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ACE Gene Polymorphism in Hypertension Patients from District Kohat

Khadija Saeed[1],Nazif Muhammad[1], Muhammad Haris[1], Sadam Hussain[1], Syeda Iqra Hussain[1], Sonila Gul[1], Iqbal Nawaz[1], Wasimullah[1], Mehak Afshan[1], Maryam Nisa[2], Aleena Javed[3],Aisha Noreen[2], Niamatullah Khan[1],Farman Ullah Khan[2], Saadullah Khan[1], Abid Jan[1], Noor Muhammad[1].

[1]Department of biotechnology & Genetic Engineering, Kohat University of Science & Technology, Kohat 26000, KPK, Pakistan
[2]Department of Zoology Kohat University of Science & Technology, Kohat 26000, KPK, Pakistan
[3]Department of Chemistry Kohat University of Science & Technology, Kohat 26000, KPK, Pakistan

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Abstract: Hypertension, an outstanding danger factor for different cardiovascular, fringe vascular and renal occasions is a significant general wellbeing challenge. Renin angiotensin framework being the most essential pathogenic system of hypertension is interceded by a key segment, the angiotensin converting enzyme. The aim of current study was to know the relationship of hypertensive patients with angiotensin converting enzyme gene polymorphism. Total 65 clinically analyzed hypertensive patients with no related sickness condition and five age and sex coordinated clearly sound controls were included in the current study. Polymerase chain reaction was used for the amplification of intron number 16 of Angiotensin converting enzyme gene harboring the 287 bp Alu repeat sequence. Homozygous Deletion/Deletion, Insertion/Insertion and heterozygous Insertion/Deletion were the three possible genotypes which were analyzed. After the amplification of products, 2% agarose gel was used for the separation of products. Statistical Package for the Social Sciences 20 (SPSS 20) was used for the statistical analysis; the P value was found non-significant and to avoid any false positive or false negative significance we cannot conclude any result.

Key words: Insertion; deletion; renin angiotensin system; total peripheral resistance; blood pressure,

Introduction

Hypertension is among the most broadly perceived conditions harrowing the number of inhabitants in the world. Due to the related terribleness, mortality and the cost to society, hypertension is a huge general prosperity challenge. From the very beginning it has been the danger factor for various cardiovascular and renal diseases in our body. Hypertension can be depicted as a persistent rise in blood pressure (Chobanian et al., 2003). A continued systolic pressure

*Correspondence to Noor Muhammad, Department of biotechnology & Genetic Engineering, Kohat University of Science & technology, Kohat 26000, Pakistan, E-mail:**noormwazir@yahoo.com**

more noteworthy than 140 mmHg and additionally a supported diastolic pressure more than 90 mmHg are commonly considered to comprise hypertension. In spite of the fact that either a raised systolic or diastolic pulse can result in hypertension, raised systolic blood pressure is a progressively critical hazard factor for controlled hypertension, coronary illness and heart failure than a raised diastolic blood pressure (Papademetriou, 2003).

Blood pressure (BP) is straight forwardly reliant on both cardiovascular output (CO), which is the volume of blood that the heart pumps per minute, and total peripheral resistance (TPR), which is the aggregate sum of resistance applied by the peripheral vasculature, explicitly the aggregate resistance of peripheral arterioles in the fundamental flow. This relationship is given by the formula:

$$BP=CO\times TPR$$

Changes in either heart yield or all out peripheral resistance can in this way adjust blood pressure El-Dorry, Pickett, MacGregor, and Soffer, 1982).

The ACE Gene

Location of angiotensin converting enzyme gene is 17q23 in human. This gene contains 25 introns and 26 exons and the size of this gene is 21 kb.To date NCBI search retrieves 160 different types of angiotensin converting enzyme gene polymorphism. Two isoforms are encoded by this gene i.e somatic angiotensin converting enzyme. Molecular weight of sACE is 170 kilo Dalton. Other is testicular angiotensin converting enzyme. Molecular weight of tACE is 100 kilo Dalton (El-Dorry et al., 1982).

