Distribution of Endothelial Nitric Oxide Synthase Gene 27 bp-VNTR Polymorphism in Turabah Region, Saudi Arabia

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Abstract: Different population distribution of 27 bp-VNTR in intron4 of eNOS gene polymorphism and its association with predisposition of certain diseases may be of geographical and ethnical important. In this study we investigated distribution of 27 bp-VNTR in intron4 of eNOS gene polymorphism in Turabah population. Genotyping of eNOS VNTR was done by PCR in 225 unrelated individuals from population living in Turabah province. The allele/and genotype frequencies of the polymorphism were tested for Hardy-Weinberg equilibrium (WBE) and compared to two Arabic populations (Egyptian and Tunisians) using Chi Square Goodness-of-Fit test. The study revealed that; the alleles and genotypes distribution of eNOS VNTR 27-bp polymorphism showed a significant difference from these expected frequencies indicating that the population is not in Hardy-Weinberg equilibrium. comparing alleles and genotypes frequencies in study population with those of Tunisian and Egyptian populations had demonstrated significant differences of genotypes frequencies between study and both Tunisian and Egyptian populations.

Key words: population / ethno genetics / eNOS/polymorphism

1. Introduction

Endothelial Nitric Oxide Synthase (eNOS) is an enzyme expressed in several tissues particularly endothelial cells, granulocytes, platelets and cardiac myocytes [1]. In mammals, there are three isoforms of nitric oxide synthases neural nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) [2 3]. Endothelial nitric oxide synthase produces Nitric Oxide under Calcium ions influx stimulation [4]. Nitric Oxide (NO) is an organic compound that plays several physiological roles in different biological situations. NO inhibits platelet aggregation and leukocyte-endothelium adhesion [5-8].However, inadequate NO levels are linked to several disorders such as Atherosclerosis, hypertension, congestive heart failure, stroke, thrombosis, diabetes mellitus and hypercholesterolemia[9-12]. Furthermore, Massion et al have reported that NO plays a key role in cardiovascular physiology [9].

In human, endothelial nitric oxide synthase is encoded by a gene located on chromosome 7 q36 position that contains 26 exons and 25 introns with approximately 21kb length [13 14]. Different polymorphisms have been identified in eNOS gene some of them are reported to be associated with atherosclerosis progression and coronary artery disease particularly a variant located on exon 7 (G984 → T) which in turn substitutes Gln298 to Asp[15-18]. In addition, T786→C polymorphism in the 5’-flanking region of the eNOS gene affects gene expression and has been linked to coronary spasm [19]. In addition, there are a high numbers of CA repeats have been identified in intron 13 of eNOS gene which are also been reported to be associated with coronary artery disease (CAD) [20]. Furthermore, two alleles (4a and 4b) have been identified in intron 4 of the eNOS gene[21]. The 4a allele is consist of four tandem 27-bp repeats while 4b is larger a bit which has five repeats[21 22]. Several studies reported a significant link of the 4b/a polymorphism with some chronic diseases such as essential hypertension, coronary artery disease, renal failure and diabetes mellitus [22-25]. Functionally, there is a significant association between the variable number of tandem repeat (VNTR) polymorphism and plasma levels of NO [26]. Populations genotypic diversity studies of eNOS gene polymorphisms is important since several authors have implicated it roles in pathogenesis of many diseases. Different population distribution of 27 bp-VNTR of eNOS gene polymorphism and its association with predisposition of certain diseases may be of geographical and ethnical important. In this study we investigated distribution of 27 bp-VNTR in intron4 of eNOS gene polymorphism in Turabah population in comparison to two Arabic populations (Egyptian and Tunisians).
2. Methods and Materials

Study subjects

Blood samples were collected from 225 unrelated individuals from population living in Turabah province. The mean age of the study subjects was 44.87 ± 10.46 years; the minimum age was 30 years and the maximum was 60 years. From the study population, 135 (60%) were men and 90 (40%) were women. The study was approved by the Ministry of Health and Research Committee, Taif University.

