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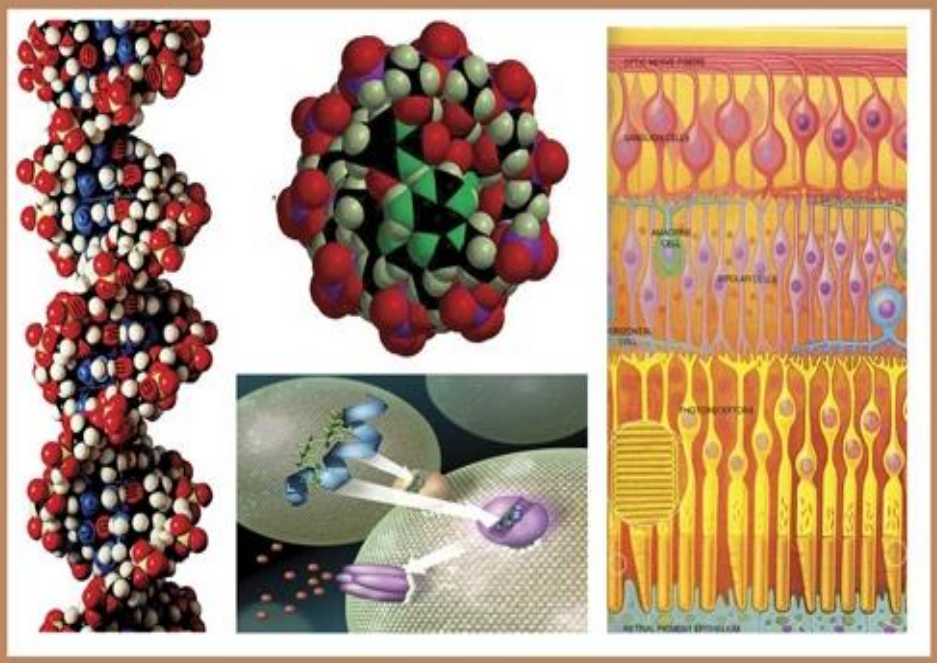

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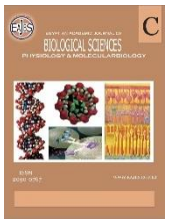

The relationship between INS/DEL of ACE Gene and the Risk of Incidence to Lymphoblastic Leukemia by GAP-PCR Technique in Iranian Population

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ABSTRACT

Background: Angiotensin-converting enzyme gene (ACE; OMIM: 106180) is located on 17q23.3 chromosomal site with functional insertion/deletion polymorphism. The frequency of cases with DD genotype is higher than that of genotypes ID and II. In this study, the association between the gene and severe lymphoblastic leukemia was investigated.

Materials and Methods: This case-control study was conducted on 50 patients with severe lymphoblastic leukemia. ACE I/D polymorphism was detected by the Gap-PCR technique. PCR products were isolated and measured by electrophoresis on 1% agarose gel. The Insertion (I) allele was observed in the band of 478 bps and the Deletion allele (D) in the 191 bps band.

Results: It was estimated that the absence of ID genotype is related to the probability of developing severe lymphoblastic leukemia. In addition, DD and II genotypes had a p-value greater than 0.05 and the hypothesis of their association with brain invasion was rejected.

Discussion and Conclusion: With respect to the results, it should be noted that these findings are the first report of the association between ACE I/D polymorphism and severe leukemia lymphoblastic. Further studies are needed to confirm these findings.

INTRODUCTION

Leukemia, also called blood cancer, is a group of cancers that usually begin in the bone marrow, resulting in a large number of unconventional white blood cells that are not fully developed and called leukemia cells(Jabbour and Kantarjian 2018). Symptoms may include bleeding and bruising, fatigue and increased risk of infection due to the absence of normal blood cells. The *ACE* gene located at 17q23 may play a major role in numerous physiological activities including cellular proliferation and metabolism of peptides owing to broad enzymatic specificity in the body (Christian et al., 2008). On other hand, this gene is also responsible for the production of two isoenzymes somatic and testicular according to alternative splicing of the single polymorphism but duplicated insertion/deletion (I/D) polymorphism located in the intron 16 of the ACE gene has found to be associated with differences of the plasma levels of ACE in the group of healthy subjects. Moreover, I/D polymorphism of the ACE gene has been linked to coronary heart disease since subjects of the homogenous deletion genotype (DD) gave the impression of increasing myocardial infection risk(Chung et al., 2005; Freeman et al., 1996).

