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Lack of Association between Angiotensin Converting Enzyme (ACE) Insertion/Deletion Polymorphism (rs4340) and Risk of Myocardial Infarction

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ABSTRACT

Background: The enzyme 'Angiotensin-Converting Enzyme (ACE)' modulates the fibrinolytic balance by converting angiotensin I into angiotensin II, which increase the activity of plasminogen activator inhibitor-1 (PAI-1). Besides, it degrades bradykinin, which an important mediator of the tissue-type plasminogen activator (t-PA). Thus, decreases fibrinolysis and may result in increased thrombotic risk. An insertion/deletion (I/D) polymorphism in the ACE gene has been identified and correlated with the enzyme serum levels. Studies concerning the association of this polymorphism and the risk of cardiovascular diseases in different populations showed inconsistent results. *Objective:* This study aimed to investigate the association between ACE I/D polymorphism and the risk of myocardial infarction among the Sudanese population. *Materials and methods:* This is a case-control study, in which blood samples were collected from a total of 100 Sudanese subjects, 50 patients with myocardial infarction and 50 age- and sex-matched healthy volunteers as a control group. Genomic DNA was extracted by guanidine chloride method and ACE I/D polymorphism was analysed by Allele-Specific Polymerase Chain Reaction (AS-PCR). Data of this study was collected using a structured interview questionnaire and analysed by statistical package for social sciences (SPSS). *Results:* The frequency D/D Genotype was higher in the control group than patients (72% vs 60%), while of the I/D genotype was higher in the patients than controls (40% vs 28%); the II genotype was absent in both study groups. The frequency of D allele was 0.80 in patients with MI and 0.86 in the control group, while the frequency of I Allele was 0.20 in the patients with MI and 0.14 in the control group. No statistically significant association was reported between ACE I/D polymorphic genotypes and MI (*P.value*= 0.29). *Conclusion:* ACE I/D polymorphism is not associated with the risk of MI among the Sudanese population.

Keywords: Myocardial infarction; Angiotensin converting enzyme; Insertion/deletion polymorphism

1. INTRODUCTION

Myocardial infarction (MI), also called a heart attack, results from reduction or blockage of blood supply to a part of the heart, and lead to damage to the heart muscle (Zhang *et al.,* 2019). It usually results from the interaction of genetic and environmental factors (Das *et al.,* 2011).

Risk factors for MI are broadly divided into modifiable and non-modifiable. Modifiable risk factors include smoking, Diabetes mellitus (DM), obesity, hypertension (HTN), inherited lipoprotein disorders, dyslipidaemia, sedentary lifestyle, stress, poor oral hygiene, and type of personality (Wilson *et al.,* 1998; Yusuf *et al.,* 2005), while non-modifiable risk factors include age, sex, and family history of coronary heart disease. However, about 50% to 60% of the major risk factors for acute MI are determined by heritability (Dai *et al.,* 2019).

The advancement in molecular genetic methods has led to the identification of many genetic variants that are associated with an increased risk of MI (Erdmann *et al.,* 2010).

Angiotensin-converting enzyme (ACE) is a metalloproteinase converts angiotensin-I to the potent vasoconstrictor angiotensin-II, which downregulates fibrinolysis by stimulating the production of plasminogen activator inhibitor type I (PAI-1). Also, ACE degrades bradykinin- an important mediator of the tissue-type plasminogen activator (t-PA)- which contributes to hypofibrinolysis (Urata *et al.,* 1990; Vaughan, 1997).

A polymorphism located on chromosome 17q23, in intron 16 of the ACE gene was identified in 1990. It consists of either the presence (Insertion- I allele) or absence (Deletion- D allele) of a 287 bp Alu repeat sequence resulting in three genotypes (D/D, I/D and I/I). This polymorphism is correlated with serum levels of ACE; the D/D genotype is associated with the highest plasma levels of ACE, I/I genotype associated with the lowest level, and I/D genotype associated with intermediate levels (Pfohl *et al.,* 1999; Zhong *et al.,* 2012). Therefore, the polymorphism is suggested to be associated with changes in the fibrinolytic system, as high levels of PAI-1 are also related to D allele (de Carvalho *et al.,* 2016); hence it could be associated with increased thrombotic risk.

Several studies have correlated ACE I/D polymorphism with cardiovascular complications (Munshi *et al.,* 2008; Jamil *et al.,* 2009) but studies concerning with the association of the polymorphism with MI risk among different populations showed controversial results, while some of them reported a significant association, others didn't (Cambien *et al.,* 1992; Arbustini *et al.,* 1995;Lindpaintner *et al.,* 1995; Pfohl *et al.,* 1999; Sayed-Tabatabaei *et al.,* 2005; Pestana *et al.,* 2010; Devi *et al.,* 2012; Semmame *et al.,* 2015; You & Shen, 2016).

