Angiotensin Converting Enzyme I/D Polymorphism and Risk of Acute Myeloid Leukemia among Sudanese Population

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Abstract

Background: Local bone marrow Rennin-Angiotensin System (RAS) has been suggested to be involved in pathological neoplastic hematopoiesis and leukogenesis, and angiotensin has been suggested to act as an autocrine growth factor for acute myeloid leukaemia (AML) cells. Objective: This study aimed to investigate the association of angiotensin converting enzyme insertion/deletion (I/D) polymorphism with risk of AML.

Materials and methods: A total of 30 patients with AML and 40 healthy volunteers were enrolled in this study. Blood samples were collected from all subjects in ethylene diamene tetra acetic acid (EDTA). DNA was extracted from whole blood using salting out method, and analyzed for ACE I/D polymorphism using allele specific polymerase chain reaction.

Results: The results showed that, the genotype DD was the most frequent among both patients and control groups, followed by the genotype ID, whereas the genotype II was present in patients and completely absent in control group. There was a significant association between I allele of ACE and risk of AML (O.R: 3.5, 95%CI: 1.2-10.0, P.value: 0.017).

Conclusion: I allele of ACE is associated with increased risk of AML.

Keywords: Angiotesin Converting Enzyme, I/D polymorphism, Acute Myloid Leukaemia

Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults and accounts for approximately 80 percent of cases [1,2]. In the United States and Europe, the incidence has been stable at three to five cases per 100,000 population. In contrast, AML accounts for less than 10 percent of acute leukemias in children less than 10 years of age [3,4]. Major Renin Angiotensin system components (RAS) and angiotensin con-ver-tig enzyme (ACE) are present in human umbilical cord blood cells [5]. Angiotensin II could serve in the erythroid and myeloid differentiation of stem cells [6]. On the other hand, there are preliminary data that local bone marrow RAS may be involved in pathological neoplastic hematopoiesis and leukogenesis [7,8,9].
Angiotensin has been suggested to act as an autocrine growth factor for AML cells [10]. The ACE gene is 21kb long, consisting of 26 exons and 25 introns and located in chromosome 17p23[11]. It is characterized by an insertion/deletion polymorphism based on the presence (insertion I) or absence (Deletion D) of a 287 base pair Alu repeat sequence in intron 16, resulting in three genotypes: DD homozygote, II homozygote and ID heterozygote[12]. This study aimed to investigate the association of ACE I/D polymorphism with AML risk.

Materials and methods

Study design and subjects

This is a hospital-based case control study, in which a total of 30 Sudanese patients with AML (13 males and 17 females) admitted at radiation and isotopes center of Khartoum (RISK) during the period from February to October 2015, and 40 healthy controls were enrolled.

Sample collection and DNA extraction

After informed consent blood samples were collected from all participants in Ethylene Diamine Tetra Acetic Acid (EDTA) and genomic DNA was extracted by salting out method.

Polymerase chain reaction (PCR)

Analysis of the ACE I/D polymorphic genotypes was performed by Allele-Specific PCR (AS-PCR). Three μL of the genomic DNA was amplified in a 25 μL reaction mixture containing five μL master mix (Maxime PCR pre mix kit (i-taq), iNtRON, Korea) and one μL of each of the forward (5’CTGGAG-ACCACCTCCATCTTCT-3’), reverse primer (5’GATGTGGCCATCATCATCG-TCAGAT-3’) and internal primer (5’TGGG-ATTACAGCGTGATACG-3), and 14 μL sterile distilled water. The amplification process consisted of initial denaturation at 94°C for 3mmin, then 30 cycles each consist of "94°C for 1 minutes, 52°C for 1 minutes, and 72°C for 1minutes", followed by final extension at 72°C for 5 minutes. PCR product was electrophoresed on 2% agarose gel stained with ethidium bromide and analyzed under UV light. Three μL of 50 bp DNA ladder was applied with each batch of patients' samples. A PCR product of 190 bp fragment was consistent with D allele, while a product of 490 bp fragment was consistent with I allele.

Statistical analysis

Data of this study was collected from patients’ medical files, and analyzed using statistical package for social sciences (SPSS), version21. Frequency of different genotypes was calculated, and correlation of genotypes with study groups was tested by Chi-square test. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit X2 test to compare the observed genotypic frequencies in normal individuals to the expected genotypic frequencies calculated from the observed allelic frequencies. Regression was used to determine the association between the genotypes and AML risk.

