



ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE T-786C POLYMORPHISM AMONG TYPE-2 DIABETES MELLITUS PATIENTS IN KHARTOUM, 2016

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ABSTRACT

Background: A single nucleotide polymorphism (T > C) rs2070744 due to transition of a thymine to a cytosine at T-786C in the promoter region of eNOS was found to reduce the rate of mRNA transcription by 50%, resulting in decreased serum NO levels which can inhibit apoptosis or stimulate tumor proliferation, angiogenesis and metastasis. **Objective:** To evaluate the association between Nitric Oxide Synthase gene T-786C polymorphism and **type-2 Diabetes mellitus patients in Khartoum**. **Material and Methods:** A total of 40 patients with **type-2 Diabetes mellitus** and 50 control subjects were enrolled in this study. DNA extractions samples previously extracted from EDTA Blood collected from SPV and stored at -20°C. Analysis of the eNOS786T > C promoter polymorphism were carried out by allele specific polymerase chain reaction method (PCR). **Results:** The present study reported that, molecular analysis showed that, the most frequent genotype in patients was TT 100% (40 sample). While in the control group there was 100% TT genotype. **Conclusion:** The present study indicates the lack of association between eNOS and type-2 Diabetes mellitus which needs to be investigated in study with larger sample.

KEYWORDS: eNOS and type-2 Diabetes mellitus.

INTRODUCTION

Nitric oxide (NO) which is catalyzed by endothelial nitric oxide synthase (eNOS), In vivo NO is synthesized during the enzymatic conversion of L-arginine to L-citrulline by three isoforms of nitric oxide synthase (NOS) enzyme, namely, neuronal NOS (nNOS or NOSI), inducible NOS (iNOS or NOSII) and endothelial NOS (eNOS or NOSIII).^[1,2] Endothelial (e) NOS, derived from vascular endothelium, is the most dominant form of these isoforms.^[3] The eNOS is encoded by a gene located on chromosome 7q35-q36, which is 21kb in size and consists of 26 exons.^[2] Additionally, promoter region of the eNOS gene harbors several transcription factor binding sites, regulating gene expression. The level of NO in the body is linked to expression of eNOS gene.^[3] The Single Nucleotide Polymorphism (SNP) (T-786C) (rs2070744) in the 5' promoter region affects the expression of eNOS gene. The T-786C allele binds the inhibitory transcription factor protein A1 resulting in a low mRNA level of eNOS and this reduces NO production and endothelial function.^[2,4] Several polymorphisms of the eNOS gene have been identified and their association with various diseases has been investigated, including coronary artery disease, myocardial infarction, coronary spasm, hypertension, end-stage renal disease (ESRD) and DN.^[5,6] T-786C substitution in the promoter region, which is strongly linked to 4b/a. The allele C of T-786C polymorphism

decreases promoter activity to less than half of normal activity, influencing thereby the progression of renal disease.^[8] Thrombotic events, both in the arterial and venous systems, are a common complication in patients with **type-2 Diabetes mellitus**. Diabetes mellitus type 2 is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Impaired nitric oxide synthase (NOS) activity in the vascular endothelial cells has been suggested as playing an important role in the pathogenesis of microangiopathic complications of type-2 Diabetes mellitus patients. Our study will be conducted to evaluate this relationship.

MATERIALS AND METHODS

This was a case control study conducted in Khartoum state during the period from November 2016 to February 2017. Sudanese patients with **type-2 Diabetes mellitus** 90 samples were enrolled in this study 40 as **type-2 Diabetes mellitus** as case group and 50 healthy volunteers as control group.

The study has been approved by the local ethics committee of Alneelain University. Selected individuals were informed with detailed objectives of the study and its importance in the future.

DAN extrication samples previously extracted from EDTA Blood collected from SPV and store at- 20C.

