ACE I/D polymorphism is not a genetic modifier of renal features in sickle cell anemia patients

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Abstract
Introduction: Sickle cell anemia (SCA) exhibits a host of complications that contribute to increased morbidity and mortality at the youngest ages.

Objectives: The aim of this investigation is to look into the association between ACE I/D polymorphism and renal function in Indian patients with SCA.

Patients and Methods: About 190 SCA patients confirmed by hemoglobin (Hb) electrophoresis were selected for this study. The severity of the disease was determined using anemia, clinical complications, total white blood cells count, and scores of blood transfusion. To define different renal function phases, estimated glomerular filtration rate (eGFR) was computed in adults and children using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) and Schwartz equations respectively. The ACE I/D polymorphism was conducted using polymerase chain reaction (PCR) and separation through agarose electrophoresis.

Results: The risk of impaired renal function was not statistically distinct between ACE I/D genotypes and alleles. Further, the genotypes of ACE I/D and the risk of disease severity was not found to be associated with each other.

Conclusion: This investigation found that ACE I/D is an insignificant genetic modifier of renal function or severity of disease in patients with SCA.

Keywords: Sickle cell anemia, ACE I/D, Renal function, Disease severity

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Introduction
Sickle cell anemia (SCA) is a hereditary blood condition that is triggered due to alteration by a single base pair in the beta globin (HBB) gene at codon 6, subsequently forming a sickle-shaped erythrocytes in the deoxygenated conditions (1). This event leads to an increase in viscosity and sickle erythrocyte adhesion to vascular walls leading to obstruction of blood flow in tiny capillaries (2). Individuals with SCA exhibit several clinical complications, such as vasocclusive crisis (VOC), splenomegaly, ocular manifestation, hepatomegaly, pulmonary hypertension, leg ulcers, sickle nephropathy, acute chest syndrome (ACS), and stroke, which contribute to mortality at an early age (3-7). Hydroxyurea treatment significantly enhanced the quality of life and survival of SCA patients, leading to an increase in the frequency of various morbidities (8).

Angiotensin II (Ang II) is known to play a greater role in proliferation of erythroid progenitors in vitro (9). The angiotensin-converting enzyme (ACE) is engaged in converting circulating angiotensin-I (Ang I) to the effector peptide Ang II. Further, ACE promotes endothelial dysfunction, which stimulates vascular inflammation by inducing vasoconstriction and thrombosis (10). Besides, ACE participates in platelet aggregation, which increases the risk of thrombosis in rats (11). As ACE-inhibition shows renoprotective properties, the ACE inhibitors (ACEIs) are being used in patients with various clinical conditions (12-14). However, there is still confusion regarding the effectiveness and safety of RAAS inhibition in achieving remission of proteinuria and renal function stabilization in SCA patients. The human gene that encodes for ACE is found on chromosome 17 (17q23) and is fundamentally expressed on most epithelial and endothelial cells (15). Intron 16 of the ACE protein sequence contains an insertion/deletion (I/D) polymorphism that is shown to influence circulating plasma and tissue ACE levels (16). Plasma ACE levels are elevated twice in people with the homozygous “D” allele as compared to people with the homozygous “I” allele (17). Multiple lines of evidence indicated that the ACE I/D is a distinct risk factor for arterial thrombotic disorders (18, 19).
Implication for health policy/practice/research/medical education

In a study on 190 sickle cell anemia patients, we found the risk of impaired renal function was not statistically distinct between ACE I/D genotypes and alleles.

Objectives

The aim of this investigation is to look into the association between ACE I/D polymorphism and renal function in Indian patients with SCA.

