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Research Article

ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE T-786C POLYMORPHISM AMONG POLYCYTHAEMIA VERA 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 Polycythemia Vera patients in Sudan.
Material and Methods: A total of 40 patients with Polycythemia Vera and 50 control subjects were enrolled this study. DAN extrication samples previously extracted from EDTA Blood collected from SPV and store at- 20C**.** 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 65% (26) followed by TC genotype 25% (10) and the reaming frequency was CC 10% (4) .While in the control group there was 100% TT genotype.
Conclusion: No significant difference was found between the distribution of the eNOS786T > C genotype in PV patients and the general population in Sudan**.**

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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 (iNOSor NOSII), and endothelial NOS (eNOS or NOSIII) (Azarpira *et al*., 2011; Nada *et al*., 2015). Endothelial (e) NOS, derived from vascular endothelium, is the most dominant form of these isoforms (Rossi *et al*., 2003).The eNOS is encoded by a gene located on chromosome 7q35-q36, which is 21kb in size and consists of 26 exons (Nada *et al*., 2015). 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 (Rossi *et al*., 2003). 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 (Nada *et al*., 2015; Rafikov *et al*., 2011).

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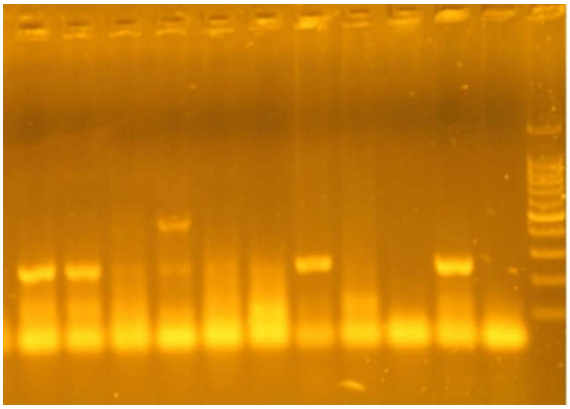
Several polymorphisms of the *eNOS* gene have been identified, and their association with various diseases has been investigated, including coronary artery disease, myocardial infarction, coronary spasm, hypertension, end-stage renal disease (ESRD), and DN (Haplotypes, 2008; Zintzaras *et al*., 2006).T-786C 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 (Asakimori *et al*., 2009). Thrombotic events, both in the arterial and venous systems, are a common complication in patients with polycythemia vera (PV) and essential thrombocythemia (ET). About 30%-50% of patients with PV and ET suffer from these complications, and vascular mortality accounts for 35%-45% of all deaths (Özgür Mehtap *et al*., 2012). Polycythemia vera (PV) is a clonal myeloproliferative disorder that leads to triliniage hyperplasia in the bone marrow with a principle clinical manifestation of erythrocytosis and plethora, The fact that these measurements are ratios of the number of red blood cells to volume of plasma must be kept in mind. An increase in hemoglobin can result from a reduction in plasma volume without a true increase in red blood cell mass (Sana Mohammed Altayeb *et al*., 2015). Phlebotomy is the mainstay of treatment for this disease, and hydroxyurea, interferon-

alpha, anagrelide drug therapies, 32P radiation therapy have been commonly used. The mortality is genes result in lack of enzymatic activity (Sana Mohammed Altayeb, 2015 Turesky, 2011).

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'



MATERIALS AND METHODS

This was case control study conducted in Khartoum state during the period from November 2016 to February 2017. Sudanese patients with Polycythemia Vera. 90 samples were enrolled in this study 40 was Polycythemia Vera 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 Thermocyler Biometra, Germany).

Three microliter (µl) of DNA was amplified in a total volume of 20 µL PCR mixture containing 1µl of each primer (Table 1) and 13µl 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 µl of PCR product was electrophoresed on 3% agarose gel containing ethidium bromide. one µl of 100 bp DNA ladder was applied with each batch of patients' samples. one µL of 100 bp DNA ladder (T-advance Thermocyler 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) The gel image with different size of bands; lane M: DNA ladder with 100 bp genotype (MI) with PCR product 250 and 176 bp, lane 1, 2

RESULTS

This was case control study, included 40 patients with Polycythemia vera,34(85%) were male, 15% (6) were female .In addition, 50 subjects as control group,60% (24) were female while 40%(16) were female. The range age of patients Polycythemia Vera (23-74) years. The molecular analysis showed that, the most frequency genotype in patients TT was 26 (65%) followed by genotype TC 10 (25%) and CC 4 (10%) While the control group showed that, 50 (100%) were TT. The frequency of *T/T*, *T/C* and *C/C* genotypes in patients were 65%, 25% and 10%, respectively where as in controls the distribution was 100% TT. The allelic frequencies were found to be 77.5% of T and 22.5 % of C in patient group, where as 100% TT in controls.

