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Angiotensin Converting Enzyme Gene I/D Polymorphism in Pakistani Rheumatic Heart Disease Patients and Healthy Controls

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ABSTRACT

Background: Valve scarring and collagen deposition are crucial in pathogenesis of Rheumatic Heart Disease (RHD), an autoimmune disorder of the heart. Angiotensin I-Converting Enzyme (ACE) plays a major role in fibrous tissue formation.
Objectives: The present research work aimed to assess the role of ACE Insertion/Deletion (I/D) polymorphism in progress of RHD.
Patients and Methods: DNA was pre pared from blood samples from 156 RHD patients (156) and 204 healthy ethnically-matched controls. Then, it was screened using sequence-specific Primers. Polymerase chain reaction and Agarose gel electrophoresis. The data were analyzed using Vassar stats (http://faculty.vassar.edu/lowry/VassarStats.html).
Results: I allele (P = 0.024, OR = 1.42) and II genotype (P = 0.001, OR = 3.07) were significantly higher in Pakistani RHD patients compared to the healthy controls. Also, a significant difference was found between the female, but not male, patients and the controls regarding I allele and II genotype.
Conclusions: The study results provided information about involvement of ACE I/D polymorphism in molecular mechanism of RHD. Thus, it can become one of the useful tools in risk assessment and help with designing strategies to combat the disease.

►*Implication for health policy/practice/research/medical education:*
This work was done to determine involvement of ACE I/D polymorphism in the pathogenesis of RHD.

1. Background

Rheumatic Heart Disease (RHD) is described as an inflammatory, autoimmune disorder, occurring as a result of *Streptococcus pyogenes (S.pyogenes)* or Group A β Hemolytic Streptococcus (GABHS) infection complicated by Rheumatic Fever (RF). The prevalence of RF and RHD is high in developing countries due to poor standards of living and inadequate medical facilities. In these countries, the occurrence rate of acute RF goes beyond 50 per 100,000 children. Overall, 15 million cases of RHD have been reported worldwide, with 282,000 new cases and 233,000 deaths per year (1). Recent studies have shown a high prevalence of RHD in both urban (22 per 1000) and rural (5.7 per 1000 population) areas of Pakistan (2, 3). The pathogenesis of RHD is quite complex; valve scarring and collagen depositions in the valves are crucial changes in RHD (4). After humoral and cellular inflammation during development of RHD, fibrous tissue formation occurs that predicts the healing process. In some individuals, however, uncontrolled fibrosis occurs that can lead to chronic rheumatic valvular disease. Renin Angiotensin System (RAS) is a vital regulatory system for keeping and maintaining normal blood pressure and balancing body fluid and electrolytes. Angiotensin I-Converting Enzyme (ACE), a zinc metallopeptidase, is a key enzyme of this system, which is also called Kininase II. The importance of ACE in balancing body circulatory system has been

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well documented. ACE gene is localized on chromosome 17q23.10 and is composed of 26 exons (5). Variability of ACE level in various individuals suggested that genetic polymorphism may be involved. In 1992, it was found that 50% of variability in ACE plasma level was due to Insertion/Deletion (I/D) polymorphism in intron 16 of this gene (6). This I/D polymorphism was due to an Insertion (I) or a Deletion (D) of a 287 non-coding base pair Alu repeat sequence (sequence (dbSNP rs4646994). II genotype results in negative remodeling and possibly triggering valve scarring and calcification, while DD genotype results in high ACE level, protecting RHD patients from valve scarring or valve calcification (7).

ACE I/D polymorphism not only affects prognosis of different diseases, but can also modify treatments given to patients, especially cardiovascular ones. This Alu polymorphism has been studied in several cardiovascular diseases (8-12).

2. Objectives

The present study aims to determine the role of ACE I/D polymorphism in development of RHD in Pakistan.

3. Patients and Methods

This was a case-control study. The RHD samples included 127 female patients and 29 male patients with the mean age of 31 ± 14.10 years selected from Shifa International Hospital (SIH), Islamabad, Pakistan Institute of Medical Sciences (PIMS), Islamabad, and Armed Forces Institute of Cardiology (AFIC), Rawalpindi. All the RHD patients had mitral valve disease with history of RF, were in quiescent phase or chronic phase, and fulfilled the two dimensional M-mode and doppler echocardiography criteria (13) for mitral valve disease.

