



ASSOCIATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE T-786C PROMOTER POLYMORPHISM WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN KHARTOUM 2016

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Article Received on 01/03/2017

Article Revised on 21/03/2017

Article Accepted on 11/04/2017

ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) refers to a group of lymphoid disorders resulting from monoclonal proliferation and expansion of lymphoid blasts in the bone marrow, blood, and other organs. Nitric oxide (NO) is a short lived vasoactive substance produced from L - arginine by the enzyme nitric oxide synthase (NOS). It is constitutively released from endothelial cells and also from macrophages and platelets. It inhibits platelet activation, keeps blood vessels dilated, controls blood pressure, and has numerous other vasoprotective effects. A mutation of *eNOS* due to transition of a thymine to a cytosine (T > C) at -786 was found to reduce the rate of mRNA transcription by 50%, resulting in decreased serum nitric oxide levels which can inhibit apoptosis or stimulate tumor proliferation, angiogenesis and metastasis. **Purpose:** This study aims to examine the association of endothelial nitric oxide synthase gene (*NOS3*) T-786C promoter polymorphism with acute lymphoblastic leukemia in Khartoum. **Materials and methods:** A total of 40 patients with acute lymphoblastic leukemia and 40 control subjects were enrolled in this study. Blood samples were collected in EDTA containers. Genomic DNA was extracted by using salting out method. *eNOS* T/C polymorphism was determined using polymerase chain reaction (PCR) method. **Results:** There is no association between acute lymphoblastic leukemia and *eNOS* (100% of patients and control subjects show T/T genotype). **Conclusion:** According to our results, no association was found between *eNOS* T/C polymorphism and the development of ALL among Sudanese patients.

KEYWORDS: endothelial nitric oxide synthase, *NOS3* T/C gene polymorphism, ALL.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a type of cancer of lymphoid progenitor cells. The two main subtypes of ALL, as categorized by immunophenotype, are B-cell ALL and T-cell ALL.^[1] The most common type of cancer in children.^[2]

Endothelial nitric oxide has been known to be a major contributor in vascular regulation, it is an important relaxing factor in the human body and it is involved in a variety of different important physiological functions.^[3] Nitric oxide causes vascular relaxation, it suppresses platelet and leukocyte adhesion to the vascular endothelium.^[4] The *eNOS* gene is located on chromosome 7q35-36 which consists of 26 exons with a total size of 21 kb and it encodes for intracellular NO production.^[5]

MATERIALS AND METHODS

This is a hospital base case control study that conducted in Khartoum state during the period from august 2016 to December 2016. Sudanese patients with acute lymphocytic leukemia attending Radiation and Isotopes Center of Khartoum (RICK) were included. We excluded patients with disease related or may related for *NOS3* polymorphisms for example gastric cancer, DM, hypertension, other leukemia in which transformation or biphenotypic are associated and cardiovascular disease were recruited for this study.

Blood samples (3ml) were collected from patients and controls in ethylene diamine tetra acetic acid (EDTA) containers and genomic DNA was extracted by salting out method.^[6] All patients and controls sampled in the study signed an informed consent approved by the Human Ethics Committee of Khartoum.

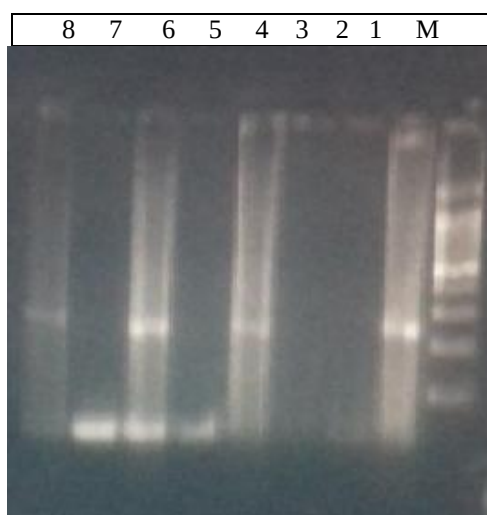
The genotyping was performed by using Allele-specific polymerase chain reaction following the protocol of Krishnaveni D *et al* using^[7] conventional PCR machine (PCR- TECHNE, TC412, UK). 4 microliter (µl) of DNA was amplified in a total volume of 20 µL PCR mixture containing 1µl of 2684C, 2684T primers, 1.5 µl of T0 and C0 primers (Table 1) and 11µl sterile distilled water. The amplification process consisted of initial denaturation at 96°C/5min; 35 cycles each one consists of 94°C for 30seconds, 60°C for 30 seconds and 72°C for 20 seconds, final extension at 72°C for 10 minutes.