Patients and Methods

Study design

This infirmary-based cross-sectional investigation was conducted at the outpatient clinic of Sickle Cell Institute Chhattisgarh (SCIC), Raipur, and the Institutional ethics committee of SCIC approved this study. About 190 SCA patients (validated by Hb electrophoresis) were appended in this investigation. Adult subjects signed written informed consent, and minors were accompanied by their parents or guardians who signed a written consent on their behalf. Information related to hematological variables and hemoglobin (Hb) fractions was obtained from the individual patient’s record. From each participant, 3 ml of plasma sample was collected in an EDTA vacutainer. An Ilab 650 automatic analyzer was used for quantifying the serum creatinine and blood urea. The estimated glomerular filtration rate (eGFR) was measured in adults and children (17 years) using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation (20) and the Schwartz equation, respectively (21,22). Further, the stage of kidney disease was determined by using eGFR and all the SCA patients were divided into four groups: glomerular hyperfiltration (GHF: eGFR >140 mL/min/1.73 m²), chronic kidney disease 1 (CKD 1: eGFR<140 to 90 mL/min/1.73 m²), chronic kidney disease 2 (CKD 2: eGFR<89 to 60 mL/min/1.73 m²) and chronic kidney disease 3 (CKD 3: eGFR<59 to 30 mL/min/1.73 m²) (23). The severity of the disease was determined using anemia, complications, total leukocyte count, and transfusion scores (24). The standard procedure was used to extract DNA from all samples (25).

Determination of ACE I/D genotypes

Polymerase chain reaction (PCR) as well as agarose electrophoresis were used to genotype the ACE I/D polymorphism. The subsequent oligonucleotide primers; 5’-CTGGAGACCACTCCCATCTCTTCT-3’ and 5’-GATGTGGCCATCACATTCGTCAGAT-3’ were used to perform amplifications. PCR amplifications were carried with the following conditions: 94°C (5 minutes) for 1 cycle, 94°C (1 minute), 58.5°C (40 seconds) and 72°C (30 seconds) for 35 cycles, and a final extension step of 72°C (7 minutes). The PCR product was resolved on 2% agarose gel. The DD and II genotypes were assigned to PCR reactions that produced single bands of 190 and 490 base pairs (bp), respectively, whereas the ID genotype was assigned to PCR reactions that produced two bands of 190 and 490 bp. To avoid mistyping the ID genotype as DD genotype, all samples with DD genotype were subjected to an additional PCR using insertion specific primers (26). A 335bp product produced by this PCR is also considered the I allele.

Statistical analysis

The distribution of clinical and biochemical variables among ACE I/D genotypes was analyzed using ANOVA. To evaluate the association between ACE I/D and renal function or disease severity, the chi-squared test was conducted. SPSS version 22 was used for all analyses (IBM Corp., Armonk, NY.). A two-tailed P value of 0.05 is deemed as statistical significance.

Results

There were 190 SCA patients investigated, including 106 men (55.8%) and 84 women (44.2%). The average age of the participants in the study was 16.5±9.3 years. According to the outcome of this investigation, ID genotype was the most common among patients with SCA, followed by the II and DD genotypes. Figure 1 depicts the distribution of ACE I/D genotypes in SCA patients based on kidney function. SCA patients with various ACE I/D genotypes had almost similar hematological profile (Table 1). The risk of impaired renal function (GHF, CKD 2 and CKD 3 stages) among SCA patients with distinct ACE I/D genotypes in codominant, dominant, and allelic models was shown in Table 2. No statistically significant variation in the risk of renal impairment among ACE genotypes and alleles was found; suggesting that ACE I/D is an insignificant modifying factor of renal function in SCA patients. Participants with normal kidney function (CKD 1 stage) and different stages of kidney damage had almost similar hematological profile (Supplementary file 1, Figure 1. Incidence of ACE I/D genotypes in SCA patients based on renal function.)
Table 1. Distribution of various hematological variables according to ACE genotypes in SCA patients

