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Effect of ACE I/D polymorphism in cardiovascular disease patients

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Abstract---Cardiovascular diseases (CVD) are the cause of the highest mortality rate among noncommunicable diseases, Some of the main risk factors for CVD include smoking, hypertension, high body mass index, lipid metabolism disorders, and diabetes mellitus, according to emerging evidence, Genetics and polymorphisms are thought to play an important role in the occurrence and progression of CVD. A number of 100 subjects were involved in this investigation, 70 of them with CVDs and the other 30 healthy individuals were used as control. The study was conducted to find out the importance of ACE gene polymorphism and biochemical variables in CVDs patients. In addition, the study aimed to investigate the effect of gene polymorphism on CVDs. The comparisons between genotypic frequency of ACE gene polymorphism in control and CVDs patients showed a significant increase ($p \leq 0.005$) in DD, ID genotype in CVDs patients compared to control, where II genotype significant decreased in CVDs patients compared to control. Our results show insertion mutation at 550bp only in CVDs patients, this mutation not show in other research.

Keywords---ACE, I/D, polymorphism, cardiovascular, patients.

Introduction

Cardiovascular diseases (CVD) are the cause of the highest mortality rate among noncommunicable diseases (NCD) (WHO,2021). Some of the main risk factors for CVD include smoking, hypertension, high body mass index, lipid metabolism disorders, and diabetes mellitus, (Iriti *et al.*, 2020 ; Gbadamosi&Tlou, 2021). according to emerging evidence, Genetics and polymorphisms are thought to play an important role in the occurrence and progression of CVD (Zhai *et al.*, 2019). A variety of investigations have reported the impact of ACE I/D polymorphism in

several cardiovascular diseases including endothelial dysfunction, atherosclerosis, and heart failure, It has been displayed that there is a relationship between ACE I/D polymorphism and the alteration of ACE activity (Amara *et al.*, 2018; AhmadYusof & Che Muhamed *et al.*,2021).

In humans, the ACE gene can be found on the long arm (q) of chromosome 17 (17q23.3). this gene is 21 kb long and consists of 26 exons and 25 introns, The ACE gene produces ACE (Ahmad Yuso & Che Muhamed ,2021). which is a key element in the renin-angiotensin (RAS) system that regulates blood pressure, water fluid balance, and tissue growth (Nehme *et al.*,2019). In a circulating RAS system, the primary role of ACE is to produce angiotensin II (ANG II), The ANG II is a potent vasopressor and aldosterone that stimulates angiotensin I peptide (ANG I), In addition, the ANG II decomposes bradykinin, which is a potent vasodilator (Phillips *et al.*,2018). It is also important to note that each individual has different levels of ACE in plasma. However, family members have a similar level of ACE plasma, suggesting that the inter individual variability in the ACE plasma level is determined by genetic factors (Ahmad Yuso & Che Muhamed ,2021). Among several polymorphisms of the ACE gene, the ACE I/D gene polymorphism has a strong association with the ACE plasma level, which accounts for 47% of the overall phenotypic variance of the ACE activity (Dos Santos *et al.*,2021) .

Material and Methods

Study population from AL-Najaf province in Iraq, we taken 70 patients with CVDs and 30 healthy persons as control, they were >50 years of age, were referred to Al-Sadder Hospital (located in kufa, Iraq) for heart center and Al Hakim Hospital in AL-Najaf province from November 2021 to February 2022., patients and control groups were approved for sampling and then informed of the results The experimental design. Evaluation criteria for patients history are considered conventional risk factors for CVDs based on high blood pressure (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg) , lipid profile (Cholesterol > 225 mg /dl , Triglyceride > 200 mg /dl , LDL >129 mg /dl , HDL <60 mg /dl).

Determination of ACE polymorphism

Extracted DNA from whole blood by using DNA isolation kit (Geniad gSYNCTM DNA Extraction Kit). the primers synthesis by (Bio Synthesis, USA) were performed for detection of I/D ACE polymorphism. forward primers were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3" reverse primer 5'- GAT GTG GCC ATC ACA TTC GTC AGA T-3'. The amplification of PCR was carried out in a total volume of 20 µl containing 7. 5 µl DNA, 2.5 µl Go Taq green master mix (PCR PreMix from Bioneer) is a premixed ready to use solution contains Taq DNA polymerase. Amplification was performed in Gradient Thermo-cycler (Agilent, USA), the program of PCR amplification were initial denaturation step at 94°C for 5 min followed by 30 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 2 min, followed by final extension step at 72°C for 5 min. then PCR products were separated on 1.5% agarose gel and visualized by ethidium bromide staining on UV transillumination

imaging system. the D allele sizes were 190 bp while the I allele 490 bp also found other I allele 550 bp.

Statistical Analysis

Mega stat software used to statistically analyzed to obtained mean \pm standard deviation (SD), the odds ratio (OR) and $P < 0.0001$ were considered significant.

Result

Detection of I/D polymorphism for the ACE gene

1.5% Agarose gel stained with ethidium bromide illustrating different band sizes in the patients group for ACE I/D polymorphism in Figure 1, the 490 bp band indicating II genotype, 190 bp indicating DD genotype, and both 490,190 bp indicating ID genotype. also appeared other insertion allele the 550 bp with ID genotype We referred to her with I*D code.

