HBB gene cluster haplotype diversity in sickle cell anemia patients of Chhattisgarh, India

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ABSTRACT
Sickle cell anemia (SCA) is one of the hereditary hemoglobin disorders in Indian populations. An exceptionally high prevalence of SCA is observed in the populations of Chhattisgarh. Restriction fragment length polymorphism (RFLP) haplotypes of the beta globin (HBB) gene cluster are important as population data, anthropological purpose for tracing migration of SCA allele and predicting the severity of SCA disease. The purpose of this study was to elucidate the HBB haplotypes and their correlation with clinical and hematological profile of SCA patients of Chhattisgarh population. The HBB gene cluster haplotypes were determined in 190 SCA patients by the polymerase chain reaction-restriction fragment length polymorphism method. Medical records of patients were reviewed to obtain pertinent clinical features, hemoglobin fractions, and other biochemical variables. Among the analyzed patients, 74% had Arab-Indian (AI) haplotype, followed by 21% atypical haplotypes. Senegal, Benin, and Cameroon types of HBB haplotypes represented 3%, 1%, and 1% of the patients, respectively. Comparison of various biochemical and hematological variables and clinical complications among various haplotypes did not reveal significant differences. The high frequency of atypical haplotypes observed may have been generated by single and double crossing-over between AI haplotype and normal HBB haplotype. Considering the Indian population’s genetic structure and diversity, the results of our study should be considered as introductory, and our study can serve as a possible tool for additional studies of SCA in India.

1. INTRODUCTION
Sickle cell anemia (SCA) is an autosomal recessive hemoglobin disorder caused by a mutation in the HBB gene. This mutation leads to substitution of valine for glutamic acid at the 6th amino acid position of the β-globin chain. SCA has been found to be more common in Africa, USA, Mediterranean region, Middle-Eastern countries, and in the Indian subcontinent [1]. In 1952, Lehmann and Cutbush [2] reported the first case of SCA in the Indian subcontinent among the Indian tribal population of Nilgiri hills. In the same year, the case of SCA was reported and documented among the tea garden laborers in upper Assam [3]. SCA is common among the populations of India and a higher prevalence is seen among the ST, SC, and OBC population of Chhattisgarh [4]. The clinical presentation of SCA is very diverse and can be modified by many factors that include age, gender, genetics, hematological, and environmental factors [5,6]. The other factors include fetal hemoglobin (HbF), HBB gene cluster haplotypes, and simultaneous presence of α-thalassemia or glucose-6-phosphate dehydrogenase deficiency [7].

The HBB gene cluster includes epsilon, gamma-G, gamma-A, delta, and beta globin genes. This HBB gene cluster spans approximately 70 kilobases on chromosome 11. Several polymorphic sites flanking the HBB gene cluster have resulted in the delineation of five HBB haplotypes [8,9]. These haplotypes are important for understanding and tracing migration of SCA allele and predicting the severity of SCA disease [10–12]. A better understanding of the HBB haplotypes that influence disease severity is important to predict the clinical outcomes in SCA patients. Hence, the purpose of this study was to elucidate the HBB haplotypes and their correlation with clinical and hematological profile of SCA patients of Chhattisgarh population.

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2. MATERIALS AND METHODS

This study was initiated after obtaining approval from the Institutional Ethics Committee of the Sickle Cell Institute, Chhattisgarh, Raipur, India. Written informed consent was obtained from study participants. A total of 190 unrelated homozygous sickle cell disease (HbSS) patients confirmed using hemoglobin electrophoresis were included in the study. Values pertaining to complete blood count and hemoglobin high performance liquid chromatography (HPLC) fractions were taken from the patient’s records. All the patients were evaluated for clinical phenotypes such as pain, anemia, jaundice, pneumonia, stroke, and osteonecrosis. Severity scores were determined using the combination of anemia, complications, total leucocyte count (TLC), and transfusion scores. A complete overview of the criteria used for assessing the severity score is given elsewhere [13].