| Variable                  | ACE II (n=59) | ACE ID (n=92) | ACE DD (n=39) | F value | P value |
|---------------------------|---------------|---------------|---------------|---------|---------|
| Age (y)                   | 16.31±9.10    | 16.73±10.05   | 16.33±7.73    | 0.046   | 0.955   |
| BMI (kg/m²)               | 15.73±2.51    | 16.29±3.12    | 16.05±4.48    | 0.516   | 0.598   |
| Hb (g/dL)                 | 8.27±1.84     | 8.49±1.64     | 8.72±1.96     | 0.782   | 0.459   |
| HbF %                     | 19.1±4.62     | 19.8±2.74     | 19.6±6.71     | 0.181   | 0.834   |
| Hematocrit %              | 24.6±5.71     | 25.2±5.17     | 25.5±4.98     | 0.380   | 0.685   |
| TLC (x10⁹/L)              | 9.7±4.74      | 11.4±6.02     | 12.5±5.61     | 3.218   | 0.041   |
| PLT (x10⁹/L)              | 302.2±172.1   | 341.7±171.1   | 334.6±150.9   | 1.041   | 0.355   |
| RBC (x10⁹/L)              | 2.94±0.66     | 3.20±2.27     | 3.02±0.75     | 0.49    | 0.614   |
| MCV (fL)                  | 85.3±10.83    | 86.7±10.17    | 86.4±9.69     | 0.309   | 0.734   |
| MCHC (g/L)                | 33.70±2.11    | 33.60±2.01    | 34.22±2.53    | 1.149   | 0.319   |
| MCH (pg)                  | 28.69±4.19    | 29.04±3.92    | 29.51±3.83    | 0.495   | 0.611   |
| RDW-CV                    | 18.42±2.63    | 18.26±2.85    | 17.90±2.70    | 0.423   | 0.656   |
| TB (mg/dL)                | 2.33±1.62     | 2.33±1.64     | 2.58±1.99     | 0.349   | 0.706   |
| DB (mg/dL)                | 0.39±0.52     | 0.40±0.61     | 0.40±0.36     | 0.016   | 0.995   |
| SGPT (U/L)                | 21.9±12.6     | 24.3±23.1     | 20.8±11.0     | 0.615   | 0.542   |
| SGOT (U/L)                | 47.1±26.5     | 50.3±32.5     | 45.1±22.5     | 0.515   | 0.599   |
| Blood urea (mg/dL)        | 16.02±6.30    | 17.36±9.7     | 18.23±16.03   | 0.567   | 0.568   |
| Serum creatinine (mg/dL)  | 0.61±0.24     | 0.64±0.21     | 0.64±0.18     | 0.083   | 0.920   |
| eGFR (mL/min/1.73 m²)     | 107.6±29.7    | 107.2±29.7    | 108.2±31.0    | 0.016   | 0.984   |
| No. of blood transfusions | 7.8±19.03     | 7.17±11.77    | 5.26±8.84     | 0.410   | 0.664   |

BMI, body mass index; Hb, hemoglobin; HbF, fetal hemoglobin; TLC, total leukocyte count; PLT, platelets; RBC, red blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; RDW-CV, variation of red cell volume distribution width; TB, total bilirubin; DB, Direct bilirubin; SGPT, serum glutamic-pyruvic transaminase; SGOT, Serum Glutamic-Oxaloacetic Transaminase; eGFR, estimated glomerular filtration rate.

Table S1). Individuals with CKD 3 stage had higher HBF levels (22.0 ± 7.4%) than those with normal renal function (18.3 ± 6.3%; P = 0.025). Additionally, CKD 2 stage and CKD 3 stage patients had substantially higher blood urea and creatinine levels than the GHF and CKD 1 groups. Higher SGOT and SGPT levels were found in CKD 2 stage and CKD 3 stage patients than CKD 1 and GHF patients.

