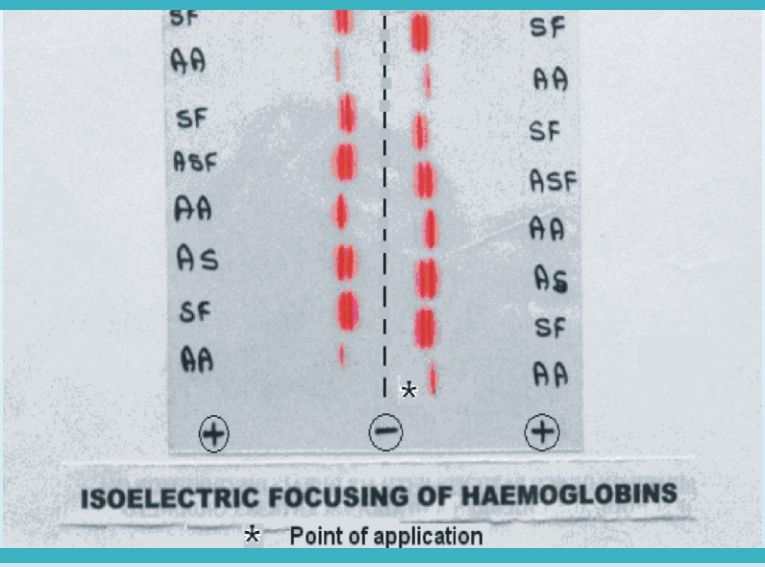
1 Genetic Studies



1.1 Prevalence of haemoglobinopathies among the scheduled tribes and scheduled castes of Shahdol district of Madhya Pradesh.

1.2 Morbidity profile of sickle cell disease in central India

1.3 Community control programme of haemoglobinopathies

1.4 Intermolecular interaction of sickle haemoglobin fiber

1.5 Impact of health education and counseling on knowledge, attitude & practices on sickle cell anaemia in Baiga tribe of Dindori district

1.1 Prevalence of haemoglobinopathies among the scheduled tribes and scheduled castes of Shahdol district of Madhya Pradesh.

Dr. R.B. Gupta

Status : Ongoing project (November 2002 - October 2004)

Objective

To find out the prevalence of haemoglobinopathies and G-6-PD deficiency among the scheduled tribes and scheduled castes of Shahdol district.

Methodolgy

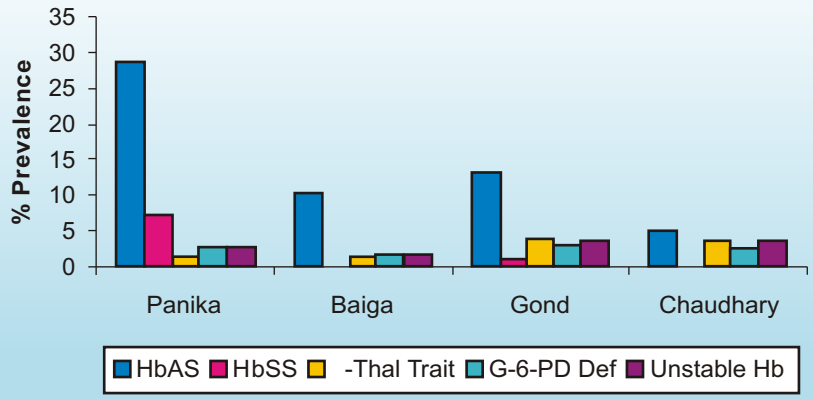
About 3 ml blood was collected from the study subjects. Complete blood count was carried out by automatic blood cell counter. HbS was identified by sickling test & electrophoresis. G-6-PD deficiency was detected by using DCIP decolourising test. -thalassaemia trait was detected using micro column chromatography. Alkali denaturation test was used to estimate foetal haemoglobin.

Salient Findings

The study was carried out in Shahdol district of M.P., where the scheduled tribe (ST) and scheduled caste (SC) population are 46.3% and 7.7% respectively (1991 Census). A total of 252 individuals from Gond tribe, 210 from Panika tribe, 219 from Baiga tribe and 195 Chaudhary from the SC group were studied. The prevalence of sickle haemoglobin as trait was higher in STs ranging from 12.0 to 28.6%, while it was 5.1% in SC group. The prevalence of sickle haemoglobin among Panikas was 28.6%, while among Gonds, 13.1%. The prevalence of -thalassaemia trait was 4% among Gonds, 3.6% among Chaudhary and 1.4% in Baigas as well as Panikas (Figure 1.1.1).