The control group, on the other hand, included 204 individuals (108 females and 96 males, mean age: 18245 ± 12.7 years) with normal echocardiograms and no history of RF. The patients and the controls were ethnically matched and were from various castes and tribes of northern Punjab and Khyber Pakhtunkhwa provinces, Pakistan. Venous blood samples were taken after gaining informed consents of the individuals or their legal guardians. The study protocol was approved by the respective institutional bioethics committee in March 2003.

Genomic DNA was prepared (14) and ACE Alu I/D polymorphism in intron 16 was studied 188 using primers sequences ACE I/D Forward 5-CTGGAGACCACTCCCATCCTTTCT-3 and ACE 189I/D Reverse 5-GATGGCCATCACATTCGTCAGAT-3 (15). A 15 µL volume was used for each 190PCR reaction. In fact, each reaction contained 1 µM of both primers, 1 x of 10x PCR buffer, 1911.5 µM of MgCl2, 200 µM of dNTPS, 1 U of Taq polymerase (Fermentas EU), and 30 ng 192of DNA. PCR cycling parameters were 1 cycle at 94 °C for 193 3 min followed by 35 cycles at 94 °C for 45 sec, at 58 °C for 1 min, and at 72 °C for 45 sec and a final cycle at 72 °C for 10 min. The amplimers were separated by using 2% agarose gel electrophoresis and after staining with ethidum bromide, agarose gels were visualized under UV transilluminator. Accordingly, the 190 and 490 bp fragments represented D allele and I allele, respectively. Besides, presence of both size bands meant ID heterozygotes. DNA ladder of 100 bp was used as the standard.

Allele and genotype frequencies were calculated by direct counting. Besides, Odds Ratios (OR), 95% Confidence Intervals (CI), and significance of the ORs were calculated by 2x2 chi-square contingency test with Yates' correction for continuity (http://faculty.vassar.edu/lowry/VassarStats.html).

4. Results

ACE I/D polymorphism was screened in 156 RHD patients and 204 controls and the results have been presented in Tables 1, 2, and 3. Accordingly, the frequency of genotype II was significantly (OR = 3.07, P = 0.01) higher among the RHD patients compared to the healthy controls (Table 1).

However, no significant difference was found between the two groups regarding the frequency of DD and ID genotypes. The frequencies of I and D alleles were also evaluated between the two groups. According to the results, the frequency of I allele was significantly higher (OR = 1.42, P = 0.024) in the RHD patients compared to the healthy controls.

Among the RHD patients, the number of females was higher than that of males. Therefore, female patients and controls were also compared in order to determine the difference between the allele and genotype frequencies of this Alu polymorphism on the basis of sex. Among the subjects, RHD patients and 106 controls were female. The frequency of II genotype was significantly higher (OR = 3.92, P = 0.002) in female patients. However, no significant difference was found between the two groups regarding DD and ID genotypes (Table 2).

In comparison of allele frequencies, the frequency of I allele was significantly higher among the female RHD patients compared to the controls (OR = 1.56, P = 0.014). Male RHD patients (N = 30) and controls (N = 90) were also compared with respect to this polymorphism. The results showed no significant difference between the two groups regarding the three genotypes or the two alleles (Table 3).

Table 1. The Results of ACE I/D Polymorphism in RHD Patients and Normal Controls

Genotype	RHD Patients	Controls	Chi-square	OR (95% CI)	P value
	N = 156, N(%)	N = 204, N(%)			
II	27(17.3)	13(6.3)	9.62	3.07(1.52 - 6.2)	0.001
DD	29(19)	51(25)	1.75	0.70(0.41 - 1.14)	0.180
ID	100(64)	140(68)	0.62	0.82(0.52 - 1.27)	0.37
Alleles					
I	154(50)	166(41)	5.04	1.42(1.06 - 1.91)	0.024
D	166(53)	242(59.3)			