***ACE* Levels and Genetic Control:**

Plasma angiotensin converting enzyme levels are steady when evaluated on and on in same individual(Alhenc-Gelas, et al., 1991). After the report of Rigat and his colleagues in 1990, further research was started for investigating ACE gene polymorphism. In their study they found the presence of a 287 bp alu sequence which they called as insertion and absence of this sequence was called as deletion in intron 16 of ACE gene. The ACE I/D polymorphism were at first identified by RFLP examination (Rigat et al., 1990). Initial PCR based detection of ACE gene polymorphism was reported by Rigat and his collegues (Rigat et al., 1992), who utilized a set of primers flanking the insertion sequence. Family based examinations were perforamed by Shanmugam et al (1993), which demonstrated the likelihood of mistyping ID heterozygous as DD homozygous with this PCR strategy. Particular amplification of the shorter D allele may cause the misclassification of roughly 4 to 5% of ID genotypes to DD. An extra PCR amplification reaction was, figured for the affirmation of DD genotypes got in the principal standard PCR, including another new sense primer that is insertion specific (Shanmugam et al., 1993). Carriers with homozygous deletion have double fluctuation of ACE levels than in other (Rigat et al., 1990). Carriers having ID genotypes had transitional dimensions showing co-dominancy. The I/D polymorphism represented around half (47%) of the watched difference in ACE dimensions in this investigation gathering. Later investigations demonstrated that the inclusion of the I/D polymorphism isn't restricted to ACE dimensions in plasma, and is additionally identified in tissue ACE dimensions (Costerousse et al.,, 1993; Danser et al., 1995).

Current study was aimed to find the association of ACE gene polymorphism with hypertension. Our statistical analysis revealed no association of ACE genotyping and hypertension however based on previous studies we strongly recommend to restudy with increased sample size.

Materials and Methods

Ethical approval was obtained from the research ethical committee of Kohat University of Science & Technology (KUST), Khyber Pakhtunkhwa, Pakistan. The research was started after obtaining written informed permission from all participating individuals.

Sample Collection

Samples(n=59) were collected from Combined Military Hospital and District Head Quarters Hospital Kohat in EDTA containing vacutainer tubes and stored at 4^0C. Among these 5 were control and 54 were patients.

DNA Extraction

Standard phenol chloroform method was used to extract the genomic DNA from peripheral blood samples(Sambrook and Russell David, 1989).

Polymerase Chain Reaction:

Intron 16 of ACE gene was amplified using PCR. Sterile PCR tubes were used and marked accordingly. MgCl2, Taq Buffer, dNTPs, Taq Polymerase, PCR Water, Reverse and Forward Primers were the requirements for amplification for total 25µl reaction. The DNA fragments were amplified by using External primers. These primers have following sequences.

Forward primer: 5′-GGGACTCTGTAAGCCACTG-3′

Reverse primer: 5′-GGCCATCACATTCGTCAGAT-3′.

Thermal cycling by PCR was performed by an initial denaturation at 95^0C for 5 min, followed by 35 cycles of denaturation at 95^0C for 1 min, annealing at 54^0C for 1 min, extension at 72^0C for 1:30 min. Then cycling was completed by final extension at 72^0C for 5 min.

A 2% agarose gel was used for the detection of ACE genotypes. Then the products amplified with external primers were purified using GeneJET PCR Purification Kit (**Thermo Scientific**). Then on these purified samples internal primer were applied and PCR was done. PCR conditions were the same used for external primers. Internal primers have the following sequences Forward Primer: 5′-GGATGGTCTCGATCTCCTGA-3′Reverse Primer:5′-GCTCACCTCTGCTTGAAAGG-3′. Amplified products were again detected on 2% gel electrophoresis using ladder. Amplified products were located at 152 bp position.

