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Case Report

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Variation of Abnormal Hemoglobins Concentrated in Durg, Chhattisgarh: A Brief Note Based on Cross-Sectional Study

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Abstract

Prevalence of different abnormal hemoglobins (Hb) in Indian tribal and nontribal population groups is well established. Sickle cell hemoglobin (HbS) is mostly concentrated in Central and South Indian states, whereas HbD and Hb E is mostly found in North-North-West and North-North-East states respectively. HbJ, an alpha globin gene variant, is earlier reported in North India whereas; its presence in the tribal Chhattisgarh state is not well understood. HbE, a beta-globin gene variant was earlier reported in North Eastern states of India. Prevalence of both these abnormal hemoglobins in the Central India specifically in Durg, Chattisgarh is incompletely understood. In this study attempts were made to analyze the presence of abnormal hemoglobins during screening for sickle cell anemia. Briefly, blood samples (N=44) were analyzed for sickle cell anemia screening and confirmatory tests by solubility tests and cellulose acetate membrane electrophoresis at alkaline pH. Two samples showed an abnormal pattern of separation on cellulose acetate membrane other than HbS. Out of total 44 tested samples, five were sickle cell carriers (HbAS), one was heterozygous HbAJ and the another one was homozygous for HbE while remaining other were normal genotypes i.e. HbAA. In brief, a case of homozygous Hemoglobin E from Kurmi caste of other backward community (OBC) and a different heterozygote pattern, i.e. Hb AJ from Brahmin community is reported from Durg, Chattisgarh, Central India. This study provides possible indication of variation of different abnormal hemoglobins, other than HbS, present in the tribal state of Chhattisgarh.

Keywords

Abnormal hemoglobins; Hemoglobin E; Hemoglobin J; Sickle cell carriers; Indian tribe; Chhattisgarh; Endemic malaria

Case Study

The origin of hemoglobinopathies and prevalence of endemic malaria are inter-linked. Natural protection from malaria pathogenesis after infection might be the most likely and highly accepted hypothesis of the origin of different alleles of normal adult hemoglobin in different population groups throughout the world, more specifically in the tropical countries where malaria is endemic. Mutation in some of the of genes encoding hemoglobin, red cell enzymes and membrane proteins are being extensively studied with reference to protection from *Plasmodium falciparum* [1]. Hemoglobin S is very common in tribal Indian states upto 35% carrier prevalence in states of Maharashtra [2].

HbE is one of the world's most common and important mutation. It results in a heterogeneous group of disorders whose phenotype range from asymptomatic to severe. HbE trait and Hb EE are mild disorders [3]. The Hb E β^{26} (Glu to Lys) is concentrated in parts of South East Asia where malaria is endemic and HbE carrier status has been shown to confer some protection against *P. falciparum* malaria [4]. Pathogenesis study suggested that patients who co-inherit a mild β- thalassemia allele with Hb E may have disease on the mild end of the spectrum while those who co-inherited severe β^{+} or β^{0} thalassaemia alleles might be more severely affected [5]. First reported in Assam with 23% carrier prevalence, HbE is widely distributed in North Eastern states of India with high prevalence amongst 46.4 % in Ahoms of Assam i.e. one of the highest for any abnormal hemoglobin reported from any population in the world. Interestingly, only 1% in Mizoram, 3-33% in West Bengal, while it is almost non-existing in South India [6]. Chhattisgarh is a tribal state and well known for the high prevalence of *P. falciparum* malaria. Presence of abnormal hemoglobin i.e. HbS which is other than normal adult hemoglobin (Hb A), is earlier reported and its prevalence pattern is being studied by different groups [7-10]. Prevalence of this abnormal hemoglobin in Central India specifically in Durg, Chhattisgarh is incompletely understood. Attempts were made to analyze urban samples during sickle cell camp at Durg for finding Hb E in the same population group.