Table 2. ACE I/D polymorphism and risk of impaired renal function among SCA patients

|                  | χ²  | OR (95% CI)     | P value |
|------------------|-----|-----------------|---------|
| **CKD1 vs. GHF** |     |                 |         |
| DD versus II     | 0.071 | 0.846 (0.248-2.879) | 0.790 |
| DD+ID versus II  | 0.142 | 0.839 (0.338-2.084) | 0.706 |
| D versus I       | 0.089 | 0.807 (0.491-1.676) | 0.755 |
| **CKD1 vs. CKD2**|     |                 |         |
| DD versus II     | 0.172 | 1.219 (0.477-3.114) | 0.679 |
| DD+ID versus II  | 0.061 | 1.096 (0.528-2.276) | 0.805 |
| D versus I       | 0.162 | 1.102 (0.687-1.766) | 0.688 |
| **CKD1 vs. CKD3**|     |                 |         |
| DD versus II     | 0.336 | 0.508 (0.049-5.216) | 0.569 |
| DD+ID versus II  | 0.157 | 0.704 (0.167-3.289) | 0.693 |
| D versus I       | 0.313 | 0.742 (0.260-2.117) | 0.577 |

Figure 2 depicts ACE I/D genotype distribution in SCA patients based on SCA severity. The risk of SCA severity associated with distinct ACE I/D genotypes in different genetic models implies that ACE I/D is not linked with SCA severity (Table 3).

Discussion

The current study found that the ACE DD genotype is the most common in patients with SCA, followed by the II
Table 3. ACE I/D polymorphism and risk of clinical outcomes of different severity among SCA patients

|                  | χ²  | OR (95% CI)    | P value |
|------------------|-----|----------------|---------|
| **Moderate vs Mild** |     |                |         |
| DD versus II     | 0.005 | 0.956 (0.263-3.481) | 0.946   |
| DD+ID versus II  | 0.062 | 1.125 (0.444-2.852)  | 0.803   |
| D versus I       | 0.002 | 1.013 (0.533-1.926)  | 0.969   |
| **Severe vs Mild** |     |                |         |
| DD versus II     | 0.864 | 1.769 (0.527-5.941)  | 0.356   |
| DD+ID versus II  | 1.009 | 1.584 (0.643-3.901)  | 0.318   |
| D versus I       | 1.003 | 1.366 (0.741-2.520)  | 0.318   |
| **Severe vs. Moderate** |     |                |         |
| DD versus II     | 1.766 | 1.850 (0.743-4.603)  | 0.186   |
| DD+ID versus II  | 0.958 | 1.407 (0.716-2.767)  | 0.322   |
| D versus I       | 1.740 | 1.349 (0.864-2.107)  | 0.188   |

ACEIs have been linked to some side effects, including a dry, irritating cough and a higher risk of lung cancer (37). According to American Society of Hematology guideline, ACEIs and ARBs use require proper follow-ups and observing toxic effects such as hyperkalemia, cough, and hypotension in SCA patients (38).

**Conclusion**

In summary, this investigation demonstrated that ACE I/D polymorphism is an insignificant genetic modifier of renal function or severity of the disease in patients with SCA.

**Limitations of the study**

The scope of the present study is limited, as we have not measured creatinine in SCA patients based on isotope dilution mass spectrometry. In addition, the nested study design adopted results in selection bias. However, unlike previous studies, the present study used well-characterized SCA patients.

**Authors’ contribution**

Conceptualization: LVKSB; Methodology: LVKSB; Data analysis: LVKSB; Writing original manuscript: LVKSB; Review and revising manuscript: SP; Funding acquisition: LVKSB. Both authors reviewed and approved the final manuscript.

**Availability of data and materials**

All data generated or analyzed during this study are included in this article and its supplementary information file.

**Conflicts of interest**

The authors declare that they have no competing interests.

**Consent to Publish**

Written informed consent obtained from each study participant is having statement to publish data without the identifiers.

**Ethical issues**

The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from each study participant. This study was approved by the Institutional Ethics Committee (IEC) of the Sickle Cell Institute of Chhattisgarh. (Letter No.29/SCIC/Ethical/2015 Raipur, Dated 16, January 2015). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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**Supplementary files**

Supplementary file 1 contains Table S1.

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