The prevalence of G-6-PD deficiency in the study area varied from 1.8 to 3.2%. The prevalence of unstable haemoglobin ranged from 1.8 to 3.6%.

Fig.1.1.1 Prevalence of haemoglobinopathies and G-6-PD deficiency in Shahdol district

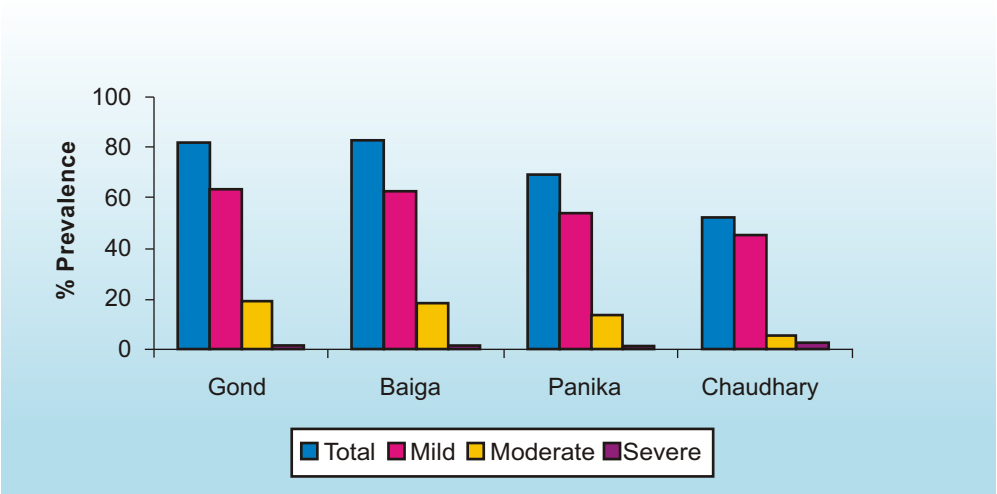


Anaemia was significantly higher (P<0.001) in STs (66.6%) than SCs (46.2%) (Figure 1.1.2). There were 10 persons with sickle cell disease (homozygous) in apparently healthy condition. Haemoglobin (Hb) level of these patients varied from 6.3 to 11.7 gm/dl (Table 1.1.1).

Table 1.1.1 Haematological parameters in sickle cell disease patients

Tribe	N	Hb (g/dl)	PCV (l/l)	TRBC ($X10^{12}$ /l)	MCV (fl)	MCH (pg)	MCHC (l/l)	HbF (%)	HbA_2 (%)
Gond	3	9.5 ±2.1	0.273 ±.066	3.7 ± 0.6	74.0 ± 5.0	25.7 ± 1.5	34.8 ± 1.0	8.7 ± 2.2	1.9 ± 0.4
Panika	7	9.2 ±1.8	0.236 ±.0 52	3.1 ± 0.6	73.6 ± 7.2	28.0 ± 3.1	38.0 ± 2.2	7.1 ± 1.4	1.6 ± 0.6

Fig. 1.1.2 Prevalence of anaemia in Shahdol district



Sickle cell disease is very common in the study area and highly prevalent especially in Panika tribe. About 0.6 - 30 per thousand population of SCs/STs is expected to suffer from sickle cell disease.

By the time the annual report is ready, this project would be completed.

Recommendations

- There is a need for suitable intervention policies for prevention and management of sickle cell disease.
- Anaemia control programme should be strengthened especially in tribal areas with a focus on children and women.

1.2 Morbidity profile of sickle cell disease in central India

Dr. R.B. Gupta, Dr. Rajiv Yadav, Dr. V. Bharadwaj*

Status : Ongoing project (October 2001 - September 2005)

Objectives

1. To study the clinical and haematological profile of sickle cell disease.
2. To develop strategies for prevention and management of sickle cell disease.

