

Angiotensin-converting enzyme insertion/deletion polymorphism is not associated with vasoocclusive complications of sickle cell anemia

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ABSTRACT

Context: Sickle cell anemia (SCA) is a group of hemoglobin disorders in which the sickle β -globin gene is inherited. It is associated with many complications; most of them are related to thrombotic events. **Aim:** This study aimed to investigate the association between angiotensin converting enzyme (ACE) insertion/deletion polymorphism and complications of SCA. **Settings and Design:** A case–control study was conducted in Khartoum state. **Subjects and Methods:** A total of 50 patients with SCA and 40 healthy volunteers as a control group were enrolled in this study. Three milliliters of ethylenediamine tetraacetic acid anticoagulated blood were collected from each subject, DNA was extracted by salting-out method, and target DNA regions of the ACE gene were amplified using allele-specific polymerase chain reaction. **Statistical Analysis Used:** Data of this study was analyzed by Statistical Package for Social Sciences. Frequency of qualitative variables was calculated, and correlation was tested by Chi-square test. Regression was used to investigate the association between the polymorphism and complications of SCA. **Results:** The frequencies of the DD, ID, and II genotypes were 42%, 50%, and 8%, respectively, for patients, whereas in the control group, it was 80% for DD genotype and 20% for ID, while II genotype was totally absent. The regression analysis showed no statistically significant association between the disease complications and each of the ACE polymorphic genotypes. **Conclusion:** No statistically significant association was found between ACE polymorphism and complications of SCA.

Key words: Angiotensin-converting enzyme, insertion/deletion, polymorphism, sickle cell anemia

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INTRODUCTION

Sickle cell anemia (SCA) is a group of hemoglobin disorders in which the sickle β -globin gene is inherited. HbS is insoluble

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and forms crystals when exposed to low oxygen tension. Deoxygenated sickle hemoglobin polymerizes into long fibers, each consisting of seven interwind double strands with cross-linking. The sickle-shaped red cells may block different areas of the microcirculation or large vessels causing infarcts of various organs.^[1] Studies in SCA have shown increased prothrombin fragment, thrombin-antithrombin complexes,

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plasma fibrinogen products, D-dimer, and decreased coagulation factor V suggesting an enhanced thrombin generation and supporting a chronic thrombophilic state in SCA patients that is further amplified during acute events.^[2]

Angiotensin I-converting enzyme (ACE), dipeptidyl peptidase, is a membrane-bound enzyme, which is present in endothelial and epithelial cells of various tissues, and innards including lungs and kidneys. ACE converts angiotensin I to II, a very potent vasoconstrictor agent.^[3] Angiotensin is a hormone as well as a locally produced cellular factor, directly affecting vascular endothelial cells and smooth muscles.^[4] Furthermore, it has been demonstrated that receptors of angiotensin II are found in the atherosclerotic vessel walls.^[5] It is pointed out that angiotensin II can promote vasoconstriction, inflammation, and thrombosis in the vascular endothelium and vessel walls.^[6] Besides being a potent vasoconstrictor, angiotensin II is a proatherogenic agent, which elevates plasminogen activator inhibitor-I levels, which results in a decrease in the fibrinolytic activity.^[7,8]

ACE also plays a role in platelet activation and aggregation.^[3] Increased ACE levels could therefore theoretically lead to an increased risk of thrombosis, a hypothesis which is supported by the finding that ACE inhibitors have an anti-thrombotic effect in rat models.^[9] The ACE I/D polymorphism is an insertion/deletion of an ALU-repeat sequence of 287 base pairs (bp) in intron 16 of the ACE gene, located at 17q23. This results in three genotypes: II, ID, and DD.^[10,11] Previous studies have reported that plasma levels of angiotensin II are closely associated with ACE insertion/deletion (I/D) polymorphism and that the serum level of ACE is likely to increase 2-fold in the presence of ACE D/D polymorphism, consequently increasing the levels of plasma angiotensin II.^[10] It has also been emphasized that the ACE I/D gene polymorphism might be an independent risk factor for thrombotic diseases.^[12-14]

This study aimed to determine the frequency of ACE genotypes (II/ID/DD) in Sudanese patients with SCA and correlate these genotypes with disease complications.

SUBJECTS AND METHODS

This is a case-control study conducted at Khartoum state, Sudan, during the period from March to May 2015. A total of 50 Sudanese patients with SCA and 40 healthy volunteers as a control group were enrolled in this study. Venous blood sample was collected from each subject in ethylenediaminetetraacetic acid blood tube, and genomic DNA was extracted from whole blood samples by salting out method. Target DNA regions of the ACE gene were amplified by allele-specific polymerase chain reaction. Primer sequences^[15] and product size are shown in Table I.

Table I: Primer sequences and product size

Primer sequences	Product size	
	D allele	I allele
Forward - 5'-CTGGAGACCACTCCCATCCTTTCT-3'		
Reverse - 5'-GATGTGGCCATCACATTCGTGAT-3'	190	480
Internal - 5'-TGGGATTACAGGCGTGATACAG-3'		160

The reaction mixture consist of template DNA 3 µl, each of primer 1 µl, primers (i-Taq) 5 µl, and distilled water 14 µl. Procedure consisted of initial denaturation at 94°C for 5 min, then denaturation at 94°C for 35 s, annealing at 56°C for 40 s, and extension at 72°C for 1 min, repeated for 35 cycles, and followed by a final extension at 72°C for 8 min. The amplified fragments were separated on 2% agarose gel containing ethidium bromide and demonstrated by gel documentation system.

