

# Retained Splenic Function in an Indian Population with Homozygous Sickle Cell Disease May Have Important Clinical Significance

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## Abstract

**Background and Objectives:** To determine whether the persistence of splenomegaly characteristic of the Asian haplotype of homozygous sickle cell (SS) disease is associated with continued splenic function, a comparison of patients from Odisha, India, and Jamaica. **Materials and Methods:** Indian patients were examined in a cross-sectional study and compared with the Jamaican Cohort Study from birth. Splenomegaly was assessed in both populations with standard methods. Splenic function was assessed in both by counts of pitted red blood cells determined by differential interference contrast microscopy in the same laboratory. **Results:** In Jamaica, the spleen became palpable in 55% of patients during the 1<sup>st</sup> year of life and the prevalence declined thereafter, whereas in Indian patients, the prevalence rose steeply after the age of 4 years. Raised pitted red cell counts, consistent with loss of splenic function, were common after 2 years in Jamaicans but did not increase in Indians until after the age of 5 years. **Interpretation and Conclusions:** The maximal risk of invasive pneumococcal infection in SS disease falls sharply after the age of 3 years, and persistence of splenic function in Odisha patients beyond this age may explain the apparent absence of pneumococcal septicemia in Indian patients and questions the role of pneumococcal prophylaxis.

**Keywords:** Asian haplotype, pitted red blood cells, sickle cell disease, splenic function, splenomegaly

## INTRODUCTION

The spleen is central to much of the early pathology of homozygous sickle cell (SS) disease as the less pliable red cells compromise its normal functions. The declining fetal hemoglobin (HbF) in postnatal life is associated with increasing levels of sickle hemoglobin (HbS), increasing intravascular sickling, and a tendency to splenomegaly. The time course of splenomegaly differs between patients of African origin and those with the Asian haplotype of the disease. In Jamaican patients, the majority develop splenomegaly in the 1<sup>st</sup> year of life followed by a progressive age-related decline in frequency<sup>[1]</sup> whereas splenomegaly in patients with the Asian haplotype develops later and persists for longer.<sup>[2,3]</sup> Splenic enlargement does not necessarily imply continuing function,<sup>[4,5]</sup> but pitted red cells, which correlate with other indices of splenic function, such as Howell–Jolly bodies and technetium (<sup>99m</sup>Tc) sulfur colloid scans,<sup>[6]</sup> provide an independent assessment of splenic function. Pitted red cell

counts rose with compromised splenic function in Jamaican patients<sup>[7]</sup> and pitted red cell counts increased later in disease of the Asian haplotype in eastern Saudi Arabia.<sup>[8]</sup> Genetic factors known to inhibit sickling such as high levels of HbF<sup>[1]</sup> and alpha-thalassemia<sup>[9]</sup> are associated with persistence of splenomegaly in Jamaican patients and both are common in the Asian haplotype of SS disease. Since early loss of splenic function renders patients prone to overwhelming septicemia and to acute splenic sequestration (ASS), it becomes important to establish the pattern of splenomegaly in the Asian haplotype and whether this splenomegaly is associated with evidence of continuing splenic function.

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## MATERIALS AND METHODS

The Jamaican SS Cohort Study recruited 311 patients with SS disease from newborn screening of 100,000 consecutive nonoperative deliveries at the main Government Maternity Hospital (Victoria Jubilee) in Kingston, Jamaica, between June 1973 and December 1981.<sup>[10]</sup> Patients with SS disease were followed prospectively with regular clinical assessments including routine hematology, estimations of HbF, and measurement of spleen size, so the age at which the spleen first became palpable was recorded. Deaths and emigration led to attrition of patient numbers, so data were depicted in 5-year groups from the age of 10 years. Of the 311 SS patients recruited, pitted red cell counts were measured in 242 patients aged 0.1–8.3 years between November 1976 and November 1984, and in those with multiple counts, the last count was selected for analysis.

Indian patients attended the SS Clinic operated at the VSS Medical College Hospital, Burla, Sambalpur, Odisha state.<sup>[2]</sup> Patients were seen once during the study period (February 19–March 6, 1986) and so data are entirely cross sectional. Clinical examinations included spleen size (cm) measured in the axis of the spleen below the left costal margin in all patients by a single observer (GRS).

In both groups, venous blood was taken by “Vacutainer” into ethylenediaminetetraacetic acid and one drop placed in 0.5 ml 10% formal saline. Pitted red cells in Indian patients were counted by differential interference contrast microscopy (Nomarski optics) by a single individual (BES) and in Jamaican patients by the same individual or one other physician. Counts of 10% or more were considered abnormal. HbF was measured by alkali denaturation<sup>[11]</sup> in both groups in the Jamaican laboratory. Values for HbF were entirely cross sectional in Indian patients, but as multiple values were available in Jamaican patients, the presented values are the mean of steady-state values over the age of 5 years avoiding the rapid age-related change in SS disease before this age.<sup>[12]</sup>

### Statistical methods

Clinical splenomegaly at any time (yes/no) was modeled using logistic regression adjusting for age at splenomegaly, then including two potential predictors: level of HbF (%) and country of residence.