Methodology

RMRCT runs a sickle cell clinic at the NSCB Medical College, Jabalpur. It identifies the patients with sickle cell disease. Detailed history was obtained from patients suspected to have sickle cell disease. They were examined for various signs of sickle cell disease. Complete blood count profile of these patients was studied using blood cell counter. Patients were treated with folic acid daily and antipyretic when required. Treatment was also given for other common ailments as and when reported by patients.

Salient Findings

A total of 287 patients were registered in sickle cell clinic till June 2004. Two patients died during the study. About three fourth of the patients were below 15 years of age, 40% belonged to SC community (Jharia, Mehara, Deharia) and another 30% to OBC (Kurmi, Lodhi, Yadav, Sahu and Bairagi) group. Among the main clinical complications for which patients sought medical intervention were painful crises of bones and joints (55.3%), recurrent fever (48%), abdominal pain/splenic pain (27.7%) and weakness (27.7%). Splenomegaly was the commonest clinical sign observed in 68.8% of the patients of all the age groups. About 4.3% patients had massive spleen and another 2.1% had undergone splenectomy. About 4.6% of the patients required multiple blood transfusions. Twenty two percent patients received first blood transfusion before the age of 5

* N.S.C.B., Medical College, Jabalpur

years. The disease was graded into three types using 13 point scale i.e. mild (1-4), moderate (5-8) and severe (9-13). The patients were followed up every fourth month in steady state and were requested to attend the clinic in between, if they fall sick. The patients were given folic acid, vitamin B-complex and antipyretic/anti-inflammatory agents. The patients and their guardians were educated about preventive measures.

One hundred and sixty five patients were followed up for one year. There was a marked reduction in the clinical severity of disease after intervention. The proportion of severe cases reduced from 15% to 7% after intervention (Fig 1.2.1).

The CBC profile of sickle cell disease patients is given in Table 1.2.1 Generally, the patients had higher level of foetal haemoglobin (above 10%) with mild to moderate level of haemolytic crises. There were wide variations in all the haematological parameters especially in Hb, MCV, MCH and MCHC.

The weights and heights of sickle cell disease patients were recorded and compared with NCHS standards (Table 1.2.2) for grading their status according to standard deviation classification. About 42% of preschool children (1-5 years) were severely affected. In 6-17 years age group, about 45% were moderately under nourished. About three fourth of the patients above 18 years of age had various degrees of chronic energy deficiency. Only 3.6 % of the patients had normal values for the BMI (Table 1.2.3).

Table 1.2.1 CBC profile of SCD patients

Group	N	Hb (g/dl)	PCV (%)	TRBC (X 10^{12}/l)	MCV (fl)	MCH (pg)	MCHC (l/l)	HbF (%)	HbA2 (%)	Retic (%)
Male	64	8.4 ± 2.5	25.2 ± 6.9	3.2 ± 0.9	79.1 ± 12.3	26.6 ± 5.2	33.4 ± 3.2	13.8 ± 6.6	3.0 ± 1.7	5.9 ± 3.9
Female	25	8.4 ± 2.0	24.7 ± 6.0	3.1 ± 0.7	79.8 ± 12.0	27.6 ± 4.8	34.1 ± 3.2	13.4 ± 4.8	3.2 ± 2.0	5.7 ± 2.4
Children	144	7.6 ± 1.9	22.9 ± 5.6	3.0 ± 1.0	80.3 ± 14.2	27.0 ± 5.2	33.4 ± 5.6	11.8 ± 4.8	2.4 ± 1.0	7.6 ± 5.5

Table 1.2.2 Distribution of nutritional status of SCD patients

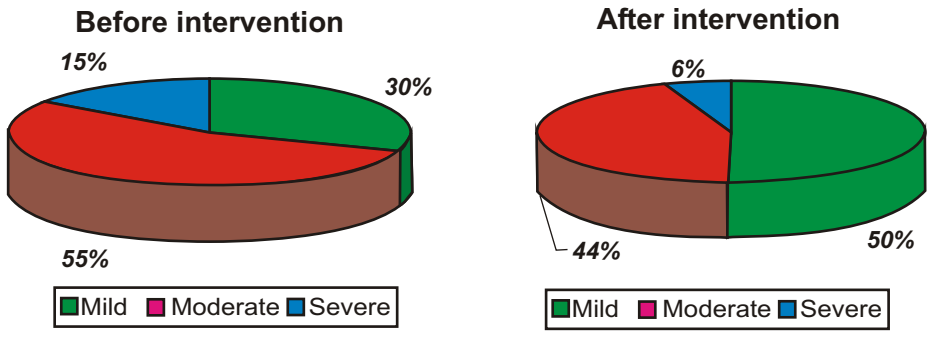
	Age group (Years)	N	Severe	Moderate	Normal
Height for age	1-5	33	42.4	43.3	15.2
	6-17	95	30.5	30.5	38.9
Weight for age	1-5	33	42.4	42.4	15.1
	6-17	93	31.2	45.2	23.6
Weight for height	1-5	33	6.1	39.4	54.5
	6-17	75	8.0	38.7	53.3