Results

A total of 30 Sudanese patients with AML
and 40 healthy volunteers were enrolled in this study to investigate the association of ACE I/D polymorphism and risk of AML among Sudanese patients.

The results showed that, the genotype DD was the most frequent among both patients and control groups, followed by the genotype ID, whereas the genotype II was present in patients and completely absent in controls (Table 1).

**Table 1. Distribution ACE I/D polymorphic genotypes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients n = 30</th>
<th>Controls n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>16 (53.3%)</td>
<td>32 (80.0%)</td>
</tr>
<tr>
<td>ID</td>
<td>9 (30.0%)</td>
<td>8 (20.0%)</td>
</tr>
<tr>
<td>II</td>
<td>5 (16.7%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

The genotype II was found to be associated with increased AML risk (O.R: 7.8, 95% CI: 0.86 - 70.7, **P.value** = 0.035). In general, patients carrying the I allele were found had 3.5 folds increased risk of AML (OR: 3.5, 95%, C.I: 1.2 - 10.0, **P.value** = 0.017).

Frequency of D allele was 0.68 in patients and 0.90 in control group, whereas frequency of I allele was 0.32 in patients and 0.10 in control group. A significant deviation from Hardy Weinberg equilibrium was found in patients with AML (\(X^2 = 12.867, df = 2, P = 0.002\)).

No statistically significant correlation was found between each of ACE I/D genotypes and gender (Table 2).

**Table 2. Correlation between ACE I/D polymorphic genotypes and gender**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gender</th>
<th><strong>P.value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>DD</td>
<td>N=13</td>
<td>N=17</td>
</tr>
<tr>
<td>ID</td>
<td>7(53.8%)</td>
<td>9(52.9%)</td>
</tr>
<tr>
<td>II</td>
<td>5(38.5%)</td>
<td>4(23.5%)</td>
</tr>
</tbody>
</table>

The present study showed that I allele (II and ID) of ACE was associated with 3.5 folds increase risk of AML. This finding was also agree with that of Akalin et al who reported that, the existence of I allele increase the risk of haematological malignancies 3.2 folds [13].

**Discussion**

In this study we investigated the association of ACE I/D gene polymorphism with risk of AML.

The results showed that, the genotype DD was the most common among both patients and control groups, followed by the genotype ID, whereas the genotype II was present in patients and completely absent in controls. On the other hand we found that the genotype ID/II frequency was significantly higher in AML patients 46.7% than control subjects (20%). There was a significant association between I allele (ID or II) of ACE gene and AML. This in agreement with a study carried out by Akalin et al who conducted study in Turkey and reported that the genotype DD was the most frequent among control group compared with leukemic (CML, AML and ALL) patients and there was significantly higher ID/II genotype frequency in patients compared to control group [13].

The fact that angiotensin II genotype stimulates the proliferation of bone marrow hematopoietic progenitors and umbilical cord blood cells support our finding that, presence of I allele increase the disease risk. Studies investigated the association between...
ACE I/D gene polymorphism and non-haematological malignancies reported conflict results. A study by Angela et al (2005) revealed that the DD carriers showed a significantly increased risk of developing breast cancer when compared with the II carriers [14]. Sun et al (2011) showed that there was no significant difference in genotype distribution (DD, ID or II) between breast cancer patients and controls [15]. A study conducted by Hibi et al (2011) found that there is no significant association between the ACE I/D polymorphism and the risk of gastric cancer [16]. Yigit et al 2007 indicated that the DD genotype may have detrimental effect and the II genotype may have protective effect on prostate cancer [17]. Recent study by Cheng et al 2015 did not find any association between the ACE gene I/D polymorphism and lung cancer in either genotype or allele distribution [18]. These different findings may reflect variations in carcinogenesis process in different types of cancer particularly between solid tumours and haematological malignancies.

Frequency of D allele was 0.68 in patients 0.90 in control group, whereas frequency of I allele was 0.32 in patients and 0.10 in control group. A significant deviation from Hardy Weinberg equilibrium was found in patients with AML (X²=12.867, df= 2, P= 0.002).

No statistically significant correlation was found between each of ACE I/D genotypes and gender.

Conclusion

I allele (ID or II) of ACE gene is associated with 3.2 folds increased risk of AML.

References


