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Role of Angiotensin-Converting Enzyme (ACE) gene polymorphism and ACE activity in predicting outcome after acute myocardial infarction

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ABSTRACT

Background: The Ace polymorphism had shown association with ACE activity, premature atherosclerosis, myocardial infarction, LV dysfunction, LV remodelling, severity and extent of CAD and mortality after MI. Though ACE I/D polymorphism has been reported to be associated with various cardiovascular diseases it remained a controversial risk factor and studies have presented conflicting results. This study was designed to determine the association between ACE) gene insertion/deletion (I/D) polymorphism, ACE activity and acute STEMI in Indian population and to determine its influence on outcome after acute MI.
Materials and methods: We investigated 934 patients diagnosed with acute STEMI who underwent thrombolysis. ACE I/D polymorphism was detected by polymerase chain reaction and ACE activity was measured in 615 patients.
Results: The prevalence of DD, ID, and II genotypes in our study group were 41.97%, 34.36%, and 23.66% respectively. The ACE polymorphism was not significantly associated with the type of myocardial infarction, the LV ejection fraction, the number of vessels diseased and patency of the vessel after thrombolysis. The polymorphism had no influence on in hospital mortality (P = 0.453). The ACE activity also showed no influence on in hospital mortality (P = 0.482). The age > 60 years, Male gender, occluded artery and severe LV dysfunction (LVEF < 35%) were predictors of in-hospital mortality on multivariate regression analysis.
Conclusion: There was no differences among ACE (I/D) polymorphism observed in STEMI population. Neither ACE I/D polymorphism nor ACE activity predicted in-hospital mortality inpatients admitted with acute STEMI.

1. Background:

Cardiovascular disease (CVD) is the number one cause of death in India and 10% of all deaths occurring due to CAD. [1] Patients in India who have acute coronary syndromes have a higher rate of STEMI than do patients in developed countries [2]. As much as 89% of the cases of all acute MI in Indians were identified with nine coronary risk factors like abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, low fruit and vegetable consumption, and lack of physical activity [3]. However, the variability in prevalence of CVD in different parts of the world suggest complex interaction between above mentioned risk factors and genetic factors.

Among the multiple genetic polymorphisms which play active role in the pathogenesis of hypertension and cardiovascular disease [4], probably the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism [5] is the most extensively studied.

A human angiotensin I-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism, due to I/D of a 287-base pair element in intron 16 of this gene, is associated with the development of AMI by modifying ACE activity and contributing to enhanced plaque vulnerability, ulceration and thrombosis [6,7]. The Ace polymorphism shown association with ACE activity [8–14], Premature atherosclerosis [15], myocardial infarction [6,7,12,16–29], LV dysfunction [30], LV remodelling [31–36], severity and extent of CAD [37] and mortality after MI [32,38,39,40]. Though ACE I/D

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polymorphism has been reported to be associated with various cardiovascular diseases it remained a controversial risk factor and studies have presented conflicting results [16,41].

There are limited studies on association of ACE polymorphism with coronary artery disease conducted in Indian population, but with conflicting results [11,25–28,42,43,44]. Moreover studies included small number of participants which may affect the results [40]. Serum ACE2 activity also correlates with development of cardiovascular diseases. It increases in hypertension and in systolic dysfunction, suggesting possible ACE2 metabolism plays a role in development of cardiovascular diseases [45]. The objective of this study is to explore the association between ACE gene polymorphism, ACE activity and acute STEMI and its influence on the in-hospital mortality in south Indian population.

2. Materials and methods

2.1. Study design

In this observational, single-center, prospective case control study conducted between January 1st 2017 and 30th June 2017. All consecutive 1000 patients older than 18 years who were admitted with diagnosis of STEMI were included. The study was approved by the institutional Ethics Committee. Informed consent for participation was obtained from all patients.

2.2. The study population:

A total of consecutive 1000 patients with diagnosis of ST elevation myocardial infarction (STEMI admitted to the coronary care unit of Sri Jayadeva Institute of Cardiovascular Sciences and Research were enrolled. The diagnosis of STEMI will be based on the third universal definition of MI [46]. The diagnosis of STEMI was based on symptoms consistent with MI in conjunction with appropriate changes on electrocardiography (ECG) (ST-segment elevation or new left bundle branch block (LBBB)) and elevation in the levels of markers of myocardial necrosis (troponin I). All patients were managed according to institutional standard STEMI protocol. Total 934 patients received thrombolysis using streptokinase and were included in the final analysis. Remaining 66 patients who didn't receive thrombolysis either due to delayed presentation or due to contraindication to thrombolysis and patients who underwent primary PCI were excluded from the final analysis. Patients with prior history of myocardial infarction, percutaneous coronary interventions or prior coronary artery bypass graft surgery or valvular heart disease were excluded. Patients who were taking ACE inhibitors/ ARB s were also excluded as it may affect ACE levels. All patients received standard dual antiplatelet agents, high dose statins, anticoagulation, ACE inhibitors/ angiotensin receptor blockers, diuretics and inotropic support where appropriate. Echocardiography was carried out during the index admission within 24 h and left ventricular ejection fraction was measured using bi-planar Simpson's method from apical two- and four chamber views. Coronary angiography was done in 513(54.9%) patients and 421(45.1%) didn't undergo coronary angiography as decided by treating cardiologist. Coronary artery disease was defined by angiographic criteria with $\geq$ 50% lumen narrowing.