Table 1.2.3 BMI status of sickle cell disease patients (above 18 years)

Body Mass Index	Grade	N	%
Below 16	III Degree CED	8	28.6
16 - 17	II Degree CED	7	25.0
17 - 18.5	I Degree CED	7	25.0
18.5 - 20	Low Normal	5	17.8
20 - 25	Normal	1	3.6
25 - 30	I Degree Obesity	0	0

(CED = Chronic Energy Deficiency)

Fig 1.2.1 Effect of intervention on severity of sickle cell disease



1.3 Community control programme of haemoglobinopathies

Dr. R.B. Gupta, Dr. Dipika Mohanthy*

Status : Ongoing project (April 2004 - March 2005)

Funding Agency : WHO

Objective

To find out the prevalence of haemoglobinopathies among women attending ANC clinic.

Methodology

About 3 ml of blood sample was drawn from pregnant women attending the ANC clinic at NSCB Medical College, Jabalpur. Complete blood count was carried out using automatic blood cell counter. HbS was identified by solubility test as well as electrophoresis on alkaline and acidic pH. -thalassaemia trait was identified by determining the raised level of HbA_2 by elution technique as well as by separating the haemolysate by HPLC on variant analysis system. Foetal haemoglobin was estimated by alkali denaturation test. Iron deficiency was determined by estimating free erythrocyte porphyrin level.

Salient Findings

The study aims to find out the prevalence of common haemoglobinopathic disorders among women attending the antenatal clinic at NSCB Medical College, Jabalpur. Three hundred and sixty three pregnant women have been screened till date. Twenty two (6.1%) women showed sickle cell trait; -thalassaemia trait was seen in 2.1% pregnant women. One woman was heterozygote for haemoglobin E and one, homozygous for sickle haemoglobin. All these women were counselled for risk of pregnancy for haemoglobnopathies and were advised to screen their husbands for haemoglobinopathies. The prevalence of anamia was 71.8%. About 7% women were severely anaemic (Hb <7g/dl) and another 22.4% were

* IIH, Mumbai

moderately anaemic. Iron deficiency was assessed by estimating free erythrocyte protoporphyrin level in blood. It was detected in 61.7% women.

A pilot survey was also carried out to know the prevalence of malaria in haemoglobinopathic heterozygotes in a high transmission tribal area of Panna district of Madhya Pradesh, which witnessed falciparum malaria epidemic recently. The study was carried out in Shyamgiri area of Panna district. In total, 287 persons were investigated for sickle haemoglobin, -thalassaemia trait and G-6-PD deficiency. The overall prevalence of sickle haemoglobin was 5.2% and G-6-PD deficiency was 0.7%. The prevalence of sickle cell haemoglobin in Gond tribe was 6.6%, which was low as compared to Gond population of various districts of Central India. In these 287 individuals, 99 episodes of *P. falciparum* malaria were recorded. The incidence of *P. falciparum* malaria did not correlate with the sickle haemoglobin status of the individual.



1.4 Intermolecular interaction of sickle haemoglobin fiber

Dr. R.P. Roy,* **Dr. R.B. Gupta**

Status : Ongoing project (September 2003 - February 2005)

Objectives

1. Construction of mutant -chain.
2. Assembly of HbS tetramers with mutant -chains and ^{s}-chain z (Glu Val).
3. Structural and functional characterization of mutant human HbS.

Methodology

Haemoglobin from sickle cell disease patients and normal persons was purified on DE-52 column and and globin chains were separated on CM-52.

