comparing lipid profile among patients with polymorphism of Glu298→Asp and patients without was performed in table 3. There was statistically significant reduction of HDL among patients with

polymorphism compared to those without (29.0± 10.8, 34.3± 14.6 respectively, P=0.0001) and significant increase in LDL in patients with polymorphism compared to those without (330.1± 152.7, 255.5± 80.2 respectively, P=0.0001), table 3. Comparing lipid profile among patients with polymorphism of polymorphism T786→C genotype and patients without was performed in table 4. The presence of polymorphism T786→C genotype was associated with significant increase in LDL (326.9± 145.5, P=0.0001).

Table 1
Comparison between demographic and laboratory results between patients and control

Parameter	Patients (n=76)	Control (n=40)	*P*
Age	59.9± 2.1*	49.1± 2.0	P=0.002
Sex			
Male	60 (78.9%)	30 (75%)	P=0.6
Female	16 (21.1%)	10 (25%)	
Smoking	50(65.7%) **	5 (12.5%)	P=0.0001
DM	27 (35.5%) **	0(0%)	P=0.0001
Hypertension	75 (98.7%) **	0(0%)	P=0.0001
Cholesterol (mg/dl)	377.4± 135.8 **	244.4± 165.5	P=0.0001
HDL (mg/dl)	26.2± 11.6 **	39.5 ± 3.3	P=0.0001
LDL (mg/dl)	367.2± 134.5 **	188.7± 22.9	P=0.0001
Triglycerides	251.8± 98.6 **	88.5± 11.5	P=0.0001
FBLG (mg/dl)	394.9± 143.6 **	114.4±15.0	P=0.0001

Significance: P< 0.05 **Significance: P< 0.001

Table 2
Comparison between the presence of genetic polymorphism of eNOS between patients and control

Type of Polymorphism	Patients (n=76)	Controls (n=40)	*P*
Polymorphism of G-Asp	30 (39.5%) *	6 (15%)	P= 0.006
Plymorphism T-C	25 (32.8%) *	4 (10%)	P=0.03

Significance: P< 0.05 **Significance: P< 0.001

Table 3
Correlation between the presence of Glu^{298}→Asp genotype and lipid profile

Lipid profile (mg/ dl)	Wild genotypes	Presence of polymorphism of Glu^{298}→Asp	*P*
Cholesterol	282.1± 91.7	317.6± 168.2	P=0.3
HDL	34.3± 14.6	29.0± 10.8 **	P=0.0001
LDL	255.5± 80.2	330.1± 152.7**	P=0.0001
TG	191± 141	212.2± 130.2	P=0.9

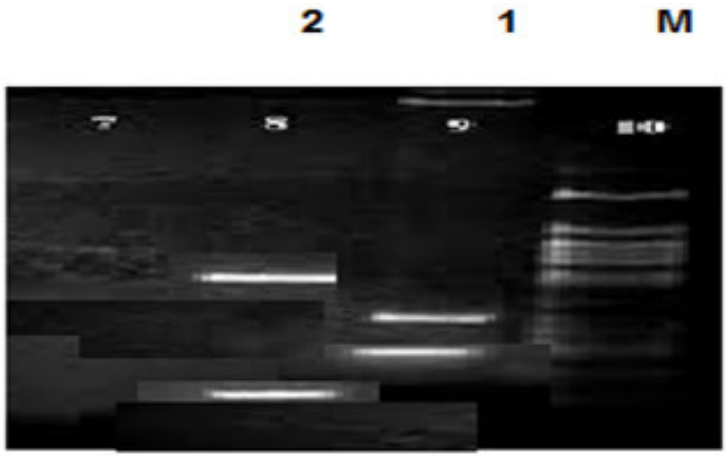
Significance: p< 0.001

Table 4
Correlation between the presence of T^{786}→C genotype and lipid profile

Lipid profile (mg/ dl)	Wild genotypes	Presence of polymorphism T^{786}→C genotype	*P*
Cholesterol	304.3± 155.1	315.1± 134.4	P=0. 1
HDL	36.6± 14.6	28.7± 10.7	P=0.2
LDL	247.3± 95.3	326.9± 145.5**	P=0.0001
TG	196.8± 118.7	197.5± 143.8	P=0. 3