Screening of 27-bp repeat polymorphism in intron 4 of the e NOS gene among study population

Genomic DNA was extracted from peripheral blood using standard procedures [27]. Genotyping of the 27-bp repeat polymorphism in intron 4 VNTR of the e NOS gene in study population was done by PCR amplification around the polymorphic region with the primers 5'- GCC CTATGG TAG TGC CTT -3’ (forward) and 5'-CTC TTAGTG CTG TGG TCA C -3’ (reverse). A 15 μl volume was used for each PCR reaction. We added 5 pmoles μl of each primer, together with 4 μl genomic DNA, 7.5 μl 2X Taq complete master mix, and 2 μl PCR grade H2O. Each reaction mixture was heated for 3 cycle at 94°C for 5 min for initialization, followed by 40 cycles of 94°C for 30 sec, 68°C for 30 sec and 72°C for 30 sec, and a final extension of 72°C for 3 min. The PCR products were separated by electrophoresis on a 3% agarose gel. The alleles 4b and 4a produced bands of 420 bp and 393 bp respectively; the 420 bp band indicated five repeats of the 27-bp consensus sequence. Lanes 1, 2, 3, 5, 6, 8 and 9 are a/b heterozygous; lanes 4, 7 and 10 are b/b homozygous. Lane 11: A 100-bp DNA marker.

Statistical analysis

The allele/and genotype frequencies 27-bp repeat polymorphism in intron 4 of the e NOS gene were tested for Hardy-Weinberg equilibrium (WBE) using the Chi Square Goodness-of-Fit test using POPGENE VERSION 1.31 for population genetic analysis. The observed homozygosity was 0.5312 and observed heterozygosity was 0.4688 while the expected ones were 0.6175 and 0.3825 respectively. There was a significant difference between observed and expected genotype distributions in the study population (Chi Square: 11.485; P value: 0.0007). This is indicating that the Study population is not in Hardy-Weinberg equilibrium (Table 1).

3. Results

Distribution of VNTR 27-bp polymorphism in intron 4 of NOS3 gene in study population is not in Hardy-Weinberg equilibrium

The allele/and genotype frequencies 27-bp repeat polymorphism in intron 4 of the NOS3 gene were determined by PCR amplification (Figure 1). The distribution of genotypes and allele frequency in study population are showing in (Figure 2). 114 (0.507) of study population were carrying 4b/4b genotypes, 106 (0.471) were heterozygote (4a/4b) genotypes while only 5 (0.022) were homozygote for 4a allele of the polymorphism, The allele frequency in study population (n= 225) was 0.743 for 4b allele and 0.257 for 0.257.

The distribution of genotypes of the polymorphism in the study population was tested for deviation from Hardy-Weinberg equilibrium by Chi Square Goodness-of-Fit Test using POPGENE VERSION 1.31 for population genetic analysis. The observed homozygosity was 0.5312 and observed heterozygosity was 0.4688 while the expected ones were 0.6175 and 0.3825 respectively. There was a significant difference between observed and expected genotype distributions in the study population (Chi Square: 11.485; P value: 0.0007). This is indicating that the Study population is not in Hardy-Weinberg equilibrium (Table 1).

Figure 1: Genotyping of VNTR 27 polymorphism in intron 4 of NOS3 gene.

The 420-bp band indicates five repeats and the 393-bp band indicates four repeats of the 27-bp (b allele), and a 393 bp band indicated four repeats (a allele) (Fig. 1).

Genotype frequencies of VNTR 27-bp polymorphism in intron 4 of NOS3 gene in study population in comparison to two Arabic populations

The Distribution of genotypes and allele frequencies 0f VNTR 27-bp polymorphism in intron 4 of NOS3 gene in study population were compared to their distributions in previously published data from
Tunisian [28] and Egyptian [29] populations. 274(0.694) of Tunisian population [28] were 4b/4b genotypes, 112(0.284 were heterozygote (4a/4b) genotypes and 9(0.023) were homozygote for 4a allele of the polymorphism, while the genotypes distribution in Egyptian [29] were 70 (0.636), 32 (0.291) and 8 (0.073) respectively (Table 2). The comparison genotypes frequencies of study population with those of Tunisian [28] and Egyptian [29] populations had demonstrated that; there was significant difference of genotypes frequencies between study and both Tunisian (Chi Square: 27.858; P value: 0.0000) and Egyptian (Chi Square: 12.943; P value: 0.002) populations as are showing in (Table 2).

Table 1. The frequency of NOS3 27-bp VNTR polymorphism in the study population compared to different ethnic populations in previous studies.