About 3 ml blood samples were collected from each patient. DNA was extracted from whole anticoagulated blood following the standard protocol [14]. DNA concentration and purity were assessed using the nanodrop spectrometer. Nucleotide sequence encompassing important polymorphic sites (5′ to ε, 5′ to Γγ, IVS II Γγ, IVS II Aγ, 5′ψβ, 3′ψβ, 5′β, and IVS II β) in HBB gene cluster were amplified by polymerase chain reaction (PCR) and the fragments were subjected to restricted digestion. The polymorphic sites analyzed; restriction enzymes used, and the consensus sequence of first eight restriction fragment length polymorphism (RFLP) sites for designating five known global HBB haplotypes are documented in Table 1. Based on the presence or absence of a restriction site at the studied polymorphic sites, we assigned haplotypes to all samples. The association of HBB haplotypes with various clinical and hematological variables was analyzed using the analysis of variance. As phase-unknown genotypes were collected, the haplotype sites and frequencies were estimated using maximum likelihood with an expectation-maximization method in Arlequin3.5 software. The descriptive data will be given as mean ± standard deviation (SD). Statistical Package for the Social Sciences software (version 22.0) was used to carry out the statistical analysis of the data.

3. RESULTS

Delineation of haplotypes encompassing the HBB gene cluster in 190 SCA patients revealed four haplotypes such as AI, Senegal, Benin, and Cameroon. Central African (Bantu) haplotype was not observed in our SCA patients. The haplotype frequencies are shown in Figure 1. The AI haplotype is the major haplotype (74.2%), followed by atypical haplotype (21.1%). Senegal, Benin, and Cameroon were found, respectively, in 2.6%, 1.1%, and 1.1% of SCA patients. The atypical haplotypes might have been generated by single and double crossing-over between AI haplotype and ancestral HBB haplotype. The mean age of SCA patients among different HBB haplotype groups is not statically different (p = 0.949) (Table 2). The comparison of mean body mass index (BMI) among haplotypes did not reveal significant differences (p = 0.697) (Table 2). Similarly, the mean number of blood transfusions is not significantly different among the haplotype groups (p = 0.160). The mean number of hospitalizations among haplotype groups is statistically different (p = 0.160). The mean number of hospitalizations in AI and Benin haplotypes is relatively lesser than in other haplotypes (Table 2).

The distribution of different clinical phenotypes among various HBB haplotypes is shown in Figure 2. Distribution of the

| S.No | Relative position on β-globin gene cluster | Restriction enzyme | Site present (+) allele (fragment sizes in bps) | Site absent (−) allele (fragment sizes in bps) | AI | Benin | Senegal | Bantu | Cameroon |
|------|------------------------------------------|-------------------|-----------------------------------------------|-----------------------------------------------|-----|-------|---------|-------|---------|
| 1    | 5′ to ε                                  | HincII            | A-ins (385+315)                               | A-del (700)                                   | +   | −     | −       | −     | −       |
| 2    | 5′ to Γγ                                 | XmnI              | T (455+202)                                   | C (657)                                       | +   | −     | +       | −     | −       |
| 3    | IVS II Γγ                                | HindIII           | T (237+91)                                    | G (328)                                       | +   | −     | +       | +     | +       |
| 4    | IVS II Aγ                                | HindIII           | T (345+308)                                   | G (653)                                       | −   | −     | −       | −     | −       |
| 5    | 5′ψβ                                     | HincII            | T (369+337)                                   | G (706)                                       | +   | −     | +       | −     | −       |
| 6    | 3′ψβ                                     | HincII            | A (517+97)                                    | G (614)                                       | +   | +     | +       | −     | −       |
| 7    | 5′β                                      | HinfII            | C (623+148)                                   | G (771)                                       | −   | −     | +       | −     | −       |
| 8    | IVS II β                                 | AvalII            | C (214+100+14)                                | G (314+14)                                    | +   | +     | +       | +     | +       |
The differences in the number of red blood cell (RBC), tHb, and HbF levels and other Red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hematocrit (HCT), and red blood cell distribution width-coefficient variation (RDW-CV) are not statistically significant between different haplotypes (Table 3). The mean HbF levels in the AI Haplotype in this study is 20.0% ± 6.6%, which is slightly higher than the Senegal (17.9 ± 10.5), Benin (13.5 ± 5.0), and Cameroon (13.1 ± 0.9) haplotypes. Levels of total bilirubin, direct bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, and creatinine in serum did not differ significantly between the different HBB haplotypes (Table 4). Distribution of different HBB haplotypes in SCA patients with various degrees of severity is shown in Table 5.
4. DISCUSSION