## RESULTS

The frequency of splenomegaly is summarized in Table 1 and graphically in Figure 1. In the Jamaican Cohort Study, splenomegaly occurred in 55% in the 1<sup>st</sup> year of life and declined progressively thereafter. In Odisha patients, splenomegaly was first seen at the age of 3 years increasing at later ages and tending to decline in frequency after 20 years. Overall, splenomegaly was significantly more frequent in Jamaican compared to Indian patients (odds ratio: 4.0, 95% confidence interval [CI]: 1.2–13.2,  $P = 0.03$ ), but this finding

**Table 1: Splenomegaly expressed as incidence in Jamaicans and prevalence in Indians from Odisha**

Age group (years)	Patients with splenomegaly, <i>n</i> (%) <sup>†</sup>	
	Jamaican data	Indian data
<1	311 (55)	-
1.0-1.9	137 (43)	-
2.0-2.9	77 (21)	2 (0)
3.0-3.9	61 (30)	4 (25)
4.0-4.9	43 (20)	12 (83)
5.0-5.9	33 (9)	6 (50)
6.0-6.9	30 (9)	5 (80)
7.0-7.9	27 (0)	12 (67)
8.0-8.9	26 (4)	7 (86)
9.0-9.9	25 (17)	2 (100)
10.0-14.9	22 (5)	17 (100)
15.0-19.9	18 (6)	19 (74)
20.0-24.9	16 (0)	13 (69)
25.0-29.9	15 (0)	11 (55)
≥30.0	13 (0)	11 (25)
Subtotal		121
Splenectomy		3
No values		7
Total		131

<sup>†</sup> represents % of those examined in each group. Denominator adjusted as seven patients did not have spleen measured

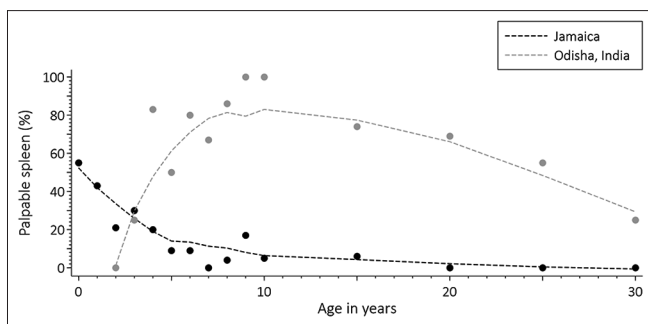
is difficult to interpret because of the different time courses of splenomegaly in the two populations. In both groups, the prevalence increased with each 1% increase in HbF level (odds ratio: 1.09, 95% CI: 1.02–1.18,  $P = 0.02$ ).

Pitted red cell counts in the two populations are compared in Table 2 and graphically in Figure 2. For Jamaicans, pitted red cell counts were not elevated in the 1<sup>st</sup> year of life but increased progressively from the 2<sup>nd</sup> year and were elevated in the majority from the age of 4 years, whereas in Odisha patients, the first elevated counts occurred at the age of 5 years and increased at later ages.

## DISCUSSION

Data from whole-genome sequencing suggest that the Asian haplotype of SS disease arose from a single occurrence of the HbS mutation approximately 7300 years ago.<sup>[13]</sup> This haplotype accounts for over 90% of the disease in the Indian subcontinent<sup>[14,15]</sup> and for almost all the diseases in the Eastern Province of Saudi Arabia.<sup>[3,14]</sup> It is associated with elevated HbF<sup>[8,14,16]</sup> and with alpha-thalassemia,<sup>[8,15,17,18]</sup> both of which inhibit sickling.

The spleen acts like a filter in the bloodstream taking out damaged red blood cells and bacteria that gain access to the blood. In SS disease, the many damaged or abnormal red cells clog up this filter causing initially an enlargement and later a progressive splenic fibrosis. The behavior of the spleen, therefore, gives information on the degree of intravascular sickling and its early loss of function renders patients prone to overwhelming infections.<sup>[19-23]</sup>



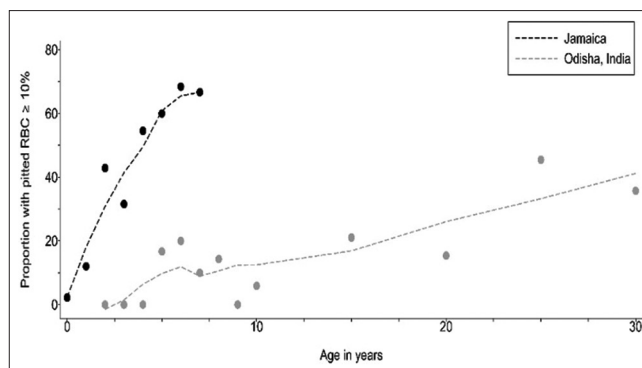
**Figure 1:** Incidence of splenomegaly in jamaicans and prevalence in indian subjects from Odisha

Splenomegaly has different time courses in Jamaican and Indian patients. Most Jamaican patients develop splenomegaly in the 1<sup>st</sup> year of life with subsequent declining prevalence so that few patients have a palpable spleen after the age of 10 years. In Odisha patients, splenomegaly occurs later and lasts for longer, and data in other Indian areas,<sup>[24]</sup> although limited, are consistent with this. It is of interest that this pattern occurs occasionally in Jamaican patients with high HbF levels<sup>[1]</sup> or alpha-thalassemia,<sup>[9]</sup> both factors ameliorating sickling, and both characteristic of the Asian haplotype of SS disease. It seems reasonable to conclude that splenic enlargement occurs later and lasts longer in Odisha patients compared with Jamaicans and probably other patients with SS disease of African origin.

