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Angiotensin-converting Enzyme Insertion/Deletion Polymorphism (rs4646994) and Susceptibility to Acute Lymphoblastic Leukemia: A Case–control Study

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Abstract

BACKGROUND: Angiotensin-converting enzyme (ACE) stimulates the proliferation of bone marrow hematopoietic progenitors and thought to be involved in pathological neoplastic hematopoiesis and leukemogenesis.

AIM: This study aimed to investigate the association between *ACE* gene I/D polymorphism and the risk of acute lymphoblastic leukemia (ALL).

MATERIALS AND METHODS: A total of 96 subjects were recruited for the study, 48 patients with ALL, and 48 apparently healthy volunteers as a control group. Genomic DNA was extracted from peripheral leukocytes and *ACE* I/D polymorphism was analysed using allele-specific polymerase chain reaction.

RESULTS: In both study groups, the *ACE* D/D polymorphic genotype was the most frequent (52.1% and 54.2%, respectively), followed by the ID genotype (47.9% and 45.8% respectively), while the II genotype was completely absent in both study groups. The distribution of the polymorphic genotypes among the study groups was not significantly different (p = 0. 0.398). The frequency of the D allele was 0.76 in the patients and 0.77 in the control group, while the frequency of I allele was 0.24 in the patients and 0.23 in the control group. No deviation from Hardy–Weinberg equilibrium was observed (χ^2 = 4.24, df = 1, p = 0.12).

CONCLUSION: ACE I/D polymorphism is not associated with susceptibility to ALL among the Sudanese population.

Introduction

Acute lymphoblastic leukemia (ALL) is a genetically heterogeneous lymphoid malignancy derived from B- and T-lymphoid bone marrow progenitors. It is a disease of children that occurs most frequently between the age of 1–4 years [1], [2].

The etiology of ALL is thought to be multi-factorial, including exogenous or endogenous exposures, genetic susceptibility, and chance. Genome-wide profiling of germline and leukemic cell DNA leads to the identification of novel structural genetic alterations and sequence mutations that play a role in leukemogenesis [3].

Diagnosis of ALL is based on the assessment of cell morphology, immunophenotyping by flow cytometry, and identification of cytogenetic-molecular abnormalities [4]. Conventional and molecular genetics methods allow the identification of chromosomal aberrations and the definition of prognostically relevant ALL subgroups with unique clinical features [5], [6].

ALL is mainly categorized into B- and T-lineage ALL, which shows differences in the prognosis and responses to therapy, that could not be discriminated completely by the current diagnostic tools, and need to be further determined by accurate description of genetic alterations [4], [6], [7].

The components of the Renin-Angiotensin System (RAS) and Angiotensin-Converting Enzyme (ACE) have been identified in human umbilical cord blood and bone marrow and angiotensin II could play a role in the regulation of hematopoiesis, erythropoiesis, myelopoiesis, thrombopoiesis, formation of monocytic, and lymphocytic lineages [8], [9], [10]. The ACE has been concerned in the pathogenesis of several cancers [11], [12], [13], [14], [15]. It is differentially expressed in several carcinomas and may affect tumor cell proliferation, migration, angiogenesis, and metastatic behaviors [16].

Inhibition of ACE activity has been reported to suppresses tumor growth and angiogenesis *in vitro* and *in vivo* of animal models; moreover, epidemiologic studies have also indicated that ACE

inhibitors might decrease the risk and mortality rate of cancers [11], [16].

The *ACE* gene located on the long arm of chromosome 17, spans 21 kb, and consists of 26 exons and 25 introns [17], [18], [19]. A polymorphism based on the insertion (I) or deletion (D) of a 287 bp Alu repeat sequence has been identified in intron 16 of *ACE* gene, resulting in three polymorphic genotypes (DD, ID, and II) [20]. A strong correlation between the genotypes and plasma activity of the ACE has been reported, the DD genotype is associated with a two-fold increase in the plasma ACE activity over that of the II genotype, with an intermediate level of the enzyme activity, is associated with the heterozygous ID genotype [20], [21].

There is some evidence that local bone marrow RAS is involved in pathological neoplastic hematopoiesis and leukemogenesis and many studies have reported an association between the *ACE* I/D polymorphism and susceptibility to different types of malignancies including some hematological malignancies [8], [9], [14], [22], [23], [24], [25], [26].

This study aimed to examine the association of *ACE* I/D gene polymorphism with susceptibility to ALL among the Sudanese population.

Materials and Methods

This was a hospital-based case–control study, conducted at Radiation and Isotopes Center, Wad Madani, Aljazeera State, and Flowcytometer centre, Khartoum, Sudan. A total of 96 Sudanese subjects were enrolled in the study, 48 patients diagnosed with ALL- using cytochemical stains and immunophenotyping- and 48 apparently healthy volunteers as a control group.