3. Data collection and blood sampling

Baseline demographic data, history of conventional coronary risk factors like diabetes, hypertension, dyslipidaemia, and smoking were obtained. The diabetes was defined by American Diabetic Association diagnostic criteria [47] hypertension was defined according to the criteria outlined in the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [48]. Smoking was defined according to National Health Interview Survey definitions [49]. Dyslipidemia was defined as LDL $\geq$ 100 mg/dl or HDL < 40md/dl in males and <50 mg/dl in females.

Before starting thrombolysis 15ML of venous blood were taken from all subjects. Blood samples were collected in tubes containing ethylene diamine tetra-acetic acid (EDTA) and aprotinin and were separated by centrifugation at 4 °C for 10 min at 3000 rpm within one hour after collection. Then, the separated plasma was used to measure ACE activity, and leukocytes used for DNA extraction. These were separated and stored at − 80 °C until assayed.

3.1. Angiotensin converting enzyme (ACE) gene polymorphism

ACE I/D (Angiotensin converting enzyme - insertion/deletion, rs4646994) polymorphism was studied by direct PCR method using forward primer5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′GAT GTG GCC ATC ACA TTC GTC AG-3′ as reverse primer. Amplification reaction was performed using reaction mixture of 25 μl containing 50–100 ng DNA template, PCR buffer (1X), 1.5mMol $MgCl_2$, 200 μMol of each dNTPs, 10pmoles each primer, and 2.5U of Taq polymerase. DNA was amplified for 35cycles with denaturation at 94 °C/30sec, annealing at 58 °C/20 sec and extension at 72 °C/30sec using Eppendorf thermal cycle (Eppendorf India. Pvt. ltd). The PCR product was analyzed on 2.0% agarose gel electrophoresis and captured using gel documentation system (Bio-rad, USA). A single band of 190 bp was observed in *DD* genotype, 490 bp in *II* genotype, and both bands in heterozygous *ID* genotype (Fig. 1).

3.2. Serum angiotensin converting enzyme activity (ACE):

ACE activity in serum was measured spectrophotometrically using N-[3-(2-Furyl) acryloyl] - L -phenylalanyl-glycyl-glycine (FAPGG) as substrate according to Simonetta Ronca-Testoni method (Clin Chem 1986. 29/6, 1093-1096). The value ACE activity was expressed as U/L. ACE activity was estimated in 615 patients and the data was analysed separately to find association with ACE gene polymorphism and its influence on in-hospital mortality after STEMI.

3.3. Statistical analysis

Dichotomous variables are reported as numbers and proportions. Continuous variables are presented as mean ± standard deviation. Differences in baseline characteristics between different categories of ACE genotypes were assessed by the chi-square ($\chi 2$) test for categorical variables and the *t*-test for continuous parameters and ANOVA test for comparison of more than two groups of continuous variables.

Potential risk factors for in-hospital mortality were investigated first by univariate logistic regression analysis and later by multivariate logistic regression model with all significant variables to estimate odds ratios (ORs) and inclusive 95% confidence bounds. All tests were performed as 2-sided at significance level of P < 0.05. Statistical analyses were performed with SPSS version 24.0 (SPSS, Inc).

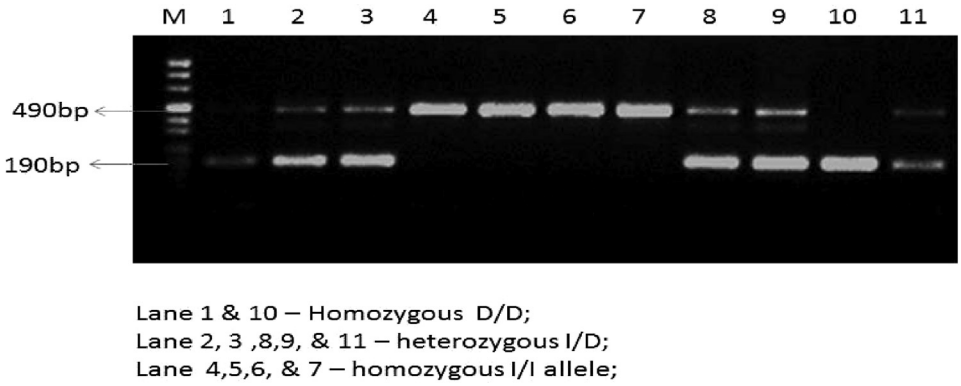

Fig. 1. Representative gel picture of *ACE I/D* polymorphism.

4. Results

4.1. General characteristics and genotype distribution

A total of 934 patients were included in the final analysis. Mean age was 54.09 ± 12.4 years (range, 22–90 years), and 11.90% of them were less than40 years of age. Majority of them (83.6%) were male. Demographic characteristics and the clinical background of patients are shown in Table 1. The mean LV ejection fraction was 44.6 ± 7.6%. The frequency of the angiotensin-converting enzyme gene genotypes was 42.00% for the DD, 34.40%, for the ID and 23.60% for the II genotype.

4.2. Association of the ACE I/D polymorphism with coronary artery risk factors and laboratory parameters in STEMI patients

There was no significant association noted between ACE I/D polymorphisms and STEMI-relevant traditional risk factors like age > 60 years, male gender, diabetes mellitus, hypertension, dyslipidemia and smoking (Table 2). There was no significant differences between mean haemoglobin, random blood sugar, serum creatinine, total cholesterol, LDL cholesterol, HDL cholesterol. There was no statistically significant difference in left ventricular ejection fraction among patients with the DD, ID or II genotypes (P = 0.252).