Analysis of \( HBB \) gene cluster haplotypes among SCA patients from the Chhattisgarh region demonstrated presence of AI, Senegal, Benin, and Cameroon haplotypes. Furthermore, several atypical haplotypes were found in this region. Hemoglobin fractions, hematological variables, and clinical complications were compared among haplotypes. To the best of our knowledge, this study is a major study involving 190 well-characterized HbSS patients. Of the 190 HbSS patients analyzed, 74% showed AI haplotype, followed by Senegal, Benin, and Cameroon with the frequencies of 3%, 1%, and 1% respectively. 21% of the chromosomes are of atypical haplotypes (slightly deviate from known haplotypes). Comparison of various biochemical and hematological variables and clinical complications among various haplotype did not reveal significant differences.
HBB haplotypes that are often distinctly associated with a particular geographical region are suggesting that the mutant HBB gene arises separately in these locations [15]. The presence of African-specific HBB haplotypes in Indian populations was documented in many studies [16–18]. Atypical HBB haplotypes that were observed in higher frequencies might have been generated by one of these underlying genetic mechanisms: (a) changes in nucleotides within the restriction site; (b) single and double crossing-over between a typical HBB haplotype and an ancestral HBB haplotype; and (c) non-reciprocal recombination between homologous DNA sequences. Atypical haplotypes are reported in many studies [18–23]. The presence of higher proportions of atypical haplotypes that observed besides AI haplotype in Relli and Thurpu Kapu populations in Andhra Pradesh is probably due to the result of gene conversion [22].

Previous studies that documented the AI haplotype in the Indian subcontinent showed that this haplotype is clinically and hematologically different from other haplotypes and might have evolved differently [15, 18]. In consistency with the previous studies, the mean HbF level of the AI haplotype is higher than the other African-specific HBB haplotypes [24–26]. Although the AI haplotype is linked with a high HbF and milder clinical presentations, vasoocclusive episodes still occur among these patients [27, 28]. The mean hemoglobin level in the study population is 8.6 g/dl, consistent with the previous studies [29, 30]. Increased SGOT/AST levels in the present study (47.42 ± 33.43 U/l) are in consensus with the increased frequency of jaundice (32.05%) and hepatomegaly (6%) occurred in these patients. The increase in total bilirubin levels found in patients with SCA in the present study is corroborated by a previous study in which they found mean values of total bilirubin of 4.85 ± 4.58 mg/dl [30]. The frequency of occurrence of hepatomegaly in the study population is 6%. The striking feature among those patients with hepatomegaly in this study group is that they are associated with an increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin [31] and may be a useful indicator of severity in sickle cell anemia [32]. The frequency of occurrence of splenomegaly in the study population is 10%. Splenomegaly is one of the main clinical findings among the AI haplotype [18].

In summary, the haplotype analysis of 190 homozygous SCA patients revealed the presence of several known HBB haplotypes. The AI haplotype was the major haplotype, followed by several atypical haplotypes. African haplotypes were found in a lesser frequency. Although our study includes more SCA patients compared to other previous studies, considering the Indian population genetic structure and diversity, our results should be considered as introductory, and our study can serve as a possible tool for future studies. Additional studies based on long-read haplotypes and statistically phased haplotypes in well-characterized SCA patients are warranted.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

6. ETHICAL APPROVALS

This study was initiated after obtaining approval from the Institutional Ethics Committee of the Sickle Cell Institute Chhattisgarh, Raipur, India.

7. CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

8. FUNDING

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