Aim of the study

This study aimed to explore whether there is an association between ACE I/D polymorphism and susceptibility to MI among the Sudanese population or not.

2. METHODOLOGY

This is an analytical case-control study, conducted at Al-Shaab teaching hospital, Khartoum, Sudan, in the period from December 2016 to January 2018. In which, 50 patients with a confirmed diagnosis of MI (based on results of troponin and echocardiography) and 50 age- and sex-matched apparently healthy volunteers- as a control group- were enrolled.

Venous blood samples were collected from all participants in Ethylenediaminetetraacetic acid (EDTA). Genomic DNA was isolated from peripheral leucocytes using conventional guanidine chloride method and stored at -30°C until PCR is carried out.

ACE I/D polymorphism was analysed by Allele-Specific Polymerase Chain Reaction (AS-PCR). A reaction mixture of 30µl was prepared for each sample, containing 2µl genomic DNA, 1µL of each of the forward (5'CTGGAGACCACTCCCATCCTTTCT-3'), reverse (5'GATGTGGCCATCACATTCGTCAGAT-3'), and internal (5'TGGGATTACAGGCG TGATACAG-3') primes (MACROGEN, KOREA), 5µL master mix (MAXIME PCR PRE-MIX KIT (I-TAQ), INTRON, KOREA), and 20µL sterile distilled water. The amplification program consists of initial denaturation at 94ºC for 3 minutes; then 30 cycles [each consists of denaturation at 94ºC for 1 minute, annealing at 52ºC for 1 min, and extension at 72ºC for 1 minute], and a final extension at 72ºC for 5 minutes.

PCR products were separated on 2% agarose gel electrophoresis containing ethidium bromide with a 50 bp DNA ladder (SOLIS BIODYEN, ESTONIA) run with each batch of samples and the size of the fragments was determined under UV transilluminator (SYNGENE, JAPAN).

A PCR fragment of 190 bp indicates the presence of D allele, while a fragment of 490 bp indicates the presence of I allele (Figure 1).

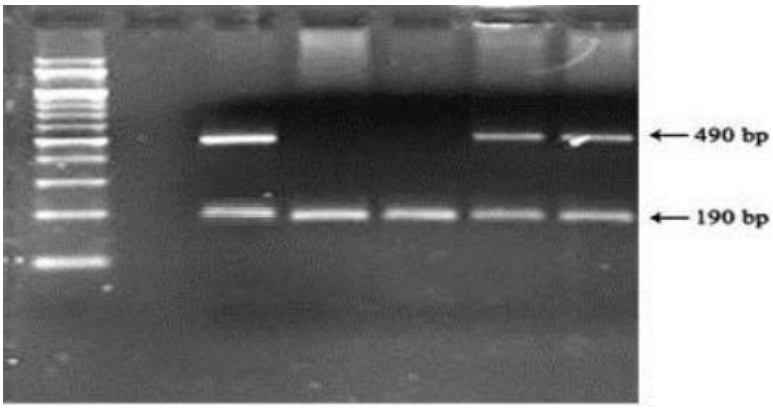

Figure 1 Amplified fragments of ACE gene

Patient's data were collected using a structured interview questionnaire and analysed by statistical package for social science (SPSS), version 21. The qualitative data were presented as frequency and percentage. Quantitative data were presented as Mean±SD. Association between qualitative variable was tested by Chi-square (X^2) and Fisher's exact tests. Multivariate logistic regression analysis was used for the examination of interaction between the polymorphism and MI risk factors. The allele frequency and Hardy Weinberg Equilibrium (HWE) were calculated using the conventional formulas.

The study was approved by the scientific research committee, faculty of medical laboratory sciences, Al Neelain University Khartoum, Sudan (#008-8/2017) and informed consent was obtained from each participant before sample collection. Patients' data was kept confidentially and only used for the purpose of the study.

3. RESULTS

A total of 100 subjects were enrolled in this study, 50 patients with MI and 50 age- and sex-matched healthy volunteers as a control group. 40 (80%) of the patients had- at least- one known risk factor for MI, 21(42%) of them had DM, 12(24%) had HTN, 11(22%) were smokers and 3(6%) had a family history of MI.

The frequency of homozygous D/D genotype was higher in healthy controls (72%) than patients (60%), while the frequency of the heterozygous I/D genotype was higher in the patients (40%) than controls (28%); the I/I genotype was absent in both study groups (Figure 3). However, there was no statistically significant association between ACE I/D polymorphic genotypes and MI (*P.value*= 0.29).

