COMPARATIVE STUDY OF I/D POLYMORPHISM OF ACE GENE IN DIABETES TYPE-2 PATIENTS AND CONTROL GROUP IN UNRELATED GUJARATI POPULATION

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Many of the previous studies indicated that the I/D polymorphism of ACE gene is associated with diabetes type-2. To validate the association of I/D polymorphism in ACE gene, a study was designed in non-diabetic (normal) and diabetic type-2 patients of unrelated Gujarati population. The random blood samples from 36 normal and 36 diabetic type -2 patients of above 45 years were collected for the studies. DNA was extracted from blood samples for PCR by using ACE specific primers. The gene and genotype frequencies were estimated for different alleles observed in diabetic as well as in normal healthy persons. In present study, all three genotypes that is, I/I (477bp), I/D (477/190bp), D/D (190bp) were observed in samples from normal and diabetic patients. Among all genotypes ID (58.3%) has maximum genotypic frequency in diabetic than Non diabetic individuals (44.4%), frequency of II (27.7%) is more in Non diabetic individuals than Diabetic individuals (19.4%) and genotypic frequency of DD (27.7%) is more in Non diabetic than Diabetic individuals (22.22%). The results were not in agreement with so many previous studies. However, recent findings of other studies conducted in different ethnic groups are similar to our findings which do not support that I/D polymorphism are associated with type-2 diabetes.

Key words: I/D polymorphism, ACE gene, DNA, PCR, Genotype and gene frequencies

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INTRODUCTION

Diabetes mellitus or diabetes is a chronic condition that affects body's ability to use the energy found in food. Majority of diabetes could be defined as type-1 or insulin dependent or juvenile-onset diabetes, type-2 or non-insulin dependent diabetes, and gestational diabetes. Type-1 diabetes is an autoimmune condition in which body immune cells due to genetic defects could not recognize and attack insulin-producing β-cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. Type-1 diabetes can occur at any age; however, it usually develops in adolescence. The first signs and symptoms of the disorder are caused by high blood sugar and may include frequent urination (polyuria), excessive thirst (polydipsia), fatigue, blurred vision, tingling or loss of feeling in the hands and feet, and weight loss. These symptoms may recur during the course of the disorder if blood sugar is not well controlled by insulin replacement therapy. Type 2 diabetes make insulin but either their pancreas do not make enough insulin or the body cannot use the insulin well enough. This is called insulin resistance or metabolic syndrome. Diseases or conditions associated with insulin resistance include: obesity, high blood pressure, abnormal cholesterol levels, heart disease, polycystic ovary syndrome and above 45 years of age. People with type-2 diabetes have high levels of insulin in the blood as a marker of the disease rather than a cause. The development of type 2 diabetes is caused by a combination of lifestyle (RIPSIN et al., 2009; ABDULLAH et al., 2010) and genetic factors (HARDER et al., 2011). Most cases of diabetes type-2 involve many genes in β-cell functions. There are a number of rare cases of diabetes that arise due to an abnormality in a single gene, monogenic condition of diabetes.