<table>
<thead>
<tr>
<th>population</th>
<th>VNTR 27-bp genotypes</th>
<th>Allele frequency</th>
<th>P HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4b/b</td>
<td>4a/b</td>
<td>4a/a</td>
</tr>
<tr>
<td>Present study</td>
<td>114 (0.507)</td>
<td>106 (0.471)</td>
<td>5 (0.022)</td>
</tr>
<tr>
<td>Saudi Arabia[36]</td>
<td>251 (0.615)</td>
<td>146 (0.358)</td>
<td>11 (0.027)</td>
</tr>
<tr>
<td>Tunisian[28]</td>
<td>274 (0.694)</td>
<td>112 (0.284)</td>
<td>9 (0.023)</td>
</tr>
<tr>
<td>Egyptian[29]</td>
<td>70 (0.636)</td>
<td>32 (0.291)</td>
<td>8 (0.073)</td>
</tr>
<tr>
<td>Turkish[37]</td>
<td>97 (0.729)</td>
<td>35 (0.263)</td>
<td>1 (0.080)</td>
</tr>
<tr>
<td>Iranian[38]</td>
<td>128 (0.810)</td>
<td>29 (0.184)</td>
<td>1 (0.006)</td>
</tr>
</tbody>
</table>

4- Discussion

Current study is to investigate the distribution of distribution of VNTR 27-bp polymorphism in intron 4 of NOS3 gene in normal (healthy) population in Turabah, Kingdom of Saudi Arabia. This study might serves as a reference for several studies on the association of that distribution with some chronic diseases that spread among population in Turabah. In addition, the comparison data of the distribution of VNTR 27-bp polymorphism in intron 4 of NOS3 gene between populations in Turabah with other societies might be a useful technical control for any related clinical investigations. Recently Droma et al have reported a significant association of eNOS gene polymorphisms with high-altitude pulmonary edema [30]. The study revealed that the alleles and genotypes distribution of VNTR 27-bp polymorphism in intron 4 of NOS3 gene in study population showed a significant difference from these expected frequencies and the population is not in Hardy-Weinberg equilibrium.

Deviation of study population from HWE could be attributed to many factors. Firstly, may be due to environmental factors and natural selection process that may affects frequencies of genes variants those interact with environmental factors and common diseases in different population. NO expressed by vascular endothelium is responsible for the vasodilator tone that is essential for the regulation of blood pressure [31]. The most important environmental selective factors are diseases with high mortality. Several previous studies on eNOS and diseases risk factors have reported strong association of the eNOS gene polymorphisms and many diseases predisposition. Variation in NO production and action have been reported by many authors as an important risk factor for cardiovascular disease. Homozygosity of the Minor allele of the polymorphism (4a4a) was reported to be associated with Coronary artery disease and essential hypertension [23 32]. The overall prevalence of CAD in Saudi Arabia is 5.5% and 26% of total hospital.
death were due CAD [33]. Never the less, several studies have implicated allele 4a of the polymorphism to be associated with recurrent spontaneous abortion in different populations, unexplained women infertility and Idiopathic asthenozoospermia and male infertility and erectile dysfunction [34]. All these select the 4a allele of the polymorphism and may affect its alleles and genotypes frequencies. This could explain the 4a allele decreased homozygosity and increased heterozygosity in the study population.

Analyses of the alleles and genotypes distribution in comparison with those published from those in Tunisian and Egyptian populations revealed that the distribution of alleles and genotypes frequencies of the polymorphism in study population are significantly different from those in Tunisian and Egyptian population. This observation is consistent that reported previously by Bolaji N. Thomas et al. Where they found extensive and clearly significant differences in the interethnic distribution and haplo-type frequency of these eNOS variants[35].

Saudi population is quite different from Tunisian and Egyptian populations. Saudi population still reserved several Arabic social habits and behaviors. Almost all Saudi subpopulations and tribes are living as small isolated and closed populations with firm restrictions in random genetic mating with high consanguineous and inbreeding. Thus, mating between individuals in such subpopulations are closed related (based on shared genes) is nearly the same as between brothers and sisters resulting in Equal fertility for all genotype groups even, with no selection or gene flow or migration of individuals in or out and this may results is decreased homozygosity and increased heterozygosity in these populations and may explains the high, negative assortative mating and Deviation of study population from HWE.

5- Conclusion

The alleles and genotypes distribution of VNTR 27-bp polymorphism in intron 4 of NOS3 gene in Turabah population is not in Hardy-Weinberg equilibrium and significantly different from Tunisian and Egyptian populations in allele and genotypes distribution.

6-References


