Association of Endothelial Nitric Cxide Synthase(eNOS) Gene Deletion with Diabetic Mellitus Type 1

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Abstract

Endothelial NOS (eNOS) is one of important antioxidant enzymes contributed in oxidative redox modulation in body researchers improved that there wear association with complicated of some diseases like diabetes mellitus, the present study was carried out to investigate relation between deletion in eNOS gene with DM type 1 in Najaf province patients, about 25 patients and 25 controls were collected from DM center with data the DNA extraction to amplified eNOS gene, the results show that there were significant elevation in deletion pattern patient (84% samples) while lower in control (16%samples) at $p< 0.0001$, also significant differences in age and fasting blood glucose, the present study concluded that it is a strong association between deletion in VNTR repetitive sequence and diabetic patients type 1.

Keywords: eNOS gene, DM 1 Najaf province, diabetic patients type 1.

Introduction

Endothelial NOS (eNOS) is enzyme known as nitric oxide synthase 3 (NOS3) it is encoded by the *NOS3* gene which located in the 7q35-7q36 region of chromosome [1,2] This enzyme synthesize nitric oxide (NO) which synthesis by three isoform included neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) . NO is molecule characterized that is a small gaseous and lipophilic which have major role in some biological activities.[3,4] eNOS is responsible for the production of NO in the vascular endothelium, [5] it has roles in regulating tone of vascular , cellular proliferation of cells , adhesion of leukocyte , and platelet aggregation [6]. thus it is essential for a healthy of cardiovascular system.

Diabetes mellitus type 1 become the highest prevalence disease in Iraq in the last years, it's also known diabetic dependent on insulin, researchers recorded that DM1 induced by several reasons such as environmental factors, stress, unbalance lifestyle and genetic predisposition. Diabetes considered as a chronic disease need medical care to avoid complications. Ammari , expected that more than 300 million people may be infected in 2025, ten percent of it having type 1 diabetes[7].

Materials and methods

1- Sample collection And Study subjects

The present study included 25 patients and 25 controls, blood samples were collected from the DM center in Al-Sadder hospital City. Data were collected from patients and control in questioner, then 2 ml of Venus blood were collected in gel tube to for DNA extraction , all samples were collected according to ethical approval of the health ministry and approval formats of Al-Sadder hospital City.

2- DNA extraction

DNA extraction according to leaflet of favogene kit, then concentration estimation by Nano drop (optizen) according to [8].

3- Primers and amplification system

eNOS primers were (f AGGCCCTATGGTAGTGCCTT and R-TCTCTTAGTGCTGT GGTCAC), it is based on the flanking VNTR sequence in intron 4, The products of PCR electrophoresis by 1% agarose , 0.5X TBE for one hour, 60 volts. The 420 bp wild-type product contained five 27 bp repeats and the 393 bp is a mutant type which these repeated deletions [9]. **PCR cycles** : 95Cº for 5 min, 95 Cº for 30 Sec, 58Cº for 30 Sec, 72Cº for 30 Sec (30 cycle), then 72Cº for 10 min [10].

Results

The result of this study shows that there were significant differences in age and fasting blood glucose and no significant differences in body mass index between patients and control, also no significant differences in gender. approximately halving of patient number suffer from hypertension.

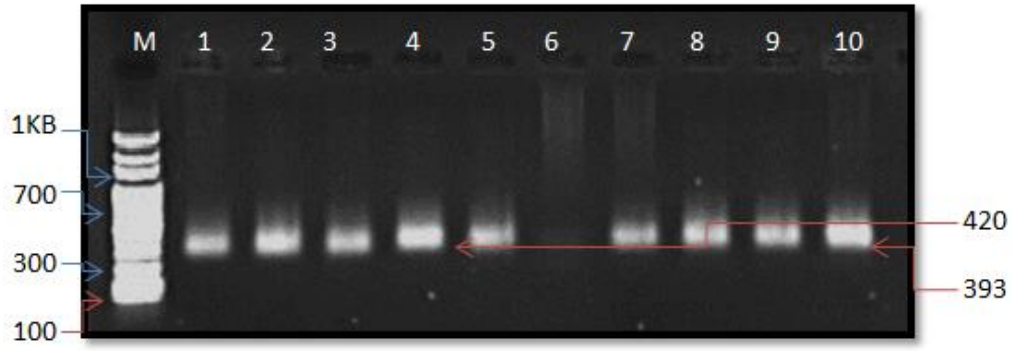
Mean of patients age was 49.24 while control was 50.5, mean of BMI was 31.07 and 25.46 for patient and controls respectively, FBG was higher in patients than control; it was 196.3 while in control was 81.0.

Table (1) means differences of demographic study of study groups.

Categories	Patients	Control	Statics	P-value
Age	49.24±12.65	50.5±6.42	*t*= 4.2472	0.0001
BMI	31.07±6.34	25.46±1.85	*T*=0.44	0.6590
FBG	196.3±90.0	81.0±40.37	*T*=5.8952	0.0001
Gender			X=3.000	0.0833
M	72%	48%		
F	28%	52%		
Hypertension				
Yes	48%	0		
No		0		
	52%			

Significant at P< 0.05

Electrophoresis of DNA show that there were clear, sharp bands. PCR product show that there were a high percentage of deletion in eNOS gene in patients than control with significant differences, 20 samples were suffering from deletion in this gene, while a low percentage in control as shown in table (2) and fig (1).



Fig(1) Electrophoresis pattern of PCR amplification, M DNA ladder, lane 1-5 patients, lane6-10 control.

Table(2) distribution of gene polymorphisms in study groups

Allele	Patients	Control	X^2	P-value
Deletion	20	4	20.513	0.0001
Normal	5	21		

Discussion

The changes in lifestyle in Iraq in last year's lead to recorded high percentage of DM, The present study one of the serious studies which designed to study some genes related with DM type 1 in the Iraqi population. Many studies were carried out about prevalence, incidence, physiological, biochemical and genetic polymorphisms were conducted in Iraq deal with DM in Iraqi population, Mansour, found that The prevalence of diabetes in Iraq was elevated from 5% in 1978 to 19.7% in 2012 [11].

Studies improved that the polymorphisms of the eNOS gene, may be related with the function of this gene, which lead to a change in gene expression and enzyme activity, Thus it studied in present study which had important role in free radical scavenger in DM patients and complications, [12] improved that sequence differences in eNOS gene is risk factor of DM nephropathy in family based study, others researches improved that it contributed in other complication like premature atherosclerosis [13,14,15]

Serious investigators performed about DM type 1 in Iraqi population to detect genetic predisposition, effect of environmental factors and antioxidant balance state related genes .Other study carried out about total antioxidant level and ROS level in DM type 1 , it shows that there were increment in mean of total antioxidant levels in whole blood while increment of ROS levels was low in patient comparison with control [16], according to previous studied and the present results we can explain the relation between elevation deletion percentage in patient in this gene, it may because unbalanced between ROS level and its scavenger from body which lead to DNA oxidation and mutants, also this deletion may be inherited from one parents which have history family of diabetic incidence, age may be effect in some DNA genes because of long exposure to different environmental factors which causes DNA mutation, deletion and insertion like heavy metals, weapons radiation and uptake

therapeutic drugs for long time, which causes increment free radical release, lifestyle also have major role in avoiding DNA changes like healthy nutrition, which contributed in activation immune system and DNA repair systems and antioxidant enzymes [8,17,18] the present study need more investigaion about gene sequence and SNPs in its promoter.

CONFLICT OF INTERESTS.
There are non-conflicts of interest.

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