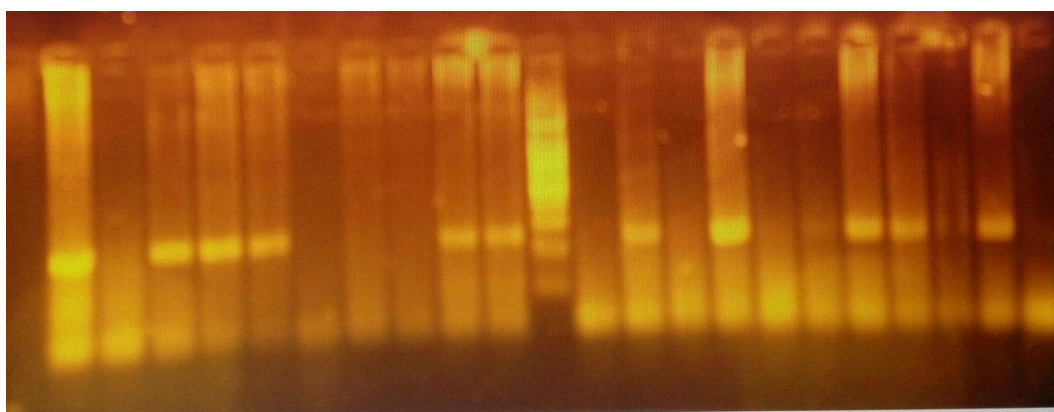
Detection of nitric oxide synthases gene (NOSs) T-786C promoter polymorphisms was done by using Allele-specific polymerase chain reaction using PCR machine (T-advance Thermocycler Biometra, Germany). one microliter (μ L) of DNA was amplified in a total volume of 20 μ L PCR mixture containing 1 μ L of each primer (Table 1) and 15 μ L sterile distilled water. Samples were amplified for 30 cycles, consisting of denaturation at 94C for 1 minute, Annealing at 60 C for 1 minute and extension at 72 C for 1 minute and the condition includes 5 minutes of initial denaturation at 94 and final extension at 72 for 10 minutes.

5 μ L of PCR product was electrophoresed on 3% agarose gel containing ethidium bromide. one μ L of 100 bp DNA ladder was applied with each batch of patients' samples. one μ L of 100 bp DNA ladder (T-advance Thermocycler Biometra, Germany) was applied with each batch of patients' samples. A PCR product of 176,250 bp fragment was consistent with C and T alleles respectively.

Data was collected by structured interview questionnaire and from patients' medical files and analyzed by statistical package for social sciences (SPSS).

Table 1 Show Primers and sequencing were used

Primer	Sequencing
CO:	5' TTT CTC CAG CCC CTC AGA TG 3';
2684C:	5' GGC AGA GGC AGG GTC AGA CG 3';
2684T:	5' CAT CAA GCT CTT CCC TGT CT 3'
TO:	5' AGG CCC AGC AAG GAT GTA GT 3'



The gel image with same size of bands; lane M: DNA ladder with 100 bp genotype (MI) with PCR product 250.

RESULTS

This was case control study, included 40 patients with **type-2 Diabetes mellitus**, 11(27.5%) were male, 29 (72.5%) were female. In addition, 50 subjects as control

group, 60% (24) were female while 40%(16) were female. The range age of patients **type-2 Diabetes mellitus** (29 - 65) years.

The molecular analysis showed that, the most frequency genotype in patients TT was 40 (100%) followed by genotype TC 0 (0%) and CC 0 (0%) While the control group showed that, 50 (100%) were TT.

Table 1

NOC789T Genotype	Patients % (n)	Control	P.Value	Odd ratio
TT	100% (40)	100% (50)		
TC	0% (0)	-		
CC	0% (0)	-		

DISCUSSION

A single nucleotide polymorphism (T > C) rs2070744 due to transition of a thymine to a cytosine at T-786C in the promoter region of eNOS was found to reduce the rate of mRNA transcription by 50%, resulting in decreased serum NO levels which can inhibit apoptosis or stimulate tumor proliferation, angiogenesis and

metastasis.^[11] This study conducted to establish the polymorphism at T-786C among **type-2 Diabetes mellitus**. The study reported the most frequency genotype TT followed by TC followed by CC.

Literature Review

A study was done in Egypt 2012 by Shoukry A1, Shalaby SM, Abdelazim S, Abdelazim M, Ramadan A, Ismail MI. among diabetic patients and they found that The C allele for -786T>C and the T allele for 894G>T were significantly more frequent in diabetics with nephropathy than in diabetics without nephropathy ($p<0.001$; odds ratio [OR] and 95% confidence interval [CI] for the C allele=1.64 [1.24-2.17] and $p<0.001$; OR and 95% CI=1.7 [1.27-2.26] for the T allele). The haplotypes CTa (with all the mutant alleles) and CTb were significantly more common in patients with DN ($p=0.01$ and 0.003 , respectively).^[23]

Another study was done in Japan 2004 by Awata T1, Neda T, Iizuka H, KuriharaS(et al.). They found that the eNOS gene polymorphisms were not significantly associated with the presence of retinopathy or with retinopathy severity or type 2 diabetes itself. However, by both association study and multiple logistic regression analysis, the T-786C and 27-bp repeat polymorphisms were significantly associated with a risk of developing macular edema with the -786C allele and the "a" allele increasing the risk.^[24]

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