Table 1
Baseline characteristics of study population (n = 934).

Variables	Study Population (n = 934)
CAD risk factors	
Age	
Mean age (years)	54.09 ± 12.4
Age < 40 years	111(11.90%)
Male gender	781(83.6%)
Hypertension	336(36%)
Diabetes	383(41%)
Smoking	447(47.9%)
Dyslipidemia	888 (95.1%)
Blood Investigations	
Serum creatinine (mg/dl)	0.98 ± 0.38
Random blood sugar (mg/dl)	169.7 ± 90.1
Total cholesterol (mg/dl)	185.1 ± 61.3
Low density lipoprotein (mg/dl)	125.7 ± 64.0
High density lipoprotein (mg/dl)	34.6 ± 16.2
Hemoglobin (G/dl)	14.1 ± 2.2
2D Echocardiography	
LVEF (%)	44 ± 7.6

LVEF = Left ventricular ejection fraction.

4.3. ACE genotypes and their association with angiographic variables and in-hospital mortality

The ACE polymorphism was not significantly associated with the type of myocardial infarction (anterior wall MI vs. non anterior wall MI; P = 0.998), the number of vessels diseased (single vessel vs double vessel vs triple vessel vs left main coronary artery disease; P = 0.778) and patency of the culprit vessel after thrombolysis (P = 0.653). The polymorphism had no influence on in hospital mortality (P = 0.453). There were 88 in-hospital deaths with mortality rate of 9.42%. There was no difference in mortality among different genotype groups (DD 9.40%, ID 8.10%, and II 11.30%). The ACE polymorphism showed no influence on in hospital mortality (P = 0.168) (Table 3).

4.4. Influence of ACE polymorphism on ACE activity in STEMI

ACE activity was measured in 615 patients. The mean ACE activity was 232.40 ± 180.704 U/L reflecting a large amount of variation (range 4–2016) in ACE activity in patients presenting with acute STEMI. The ACE polymorphism was not significantly associated with the Ace activity as shown in Table 4. There was wide variation of ACE activity within the same genotypes.

4.5. Predictors of mortality after acute STEMI

There were 88 in-hospital deaths in study population (Mortality rate = 9.42%). Distribution of various demographic, clinical, biochemical, angiographic and ACE genotypes and ACE activity were studied between two groups (Table 5).

Subjects in mortality group were more frequently aged >60 years, male gender, smoker, diabetic and had dyslipidemia. Patients who had mortality were frequently noted to have occluded artery. Patients in mortality group more frequently had LVEF < 35%. There was no statistical difference in various ACE genotypes between mortality and survivors. Neither higher nor lower ACE activity were statistically different between the groups.

On univariate regression analysis (Table 6) between two groups age > 60 years, male gender, smoking, diabetes, RBS > 200, reduced HDL (<40 mg/dl in males and < 50 mg/dl in females), LVEF < 35% and occluded culprit coronary artery were noted as strong predictor of in-hospital mortality. Neither ACE genotype nor ACE activity were found to predict in-hospital mortality after STEMI.

On step-wise multivariate regression analysis (Table 7) age > 60 years (OR = 3.442; 95% CI: 1.072–11.047; P = 0.038), male gender(OR = 3.926; 95% CI: 1.030–14.973; P = 0.045), severe LV

Table 2
Baseline characteristics in different ACE gene polymorphism categories (n = 934).

Variables	Genotype DD (n-392; 42.0%)	Genotype ID (n-321; 34.4%)	Genotype II (n-221; 23.6%)	p-value
CAD risk factors				
Age				
Mean age (years)	53.7 ± 12.7	54.9 ± 12.3	53.6 ± 12.2	0.374
Age < 40 years	50 (12.8)	35 (10.9)	26 (11.8)	0.748
Age > 60 years	107 (27.3)	104 (32.4)	85 (29.4)	0.331
Male gender	3318 (81.7)	273 (85)	190 (86.0)	0.206
Hypertension	148 (37.8)	117 (36.4)	71 (32.1)	0.369
Diabetes	165 (42.09)	129(40.18)	89(40.27)	0.848
Smoking	211 (53.8)	170 (53)	106 (48)	0.354
Dyslipidemia	368 (93.9)	311 (96.9)	209 (94.9)	0.168
Blood reports:				
Hemoglobin (G/dl)	14.0 ± 2.1	14.2 ± 2.2	13.9 ± 2.3	0.372
Serum creatinine	0.98 ± 0.44	0.99 ± 0.37	0.95 ± 0.27	0.299
Total cholesterol (mg/dl)	188.5 ± 79.2	185.0 ± 45.3	178.9 ± 40.7	0.175
Low density lipoprotein (mg/dl)	128.6 ± 88.4	125.9 ± 37.7	120.1 ± 37.1	0.283
High density lipoprotein (mg/dl)	34.1 ± 9.9	34.6 ± 9.6	34.0 ± 10.3	0.738
2D Echocardiography				
LVEF (%)	44.2 ± 7.7	45.1 ± 7.3	44.4 ± 7.9	0.252

LVEF = Left ventricular ejection fraction.

Table 3
Clinical diagnosis and Angiographic characteristics in different ACE gene polymorphism categories (n = 934).