Blood sample collection and genomic DNA extraction

After informed consent, a blood sample (2.5 ml) was collected from each participant in an EDTA blood tube and genomic DNA was isolated using the non-enzymatic salting-out method: 900 μl of TKM 1 buffer (0.605 g of Tris-HCl [10 mM] pH 7.6, 0.372 g of KCl [10 mM], 1.016 g of $MgCl_2$ [10 mM], and 0.372 g of EDTA [2 mM] were dissolved in 500 ml of distilled water) and 50 μl of 1x Triton-X were added to 300 μl of whole blood in 1.5 ml sterile Eppendorf tube; the mixture was centrifuged at 8000 rpm for 3 min and the supernatant was discarded; this step was repeated three times until RBCs lysis was complete and a white pellet of WBCs was obtained. Then, 300 μl of TKM 2 buffer (0.121) g of Tris-HCl (10 mM) pH 7.6, 0.074 g of KCl (10 mM), 1.203 g of $MgCl_2$, 0.074 g EDTA (2 mM), and 0.467 g of NaCl (0.4 M) were dissolved in 100 ml of distilled water] and 40 μl of 10% Sodium dodecyl sulphate (SDS) were added, mixed thoroughly, and incubated at 37°C for 5 min. At the end of incubation, 100 μl of 6 M NaCl was added, vortexed, and centrifuged at 8000 rpm for 5 min to precipitate the proteins. For DNA precipitation, the supernatant was transferred into a new Eppendorf tube containing 300 μl of isopropanol and DNA was precipitated by inverting the Eppendorf tube slowly. Further, the Eppendorf tube was centrifuged at 8000 rpm for 10 min to pellet down the DNA, the supernatant was discarded, and 70% ethanol was added and mixed slowly to remove any excess salts. Finally, the tube was centrifuged at 8000 rpm for 5 min to pellet down the DNA, the supernatant was discarded, and the DNA air-dried. After thorough drying, 50 μl of TE buffer was added to dissolve the DNA [27].

Polymerase chain reaction (PCR)

Allele-specific PCR was used for the analysis of *ACE* I/D polymorphism. A reaction mixture of 20 μl was prepared for each sample, consisting of 4 μl ready-to-load master mix, 2 μl genomic DNA, 1 μl of each of the forward (5’-CTG GAG ACC ACTCCCATCCTT TCT- 3’), reverse (5’-GATGTGGCCATCACATTCGTCGTCAGAT-3’), and internal (5’-TGG G A T T ACAGG CGTGATACA G- 3’) primers, and 11 μl distilled water.

The amplification process was set as follows: Initial denaturation at 94°C for 3 min, then 35 cycles each consisting of (94°C for 30 s, 57°C for 30 s, and 72°C for 30 s) and final extension at 72°C for 5 min.

Agarose gel electrophoresis

PCR products were separated on 2% agarose gel stained with ethidium bromide and analyzed under a UV transilluminator. DNA ladder (100 bp) was applied with each batch of patients’ samples. PCR fragment of 490 bp was consistent with the I allele and that of 190 bp was consistent with the D allele.

Statistical analysis

Data were collected using a structured interview questionnaire and analyzed by the Statistical Package for the Social Sciences (SPSS), version 20. Qualitative data were represented as frequency and percentage and quantitative data as mean±SD. Means of quantitative variables were compared using the independent two-sample t-test. The association between qualitative variables was tested using Pearson’s Chi-square (χ^2) and Fisher’s exact tests. The frequency of alleles and Hardy–Weinberg equilibrium were calculated using an online calculator [28].

Ethical considerations

This study was extracted from the M.Sc complementary research of Al Romisa Ahmed Abdulaziz. It was approved by the scientific research committee, Sudan University of Science and Technology. The protocol and procedure applied in this study complied with the Helsinki Declaration. Informed consent was obtained from each participant before samples collection; for children, the consent was obtained from their guardians. Patients' data were kept confidentially and used only for this study.

Results

Demographic data

A total of 96 Sudanese subjects were enrolled in this study, 48 patients diagnosed with ALL and 48 age- and sex-matched healthy volunteers as a control (Table 1).

Table 1: Demographic data of the study participants

Characteristics	Patients	Control
Age		
Mean	14.0	17.0
SD	16.2	17.1
Gender		
Male	36 (75%)	30 (62%)
Female	12 (25%)	18 (37%)

Genotyping of ACE I/D polymorphism

The molecular analysis showed that the *ACE* D/D genotype was the most frequent in both study groups, followed by the I/D genotype, whereas the II genotype was completely absent in both study groups. The distribution of polymorphic genotypes showed no statistically significant difference (Table 2).

Table 2: Distribution of *ACE* I/D polymorphic genotypes among the study groups

Genotype	Patients	Control	p value*
D/D	25 (52.1%)	26 (54.2%)	0.398
I/D	23 (47.9%)	22 (45.8%)	

p value considered significant at < 0.05.

The results showed, no statistically significant difference in the distribution of *ACE* I/D polymorphic genotypes in ALL patients according to gender (Table 3).