There are more than 50 hemoglobin J variants described in the literature [11]. They all have an electrophoretic mobility "faster" than HbA towards anode in common. All are classified under "variants of the alpha or beta chains" (single or multiple base changes) or "hemoglobins with more than one amino acid substitution in the alpha chain." Hemoglobin J, depending on its type, have different characteristics and functions. Indian variant of Hemoglobin J was reported earlier in North Indian region [12]. Previously Hemoglobin J has been noted by many researchers in various countries. The case of HbJ-Rajappen was reported by Hyde et. al. and later by Henthorn et. Al. in their results of a 10-year program in an English Health region [13,14]. HbJ Baltimore was first described in 1963 in an African-American family. Since then, several cases have been reported in distinct racial groups and also incidentally during the study of other entities, such as thalassemia [15]. More recently, the increasingly frequent determination of HbA1c in diabetic persons has contributed to the appearance of cases of Hb J-Baltimore associated with anomalous HbA1c values [16,17].

In brief, separation of hemoglobin composition was carried out by cellulose acetate membrane electrophoresis method after solubility tests. Briefly, peripheral blood samples (N=44) after red cell washing and solubility testing for screening HbS were processed for hemolysate preparation by osmotic shock method. Pure hemoglobin from hemolysate was subjected to cellulose acetate membrane electrophoresis in Tris-Glycine buffer (pH 8.6). After 45 minutes, resultant hemoglobin pattern was stained by Ponceau S red

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dye followed by destaining by 5% acetic acid. Appropriate known controls i.e. sickle cell carrier samples were applied every time new samples loaded on cellulose acetate membrane for electrophoretic separation of samples.

Out of 44 samples as shown in Table 1, five samples showed sickle cell carrier status with one fast migrating (HbA) and one slow migrating band (HbS). Two samples i.e. #17 and #27 (Figures 1 and 2) showed mobility pattern different than HbS. Based on mobility pattern, both samples were compared with normally fast migrating HbA and slow migrating HbS. Homozygous Hemoglobin E formed

Table 1: All three abnormal hemoglobin's along with normal pattern (Hb A+A) shown, around 15% abnormal hemoglobin's (Hb A+S, Hb E+E and Hb A+J) were found in the study.

Gender	Mean age in years	Hemoglobin electrophoresis on cellulose acetate membrane in alkaline conditions			
		HbA+A	HbA+S	HbA+J	HbE+E
Male (n=09)	26.77	09	0	0	0
Female (n=35)	22.08	28	5	1	1
Total (n=44)	23.04	37	5	1	1
Percent prevalence		84.09	11.36	2.27	2.27