5 µl of PCR product was electrophoresed on 2% agarose gel containing 0.3 ethidium bromide. 3 µL of 100 bp DNA ladder (Promega, USA) was applied with each batch of patients' samples. A PCR product of 250 bp fragment was consistent with T allele, while a product of 179 bp fragment was consistent with C allele.

Data was collected by structured interview questionnaire and from patients' medical files and analyzed by statistical package for social sciences (SPSS), version 21.

Table (1) Oligonucleotides Sequences for NO:

Primers	Primer direction	Sequence
Mutant	Forward primer C0	5' TTT CTC CAG CCC CTC AGA TG 3'
	Reverse primer 2684C	5' GGC AGA GGC AGG GTC AGA CG 3'
Wilde type	Forward primer T0	5' AGG CCC AGC AAG GAT GTA GT 3'
	Reverse primer 2684T	5' CAT CAA GCT CTT CCC TGT CT 3'



The gel image with size of bands; Lane M: DNA ladder with 100 bp, Lane1, 4, 6 and 8: NOS3 genotype (TT) with PCR products 250 bp.

RESULTS

This is a case-control study, included 40 ALL patients, 28 (70%) of them were males and 12 (30%) were females. In addition, 40 healthy subjects were included in the study as control group who were healthy and without medical history, 24 (60%) were males and 16 (40%) were females.

The molecular analysis showed that all genotypes of ALL patients and control subjects were T/T, while the genotypes T/C and C/C were totally absent (Table 2).

According to our result, no association between ALL and eNOS gene mutation was found.

Table (2) eNOS T/C polymorphism in ALL patients:

Group	NOS3 Polymorphism		
	T/T	T/C	C/C
Patient	40 (100%)	0 (0%)	0 (0%)
Control	40 (100%)	0 (0%)	0 (0%)

DISCUSSION

Nitric oxide synthase (NOS3) polymorphism was found to reduce the transcription rate resulting in decreased serum NO levels which can inhibit apoptosis or stimulate tumor proliferation, angiogenesis and metastasis. We found that no association between NOS3 gene polymorphism mutation and the development of ALL among Sudanese patients, only one genotype T/T was detected.

We did not find any research similar to our study except Demacq C, *et al*.^[8] who disagrees with our findings and reported that the endothelial nitric oxide synthase

(NOS3) polymorphism is associated with high relapse risk in childhood acute lymphoblastic leukemia.

In comparison with other diseases, this study agrees with the findings of Paradossi *et al*.^[9] who found no significant association of T > C promoter polymorphism with atherosclerosis in Italian population.

In contrary to our results, an association of C allele and C/C genotype with coronary artery disease (CAD) was demonstrated in the Iranian population by Khaki-Khatibi *et al*.^[10] In the study of Ghilardi *et al*.^[11] the -786T > C polymorphism was found to be associated with vascular invasion in breast cancer^[11] Another study by Jang *et*

al^[12] on South Korean population highlighted that TC+CC genotype of the -786T > C polymorphism was significantly associated with an increased risk of colorectal cancer.

The difference of the findings may relate to the variation in ethnic origin, the demographic factors and number of tested samples that reduced to 40 patients and 40 controls due to limitation in cost and time.

CONCLUSION

Based on our results no association was found between NOS3 T/C polymorphism and the development of ALL among Sudanese patients.

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