Variables	Genotype DD (n-392; 42.0%)	Genotype ID (n-321; 34.4%)	Genotype II (n-221; 23.6%)	p-value
Coronary artery disease (n-934)				
Anterior wall MI (509)	214 (54.6)	175 (54.5)	120 (54.3)	0.998
Inferior wall MI (425)	178 (45.4)	146 (45.5)	101 (45.7)	
Killip Class IV (63)	26(6.6)	21(6.5)	16(7.2)	
Coronary angiography(n-513)				
Single vessel disease (318)	120 (58.3)	120 (65.9)	78(62.4)	0.778
Double vessel disease (121)	52 (25.2)	40 (22)	29 (23.2)	
Triple vessel disease (67)	30 (14.6)	20 (11)	17 (13.6)	
Left main disease (7)	4 (1.9)	2 (1.1)	1 (0.8)	
Flowing culprit artery (438)	179 (40.9)	153 (34.9)	106(24.2)	0.653
Occluded culprit artery (76)	27 (13.1)	30 (16.5)	19 (15.2)	
Death (88)	**37 (9.4)**	**26 (8.1)**	**25 (11.3)**	**0.168**

Table 4
ACE activity across different ACE gene polymorphism categories (n = 615).

Variables	Genotype DD	Genotype ID	Genotype II	p-trend
ACE activity (U/L)	235.7 ± 179.7	213.5 ± 141.2	253.4 ± 225.1	0.110

dysfunction (LVEF < 35%) (OR = 28.255; 95% CI: 6.816–117.128; P< <0.001) and occluded culprit vessel (OR = 5.090; 95% CI: 1.522–17.019; P = 0.008) emerged as independent predictor of in-hospital mortality after STEMI.

5. Discussion

Though several conventional risk factors are proven beyond doubt in causation of coronary artery disease, the complex interplay of genetic factors aroused great curiosity. However, the contribution of ACE gene polymorphism as a risk factor of CAD was investigated in several studies but with conflicting results [19,32]. In our study no differences among ACE genotypes have been observed in a STEMI population. It also shows no significant association between conventional risk factors of CAD like advanced age, diabetes mellitus, hypertension, dyslipidemia or smoking and ACE genotype. Though ethnic background might to have influence on expression of different ACE genotypes, the studies conducted in Indian population inconsistent in confirming the association. Since majority of these tests involved smaller study cohorts, the results might have been driven only by statistical difference rather than clinical relevance. Our study involved large clinical cohort (n = 934) with clinical diagnosis of acute STEMI and it showed that there is no association between different ACE genotypes and STEMI.

Studies have shown association between ACE genotypes and diabetes mellitus and its complications [50–53], dyslipidemia [53] and hypertension [54–56]. But in our study cohort there was no association between ACE genotypes and coronary risk factors like diabetes, dyslipidemia and hypertension as observed in a study by Harrap SB et al [57].

Table 5
Comparative demographic, clinical, angiographic, biochemical parameters and genotype distribution among patients who survived and expired in hospital (n = 934).

Variables	Survival (n = 846)(%)	In hospital death (n = 88)(%)	p-value
Clinical parameters:			
Age > 60 years (276)	228 (27)	48 (54.5)	<0.001
Age < 40 years (111)	107 (12.6)	4 (4.5)	<0.001
Male gender	728 (86.1)	53 (60.2)	<0.001
Smoking	425 (50.2)	62(70.5)	<0.001
Hypertension	299 (35.3)	37(42.0)	0.243
Diabetes	331 (39.2)	52 (59.1)	<0.001
Laboratory Parameters			
Random Blood Sugar > 200 mg/dl	221 (26.1)	43 (48.9)	<0.001
Dyslipidemia			
LDL-c > 100 mg/dl	597 (70.7)	54 (51.4)	0.048
HDL-c < 40 mg/dl in males, <50 mg/dl in females	674 (79.7)	79 (89.8)	0.012
LV Ejection Fraction < 35%)	**30 (3.5)**	**33 (37.5)**	**<0.001**
Angiographic Findings:			
Single vessel disease	310 (62.1)	8 (57.1)	0.330
Double vessel disease	119 (23.8)	2 (14.3)	
Triple vessel disease	63 (12.6)	4 (28.6)	
Left main coronary artery	7 (1.4)	0 (0)	
Flowing culprit artery	429(86.0)	8 (57.0)	**0.014**
Occluded culprit artery	70 (14.0)	6 (43.0)	
ACE Genotypes			
Genotype DD	355 (42)	37 (42)	0.453
Genotype ID	295 (34.9)	26 (29.5)	
Genotype II	196 (23.2)	25 (28.4)	
ACE Activity	**234.2 ± 183.3**	**217.6 ± 158.4**	**0.482**
Median ACE Activity (≤194.4.U/L)	274 (49.9)	35 (53)	0.364
Median ACE Activity (>194.4.U/L)	275 (50.1)	31 (47)	
Mean ACE Activity (≤232.4.U/L)	333 (60.7)	43 (65.2)	0.285
Mean ACE Activity (>232.4.U/L)	216 (39.3)	23 (34.8)	

LDL = low density lipoprotein; HDL = high density lipoprotein.

Table 6
Stepwise univariate logistic regression analysis for predictors of mortality after STEMI (n = 934).