ACE has also been found in human monocytes/macrophages, and in the T-lymphocyte population. ACE likewise other ectopeptidases, may participate in the regulation of lymphocyte functions(Friis et al., 2001; Rigat et al., 1990). The T-lymphocyte ACE levels of a given subject are highly reproducible when measured on two different occasions, but may vary widely between individuals in association with I/D polymorphism of the ACE gene(Rinaldi, 2012; Saraswathy *et al.,* 2009; Sayed-Tabatabaei *et al.,* 2006). There is a common 287 base pair Ins/Del (I/D) polymorphism (ALU repeat sequence) that has been reported in intron 16 of ACE gene and known to be associated with serum levels of circulating ACE(Schachter et al., 1994). Subjects having the extra fragment (Insertion allele) is associated with lower circulating ACE and tissue activity, and the absence of this fragment (Deletion allele) is associated with a comparatively higher ACE activity(Shanmuganathan *et al.,* 2014; Bosnyak *et al.,* 2011). However, heterozygous (Ins/Del) subjects display an intermediate level of ACE activity. Moreover, with the aim of linking the insertion/deletion of gene polymorphism, conducted a meta-analysis of gastrointestinal cancers with an ACE risk of 2903 cases per year with Sixteen groups analyzed the control sample analysis. The overall analysis failed to show any sign of 10833 gastrointestinal tracts and risk of gastrointestinal cancers. In the analysis of the subgroups, the 15% risk genotype was associated with a decrease in ACE III(Randhawa et al., 2006; Carter and Lin, 1993; Chow *et al.,* 2009). By controlling the sources, the allele was associated with a 2% high risk of colon cancer. However, with an increase in gastric cancer, it appeared to be a protective agent against gastrointestinal cancers in Population-based studies. However, it is a risk factor for studies in the hospital. Their findings indicated that the ACE gene 1 allele may be a protective agent against cancer, which needs to be confirmed in studies with a high population density(Freitas-Silva *et al.,* 2004).In the current study, we aimed to respond to two objectives, a)is there any associated between INS/DEL of ACE gene and the risk of incidence to lymphoblastic leukemia by GAP-PCR technique . b) may be there isn't associated between INS/DEL of ACE gene and the risk of incidence to lymphoblastic leukemia by GAP-PCR technique. C) GAP-PCR is suitable for finding INS/DEL mutations in this gene.

MATERAILS AND METHODS

Blood Samples and DNA Extraction:

In this study, two hundred blood samples including a hundred normal groups and case groups were collected from hospitals in Iran. Genomic DNA was extracted by standard kit GENET Bio from 200 samples. 1.5% Agarose gel was used for determining total Genomic DNA. The concentration of Genomic DNA was calculated and is ready for doing PCR.

How Can Designing Primers for GAP-PCR Technique:

In this method, only mutations that are studied to be known and also have a range of one kb are considered. Hence, for a healthy allele, a primer that was able to connect to the deletion region was used up to 287 pairs primer (487 bp) and a band with a difference with the removed allele (198 pairs of primer). The primers showed the following;

Forward primer;
5'GATGTGGCCATCACATTCGTCAGAT 3'.

Reverse primer,
5'CTGGAGACCACTCCCATCCTTTCT3'.

PCR products were run on 1.5 percent agarose gel to determine the quality of PCR products. Purified samples were sent for sequencing.

Statistical and Molecular Analysis of ACE Gene:

The situation of PCR product on the gel electrophoresis (the number of bands) was analysed SPSS version 21 program.

RESULTS

Investigating the Relationship Between ACE Gene Insertion/Deletion Polymorphism and Acute Leukemia:

In this study, GAP-PCR technique was used to analyze the *ACE* gene insertion/deletion polymorphism. Two fragments of 191 and 487 base pairs related to deletion (allele D) and insertion (allele I) were observed respectively. Genotypes of II, ID and DD of ACE gene amplified by GAP-PCR are shown in Figure 1.

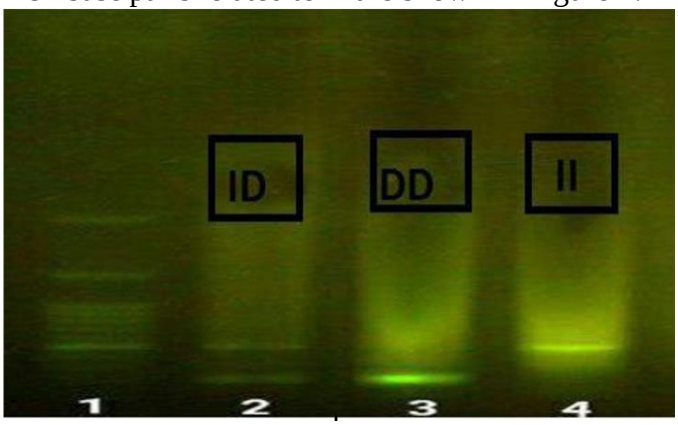

Fig. 1. The result of insertion-deletion ACE of gene. ID, (487 and 191bp), DD,(191bp) and II(487bp). Lane 1 is ladder.

Statistical Analysis:

Based on statistical calculations and genotypic distribution between the two groups of patients and control, which is measured by the p-confidence coefficient value, the meaningfulness-calculated is such that for various genotypes if the value is considered to be the acceptance of the hypothesis is smaller than 0.05, if it is greater than 0.05 meaning the rejection hypothesis means if accepted there is a relationship between INS/DEL of ACE gene and the risk of incidence to lymphoblastic leukemia. In this regard from 50 patients with 21(II), 2.0(ID) and 27(DD) and 50 samples under controls with 20(II), 2.0(ID) and 28(DD), P-value revealed insignificant variation between INS/DEL of ACE gene and Leukemia. In this regards the result of Table 1 showed that from 50 patients and 50 samples under controls, 20(II), 2.0(ID) and 28(DD) for the control group and 21(II), 2.0(ID) and 27(DD) for patients group. P-value of our result showed insignificant variation between INS/DEL of ACE gene and Leukemia.