Table 2. ACE I/D Polymorphism in Female RHD Patients and Female Normal Controls

Genotypes	RHD patients N = 126, N (%)	Controls N = 106, N (%)	Chi-square	OR (95% CI)	P value
II	23(18)	6(0.06)	9.62	3.92(1.53 - 10.0)	0.002
DD	30(28)	30(28)	1.75	0.62(0.34 - 1.15)	0.131
ID	79(63)	70(66)	0.62	0.83(0.48 - 1.43)	0.511
Alleles					
I	125(50)	82(39)	5.97	1.56(1.14 - 2.30)	0.014
D	139(50)	130(61)			

Table 3. ACE Insertion/Deletion Polymorphism in Male RHD Patients and Male Normal Controls

Genotypes	RHD patients N = 98, N (%)	Controls N = 30, N (%)	Chi-square	OR (95% CI)	P value
II	4(13)	7(0.07)	-	2(0.54 - 7.36)	
DD	4(13)	21(21)	0.51	0.6(0.18 - 1.91)	0.47
ID	22(74)	70(71)	0	1.1(0.44 - 2.76)	1
Alleles					
I	154(50)	84(43)	0.68	0.75(0.42 - 1.13)	0.41
D	166(53)	112(57)			

5. Discussion

The concentration of ACE is quite high in valve matrix of the heart (16), which may contribute to collagen synthesis at these sites, resulting in proper regulation of body circulatory system. ACE levels vary in individuals, and it was found that 50% of its variability was due to an Alu insertion in intron 16 of ACE gene. The present study amid to find out whether ACE I/D polymorphism has any role in prognosis of RHD. Our results showed that the frequency of allele I and genotype II was significantly higher in Pakistani RHD patients compared to the control group. These findings were supported by a previous study on Taiwan-Chinese population, in which the frequency of I allele and II genotype was appreciably higher in the patients than in the controls (17). Another study on Turkish RHD samples also indicated that II genotype was a risk genotype in RHD patients (7). However, a study on Egyptian children with RHD showed that the frequency of DD genotype was significantly higher in the patients in comparison to the controls (18). Similarly, two other studies on Turkish RHD patients revealed DD homozygotes as the risk genotype (19, 20). The findings of these studies were in contrast to those of the current study. These differences can be attributed to many factors, one of which being ethnicity. Data from different geographical regions confirm the importance of this factor (21). Therefore, special attention should be paid to ethnic foundation in case of association studies, such as ACE I/D studies. Moreover, results from one group with different ethnic background cannot be applied to another group.

I allele of ACE I/D polymorphism has been found to be associated with hypertension (9), insulin resistance (22), metabolic syndrome (23), mitral valve prolapsed syndrome (17), and arterial fibrillation with hypertrophic cardiomyopathy (24). Therefore, ACE II genotype can be considered as a genetic risk factor for various cardiovascular diseases.

With the help of several research works, it was found that the difference among association studies could also be due to selection of samples. In the study on Turkish RHD patients, DD genotype was found to be the risk allele for the disease. In that study, the patients had RF (19) and they were not in the chronic phase of the disease. However, our patients were in the chronic phase of the disease. Sample size and study design may also serve as the reason for discrepancies seen among the results.

In the current study, the samples were compared on the basis of sex. According to the results, the frequency of II genotype and I allele was significantly higher in female RHD patients compared to the female healthy controls. However, no significant difference was found between the male patients and the male controls in this regard. This can be attributed to the small number of male patients in the current study.

5.1. Conclusions

The present study revealed a significant difference between the patients and controls regarding the frequency of ACE I/D II genotype and I allele. Also, a significant difference was found between the female patients and controls in this regard. These results support the fact that ACE I/D polymorphism might play a role in prognosis of RHD in Pakistani population.

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Authors' Contribution

Sadia Rehman, Nusrat Saba, Saeeda Munir and Azra Khanum were involved in conceiving the idea of this research. Sadia Rehman, Nusrat saba and Saeda Munir performed the experimental work. Waqar Ahmed helped in sample collection and made the clinical diagnosis. Statistical analysis and writing was done by Sadia Rehman.

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