Table 3: Association of *ACE* I/D polymorphic genotypes with gender

Genotype	Male	Female	p value*
D/D	20 (41.7%)	5 (10.4%)	0.404
I/D	16 (33.3%)	7 (14.6%)	

p value considered significant at < 0.05.

The comparison of the mean age in patients with DD and ID genotypes showed no statistically significant difference (mean ± SD: 12.7 ± 11.5 and 14.3 ± 20.4 for DD and ID genotypes, respectively, P value = 0.726).

The frequency of the D allele was 0.76 in the patients and 0.77 in the control group, while the frequency of I allele was 0.24 in the patients and 0.23 in the control group. No deviation from Hardy–Weinberg equilibrium was observed (χ^2 = 4.24, df = 1, p = 0.12).

Discussion

Targeting the actions of local RAS is proposed to represent a valuable therapeutic option for the management of cancer [29]. This study investigated the association of *ACE* I/D gene polymorphism with the susceptibility to ALL among Sudanese. The results showed that the *ACE* D/D genotype was the most frequent among both patients and control groups, followed by the genotype ID, whereas the genotype II was completely absent in both groups. The distribution of genotypes among the two study groups was not different significantly. A similar finding was reported by Rezaei and Ladmakhi (2020) who conducted a study on Iranian patients with lymphoblastic leukemia and found no significant association between *ACE* I/D polymorphism and ALL risk [30].

A study by Akalin *et al.* (2011) included different types of hematological malignancies revealed that the risk of hematological diseases increases 3.2 times in Turkish patients who carry the I allele [22]. However, this study included different types of hematological malignancies and ALL represent only 17% of cases, they did not estimate the risk of each type of hematological malignancies separately.

The results of the previous studies concerning the association of *ACE* I/D polymorphism and other types of leukemia among the Sudanese population were inconsistent with the result of the current study. Adam *et al.* (2019) reported that the *ACE* I/I genotype was the most frequent among chronic lymphocytic leukemia (CLL) patients followed by the genotypes I/D and D/D consequently, while in the control group, D/D was the most frequent genotype, followed by the I/D genotype, while the I/I genotype was absent, they reported a significant association between *ACE* I/D polymorphism and CLL among the Sudanese patients [25]. Furthermore, another study by Elmubarak *et al.* (2016) reported a significant association between the I allele of *ACE* and the risk of acute myeloblastic leukemia (AML) among the Sudanese population [24].

Furthermore, a study in Poland by Zmorzynski *et al.* (2019) confirmed the significant relationship between *ACE* (I/D) polymorphism and the risk of multiple myeloma (MM) development [26]. The inconsistency between our study and the three mentioned studies

may be due to the genetic hetergenity of hematological malignancies which results in different leukemogensis mechanisms in different types of leukemia; furthermore, in our study, we did not classify ALL into different subtypes, as both B-cell and T-cell ALL have distnict molecular basis and include several subtypes harboring distinct groups of somatic structural DNA rearrangements and sequence mutations [31].

Studies that investigated the association between *ACE* I/D gene polymorphism and non-hematological malignancies reported conflict results, while two studies revealed that the DD carriers had a significantly increased risk of developing breast cancer and prostate cancer when compared with the II carriers [14], [23], other studies reported no significant association with gastric cancer and lung cancer [32]. Variation in the association of the *ACE* I/D polymorphism and various types of cancer (hematological and non-hematological) may reflect the genetic heterogeneity of cancer which results from multilineage somatic evolution of genetically unstable cancer cells and variations in the carcinogenesis process [33], [34].

The results of the present study showed no statistically significant difference in the distribution of *ACE* I/D polymorphic genotypes according to gender. This finding agrees with the findings of two previous studies conducted on Sudanese patients with AML and CLL, both of them also reported no association between *ACE* I/D polymorphism and gender in leukemic patients [24], [25].

In the present study, the comparison of the mean age in the patients with DD genotype and those with ID showed no statistically significant difference, meaning that *ACE* I/D polymorphism does not affect the age of incidence of ALL. This finding disagrees with the finding of Alkalin *et al.* (2011) who reported that the I allele increases the leukemia risk in patients under 50-years-old [22]. However, in our study, none of the ACE alleles were associated with the risk of the disease regardless of the age group or any other factor; further, their study population included patients with different types of hematological malignancies while our study included patients with ALL.

The limitations of this study was the small sample size and lack of stratification based on ALL subtypes; furher study with increased sample size and take in consideration the subtypes of ALL may be benfecial to confirm the association between *ACE* I/D polymorphism and susceptibility to ALL.

Conclusion

ACE I/D polymorphism is not associated with susciptibility to ALL among the Sudanese population.

Authors’ Contributions

A. A. A collected sample and data, performed practical work, analysed data, and wrote the draft; E.W.A. stated the study design, supervised all the processes; interpreted the findings, reviewed, and approved the final version of manuscript.

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