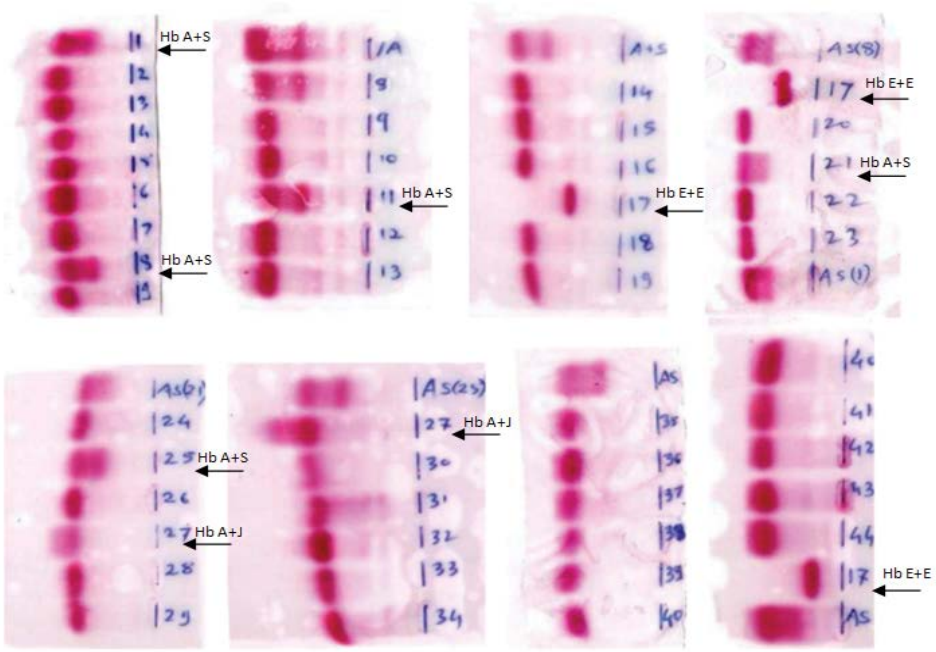

Figure 1: The family tree was shown. The man pointed by an arrow is the proband. The man A had a renal disease but the detail is unclear. The woman B suffers from recurrent self-limiting febrile episodes and arthralgia but the diagnosis is not made. The man C is on hemodialysis. The woman D had a renal transplant.

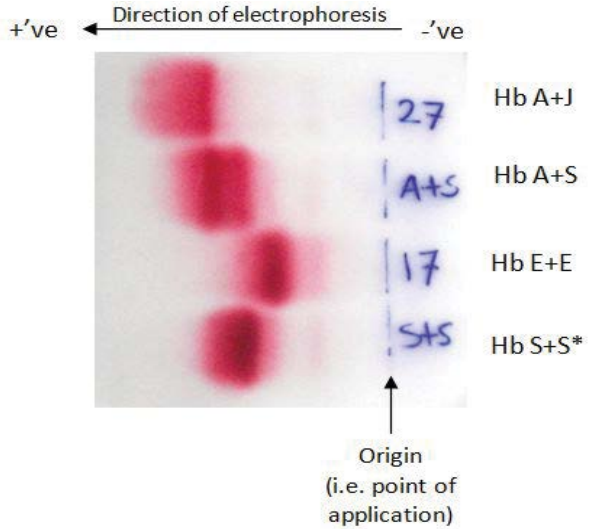

Figure 2: Hemoglobin electrophoresis for sample # 17 and 27. Known A+S sample was applied in between #17 and #27 as appropriate control for referring position of HbA, and HbS on the gel. One homozygous sickle cell disease sample (HbS+S) available in the lab from earlier studies* was also applied below sample #17 as a second positive control for examining position of HbE+E and first positive control HbA+S.

a single band and was very slow during electrophoresis and resolved before HbS whereas, heterozygous HbAJ formed one band with similar mobility pattern like HbA for its majority of fractions and second fast migrating band faster than HbA. Separation pattern of sample no. 17 was different from that of HbA and HbS. After comparison with these abnormal hemoglobins, known mobility of Hb E from review of literature and repeated application during cellulose acetate membrane electrophoresis, it is confirmed that this is Hb E.

In brief, in the present study, a case of homozygous HbE from Kurmi caste of OBC community is found in one 21 year old normal healthy female in Durg Chhattisgarh, Central India. Along with this one abnormal heterozygous case of abnormal hemoglobin variant, HbAJ was also found in one 20 year old healthy female from the Brahmin community without any associated clinical manifestations. As this study was based on very small sample size, the presence of different hemoglobin variants such as HbE and HbJ in single study provides possible indication of variation of different abnormal hemoglobins, other than HbS, present in the tribal state of Chhattisgarh. This initial finding of the presence of abnormal hemoglobin variant in the tribal Chhattisgarh state may prove a significant step for further careful observation of routine sickle cell anemia screening.

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Author contributions

Devendra Lingojwar (DL) contributed to the conception and design of the project, prepared the manuscript and revised it critically for intellectual content. PG, SL and NM has acquired, analyzed and interpreted the data, contributed to data collection and analysis. DL, SB and AK has contributed in image analysis and manuscript preparation. All authors approved the final version of the article.

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