Variables	Odds Ratio	(95% CI)	p-value
Age (years)	1.07	1.045–1.087	<0.001
Age > 60 years	3.253	2.082–5.082	<0.001
Age < 40 years	3.041	1.093–8.460	0.033
Male gender	4.074	2.549–6.513	<0.001
Smoking	2.363	1.466–3.807	<0.001
Hypertension	1.327	0.850–2.073	<0.241
Diabetes	2.247	1.438–3.571	<0.001
LDL-c > 100 mg/dl	0.660	0.419–1.039	0.073
HDL-c < 40 mg/dl (males) <50 mg/dl (females)	2.243	1.103–4.567	0.026
LVEF < 35%)	24.043	13.203–43.787	<0.001
Single vessel disease			
Double vessel disease	0.651	1.36–3.111	0.591
Triple vessel disease + LMCA	2.214	0.649–7.560	0.205
Occluded culprit artery vs Flowing culprit artery	4.086	1.411–11.834	0.009
Genotype DD			
Genotype ID	0.84	0.500–4.439	0.531
Genotype II	1.224	0.716–2.093	0.461
ACE Activity U/I	0.999	0.998–1.001	0.480
Median ACE Activity (>194.4 .U/L)	0.882	0.529–1.472	0.632
Mean ACE Activity (>232.4 s.U/L)	0.825	0.483–1.407	0.479

LDL = low density lipoprotein; HDL = high density lipoprotein, LVEF = Left ventricular ejection fraction, LM = Left main coronary artery.

Table 7
Stepwise multivariate logistic regression analysis for predictors of mortality after STEMI (n = 934).

Variables	Odds Ratio	(95% CI)	p-value
Occluded culprit artery	5.090	1.522–17.019	0.008
Age > 60 years	3.442	1.072–11.047	0.038
Diabetes	1.556	0.492–4.923	0.452
Low HDL	1.566	0.374–6.555	0.539
Low LVEF	28.255	6.816–117.128	<0.001
Male	3.926	1.030–14.973	0.045
Smoking	1.558	0.368–6.600	0.547

HDL = high density lipoprotein, LVEF = Left ventricular ejection fraction.

One of the proposed mechanisms of influence of ACE gene polymorphism on CAD is through variability in ACE activity/ ACE level [8–14]. However, in our study a wide variation was noted in ACE activity among patients with STEMI. There was great individual variation even between those with the same genotype as observed in a study by Ljungberg L et al [58].

The in-hospital mortality in our study cohort was 9.42%, which probably due to all our study cohorts received pharmaco-invasive therapy and patients who underwent primary PCI were excluded. However, in 513 patients who underwent coronary angiography 437 (85.18%) patients had either flowing culprit vessel. However, this may be falsely overestimated as very sick patients and majority of patients who died didn't undergo angiography. In those 513 patients cohort who underwent angiography no association was observed between ACE genotypes and severity of coronary artery disease or culprit artery patency rate. This observation in contrast with a study by Dakik H.A et al. [37] which showed larger ischemic defects and occluded infarct related artery in DD genotypes.

In patients who had in-hospital mortality there was no significant association between different ACE genotypes and mortality. This observation correlated with previous studies [19,30,40] which investigated influence of ACE genotypes on mortality after AMI. However, these findings were in contrast to Palmer BR et al [32], Yoshida M et al [38] and Evans AE et al [39] which showed higher risk of mortality associated with DD/ID genotypes.

On univariate and multivariate logistic regression analysis age > 60 years, male gender, severe LV dysfunction and occluded culprit coronary artery on angiography were predictors of in-hospital mortality.

The wide variation and inconsistency in the observations could be due to statistical significance which primarily depends on the sample size as highlighted by a meta-analysis reported by Agerholm-Larsen et al [16] which showed difference in the results between the small and large studies. It could also be due to publication bias resulting from non-publication of small precise studies with negative results. Moreover case control studies investigating association of risk factor and disease are prone to spurious results and do not establish causality.

6. Study limitations

In our study the cohorts consisted only of acute STEMI patients receiving thrombolysis as first-line therapy. Hence the mortality rate may not represent patients who undergo primary PCI, which is standard of care in STEMI in developed countries. The majority of deaths occurred within 24 h after admission which could be due to cardiogenic shock or arrhythmias. Only 14 out of 88 patients who died underwent coronary angiography, hence coronary anatomy of these patients remained unknown. As the study analysed only in-hospital mortality the influence of ACE polymorphism on residual LV dysfunction and LV remodelling which could affect long mortality and morbidity remained unanswered. However, our study included only STEMI cohorts without control arm which may avoid spurious results of association.

7. Conclusion

There is no differences between different ACE polymorphism and risk of acute STEMI. Neither ACE genotype nor ACE activity predicted in-hospital mortality. Hence knowledge of ACE polymorphism and Ace activity in not useful in predicting STEMI or mortality after STEMI.

