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# Status of HbS Distribution and β-Globin Gene Haplotypes among Scheduled Caste and Scheduled Tribe Populations of Odisha

***Abstract*: The present study aims to assess sickle cell hemoglobin (HbS) status and associated haplotypes within the β-globin gene cluster among individuals in Koraput district of Odisha. The prevalence of HbAS was found to be 11.18%. Molecular screening was conducted at 4 specific sites, i.e.,HbA/S, HincII-ψβ, HincII-3´ ψβ, and HinfI 5´ β. The Arab-Indian haplotype was identified as the most frequent haplotype associated with HbS. Atypical haplotypes such as Senegal, Benin, and Bantu were also observed at low frequencies.**

**Keywords:** Sickle cell hemoglobin, β-globin gene, Haplotypes, RFLP, Odisha

Sickle cell disease (SCD) is an autosomal recessive blood disorder caused by a single nucleotide mutation in the β-globin gene, resulting in the production of abnormal hemoglobin S (HbS). The global population of sickle cell carriers exceeds 300 million individuals, with notable concentrations found in Africa, the Arab Peninsula, India, the Mediterranean, and the southern United States[1]. Within the genome's specific region, the β globin gene cluster contains additional globin genes such as ε, γ, ψβ, and δ. The presence of various neutral polymorphic restriction sites within the β globin gene cluster allows for the reform of haplotypes, which hold both evolutionary and clinical importance[2]. Five geographically confined and significant haplotypes have been traced, i.e., Senegal (Atlantic West Africa), Benin (Central West Africa), Bantu or CAR (South Central and Eastern Africa), Cameroon (Cameroon), and Arab Indian (Mediterranean, Saudi Arabia, and India). Among these, the Arab-Indian haplotype has been stated as the most commonly linked with the HbS allele in India[3,4]. In addition to these commonly observed haplotypes, numerous atypical haplotypes have also been described in association with the HbS allele in India[3-6]. Understanding the prevalence and diversity of HbS haplotypes is crucial for improving interventions and healthcare strategies.

The study was conducted in Koraput district of Odisha state, which is characterized as an underdeveloped region primarily inhabited by tribal and scheduled caste communities. These communities face a significant burden of socioeconomic disadvantage and frequently experience limited access to healthcare services[7]. Most importantly, to the best of our knowledge, no study has been found that reports the distribution of HbS and â-globin gene haplotypes.

Therefore, the aim of the present study is to assess the distribution patterns of HbS and its associated haplotypes within the β-globin gene cluster among individuals in Koraput district of Odisha.

***Materials and Methods:*** This study was carried out in two phases in Koraput district of Odisha. In the first phase, a total of 152 unrelated scheduled caste (n=98; *Gadaba and Paraja*) and scheduled tribe (n=54) individuals, aged 30 years and above, were screened for HbS. Informed consent was obtained from each participant before collecting samples through finger pricks. The blood samples were tested by the sickle slide test technique described by Daland and Castle to determine the presence of sickling red cells[8].

Moving to the second phase, informed consent was obtained from58 individuals who were related to the respondents identified as HbS positive in the 1st phase. A 5ml intravenous blood sample was drawn in ACD from each participant. This phase also aimed to determine the prevalence of HbS among the family members with HbS. Since respondents related to sickle cell carriers have a greater possibility of having HbS, raising awareness and providing education and counselling regarding their HbS status as well as its implications was essential.DNA extraction of the samples (n=58) was conducted using the salting-out method described by Miller et al.[9]. The β globin haplotypes were classified based on the analysis of three restriction fragment length polymorphisms (RFLPs):HinfI 5' β, HincII-3' ψβ, and HincII-ψβ. Further, amplification of the HbA/S site was performed using ASPCR.

The gene counting method was used to determine allele frequencies. All four loci (HB A/S, HinfI 5′ β, HincII-3′ ψβ, HincII-ψβ) were examined for Hardy-Weinberg equilibrium. The HaploPOPtool, which employs the maximum likelihood estimation method, was used to calculate haplotype frequencies. The LINKED software was utilized to calculate the maximum likelihood of the coefficient of linkage disequilibrium (LD) between pairs of loci.The guidelines provided in the Helsinki declaration were followed throughout the research to ensure ethical standards were upheld.

