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 in patients admitted with acute STEMI.

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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
ACE 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 inhospital 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 ≥ 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 theJoint 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]. Dyslipidaemia was defined as LDL ≥ 100 mg/dl or HDL < 40mg/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 GAC ACC ACT CCC ATC CTT TCT-3’ and5’GAT GTG GCC ATC ACA TTC GTG AC-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 MgCl2, 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 (χ2) test for categorical variables and the t-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).
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 than 40 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).

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...
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 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 (42.0)                  | 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:

| Variables                  | Genotype DD  (n=392; 42.0%) | Genotype ID  (n=321; 34.4%) | Genotype II  (n=221; 23.6%) | p-value |
|----------------------------|------------------------------|-----------------------------|-----------------------------|---------|
| 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)  | 185.5 ± 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

| Variables                  | Genotype DD (n=392; 42.0%) | Genotype ID (n=321; 34.4%) | Genotype II (n=221; 23.6%) | p-value |
|----------------------------|------------------------------|-----------------------------|-----------------------------|---------|
| LVEF (%)                   | 44.2 ± 7.7                   | 45.1 ± 7.3                  | 44.4 ± 7.9                  | 0.252   |

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 (61)       | 266(66)                      | 21(6.5)                     | 167(2.2)                    |         |
| 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 (n=415) | Genotype ID (n=312) | Genotype II (n=188) | p-trend |
|----------------------------|---------------------|---------------------|---------------------|---------|
| ACE activity (U/L)         | 235.7 ± 179.7       | 213.5 ± 141.2       | 253.4 ± 225.1       | 0.110   |

LVEF = Left ventricular ejection fraction.
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)         | 2.243 (1.103–4.567) | 0.026   |
| LV Ejection Fraction < 35%      | 24.043 (13.203–43.787) | <0.001  |

| Variables                        | Odds Ratio (95% CI) | p-value |
|----------------------------------|---------------------|---------|
| Age > 60 years                   | 3.442 (1.072–11.047) | 0.038   |
| Diabetes                         | 1.556 (0.492–4.923) | 0.452   |
| Low HDL                          | 1.566 (0.374–6.535) | 0.539   |
| Low LV EEF                       | 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   |

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 pharma-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 inhospital 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 spuriously 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 unanswer. 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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