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References

[1] S. Guha, R. Sethi, S. Ray, V.K. Bahl, S. Shanmugasundaram, P. Kerkar, et al., Cardiological Society of India: position statement for the management of ST elevation myocardial infarction in India, Indian Heart J. 69 (2017) S63–S97.
[2] D. Xavier, P. Pais, P.J. Devereaux, C. Xie, D. Prabhakaran, K.S. Reddy, R. Gupta, P. Joshi, P. Kerkar, S. Thanikachalam, K.K. Haridas, T.M. Jaison, S. Naik, A.K. Maity, S. Yusuf, CREATE registry investigators. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. Lancet. 2008; 371:1435–1442.
[3] S. Yusuf, S. Hawken, S. Ounpuu, T. Dans, A. Avezum, F. Lanas, M. McQueen, A. Budaj, P. Pais, J. Varigos, L. Lisheng, Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study, Lancet 364 (2004) 937–952.
[4] T. Inagami, A memorial to Robert Tiegerstedt: the centennial of renin discovery, Hypertension 32 (1998) 953–957.
[5] D.K. Nagi, C.A. Foy, V. Mohamed-Ali, J.S. Yudkin, P.J. Grant, W.C. Knowler, Angiotensin-1-converting enzyme (ACE) gene polymorphism, plasma ACE levels, and their association with the metabolic syndrome and electrocardiographic coronary artery disease in Pima Indians, Metabolism 47 (1998) 622–626.
[6] F. Cambien, O. Costerousse, L. Tiret, O. Poirier, L. Lecerf, M.F. Gonzales, A. Evans, D. Arveiler, J.P. Cambou, G. Luc, R. Rakotovao, P. Ducimetiere, F. Soubrier, F. Alhenc-Gelas, Plasma level and gene polymorphism of angiotensin-converting enzyme in relation to myocardial infarction, Circulation 90 (1994) 669–676.
[7] Y. Chen, S. Dong, M. He, T. Qi, W. Zhu, Angiotensin-converting enzyme insertion/deletion polymorphism and risk of myocardial infarction in an updated meta-analysis based on 34993 participants, Gene 522 (2) (2013) 196–205.
[8] B. Rigart, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, F. Soubrier, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels, J. Clin. Invest. 86 (1990) 1343–1346.
[9] A.H. Danser, M.A. Schalekamp, W.A. Bax, A.M. van den Brink, P.R. Saxena, G.A. Riegger, H. Schunkert, Angiotensin-converting enzyme in the human heart: effect of the deletion/insertion polymorphism, Circulation 92 (1995) 1387–1388.
[10] S. Dai, M. Ding, N. Liang, Z. Li, D. Li, L. Guan, H. Liu, Associations of ACE I/D polymorphism with the levels of ACE, kallikrein, angiotensin II and interleukin-6 in STEMI patients, Sci. Rep. 9 (2019) 19719, https://doi.org/10.1038/s41598-019-56263-8.
[11] B. Pulla Reddy, B.M. Srikanth Babu, K. Venkata Karunakar, J. Yasovanthi, A. Munshi, P. Sampath Kumar, A. Sharath, A. Jyothy, Angiotensin-converting enzyme gene variant and its levels: risk factors for myocardial infarction in a South Indian population, Singapore Med. J. 51 (7) (2010) 576–581.
[12] S. Mehri, B. Baudin, S. Mahjoub, Angiotensin-converting enzyme insertion/deletion gene polymorphism in a Tunisian healthy and acute myocardial infarction population, Genet. Test. Mol. Biomarkers. 14 (1) (2010) 85–91.
[13] L. Tiret, B. Rigat, S. Visvikis, C. Breda, P. Corvol, F. Cambien, et al., Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels, Am. J. Hum. Genet. 51 (1992) 197–205.
[14] B. Agerholm-Larsen, A. Tybjærg-Hansen, P. Schnohr, B.G. Nordestgaard, ACE gene polymorphism explains 30–40% of variability in serum ACE activity in both women and men in the population at large: the Copenhagen City Heart Study, Atherosclerosis 147 (1999) 425–427.
[15] C. Sekuri, F.S. Cam, E. Ercan, I. Tengiz, A. Sagcan, E. Eser, A. Berdeli, M. Akin, Renin-angiotensin system gene polymorphisms and premature coronary heart disease, J. Renin Angiotensin Aldosterone Syst. 6 (2005) 38–42.
[16] Agerholm-Larsen, B. Nordestgaard, A. Tybjaerg-Hansen, ACE gene polymorphism in cardiovascular disease: meta analysis of small and large studies. Arterioscler Thromb Vasc Biol. 2000;20:484–492.
[17] S.H. Dai, J.F. Li, J.B. Feng, R.J. Li, C.B. Li, Z. Li, Y. Zhang, D.Q. Li, Association of serum levels of AngII, KLK1, and ACE/KLK1 polymorphisms with acute myocardial infarction induced by coronary artery stenosis. J Renin Angiotensin Aldosterone Syst. 2016; 21; 17(2):1470320316655037. doi: 10.1177/1470320316655037.
[18] J.A. Staessen, J.G. Wang, G. Ginocchio, V. Petrov, A.P. Saavedra, F. Soubrier, R. Vlietinck, R. Fagard, The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular-renal risk, J. Hypertens. 15 (1997) 1579–1592.
[19] N.J. Samani, J.R. Thompson, L. O'Toole, K. Channer, K.L. Woods, A metaanalysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction, Circulation 94 (1996) 708–712.
[20] M. Pfohl, A. Athanasiadis, M. Koch, P. Clemens, et al., Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene is associated with coronary artery plaque calcification as assessed by intravascular ultrasound, J. Am. Coll. Cardiol. 31 (1998) 987–991.
[21] A. Gardemann, M. Fink, J. Stricker, Q.D. Nguyen, et al., ACE I/D gene polymorphism: presence of the ACE D allele increases the risk of coronary artery disease in younger individuals, Atherosclerosis 139 (1998) 153–159.