***Results:*** The present study was carried out in 2 phases. In the 1st phase, 152 unrelated subjects were screened for HbS, among which the HbAS genotype was found to be 11.18%. HbA/S locus was identified to be polymorphic with the 2 alleles 'A' and 'S'with frequencies of 0.901 and 0.099, respectively. The prevalence of HbAS was found to be 48.28% among the individuals related to HbS family members, which was significantly higher as compared to the prevalence of the unrelated individuals ($p<0.001$). However, no SS genotype was found in the studied population (Table 1). The chi-square test revealed a minor variation from the Hardy-Weinberg equilibrium.

**Table 1. Prevalence of HbS carriers (HbAS) among the individuals in Koraput district of Odisha**

| HbS prevalence | No. screened | HbAS individuals | Prevalence (%) | p-value |
|---|---|---|---|---|
| Unrelated individuals | 152 | 17 | 11.18 | <0.001 |
| Individuals related to HbS family members | 58 | 28 | 48.28 | |

Table 2 depicts the allele frequency at the HbA/S and the 3 RFLPs loci from the 2nd phase of the study. Due to the technical errors, the disparity was found in the number of samples tested. The presence and absence of the restriction digestion sites of the relevant enzymes are indicated by '+' and '-' alleles at Hinc II-ψβ, Hinc II-3′ ψβ, and Hinf I 5′ β Hb-sites.At the HbAS locus, the '+' allele denotes the 'A' allele, whereas the '-' allele corresponds to the 'S' allele. All the loci in the sample were determined to be polymorphic, with the extent of polymorphism varying among different loci. The HbS allele frequency was found to be 0.305 in the present study. Among the 3 RFLP loci, HinfI5′ β had a relatively greater frequency of '+' allele(0.517) as compared to its '-' allele (0.483). HincII-32 ψβ and HincII-ψβ had higher frequency of '-' alleles (0.597 and 0.711, respectively) as compared to its'+' alleles. Pair-wise LD was also calculated among the 4 sites and the $\chi 2$ value was significant between HbA/S and HincII 3′ ψβ loci, and HincII ψβ and HincII 3′ ψβ loci.

**Table 2. Allele frequencies at the four HB sites (Hb A/S locus, HinfI 5′ β, HincII-3′ ψβ, and HincII-ψβ)**

| Site | No. of individuals tested | Allele frequency |
|---|---|---|
| HbA/S | 58 | + 0.695 |
| | | - 0.305 |
| HinfI 5′ β | 54 | + 0.517 |
| | | - 0.483 |
| HincII-3′ ψβ | 55 | + 0.403 |
| | | - 0.597 |
| HincII-ψβ | 55 | + 0.289 |
| | | - 0.711 |

Out of the 16 possible haplotypes (8 with HbA and 8 with HbS), 13 haplotypes were obtained in the studied population (Table 3). Genotype data for all 4 sites (HB A/S, HinfI 5′ β, HincII-3′ ψβ, HincII-ψβ) were available for 53 individuals. Of the 13 haplotypes, -++ was the most frequent haplotype linked with the HbS allele (0.306), which is part of the Arab-Indian haplotype. Further, +++ was identified to be the second most HbS-associated haplotype (0.086), which is part ofSenegal haplotype. Among a few individuals, Benin (S-+-) and Bantu (—) haplotypes were observed.However, Cameroon haplotype (S++-) was not found in the studied population.