ACE gene synthesizes Angiotensin I Converting Enzyme which able to cleave proteins. The enzyme indirectly increases blood pressure by causing blood vessels to constrict. It does that by converting angiotensin I to angiotensin II, which constricts the vessels. Angiotensin I and angiotensin II are part of the renin-angiotensin system (RAS), which controls blood pressure by regulating the volume of fluids in the body. ACE is secreted in the lungs and kidneys by cells in the endothelium (inner layer) of blood vessels. Precisely, ACE has two primary functions; it catalyses the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor in a substrate concentration-dependent manner (ZHANG et al., 2000) and degrades bradykinin, a potent vasodilator, and other vasoactive peptides (IMIG, 2004). These two actions make ACE inhibition a goal in the treatment of conditions such as high blood pressure, heart failure, diabetic nephropathy, and type 2 diabetes mellitus. Inhibition of ACE by inhibitors results in the decreased formation of angiotensin II and decreased metabolism of bradykinin, leading to systematic dilation of the arteries and veins which in turn reduces blood pressure. In addition, inhibiting angiotensin II formation diminishes angiotensin II-mediated aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extracellular volume (WANG et al., 2008). A certain variation in the ACE gene has been associated with increased risk of several conditions, including stroke and complications of diabetes. ACE gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. This genetic variation, called the ACE I/D polymorphism, involves a region of DNA that spans 287 nucleotides. One variant of the ACE gene includes this region; it is called the insertion, or I, allele. Another variant is missing this region of DNA and is called the deletion, or D, allele (WUYTS et al., 1997; ZHANG et al., 2012). Therefore, each individual can be homozygous for I alleles (II) or D alleles (DD), and heterozygous (ID). The individuals with DD pattern are associated with higher levels of angiotensin-converting enzyme than the II pattern. The ID pattern is associated with intermediate levels. Hence, people with the DD pattern of alleles have an increased risk of stroke. Stroke can be
caused by a lack of blood flow to the brain or bleeding in the brain or intracerebral hemorrhage (HUANG et al., 2014). However, many genetic and environmental risk factors likely contribute to this complex condition. In people with type-1 or type-2 diabetes, the DD pattern of alleles is associated with an increased risk of developing a kidney disorder called diabetic nephropathy (GUBLER and ANTIGNAC, 2010). Damage to the kidneys caused by this condition worsens over time and can lead to kidney failure.

MATERIALS AND METHODS

A set of 72 Blood samples comprising 36 diabetic and 36 normal were collected from unrelated normal and diabetic individuals above 45 years of age from the Gujarati population with prior consent, in vacutainer (EDTA, K₃) blood collecting tubes. Approximately, 2ml blood was taken which were transported to the laboratory in cool condition. DNA was extracted from blood samples with little modifications in previous protocol (JOHN et al., 1991). The quality and quantity of DNA were determined using agarose gel electrophoresis and Nanodrop ND-1000 Spectrophotometer. Nanodrop and the samples were diluted to 50 ng/µl by autoclaved MilliQ water for final concentration and 3 µl of DNA was used as template for PCR reaction. For detection of I/D polymorphism in an ACE gene coding for Angiotensin I Converting Enzyme, the 190bp or and 477bp because of insertion of 287bp, DNA fragment was amplified by PCR, which was set by adding forward and reverse primers (CTGGAGACCACCTCCCATCCTTTCT) (GATGTGGCCATCACATTCGTCAGAT). The PCR mix containing 3 µl (30ng/µl) genomic DNA, PCR Master mix (Cat no. K0171, MBI Fermentas) containing 0.05U/µl Taq DNA polymerase (recombinant) in reaction buffer, MgCl₂ (4mM) and dNTPs (0.4 mM of each), and finally added with sterilized distilled water to make a final volume of 25 µl. The PCR reaction included the following steps: Predenaturation for 5 minutes at 94°C followed by 30 cycles of 1 minute at 94°C, 1.45 minutes at 60°C, and one minute at 72°C and final extension for 10 minutes at 72°C for utilization of extra dNTPs in mixture. The amplified product 190bp and 477bp were visualized on 1.5% agarose gel. Genotypic and allelic frequencies of I/D ACE gene polymorphism in normal as well as diabetic patients in Gujarati population were determined manually.

RESULTS AND DISCUSSION

DNA was successfully extracted from all samples. The quality and quantity of DNA was estimated on agarose gel electrophoresis and Nanodrop spectrophotometric analysis. Out of 96 samples, 72 DNA templates were successfully amplified and were electrophoresed on 2.0% agarose along with the 100bp DNA molecular weight marker and were visualized using transilluminator. The size of the PCR amplicons was analyzed by comparing them with that of the 100bp DNA molecular weight marker (Fig 1). In present study, all three genotypes that is, II (477bp), ID (477/190bp), DD (190bp) were observed in samples (Table-1). Among all genotypes ID (58.3%) has maximum genotypic frequency in diabetic than Non diabetic individuals (44.4%), frequency of II (27.7%) is more in Non diabetic individuals than Diabetic individuals. (19.4) and genotypic frequency of DD (27.7%) is more in Non diabetic than Diabetic individuals (22.2%).