[22] Cardoso R., Nogueira A., Salis L., Urmenyi T., Silva R., Moura-Neto R., Pereira B., Rondinelli E. and Silva N. (2008) "The association of ACE gene D/I polymorphism with cardiovascular risk factors in a population from Rio de Janeiro". Brazilian Journal of Medical and Biological Research, (41) 512-518.
[23] F. Cambien, O. Poirier, L. Lecerf, A. Evans, J.P. Cambou, D. Arveiler, et al., Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction, Nature 359 (1992) 641–644.
[24] D.R. Singer, C.G. Missouris, S. Jeffery, Angiotensin-converting enzyme gene polymorphism: what to do about all the confusion, Circulation 94 (1996) 236–239.
[25] Metta S, Uppala S, Basalingappa DR, Badeti SR, Mitta G, Mohanty S, Subhadra P and Hasan Q. Association of Angiotensin converting enzyme gene insertion/deletion polymorphism with risk of ischemic heart disease in a population of smokers in southern India. J Clin Diagn Res 2015; 9: GC01-04.
[26] M. Ganesan, S. Bhaskar, R. Mani, M.M. Idris, N. Khaja, S. Gulla, et al., The relationship of ACE and CETP gene polymorphisms with cardiovascular disease in a cohort of Asian Indian patients with and those without type 2 diabetes, J. Diabetes Complications 25 (2011) 303–308.
[27] D. Mani, R. Chinniah, P. Ravi, K. Swaminathan, R.A. Janarthanan, M. Vijayan, et al., Predisposition of angiotensin-converting enzyme deletion/deletion genotype to coronary artery disease with type 2 diabetes mellitus in South India, Indian J. Endocr. Metab. 21 (2017) 882–885.
[28] G.K. Bhatti, J.S. Bhatti, R. Vijayvergiya, B. Singh, Implications of ACE (I/D) gene variants to the genetic susceptibility of coronary artery disease in Asian Indians, Indian J. Clin. Biochem. 32 (2017) 163–170.
[29] L. Tiret, A. Bonnardeaux, O. Poirier, S. Ricard, P. Marques-Vidal, A. Evans, D. Arveiler, et al., Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction, Lancet 344 (1994) 910–913.
[30] J. Parenica, M.P. Goldbergova, P. Kala, J. Jarkovsky, M. Poloczek, J. Manousek, et al., ACE gene insertion/deletion polymorphism has a mild influence on the acute development of left ventricular dysfunction in patients with ST elevation myocardial infarction treated with primary PCI. BMC Cardiovasc Disord. 2010, 10: 60-10.1186/1471-2261-10-60.
[31] N. Ohmichi, N. Iwai, Y. Nakamura, M. Kinoshita, The genotype of the angiotensin-converting enzyme gene and global left ventricular dysfunction after myocardial infarction, Am. J. Cardiol. 76 (1995) 326–329.
[32] B.R. Palmer, A.P. Pilbrow, T.G. Yandle, C.M. Frampton, A.M. Richards, M.G. Nicholls, et al., Angiotensin-converting enzyme gene polymorphism interacts with left ventricular ejection fraction and brain natriuretic peptide levels to predict mortality after myocardial infarction, J. Am. Coll. Cardiol. 41 (2003) 729–736.
[33] M.S. Ulgen, O. Ozturk, S. Alan, M. Kayrak, Y. Turan, S. Tekes, et al., The relationship between angiotensin-converting enzyme (insertion/deletion) gene polymorphism and left ventricular remodeling in acute myocardial infarction, Coron. Artery Dis. 18 (2007).
[34] J. Nagashima, H. Musha, T. So, T. Kunishima, S. Nobuoka, M. Murayama, Effect of angiotensin-converting enzyme gene polymorphism on left ventricular remodeling after anteroseptal infarction, Clin. Cardiol. 22 (1999) 587–590.
[35] N. Ohmichi, N. Iwai, K. Maeda, H. Shimoike, Y. Nakamura, M. Izumi, et al., Genetic basis of left ventricular remodeling after myocardial infarction, Int. J. Cardiol. 53 (1996) 265–272.
[36] Y.M. Pinto, W.H. Van Gilst, J. Herre Kingma, H. Schunkert, Deletion-type allele of the angiotensin-converting enzyme gene is associated with progressive ventricular dilation after anterior myocardial infarction, J. Am. Coll. Cardiol. 25 (1995) 1622–1626.
[37] H.A. Dakik, J.J. Mahmarian, M.S. Verani, J.A. Farmer, G. Zhao, A.J. Marian, Association of angiotensin I–converting enzyme gene polymorphism with myocardial ischemia and patency of infarct-related artery in patients with acute myocardial infarction, J. Am. Coll Cardiol. 29 (1997) 1468–1473.
[38] M. Yoshida, N. Iwai, N. Ohmichi, M. Izumi, Y. Nakamura, M. Kinoshita, D allele of the angiotensin-converting enzyme gene is a risk factor for secondary cardiac events after myocardial infarction, Int. J. Cardiol. 70 (1999) 119–125.
[39] A.E. Evans, O. Poirier, F. Kee, L. Lecerf, E. McCrum, T. Falconer, J. Crane, D.F. O'Rourke, F. Cambien, Polymorphism of the angiotensin-convertingenzyme gene in subjects who die from coronary heart-disease, Q J Med. 87 (1994) 211–214.
[40] G.K. Andrikopoulos, S.M. Tzeis, E.W. Needham, D.J. Richter, M.N. Zairis, E.J. Gialafos, F.G. Kardaras, S.G. Foussas, C.I. Stefanadis, P.K. Toutouzas, R. Mattu R, 2005. Lack of Association between Common Polymorphisms in Genes of the Renin-Angiotensin System and Mortality after Myocardial Infarction. Cardiology 2005; 103:185–188.