**Table 3. Haplotype frequencies at the four loci in β globin gene cluster (order of loci: Hb A/S locus, HinfI 52 β, HincII-32 ψβ, and HincII-ψβ) (n=53; 106 chromosomes)**

| Hb Aassosiated haplotypes | Haplotypes frequency | Hb Sassosiated haplotypes | Haplotypes frequency |
|---|---|---|---|
| A+++ | 0.019 | S+++(Senegal) | 0.086 |
| A-++ | 0.066 | S-++(Arab-Indian) | 0.306 |
| A+-+ | 0.06 | S+-+ | 0.008 |
| A--+ | 0 | S--+ | 0.080 |
| A++- | 0.121 | S++-(Cameroon) | 0 |
| A-+- | 0.001 | S-+-(Benin) | 0.018 |
| A+--- | 0.053 | S+--- | 0.096 |
| A--- | 0 | S--- (Bantu) | 0.011 |

***Discussion:*** In the present study, the prevalence of HbS carriers and the distribution pattern of its associated haplotypes in the β-globin gene cluster have been reported

among the SCs and STs in Koraput district of Odisha. The distribution pattern of HbS and β-globin gene haplotypes provides valuable insights into the epidemiology and genetic diversity of SCD in different populations. Understanding the prevalence and diversity of HbS haplotypes is crucial for improving interventions and healthcare strategies.

The prevalence of HbAS was found to be 11.18% in the studied population.However, no individuals with HbSS were found, as the age group of the respondents was 30 years and above. Previous studies have also reported similar prevalence rates for the HbS allele in India[11-13]. According to a recent survey by the Indian Council of Medical Research (ICMR), sickle cell carrier prevalence ranges up to 40% among various populations in India[14]. However, to the best of our knowledge, no published literature was found reporting the prevalence of HbS in the studied area over the past five decades, except our recently published study that reported the prevalence of sickle cell carriers[10]. The last study conducted in the districtin 1967 screened only 225 tribal individuals, reporting an HbAS prevalence of 12.4%[15]. Notably, populations other than the tribal communities were not screened for HbS in this region. Therefore, this study is the first to include scheduled caste and scheduled tribe populations in HbS carrier screening. Further, the prevalence of consanguineous marriage was found to be almost 12% in the studied population. This may be attributed to the lethal nature of the condition, regardless of the presence or prevalence of malaria, suggesting a genetic burden associated with the presence of HbS.

In the present study, the Arab-Indian haplotype was identified as the most frequent haplotype linked with HbS. Additionally, atypical haplotypes such as Senegal, Benin, and Bantu were also detected at minor frequencies. Most importantly, no study was found in Koraput district that reported on â-globin gene haplotypes. However,A similar study conducted inKoya Dora of Andhra Pradesh reported similaroutcomes to the present study[2].Among the population studied here, the most common haplotype associated with HbS was found to be -++, which aligns with previous findings in Andhra Pradesh and other parts of India[4-6, 16] and is part of the Arab Indian haplotype. However, in the present study group, three other atypical haplotypes, namely Benin, Bantu, and Senega were also observed in low frequencies. The presence of the Bantu haplotype had been previously reported in the Mala caste population from Andhra Pradesh[6]. Majumder et al. (1999) found the presence of the Benin haplotype (+++) in a study of four ethnic communities in eastern India[5]. The Arab Indian haplotype (0.306) was found to be the most frequent haplotype linked with HbS in the present study, followed by the Senegal haplotype (0.086) as the second most prevalent. A previous study also reported the occurrence of the Senegal haplotype in south India[4]. However, the presence of the Cameroon haplotype was not observed in the present study, which is considered an uncommon haplotype and has been reported in other studies conducted among Indian populations[5-6].

The occurrence of atypical haplotypes (Senegal, Benin, and Bantu) can be explained by methods such as probable admixture, gene alteration, or chance mutation at the studied RFLP loci[6]. However, a possible explanation appears to be recombination events between the frequent haplotypes linked with the HbA and HbS alleles[5]. The â-globin cluster haplotypes are recognize to be potential modifiers of therapeutic hazards in sickle cell disease. Various haplotypes have been interrelated to different clinical phenotypes among individuals with sickle cell anemia[17], although this remains a topic of debate. To gain a clearer understanding of the association between haplotypes and disease severity, further investigations are required. These studies should focus on various haematological factors in SCD patients, taking into account the distribution patterns of â-globin haplotypes. Additionally, demographic variables like fertility factors, neonatal and offspring mortality should be considered to assess the potential genetic burden because of HbS allele. Such evidences are crucial for effective counselling of individuals affected by sickle cell disease. ❐

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