Table 1. Genotyping of insertion/deletion of ACE gene. The result showed an insignificant value between ACE gene and the risk of leukemia.

Genotypes	Patient group(n=24)	Control group(n=14)	p-value
II	20.0	21.0	0.585^{NS}
1D	2.0	2.0	0.22^{NS}
DD	28.0	27.0	0.561^{NS}

NS. Non significant, p-value>0.05.

Evaluation of Allele Frequency of Patients and Their Control and Their Relation with Acute Leukemia:

The allelic distribution of this polymorphism was also calculated for patients and controls in Table 2. The frequency of the allele I for the leukemia was 40% and for the control group was 27.5%. The frequency of allele D for the patient group was 60% and in the control group was 72.5%.

Table 2. Allelic frequencies of INS/DEL of ACE gene. Result showed insignificant value were observed.

Alleles	Cases	Control	Odd ratio	p-value
I (%)	16(40%)	11(27.5%)	2.17	0.06^{NS}
D (%)	24(60%)	29(72.5%)	0461	0.06^{NS}

NS. Non significant, p-value>0.05.

DISCUSSION

ACE polymorphism has recently been associated with many other cancers. A case-control study showed that ACE I / D polymorphism plays a very important role in prostate cancer. DD genotype was associated with patients with an invasive degree of prostate cancer. A Mexican-based case-control study showed that the D allele with ACE gene was clearly associated with an increased risk in the formation of prostate cancer(Freitas-Silva *et al.*, 2004). A case-control study of ACE I / D may be associated with the progression of human prostate cancer, a demonstration of polymorphism. This case revealed that DD genotype was seen in 31 out of 78 cases of advanced disease and in 19 out of 82 cases of cross-sectional disease, and these differences were statistically significant(Singh M, Singh AK, Singh, *et al.*, 2016). A case-control study suggested that ACE I / D polymorphism can play an important role in the development of chronic pancreatitis and pancreatic cancer by interacting with other genetic and environmental factors. MO and colleagues conducted a meta-analysis study to assess the relationship between the insertion/deletion polymorphism of the angiotensin-converting enzyme gene and the risk of lung cancer(Woods *et al.*, 2001). They stated that there was no significant association between D allele D genotype and lung cancer susceptibility. While genotype II does not protect against the risk of lung cancer in the population. In addition, they also found that the insertion/deletion polymorphism of the angiotensin-converting enzyme gene with susceptible lung cancer in the Asian and Caucasian populations is not relevant. Finally, the conclusion was that the insertion/deletion of the angiotensin-converting enzyme polymorphism is not related to the susceptibility to lung cancer. However, they suggested that further research is needed to clarify the link(Akhtar *et al.*, 2005; Arnon *et al.*, 2008).

Zarouk *et al.*, 2011, investigated the possible association of ACE gene polymorphisms with the risk of basal cell carcinoma(BCC). To this end, they studied the ACE gene I / D polymorphism in DNA samples of 92 patients with BCC and 103 individuals of Greek origin and compared their age and sex(Aroni *et al.*, 2011; Benndorf *et al.*, 2003). To compare the allele frequency and genotype between the control group and the patients, FISHER's exact test was used and found that the frequency of allele I in the BCC group was significantly reduced compared to the control group. The risk of BCC in heterozygous ID was lower compared to homozygote DD. Also, the protective role of allele I was particularly prominent in women, while it was shown to men at a lower level. These findings showed that the low expression of the ACE allele I had a lower risk for BCC. Our study found that none of the patients had this genotype(Zarouk *et al.*, 2011; Ager *et al.*, 2008). Our results, although biologically and logically, are still ambiguous about the exact mechanism of cancer detection (Tables 1 and 2). Some of the limitations of this study should be considered; First, interactions between the gene, the gene-environment and even the different polymorphic leukemia of the same gene may play a role in the risk of acute leukemia. Second, this case study is an overview of the hospital, so selective criteria are inevitable, and the cases may not represent the general population. Third, these results should be

interpreted with particular considerations because the present population is only related to the Iranian population, which reduces the probability of mixing the gene with other ethnic groups. Hence, these results cannot be extrapolated to other ethnic groups.

Conclusion

The results of this study showed that the absence of the genotype ID of the ACE gene in people at risk for acute leukemia is significant. In addition, DD and II genotypes were observed in both healthy and healthy populations, which were statistically analyzed and showed no significant correlation with acute leukemia. In finally proposed that considering the extensive studies of ACE gene and multiple reports of meaningful ID polymorphisms associated with them, it is suggested that in the studies of the ACE gene, the records of each individual's case should be investigated in order to the inability of the gene to survive the previous illness is ensured. It is also suggested that in studies of acute leukemia, the presence or absence of a patient should be guaranteed for other cancers. In the end, it is suggested that the number of more people be studied in subsequent studies; because the larger the statistical society is the results will be more accurate and more reliable.

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