In hematologically normal people, HbF declines from the high levels at birth to mean levels below 1% by 1 year of age. In SS disease, this decline is slower and age related, so comparisons should be matched for age as closely as possible. HbF values in older children and adults in Gujarat<sup>[18]</sup> and Odisha,<sup>[2]</sup> in children in Akola,<sup>[25]</sup> and at the age of 1 year in Nagpur<sup>[24]</sup> are all significantly higher than for comparable age groups in Jamaica. The observed prevalence of alpha-thalassemia (heterozygous and homozygous combined) was 37% in Jamaica, 16% in Akola,<sup>[25]</sup> 16% in Nagpur,<sup>[24]</sup> and 53% in Odisha.<sup>[2]</sup> Of these two genetic factors associated with the Asian haplotype, the consistently elevated HbF levels seem a more likely candidate influencing splenic natural history than the variable prevalence of alpha-thalassemia.

Splenomegaly does not necessarily imply continued splenic function<sup>[4,5]</sup> although low pitted red cell counts consistent with continuing splenic function were found in 17/24 (71%) patients with a mean age of 13.7 years in the Eastern Province of Saudi Arabia.<sup>[8]</sup> Over half of the Jamaican cohort had abnormal pitted red cell counts by the age of 5 years, but in Odisha patients, abnormal counts began in the 6<sup>th</sup> year and increased markedly after the age of 15 years.

The persistence of splenic function in Indian patients may have profound implications for clinical management. In SS disease of African origin, the early loss of splenic function renders patients prone to pneumococcal septicemia and ASS, so pneumococcal prophylaxis<sup>[26,27]</sup> and parental education on early detection of ASS<sup>[28]</sup> are now routine



**Figure 2:** Pitted red blood cells in jamaicans and indian subjects from Odisha

**Table 2: Pitted red blood cells in Jamaicans and Indians from Odisha**

Age group (years)	Patients with raised pitted RBC, n (%) <sup>†</sup>	
	Jamaican data	Indian data
<1.0	43 (0)	-
1.0-1.9	45 (9)	-
2.0-2.9	30 (37)	2 (0)
3.0-3.9	42 (36)	5 (0)
4.0-4.9	26 (54)	12 (0)
5.0-5.9	29 (59)	6 (17)
6.0-6.9	23 (65)	5 (20)
7.0-7.9	3 (67)	10 (10)
8.0-8.9	1	7 (14)
9.0-9.9	-	2 (0)
10.0-14.9	-	17 (6)
15.0-19.9	-	19 (21)
20.0-24.9	-	13 (15)
25.0-29.9	-	11 (45)
≥30.0	-	14 (36)
Subtotal	242	123
Splenectomy	4	3
No values	65	5
Total	311	131

<sup>†</sup>represents % of those examined in each group. RBC: Red blood cells

in the clinical management. Since the risk of invasive pneumococcal disease falls sharply after the age of 3 years,<sup>[19-23]</sup> the persistence of splenic function beyond this age may confer protection against the pneumococcus. This may account for the apparent absence of pneumococcal septicemia in Indian SS disease either in the published literature or from discussions with pediatricians expert in the disease in India. ASS also dominates in the first 3 years of life in disease of African origin,<sup>[28]</sup> and there is a need to document the natural history of this complication in Indian patients.

Major strengths of the present study are that assessments of spleen size were performed by the same clinician GRS), the pitted red cell counts were performed by the same individual (BES), and that estimations of HbF were performed

by the same techniques in the same laboratory. A weakness is the small number of Odisha patients before the age of 4 years, but it seems unlikely that younger patients would manifest earlier loss of splenic function. In view of the more severe disease reported from Central India,<sup>[25,29]</sup> it could be argued that lessons from Odisha may not be representative of other areas of India.

## CONCLUSIONS

Compared to the predominantly Benin haplotype of Jamaica, the Asian haplotype of SS disease in Odisha, India, is associated with the later occurrence of splenomegaly and retention of splenic function beyond the high-risk period for invasive pneumococcal disease. This may account for the absence of this complication in Indian SS disease in the literature and casts doubt on the role of pneumococcal prophylaxis. The persistence of splenic function probably reflects the high HbF levels associated with this haplotype. It is unclear whether these observations may be extrapolated to the more severe disease in Central India. The significance of these observations on other splenic complications such as ASS and hypersplenism remains to be clarified.

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## Conflicts of interest

There are no conflicts of interest.

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