[41] E. Zintzaras, G. Raman, G. Kitsios, J. Lau, Angiotensin-converting enzyme insertion/deletion gene polymorphic variant as a marker of coronary artery disease: a meta-analysis, Arch. Intern. Med. 168 (2008) 1077–1089, https://doi.org/10.1001/archinte.168.10.1077.
[42] S. Dhar, S. Ray, A. Dutta, B. Sengupta, S. Chakrabarti, Polymorphism of ACE gene as the genetic predisposition of coronary artery disease in Eastern India, Indian Heart J 64 (2012) 576–581, https://doi.org/10.1016/j.ihj.2012.08.005.
[43] U. Pandey, R. Kumari, B. Nath, Association of angiotensin- converting enzyme, methylene tetrahydrofolate reductase and paraoxonase gene polymorphism and coronary artery disease in an Indian population, Cardiol J. 2011;18:385–394.
[44] R.C. Sobti, N. Maithil, H. Thakur, Y. Sharma, K.K. Talwar, Association of ACE and FACTOR VII gene variability with the risk of coronary heart disease in north Indian population, Mol. Cell Biochem. 341 (1–2) (2010) 87–98.
[45] K. Uri, M. Fagyas, A. Kertesz, A. Borbely, C. Jenei, O. Bene, Z. Csanádi, W.J. Paulus, I. Édes, Z. Papp, et al., Circulating ACE2 activity correlates with cardiovascular disease development, J. Renin Angiotensin Aldosterone Syst. 17 (2016), 1470320316668435.
[46] K. Thygesen, J.S. Alpert, A.S. Jaffe, M.L. Simoons, B.R. Chaitman, H.D. White. the Writing Group on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. Third universal definition of myocardial infarction. Circulation. 2012; 126:2020 –2035.
[47] Diagnosis and classification of diabetes mellitus, Diabetes Care 34 (suppl 1) (2011) S62–S69.
[48] C. Lenfant, A.V. Chobanian, D.W. Jones, E.J. Roccella, Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7): resetting the hypertension sails. Hypertension. 2003;41(6):1178–1179.
[49] D.E. Nelson, E. Powell-Griner, M. Town, M.G. Kovar, A comparison of national estimates from the National Health Interview Survey and the Behavioral Risk Factor Surveillance System, Am. J. Public Health. 93 (8) (2003) 1335–1341.
[50] Y. Wang, W. Peng, X. Zhang, H. Qiao, L. Wang, Z. Xu, C. Wu, The association of ACE gene polymorphism with diabetic kidney disease and renoprotective efficacy of valsartan, J. Renin Angiotensin Aldosterone Syst. 17 (2016).
[51] S. Schmidt, N. Schone, E. Ritz Association of ACE gene polymorphism and diabetic nephropathy? The Diabetic Nephropathy Study Group Kidney Int, 47 (1995), pp. 1176-1181.
[52] S.K. Ha, ACE insertion/deletion polymorphism and diabetic nephropathy: clinical implications of genetic information, J. Diabetes Res. 2014 (2014) 846068.
[53] U.N. Mahwish, K.C. Ponnaluri, B. Heera, S.R. Alavala, K.R. Devi, S.B. Raju, G.S. Latha, P. Jahan, Link between ACE I/D gene polymorphism and dyslipidemia in diabetic nephropathy: A case-control study from Hyderabad, India, Indian J. Nephrol. 30 (2020) 77–84.
[54] P.K. Borah, P. Shankarishan, N.C. Hazarika, J. Mahanta, Hypertension subtypes and angiotensin converting enzyme (ACE) gene polymorphism in Indian population, J. Assoc. Physicians India 60 (2012) 11–17.
[55] K. Siddharth, S. Kapur, C.V. Ram, Angiotensin converting enzyme gene polymorphism and hypertension: no ace yet in the pack of cards, J. Assoc. Phys. India 60 (2012) 9–10.
[56] R. Krishnana, D. Sekara, S. Karunanithyb, Subramaniumb S Association of angiotensin converting enzyme gene insertion/deletion polymorphism with essential hypertension in south Indian population, Genes Diseases 3 (2) (2016) 159–163.
[57] S.B. Harrap, C. Tzourio, F. Cambien, O. Poirier, S. Raoux, J. Chalmers, N. Chapman, S. Colman, S. Leguennec, S. MacMahon, B. Neal, T. Ohkubo, M. Woodward, PROGRESS Collaborative Group. The ACE gene I/D polymorphism is not associated with the blood pressure and cardiovascular benefits of ACE inhibition. Hypertension 42 (2003) 297–303.
[58] L. Ljungberg, U. Alehagen, T. Lanne, H. Bjorck, R. De Basso, U. Dahlstrom, et al., The association between circulating angiotensin- converting enzyme and cardiovascular risk in the elderly: a cross-sectional study, J. Renin-Angiotensin-Aldosterone Syst JRAAS. 12(3) (2011) 281–9.