Result

External primer was applied on 63 DNA samples. Insertion alleles were located at 495 bp position, deletion alleles were located at 205 bp position while insertion/deletion alleles were located at both 495 and 205 bp positions (Figure 2).Out of 63 samples 40 gave positive result. In patients insertion alleles (I/I) were 11, deletion alleles (D/D) alleles were (0) and insertion/deletion (I/D) 29. In control 4 samples out of 5 gave I/I, D/D alleles were 0 while I/D was 1 (Table 1). Then we amplified the 40 samples (which gave positive result in EXL) by using the INL primer, all of them gave positive results so the D/D genotype are zero(0) after using internal primer because of mistyping of I/D as D/D, so the D/D allele is actually the I/D allele (preferred amplification) in our research results (Figure 3).

Table 1. Frequency distribution of ACE alleles in control and hypertensive patients

Type of ACE polymorphism	Patients n=40 (%)	Control n=5 (%)
I/I	11 (27.5)	4 (80)
D/D	0(0)	0 (0)
I/D	29 (72.5)	1 (20)

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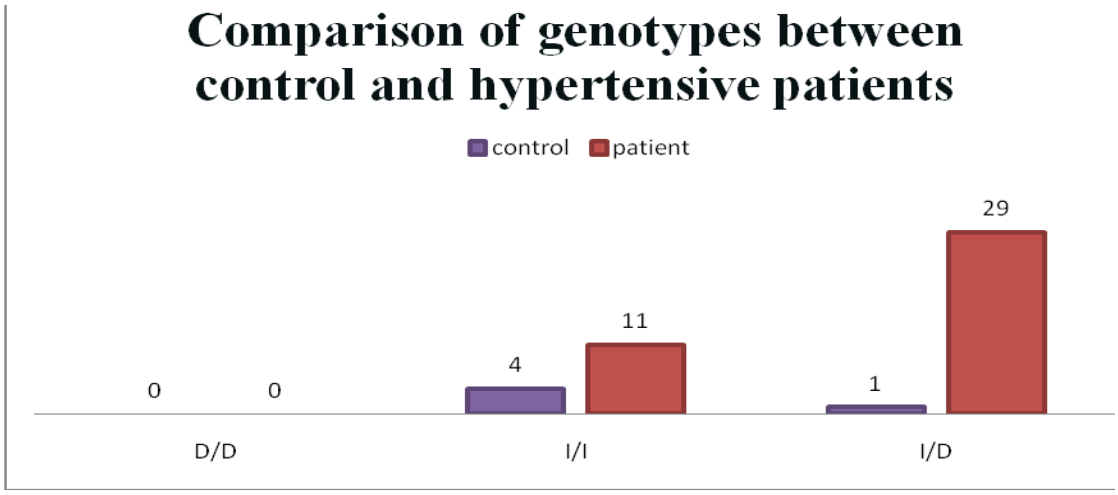

Figure 1. Comparison of genotypes between controls and hypertensive patients.

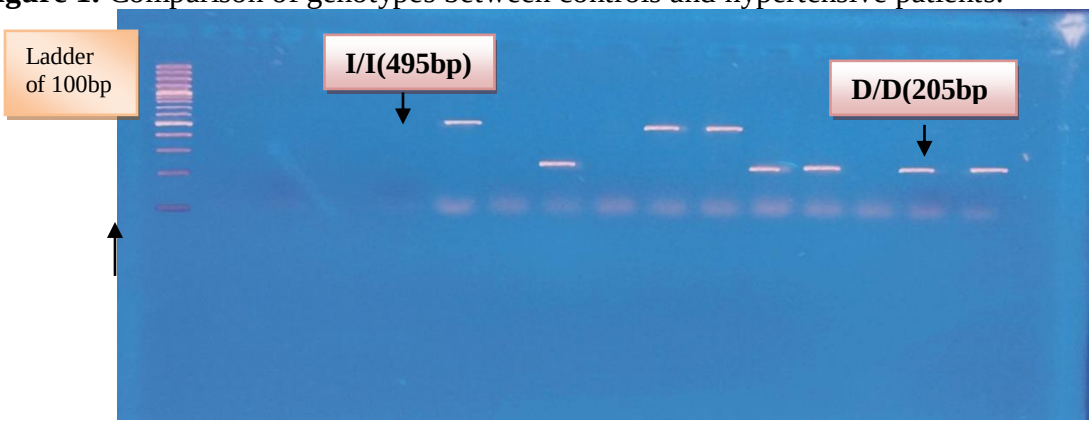

Figure 2. PCR products showing ACE polymorphism where I/I represents homozygous insertion with product size of 495bp and D/D represents homozygous deletion with the product size of 205bp.