This study was approved by the Faculty of Medical Laboratory Sciences, and informed consent was taken from each participant before sample collection. Patients' data were collected using structural interview questionnaire and analyzed by the Statistical Package for Social Sciences (IBM SPSS statistics for Windows, version 21.0. Armonk, NY: IBM corp.).

RESULTS

This is a case-control study, included 50 SCA patients, 31 (62%) of them were males and 19 (38%) were females. In addition, 40 healthy volunteers were included as a control group, of them 19 (47.5%) were males and 21 (52.5%) were females.

Complications of SCA in study subjects were included hand-foot syndrome (6%), acute chest syndrome (2%), and pain crisis (6%). About 46% of patients had been transfused with blood in the last year (1-4 times).

The molecular analysis showed that the genotype I/D was the most common in patients with SCA, followed by the genotype D/D and I/I consequently; whereas in the control group, the D/D genotype was the most frequent followed by the genotype I/D, while the genotype I/I was totally absent [Table 2].

SCA patients with complications were found to have either genotype D/D or I/D, and none of them had genotype I/I. However, the correlation was not statistically significant [Table 3].

The regression analysis showed no statistically significant association between the disease complications and each of the genotypes D/D (odds ratio [OR]: 1.069, 95% confidence interval [CI]: 0.167-7.216, *P* = 0.924) and I/D (OR: 0.638, 95% CI: 0.097-4.188, *P* = 0.639)

Table 2: Angiotensin-converting enzyme genotypes frequency in study subjects

Genotype	Frequency (%)	
	Patients	Controls
D/D	21 (42)	32 (80)
I/D	25 (50)	8 (20)
I/I	4 (8)	0 (0)

Table 3: Correlation between angiotensin I-converting enzyme genotypes and disease complications

Complication	n	D/D (%)	I/D (%)	I/I (%)	P
Pain crisis					
Yes	2	1 (50)	1 (50)	0 (0)	0.906
No	48	20 (41.66)	26 (54.16)	2 (4.166)	
Hand-food syndrome					
Yes	3	2 (66.66)	1 (33.33)	0 (0)	0.539
No	47	19 (40.43)	25 (53.19)	3 (6.38)	
Acute chest syndrome					
Yes	1	1 (100)	0 (0)	0 (0)	0.292
No	49	19 (38.77)	26 (53.06)	4 (8.16)	

P considered statistically significant if <0.05

Moreover, no statistically significant correlation was found between ACE genotypes and frequency of each of hospitalization ($P = 0.966$) and blood transfusion ($P = 0.684$) in the last year.

DISCUSSION

SCA is a genetic disease characterized by hypercoagulable state and increased risk of thromboembolic events, complications of SCA are most likely caused by the obstruction of the blood supply to body organs, mainly due to the sickling shape of red cells.^[2] Many another factors also have been reported to contribute to the hypercoagulable state of patients with SCA such as hyperfibrinogenemia, increased concentration of von Will brand factor and decreased plasma levels of protein C, protein S, and antithrombin III, increased prothrombin fragment, thrombin-antithrombin complexes, plasma fibrinogen products, D-dimer, and decreased coagulation factor V.^[16]

The ACE I/D polymorphism is an insertion/deletion of an ALU-repeat sequence of 287 bp in intron 16 of the ACE gene, located at 17q23. This results in three genotypes: II, ID, and DD; the DD genotype is associated with a 2-fold increase in plasma ACE activity over that of II genotype, with intermediate level of heterozygote I/D.^[10] This study aimed to determine the frequency of ACE genotypes (II/ID/DD) in Sudanese patients with SCA and correlate these genotypes with disease complications. The results of the present study showed that the most frequent genotype in patients with SCA was I/D genotype followed by the genotypes D/D and I/I consequently. In the control group, the genotype D/D was the most frequent

followed by the genotype I/D while the genotype I/I was completely absent. Patients with complications were found to have either D/D or I/D genotype. The regression analysis showed no statistically significant association between the SCA complications and each of the genotypes. These findings agree with many studies concerning with ACE polymorphism in patients with thrombotic disorders; Jackson *et al.* conducted a case-control study of more than 500 unselected patients, I/D polymorphism in the ACE gene was not a risk factor for venous thromboembolism. Moreover, no correlation between ACE genotypes and venous thrombosis was found by González Ordóñez *et al.*^[14] These findings disagree with the study concerning with ACE polymorphism by Dilley *et al.* who studied African-Americans with venous thrombosis and reported a moderate increase of venous thrombosis risk in male patients with the D/D genotype.^[17] This variation can be due to the difference in the study population.

CONCLUSION

No statistically significant association was found between ACE polymorphism and complications of SCA among Sudanese patients.

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Conflicts of interest

There are no conflicts of interest.

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