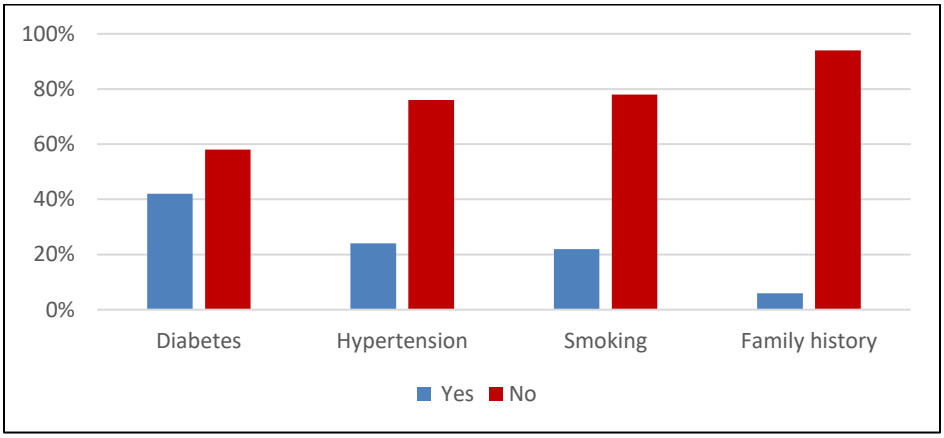

Figure 2 Frequency of MI risk factor among patients' group

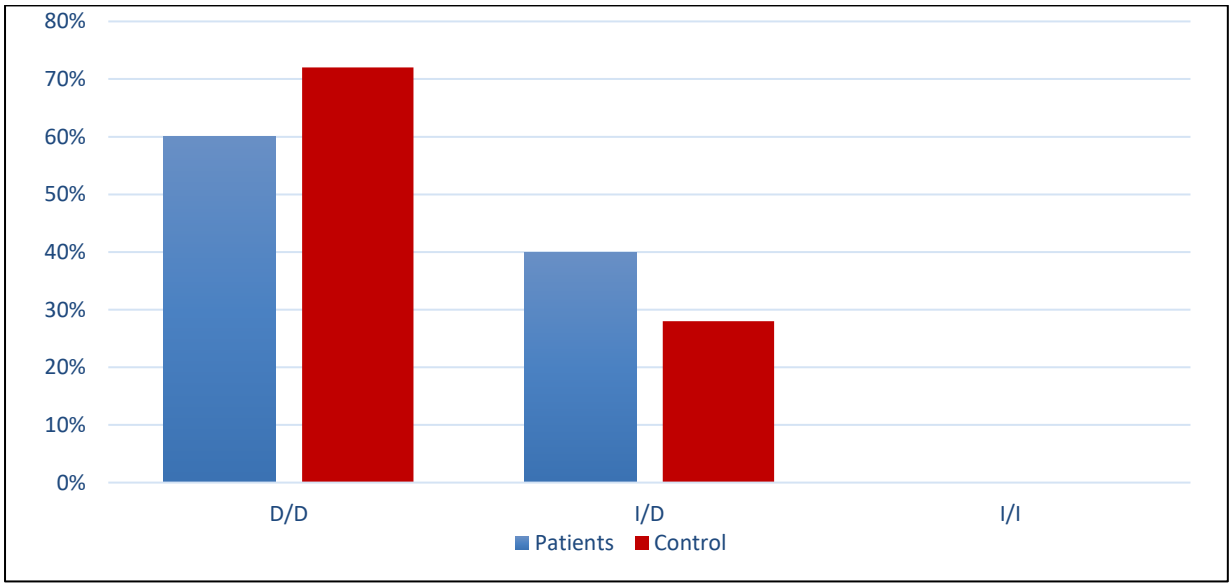

Figure 3 Frequency of ACE I/D polymorphic genotypes

There was no statistically significant difference in the mean age of patients with D/D genotype compared to those with I/D genotype (Mean±SD: 51±12.9 and 51.5±15.0 respectively, *P.value*= 0.86). The results of the current study showed no statistically significant association between the genotypes and patients' gender (*P.value*= 0.06). The Multivariate regression analysis revealed no interaction between ACE I/D polymorphic variants and known MI risk factors (Table 2).