The role of an ACE gene insertion/deletion (I/D) polymorphism in type 2 diabetes in northern China was studied (FENG et al., 2002). They observed genotype frequencies for II, ID, and DD; 39.8, 39.8, and 20.3%, respectively, in the diabetic group and 44.8, 44.8, and 10.4% in the control group. The DD frequency was significantly higher in the diabetic group than in the control group, suggesting that the DD genotype is associated with an increased susceptibility to
type-2 diabetes in their population. Similar study showed that the D allele were risk factors for type 2 diabetes even when adjusting for age, sex, hypertension and serum total cholesterol levels in Japanese population (DAIMON et al., 2003). The same observations were also observed in Caucasian group (STEPHENS et al., 2005). On the contrary in our study, the frequency of DD allele in more in control group (0.277) then diabetic group (0.222), supporting no association of I/D polymorphism and type-2 diabetes. However, recent findings are similar to our findings which do not support that I/D polymorphism are associated with type-2 diabetes. For example, a large follow up of 10.2 years in 24,309 Caucasian women free from diabetes at baseline failed to show any association of ACE genotype with diabetes (CONEN et al., 2007). This result was replicated in many other studies in different ethnic groups, both in patients with and without nephropathy (ARFA et al., 2008; EROGLU et al., 2008; VAN-VALKENGOED et al., 2008). Hence, ACE gene polymorphism is not associated as an independent factor responsible for diabetes. Anyhow, further research can investigate other effective genetic factors and environmental factors to find out the possible role of ACE in the onset of diabetes.

Table-1: Genotypic and allelic frequency of ACE gene. No of individuals in parentheses

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypic Frequency</th>
<th>Allelic Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD</td>
<td>I</td>
</tr>
<tr>
<td>Diabetic</td>
<td>22.2% (n=8)</td>
<td>0.486</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>58.3% (n=21)</td>
<td>0.514</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.4% (n=7)</td>
<td></td>
</tr>
<tr>
<td>Non Diabetic</td>
<td>27.7% (n=10)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.4% (n=16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.7% (n=10)</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1: Lane # 5, 10, 13, 18 & 21 – Genotype II (499bp), lane # 12, 17 & 24 – Genotype DD (190bp), lane # 1, 2, 3, 6, 8, 9, 11,14, 15, 16, 20, 22 & 23 – Genotype ID (477/190 bp), lane # 4 is control (blank) and lane # 7 & 19 DNA ladder (100bp).
Fig 2: Genotypic Frequency of Diabetic and Non Diabetic Individuals

Fig 3: Allelic Frequency of Diabetic and Non Diabetic Individuals

REFERENCES


KOMPARATIVNA ISTRAŽIVANJA I/D POLIMORFIZMA U ACE GENU KOD PACIJENATA SA DIABETESOM TIP – 2 I KONTROLNOJ GRUPI NEZAVISNE GUJARATI POPULACIJE

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Izvod
U cilju utvrđivanja validnosti asocijacije I/D polimorfizma u ACE genu vršena su ispitivanja kod normalnih – bez dijabetesa i pacijenata sa dijabetesom tipa 2 u nesrodnoj Gujarati populaciji. Uzeti su slučajnim izborom uzorci od 36 zdravih i 36 pacijenata sa dijabetesom tipa 2, starosti oko 45 godina, korišćenjem ACE specifičnog prajmera. Učestalost gena i genotipa je utvrđena za različite allele u obe ispitivane grupe pacijenata. Utvrđena su sva tri genotipa (I/I (477bp), I/D (477/190bp), D/D (190bp) u uzorcima normalnih i pacijenata sa dijabetesom. Od svih genotipova ID (58.3%) je imao maksimalnu genotipsku frekvencu. Frekvencija genotipa DD (27 %) je veća kod individua bez dijabetesa, nego kod individua sa dijabetesom (22.22%). Dobijeni rezultati nisu u saglasnosti sa rezultatima mnogih ranijih studija. Međutim, skorašnji rezultati dobijeni u različitim etničkim grupama su slični našim rezultatima koji ne podržavaju da je I/D polimorfizam udružen sa tipom-2 dijabetesa.

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