1-30 analogues peptides were synthesized by semi-automatic peptide synthesizer. Mutant -globin chains were constructed by V8 protease mediated semisynthesis and monitored by RPHPLC. The heme free mutant globin chains were reconstituted with ^{S} chain and heme to tetrameric mutant HbS through the Alloplex route. CD spectra of HbS will be recorded on J710 spectrophotometer. Oxygen affinity of the mutant/ synthesized hemoglobin will be carried out on Hemox Analyser.

Salient Findings

The project aims to study the role of substituted coded and non coded amino acids in the dynamics of deoxyhaemoglobin HbS fiber assembly. We also plan to study the short or long range addivity or nonaddivity of mutational effect to delineate interaction-linkage between selected contact regions of HbS fiber. Till date we have examined the role of dynamics of AB/GH region of HbS

* NII, New Delhi

polymerization through simultaneous replacement of non-contact Ala[19] and Ala[21] of the AB corner with more flexible glycine or rigid -aminoisobutyric acid (Aib) residues. The polymerization behavior of HbS with Aib substitution was similar to native HbS. But the substitution by glycine inhibited HbS polymerization. Molecular dynamics simulation studies of -chain indicated that coordinated motion of AB and GH region residues present in native as well as Aib mutant was disrupted in the Glycine mutant. The inhibitory effect due to Gly substitutions was further explored in the triple mutants that included mutation of an inter-double strand contact (Asn[78] His or Gln) at the EF corner. Although the inhibitory effect of Gly substitutions in the triple mutant at [78] position was unaffected by the presence of Gln[78] but His at this site almost abrogated its inhibitory potential.



A patient of sickle cell disease having splenomegaly

1.5 Impact of health education and counseling on knowledge, attitude & practices on sickle cell anaemia in Baiga tribe of Dindori district

P. L. Pande

Status : Ongoing project (October 2002 - September 2007)

Objectives

1. To screen the population for sickle cell anaemia.
2. To impart health education to the community and high-risk couples using IEC strategies.
3. To assess the impact of intervention on prevalence of sickle cell anemia.

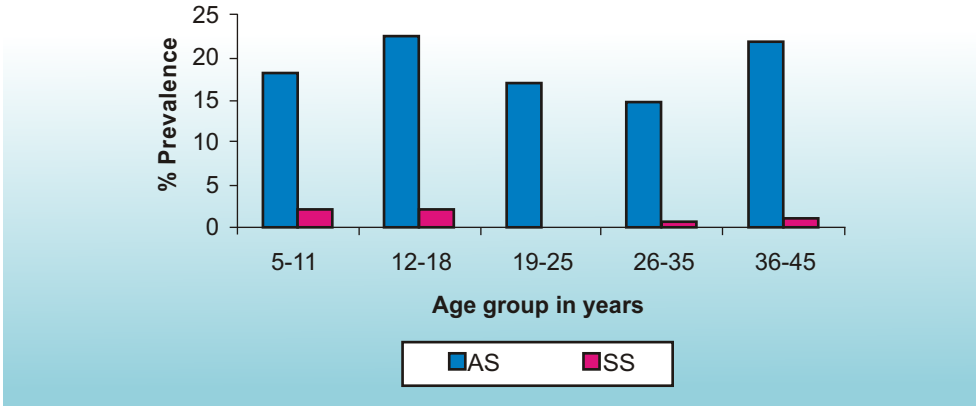
Salient Findings

The study is being conducted in Baiga tribe of Dindori district. Nine hundred and ninety individuals, who were selected randomly, formed the study group. They were screened for the prevalence of sickle cell anaemia. A structured schedule was used to know their knowledge, attitude and practices about sickle cell anaemia.

Among the screened population, 20% showed the presence of sickle cell disorder. The age specific distribution of the sickle cell disorder is shown in Fig 1.5.1.

The basic demographic characteristic relevant in understanding sickle cell anaemia as revealed from the survey is that most of the marriages take place at the age of 18 to 21 years within a radius of 20 km of the villages. Marriage endogamy is usually practiced. About 38% of the marriages were found to be consanguineous. Not a single villager was found to be aware of sickle cell disease. As a part of intervention, door-to-door health education on sickle cell anaemia is in progress.

Fig. 1.5.1 Sickle cell disease in Baigas





Study area