INTRODUCTION
Nitric oxide synthases (NOSs) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule. It helps modulate vascular tone, inhibit leukocytes adhesion to endothelium and also inhibits platelets adhesion to vascular endothelium. Also NO protects against platelets aggregation. All of these processes are important events during atherogenesis. Dysfunction of this mechanism may promote atherogenesis and increase risk of thrombosis leading to acute myocardial infarction. There are several forms of nitric oxide synthase such as neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS). The vascular nitric oxide (NO), mainly produced by eNOS, is a critical molecule in regulating the vascular system, including the inhibition of the platelet aggregation and adhesion and reduction of vascular smooth muscle cell proliferation. Furthermore, overproduction of NO can inhibit DNA repair and cause DNA damage, which plays an important role in the occurrence of MI. NO regulation may result from the functional eNOS genetic polymorphisms. The eNOS gene is mapped on human chromosome 7q35–36 and contains 26 exons and 25 introns. 786C–T substitution in the promoter region, which is strongly linked to 4b/a. The allele C of T-786C polymorphism is associated with re-infarction. While up to 90% of all MI patients are known to survive at the first MI, mortality associated with re-infarction is very high. MI results from thrombus formation in coronary blood vessels. Such thrombus may cause a stable coronary atherosclerotic lesion to transform into a ruptured plaque. Polymorphisms of the endothelial nitric oxide synthase (eNOS) gene are reportedly associated with myocardial infarction. 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, therefore this study was aimed to evaluate the association...
between Nitric Oxide Synthase gene T-786C polymorphism and myocardial infarction patients

MATERIALS AND METHODS

This was case control study conducted in Khartoum state during the period from November 2016 to February 2017. Sudanese patients with Myocardial infarction attending Almawada and Alshaab hospital were recruited for this study. 90 subjects were enrolled in this study. 40 was Myocardial infarction as case group, while 50 healthy as control group.

Blood samples (3ml) were collected from patients and control- in ethylene diamine tetra acetic acid (EDTA) containers and genomic DNA was extracted by Guanidine chloride methods.

Analysis of the eNOS786T > C promoter polymorphism were carried out by allele specific polymerase chain reaction method. The oligonucleotide primers used in the reaction were: C0: 5’ TTT CTC CAG CCC CTC AGA TG 3’; 2684C: 5’ GGC AGA GGC AGG GTC AGA CG 3’; 2684 T: 5’ CAT CAA GCT CTT CCC TGT CT 3’ and T0: 5’ AGG CCC AGC AAG GAT GTA GA 3’.

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.

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). Three microliter (μl) of DNA was amplified in a total volume of 20 μl PCR mixture containing 1μl from each primer (Table 1) and 13μl sterile distilled water. Samples were amplified for 30 cycles, consisting of denaturation at 94 C 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 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).

RESULTS

Table. 1: Show Primers and sequencing were used.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequencing</th>
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<tr>
<td>C0: 5’ TT CTC CAG CCC CTC AGA TG 3’</td>
<td>100% TT genotype</td>
</tr>
<tr>
<td>2684C: 5’ GGC AGA GGC AGG GTC AGA CG 3’</td>
<td>75.5% TT genotype</td>
</tr>
<tr>
<td>2684 T: 5’ CAT CAA GCT CTT CCC TGT CT 3’</td>
<td>75% TT genotype</td>
</tr>
<tr>
<td>T0: 5’ AGG CCC AGC AAG GAT GTA GA 3’</td>
<td>25% TT genotype</td>
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This was case control study, included 40 myocardial infarction patients, 21(52.5%) were male while 19(47.5%) were female. The patients age range from 47 to 75 years In addition, 50 subjects as control group, 24 (60%) were female while 16(40%) were female. The range age of myocardial infarction patients 47-75 Years. The molecular analysis showed that, the most frequent genotype in patients was TT 87.5% (35) followed by TC genotype 12.5% (5). While in the control group there was 100% TT genotype.

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. This study conducted to establish the polymorphism at T-786C among myocardial infarction. The study reported the most frequency genotype TT followed by TC.

Pervious study by Aggeliki-Maria Zigraa and Loukianos S on eNOS gene variants and the risk of premature myocardial infarction reported that the T-786C genetic polymorphisms seem to be associated with the development of MI.

CONCLUSION

The present study indicates the lack of association between eNOS and myocardial infarction.

REFERENCES

4. Qi Wang, Shao-Bo Zhou, Li-Jie Wang, Ming-Ming Lei, Yong Wang, Chi Miao, Yuan-Zhe Jin, Seven