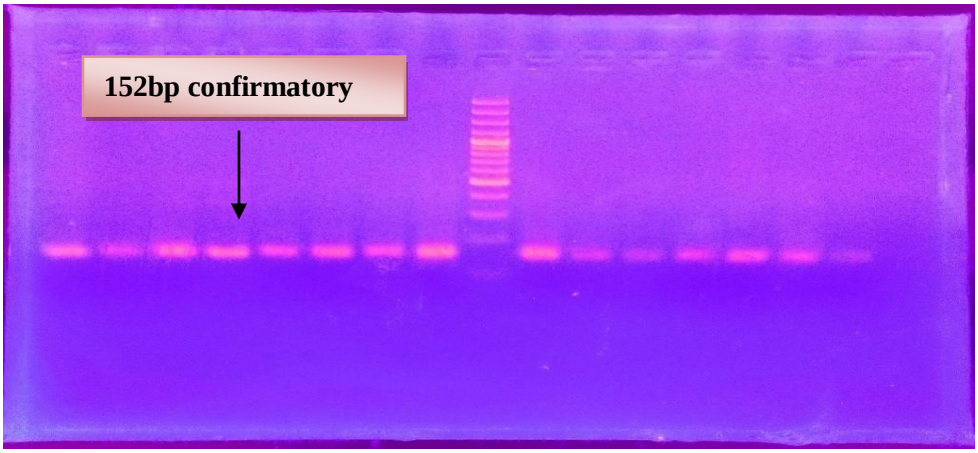

Figure 3. PCR product for the confirmation of I/D allele with internal primer which were preferentially amplified as D/D.

Discussion

Cardiovascular disorders are rapidly rising as a noteworthy wellbeing worry in most creating nations. Hypertension is a noteworthy modifiable hazard factor of morbidity and mortality, in charge of 50 % of passing because of stroke and 24 % because of all other coronary vein sickness (Gupta, 2004). South Asians have an expanded danger of coronary illness, in contrast with Europeans. The RAS has a focal job in controlling pulse and homeostasis of sodium. Angiotensin converting enzyme is a key enzyme involved in the conversion of Angiotensin I to Angiotensin II, which is an intense vasodepressor. Gene which encodes segments of renin angiotensin system, which also includes Angiotensinogen, angiotensin converting enzyme, Angiotensin gen II type-1 receptor and Renin have been broadly explored as hereditary factors of basic hypertension (Manunta and Bianchi, 2002; Thiel and Weder, 2000). The I/D polymorphism in the angiotensin converting enzyme gene is associated with circling angiotensin converting enzyme dimensions. People with the Deletion/Deletion genotype have the most noteworthy coursing angiotensin converting enzyme dimensions when contrasted with the I/I genotype. It has been projected that the relationship between the angiotensin converting enzyme Insertion/Deletion polymorphism and hypertension may be identified with gender and ethnicity.

In the current study 65 hypertensive patients and 5 age and sex coordinated controls were taken. The distribution of all these factors between the hypertensive and controls were considered and their association with the three genotypes Insertion/Insertion, Insertion/Deletion and Deletion/Deletion were evaluated. After the analysis of genotypes frequency of Insertion/Insertion, Insertion/Deletion and D/D polymorphism among non-hypertensive (controls) 80%, 0% and 20%. Frequency among hypertensive (cases) was 27.5%, 72.5% and 0%. SPSS 20 was used for the statistical analysis; the P value was found non-significant. To avoid any false positive or false negative significance we cannot conclude any result.

Conclusion

Our statistical analysis reveals no association of ACE genotyping and hypertension however based on previous studies we strongly recommend to restudy with increased sample size.

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