Table 1. Distribution of genotype, allelic frequencies and odds risk estimates in patients compared to control subjects

NOC789T Genotype	Patients % (n)	Control % (n)
TT	65% (26)	100% (50)
TC	25% (10)	0
CC	10% (4)	0
Alleles		
T	77.5%	100%
C	22.5%	0

DISCUSSION

The (T > C) rs2070744 polymorphism was found to reduce the transcription rate resulting in decreased serum NO levels which can inhibit apoptosis or stimulate tumour proliferation, angiogenesis and metastasis. The C allele of T > C polymorphism may influence the expression and activity of the NOS enzyme and shown to increase the risk for the development of various diseases (Koh *et al*., 1999).This study was conducted to establish the polymorphism at T-786C among Polycythemia vera. The present study reported that the most frequent genotype among PV patients was TT followed by TC and CC with no significant difference between patients and control group.

Conclusion

No significant difference was found between the distribution of the eNOS786T > C genotype in PV patients and the general population in Sudan.

REFERENCES

Asakimori, Y., Yorioka, N., Taniguchi, Y. *et al*. 2002. T (786) C polymorphism of the endothelial nitric oxide synthase gene influences the progression of renal disease. *Nephron* 2002; 91:747–751.

Azarpira, N., Geramizadeh, B., Nikeghbalian, S., Bahador, A. 2011. Endothelial Nitric Oxide Synthase Gene T-786C Polymorphism in Renal Transplant Recipients, *International Journal Organ Transplantation Medicine* 2(2): 87–92.

Haplotypes and diabetic nephropathy among Asian Indians. *Mol Cell Biochem* 2008;314:9 –17.

International Journal of Information Research and Review, 2015;2(07),pp. 880-882.

Koh, E., Noh, S.H., Lee, Y.D., Lee, H.Y., Han, J.W., Lee, H.W., Hong, S. 1999. Differential expression of nitric oxide synthase in human stomach cancer. Cancer Lett. 146:173–180.

Nada, H. Eltayeb, Mohamed A. M. Salih, Abdel Rahim M. Muddathir, Endothelial Nitric Oxide Synthase Gene Polymorphism (T-786 C) in Sudanese Patients with Sickle Cell Anaemia**,** American Journal of Medicine and Medical Sciences, 2015; 5(5): 231-234

Özgür Mehtap, Elif Birtaş Ateşoğlu, Pınar Tarkun, Emel Gönüllü, The Association Between Gene Polymorphisms and Leukocytosis with Thrombotic Complications in Patients with Essential Thrombocythemia and Polycythemia Vera, Turkish Journal of Hematology, 2012;29(2), 162–169.

Rafikov, R., Fonseca, F.V., Kumar, S. *et al*. 2011. eNOS activation and NO func-tion: Structural motifs responsible for the posttranslational control of endothelial nitric oxide synthase activity." *Journal of Endocrinal,* 45 (12) 271–284.

Rossi, G.P., Taddei, S., Virdis, A., Cavallin, M., Ghiadoni, L., Favilla, S. *et al*. 2003. "The T-786C and Glu 298 Asp polymorphisms of the endothelial nitric oxide gene affect the forearm blood flow responses of Caucasian hypertensive patients." Jam CollCardiol 41(6): 938 – 945.

Sana Mohammed Altayeb, Mohamed El-Fatih and Ibrahim Khider Ibrahim, GSTT1 Polymorphism In Sudanese Patients With Polycythaemia VeraTuresky, R.J. and Marchand, L.L 2011. Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: Lessons learned from aromatic amines. Chem Res Toxicol, 24: 1169-1214.

Zintzaras, E., Kitsios, G., Stefanidis, I. 2006. Endothelial NO synthase gene polymorphisms and hypertension: a meta-analysis. *Hypertension,* 48:700– 710.

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