Significance: p< 0.001

Figure 1
Polymorphism of eNOS. M: marker, lane 1 positive sample for Glu^{298}→Asp, lane 2positive sample for T^{786}→C



DISCUSSION

Cardiovascular disorders are a well-known etiology of marked morbidity and mortality. Among the studied patients older age in male gender associated hypertension, DM, smoking and elevated cholesterol and LDL were significantly the present Findings. Similar results were reported previously.[13, 14] Moreover, the modification of these risk factors like corrections of high blood pressure and blood glucose levels and treatment of dyslipidemia [15-17] is associated with correction of both morbidity and mortality of this condition. The genetic susceptibility to coronary artery diseases is another well-known risk factor. Among genes playing role in such condition is the polymorphism in NOs genes because nitric oxide plays a remarkable role in preventing events leading to atherosclerosis as mentioned before. [18, 19]In the present study, the presence of polymorphism of G-Asp was detected in patients in significantly higher rates than control (39.5%&15% respectively, P=0.006). Also, polymorphism T-C was significantly detected in patients compared to control (32.8%& 10% respectively, P=0.03). These results are online with previous studies reporting strong association of genetic polymorphism of eNOS gene at Gu298Asp and T786C and occurrence of ischemic cardiovascular disorders. [5,6,9,20,21] However, previous study from Egypt found no association between genetic polymorphism of eNOS and occurrence of myocardial infarction. [22]The difference between our results and the other study from Egypt can be attributed to the difference in the number studied and the difference in group of patients present in the study. [23]Generally the association between genetic polymorphism of eNOS and cardiovascular diseases can be explained by two theories. The first theory described that the mutations of eNOs genes is associated with significant reduction of the produced nitric oxidase enzyme or reduced in its enzymatic activity. [24, 25]The other theory demonstrated that genetic mutations result in enhanced intracellular cleavage of nitric oxidase enzyme. [26]The endothelial dysfunction precedes the development of atherosclerosis leading to cardiovascular diseases [27, 28]. There was statistically significant reduction of HDL among patients with polymorphism compared to those without (29.0± 10.8, 34.3± 14.6 respectively, P=0.0001) and significant increase in LDL in patients with polymorphism compared to those without (330.1± 152.7, 255.5± 80.2 respectively, P=0.0001), Comparing lipid profile among patients with polymorphism of polymorphism T786→C genotype and patients without was performed in table 4. The presence of polymorphism T786→C genotype was associated with significant increase in LDL (326.9± 145.5, P=0.0001). These results were online with results reported previously with the association of polymorphism of eNOS with dyslipidemia.[29, 30]The exact etiology of dyslipidemia associated with polymorphism of eNOS is not well defined but it is assumed to be due to the reduction of fatty acid oxidation and energy production. Also, there was reduction of HDL-cholesterol with its protective role with consequent reduction in endothelium vasodilation.[31] This again is explained by the theory that polymorphism of the eNOS genes results in reduction of enzymatic activity of nitric oxidase and even its activation by physical activity.[32] Even after physical activity trial to reduce lipid concentrations, patients with polymorphism in eNOS genes, fail to have reduced lipid levels compared to patients without polymorphism.[33]Study have shown that NO has lowering effect on cholesterol level through modifying its metabolism. Even introducing NO as therapeutic trials in animals revealed lowering effects on LDL-cholesterol in. [34] We can conclude that our study highlights that Glu298Asp and T786C genetic polymorphism of endothelial nitric oxide synthase is a common finding among patients with ischemic heart disease. The presence of polymorphism is associated with dyslipidemia. Further studies are required to establish this finding.

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