ABSTRACT
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. To evaluate the association between Nitric Oxide Synthase Gene T-786C polymorphism and pregnant woman in Sudan. A total of 40 patients pregnant woman and 50 control subjects were enrolled this study. DAN extraction samples were extracted from EDTA Blood using guanidine chloride method. Analysis of the eNOS786T > C polymorphism were carried out by allele specific polymerase chain reaction method (PCR). The present study reported that, molecular analysis showed that, the most frequent genotype in patients was TT 95% (38) followed by TC genotype 5% (2). While in the control group there was 100% TT genotype. The present study indicates the lack of association between eNOS and pregnant woman which needs to be investigated in a study with larger samples.

KEYWORDS: Polymorphism, Investigated, Polymorphism and Pregnant.

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] Nitric oxide (NO) contributes to maternal systemic vasodilation during pregnancy, regulates uterine and fetoplacental blood flow, and is involved in uterine quiescence prior to parturition. Also, whether a deficiency of NO contributes to the hypertensive disorder of pregnancy, preeclampsia, will be considered. The biosynthesis of NO increases in gravid rats and sheep, but the status in normal human pregnancy and preeclampsia is controversial. NO contributes to maternal systemic vasodilation and reduced vascular reactivity during normal pregnancy, however, whether these mediate uterine vasodilation during pregnancy remains to be established. NOS is expressed in the human placental syncytiotrophoblast and in the fetoplacental and umbilical vascular endothelium where basal production of NO contributes to low fetoplacental vascular resistance. Controversy exists over the status of placental NOS in preeclampsia, although an abnormality of umbilical vascular activity is likely. Finally, the uterus has NOS activity, which decreases at the end of gestation, and exogenous NO relaxes the myometrium, but whether endogenous NO contributes to uterine quiescence during pregnancy has yet to be confirmed.
MATERIALS AND METHODS
This was case control study conducted in Khartoum state during the period from January 2017 to October 2017. Sudanese patients were pregnant woman. 90 samples were enrolled in this study. 40 was pregnant woman as case group. And 50 the controls faced nonpregnant and without complications and they were free of hypertension history. 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. DNA extraction samples was extracted from EDTA whole Blood using guanidine chloride method. 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 1 μl of 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 100bp DNA ladder was applied with each batch of patients' samples. One μl of 100pb DNA ladder (T-advance Thermocyler Biometra, Germany) was applied with each batch of patients’ samples. A PCR product of 176,250bp 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 bands; lane M: DNA ladder with 100 bp genotype (MI) with PCR product 250.

RESULTS
A total of 40 cases was pregnant woman and 50 controls were analyzed. The mean age was (19-40) for cases and controls. All subjects were females 100%. The molecular analysis showed that, the most frequency genotype in patients TT was 38 (95%) followed by genotype TC 1 (5%) and CC 0 (0%) While the control group showed that, 50(100%) were TT.

Table 1. Show Primers and sequencing were used.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequencing</th>
</tr>
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<tbody>
<tr>
<td>CO</td>
<td>5’T TT TCT CAG CCC CTC AGA TG 3’;</td>
</tr>
<tr>
<td>2684C</td>
<td>5’ GGC AGA AGC AGG GTC AGA CG 3’;</td>
</tr>
<tr>
<td>2684T</td>
<td>5’ CAT CAA GCT CTT CCC TGT CT 3’</td>
</tr>
<tr>
<td>TO</td>
<td>5’ AGG CCC AGC AAG GAT GTA GT 3’</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>NOC789T Genotype</th>
<th>Patients% (n)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>95%(38)</td>
<td>100%(50)</td>
</tr>
<tr>
<td>TC</td>
<td>5% (2)</td>
<td>-</td>
</tr>
<tr>
<td>CC</td>
<td>0% (0)</td>
<td>-</td>
</tr>
</tbody>
</table>

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 hypertensive pregnant Sudanese woman. The study reported the most frequency genotype TT followed by TC and no subject with CC genotype.

CONCLUSION
The present study indicates the lack of association between eNOS and pregnant woman which needs to be investigated in a study with larger samples.

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CONFLICT OF INTEREST: Nil.

REFERENCES
4. Rafikov R, Fonseca FV, Kumar S, et al. eNOS activation and NO function: Structural motifs responsible for the posttranslational control of