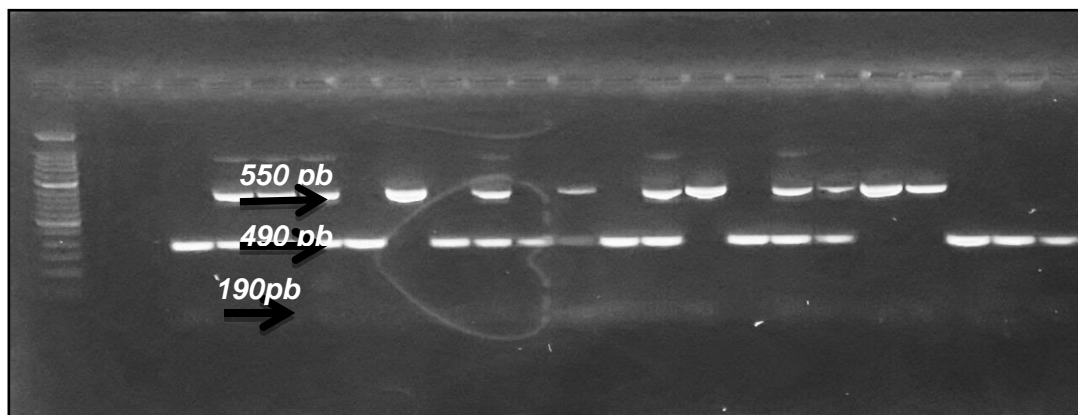


Figure 1. Electrophoresis of ACE (I/D) polymorphism in a 1.5% agarose gel. The PCR products of patient were illustrated under UV light

Table 1
Comparison of different ACE gene polymorphism

		patients n=70	Control n=30	OR(95 % CI)	
DD		(24) 34.3%	(4) 13.3%	3.3913(1.0603 to 10.8466)	0.0395*
II		(18) 25.7%	(16) 53.3%	0.3029(0.2776 to 1.4886)	0.3024ns
ID		(4) 5.7%	(10) 33.4%	0.1429(0.0402 to 0.5072)	0.0026**
I*D		(24) 34.3%	(0) 0%	32.1398(1.8834 to 548.4492)	0.0165*
Allele frequency	D	76(52.3%)	18(30%)	2.7708 (1.4544 to 5.2789)	0.0019**
	I	64(47.7%)	42(70%)	0.3609(0.1894 to 0.6876)	
*(P<0.05): significant , **or*** (P<0.05) higher significant					

Table 2
Risk Relative risk of different ACE gene polymorphism

	patients n=70	Control n=30	Relative risk (95 % CI)	NNT (95 % CI)	
DD	(24) 34.3%	(4) 13.3%	1.3416 (1.0657 to 1.6890)	4.582(43.783 (Harm) to 2.417 (Harm))	0.0124*
II	(18) 25.7%	(16) 53.3%	0.6538 (0.4590 to 0.9315)	3.667 (2.188 (Benefit) to 40.087 (Benefit))	0.0519 ns
ID	(4) 5.7%	(10) 33.4%	0. 3878 (0.1676 to 0.0269)	2.217 (1.422 (Benefit) to 5.023 (Benefit))	0.0269*
I*D	(24) 34.3%	(0) 0%	1.5556 (1.2797 to 1.8909)	2.800 (6.044 (Harm) to 1.822 (Harm))	< 0.0001** *
*(P<0.05): significant , **or*** (P<0.05) higher significant				NNT=	number needed to treat

Based on the electrophoresis method of genotyping figure 1 found three genotypes ACE gene were determined as numbers and percentages for healthy individuals (control) and patients. OR and 95% CI were calculated to find the significance differences between groups, Table1 Deletion (DD) in ACE gene was 13.3 % and 34.3% in control and patients respectively. The occurrence of deleted allele in patients more than in control with 95% CI between 1.0603 to 10.8466. Insertion (II) in ACE gene was 25.7 % and 53.3% in control and patients respectively. The occurrence of Insertion gene in patients was more than in control with 95% CI between 0.2776 to 1.4886. both Deletion and Insertion together (ID) in ACE gene was 33.4% and 5.7% in control and patients respectively. The occurrence of ID together in control was more than in patients with 95% CI between 0.0402 to 0.5072. And in my study I were found a mutation at 550pb (I*D) in ACE gene was 0% and 34.3% in control and patients respectively. The occurrence of I*D in patients only with 95% CI between 1.8834 to 548.4492.

Discussion

Our results indicated that DD allele was associated with CVDs comparing with control. It was clear from higher OR, that presence of DD allele was associated with the rise in the susceptibility to CVDs. This finding was supported by the results of Duque *et al.*, (2016) and agreed with Malueka *et al.*, (2018) who suggested Higher serum levels of ACE and angiotensin II in patients with DD allele can be related to worse outcome for those patients. But Shafiee *et al.*, (2010) conflict this result he was found in his study showed that DD genotype does not increase the CAD susceptibility in the studied Iranian population and may not be as a risk factor.

Also our result indicated that ID, II allele was not associated with CVDs comparing with control. This result also was found by Kato *et al.*, (2010) he was mention the ACE ID , II genotype was not a predictor of CVD, nor did it modify the response to antihypertensive treatment but he also said the DD allele has functioned as a risk factor for CVD in Japan , he was Explain confirmed this risk, Subjects with the DD genotype exhibited higher tissue ACE activity and

increased ACE expression in the plaque of acute coronary syndrome, observations that may explain why hypertensive patients with the DD genotype have a higher incidence of cardiovascular disease. These results were similar to those of our previous study. These results were similar to those of our previous study suggest that D/D genotype of ACE gene associated with susceptibility to diabetes and DN than I/I genotype also we found there is relation between level of serum ACE in D/D genotype carriers (Al-Nahi & Al-Barqaawee, 2019) . In my current study was found the insertion allele at 550 pb , I think it implicated in CVDs comparing with control. It was clear from higher OR, I did not find studies indicating signs of this mutation, perhaps it was only among Najaf patients, so please study it in future researches.

Conclusion

II genotype significant decreased in CVDs patients compared to control. Our results show insertion mutation at 550bp only in CVDs patients, this mutation not show in other research.

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