Table 2 Interaction between ACE I/D polymorphism and known MI risk factors

Risk factor	Odd ratio	95% confidence intervals		*P.value
		Lower	Upper	
Diabetes mellitus	1.467	0.383	5.610	0.576
Hypertension	2.515	0.530	11.940	0.246
Smoking	0.857	0.189	3.880	0.841
Family history	0.333	0.026	4.193	0.395

**P.value is significant at < 0.05*

The frequency of D allele was 0.80 in the patients with MI and 0.86 in the control group, while the frequency of I Allele was 0.20 in the patients with MI and 0.14 in the control group. No deviation from HWE observed when tested for the control group (X^2= 1.33, df=1, *P.value*=0.25).

4. DISCUSSION

The present study was conducted to investigate the association between ACE I/D Polymorphism and the risk of MI among the Sudanese population. The results showed that D/D genotype was more frequent in healthy controls than patients, while the I/D genotype was more frequent in patients than controls; the I/I genotype was absent in both study groups. There was no statistically significant association between ACE I/D polymorphism and MI. Although the frequency of the D allele was higher in the control group, while the frequency of the I allele was higher in the patients, no deviation from HWE was observed when tested for the control group. Many studies concerning with ACE I/D polymorphism and risk of MI in different populations showed inconsistent results. Two studies in Germany by Winkelmann *et al.,* (1996) and Pfohl *et al.,* (1999) reported no association between ACE I/D gene polymorphism and the prevalence of MI. A prospective case-control study by Lindpaintner *et al.,* (1995) including a white population of male US physicians failed to detect an association between the D allele of ACE and incidence of MI. Sayed-Tabatabaei *et al.,* (2005) conducted a population-based cohort study in the Netherland and concluded that ACE I/D polymorphism is not an independent risk factor for MI. Also, a study in Venezuela by Pestana *et al.,* (2010) found no statistically significant association between ACE genotype and MI. Furthermore, two meta-analysis evaluated the correlation between ACE I/D polymorphism and MI risk, included a large number of studies, concluded that ACE I/D polymorphism is not associated with increased risk of MI (Agerholm-Larsen *et al.,* 2000; You & Shen, 2016).

On the other hand, there are many studies disagree with our finding, all of them reported a significant association between D/D genotype and risk of MI (Cambien *et al.,* 1992; Arbustini *et al.,* 1996;Devi *et al.,* 2012; Semmame *et al.,* 2015).

The result of the present study showed no statistically significant difference in the mean age of patients with different ACE I/D polymorphic variants, this indicating that ACE I/D polymorphism does not affect the age of incidence of MI. Also, no statistically significant association was found between gender and ACE genotypes. Similar results were reported by Semmame *et al.,* (2015) & Pfohl *et al.,* (1999), both reported no association between age, gender and ACE genotypes in patients with MI. In contrast. Hmimech *et al.,* (2017) suggesting that the I/D ACE polymorphism may be associated with MI occurrence among younger patients (< 45 years of age).

In the present study, no interaction between ACE I/D polymorphism and the conventional MI risk factors (DM, Family history, HTN, and smoking) was reported. This finding wasin agreement with a study by Chen *et al.,* (2012) who also reported no significant association between ACE I/D polymorphisms and any other risk factor among patients with acute coronary syndrome. Also, Semmame *et al.,* (2015) found no association between ACE genotype and diabetes and hypertension in MI patients. Our result disagrees with the finding of Semmame *et al.,* (2015) and Sayed-Tabatabaei *et al.,* (2005) both reported an interaction between the polymorphism and smoking. Variations of results regarding the association of the polymorphism and MI in different populations can be interpreted by what is reported by Takahashi *et al.,* (1995) that, differences in ACE genotypes are due to importance of ethnic consideration in gene-association.

5. CONCLUSION

The homozygous D/D genotype of ACE gene was more frequent in the healthy controls, while the heterozygous I/D genotype was more frequent in MI patients; the homozygous I/I genotype was absent in both study groups. However, There was no statistically significant association between ACE I/D polymorphism and risk of MI among the Sudanese population.

Author contributions

Safa A.M.I. collected samples and data, carryout practical work, analysed data, and prepare the draft. Elshazali W.A. state the study design, supervised the practical work and solve problems, interpreted the findings, and reviewed and approved the final version of the manuscript.

Ethical approval

The study was approved by the scientific research committee, faculty of medical laboratory sciences, Al Neelain University, Khartoum, Sudan.

Informed consent

An informed consent was obtained from each participant before sample collection.

Funding statement

The study has not received any grant from funding agencies.

Conflict of interest

The authors declare that there is no conflict of interest.

Data and materials availability

All data associated with this study are available upon request to the corresponding author.

Peer-review

External peer-review was done through double-blind method.

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