Correlation between Clinical and Pathological Findings in Sickle Hemoglobin (HbS) Disease

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Abstract

Introduction: Present study relates to the results of clinical examination of 10 patients with sickle hemoglobin.
Material and Methods: All the patients belonged to TharuChaudhary community of Nepal. The patients were clinically examined. Sickling test was done using Sodium metasulphite. Hemoglobin agarose gel electrophoresis was done at alkaline pH. Total hemoglobin was estimated by cyanhemoglobin method.
Results: Sickling test was positive in all the patients. In addition, electrophoresis revealed detection of HbS in all the patients. Four of 10 patients had homozygous sickle cell disease (βglobin genotype SS). Five other patients had heterozygous sickle cell trait (βglobin genotype AS). Another patient had double heterozygous sickle cell β+thalassemia; high level of fetal hemoglobin (16.4%) was also detected in this patient.
Discussion: Capillary thromboses might have resulted in minor infarcts, resulting in pain in chest and abdomen.
Conclusion: The patients belonged to mid-western region of Nepal; this region is infested with vivax malaria. High frequency of malaria in this region might have resulted in biological advantage for sickle hemoglobin patients.

Keywords:
Sickle cell disease , Nepal

Introduction

Two patients with sickle cell disease (SCD) have been reported earlier from Nepal; one of the patients had sickle cell-β thalassemia and another patient had homozygous sickle cell disease1. In addition, a patient with SCD and avascular necrosis of femoral head was reported from Nawalpasi district of Nepal2.

Present study was done in mid-western region of Nepal. Surprisingly, many cases with abnormal hemoglobins were detected. Present study relates to the results of clinical and hematological examination of these patients.

Materials and methods

While investigating the etiopathogenesis of 30 subjects with anemia, we came across with 10 subjects with HbS. Herewith, we report the clinical findings of 10 patients with HbS.

Study population Present study relates to the results of clinical and hematological examination of 10 patients with HbS. Age of the patients ranged from 9 to 60 years (median 23). Male to female ratio was 1:1. Six of 10 patients belonged to Banke district. Three other patients came from Bardiya. Another patient came from Dhangadhi. All the patients belonged to TharuChaudhary community.

Venous blood samples were collected and smears were prepared. About 2.5 ml of blood was collected in a tube containing EDTA as an anticoagulant. It was mixed well and analysed. Smears were stained by Leishman method and examined. Total hemoglobin was estimated by hemoglobincyanide method. Sickle cell test was also done. It is a slide based test for sickling using Sodium metasulphite. CBC was done using automatic cell counter.

Preparation of hemolysate Two ml of EDTA blood was washed × 3 in normal saline. The packed cells were lysed with distilled H2O and centrifuged. Supernatant was collected as hemolysate and stored at +4°C.

Agarose gel electrophoresis at alkaline pH 8.6 Five µl of hemolysate was transferred into well plates using an unit applicator, the sample was applied into agarose gel along with suitable controls and immediately placed in the electrophoresis chamber. The chamber was connected to a power supply and electrophoresed for 45 minutes at 100V. Separation of charged molecules at alkaline pH depends upon their different electrophoretic mobility, electrolyte pH and electro-osmotic flow. Each peak appears in a definite zone. Thus concentration of each hemoglobin variant can be obtained. The equipment was supplied by Biotech-Fischer GMBH Maestro 101, Germany.

Reticulocyte index (RI) was calculated using the following formula.

$$RI = \text{reticulocyte count} \times \text{Patient's Hct} \times \frac{1}{45} \times \frac{}{1.85}$$

45 = normal Hct, 1.85 number of days for reticulocyte to mature

Results

On the basis of results of hemoglobin electrophoresis, 3 groups of patients were detected. Four of 10 patients had HbS concentration of >94% suggesting homozygous sickle cell disease (βgenotype SS). Five other patients had mild rise in HbS (<34%), suggesting a diagnosis of heterozygous sickle cell trait (βgenotype AS).

Another patient had moderate rise in HbS (66.4%) and mild rise in fetal hemoglobin (16.4%) and HbA2 (5.7%). In addition, this patient also had severe reduction in HbA (6.5%). These findings suggested a diagnosis of compound double heterozygous sickle cell β^{+}thalassemia.

(A) **Patients with homozygous sickle cell disease (βgenotype SS) :** This group included 4 patients. Age of the patients varied from 9 to 23 (median 21) years. Male female ratio was 1:3. All the patients had generalized pallor, fever and arthralgia. One of the patients complained of pain both in chest and abdomen. Two patients had jaundice as well as splenomegaly (Table 1).

Total hemoglobin of patients ranged from 4.7 to 10.5 (median 8.4) gm/dl. Three of 4 patients had sickled red cells in blood smears (Table 2).

Sickle cell test was positive in all the patients. Hemoglobin electrophoresis revealed severe rise in HbS concentration (>94%) in 4 patients (Table 3).

(B) **Patients with heterozygous sickle cell trait (βgenotype AS) :** This group included 5 patients. Age of the patients ranged from 12 to 64 (median 32) years. Female male ratio was 1:1.5. Generalized pallor was seen in 3 patients. Both fever and arthralgia were seen in 3 patients. One of the patients had pain in chest and abdomen. Another patient (no.6) had both jaundice and splenomegaly. Moreover, another patient had jaundice alone (Table 4).

Total hemoglobin concentration ranged from 6.2 to 13.9 (median 10) gm/dl.
Sickled red cells were seen in blood smears of 2 patients (Table 5).
Sickling test was positive in all the patients. Moreover HbA was detected in all the patients (Table 6).

(C) **Compound double heterozygous sickle β+thalassemia :** It was detected in a patient. He was a male, aged 31 years. He had fever, arthralgia, pain both in chest and abdomen and jaundice. He alsohad a palpable spleen 4 cms below left subcostal margin. He had moderate anemia (Hb 8 gm/dl). Blood smear showed 2% sickled cells.

Table 7 shows mean values of hematological parameters in 3 groups of patients. B subgroup patients with sickle cell trait had higher mean Hb%, Hct, RBC and MCV suggesting better hematological status as compared to patients of subgroup A with homologous sickle cell disease. In addition, high HbA levels in sickle cell trait patients might have prevented the polymerization of sickle hemoglobin (Table 8).

Discussion

For the first time the presence of HbS gene was detected in aIrula boy in Nilgiri hills[3] in 1952. Later, the HbS gene was found in sickle cell belts of central India[4] and in certain parts of south India[5]. This study describes the some findings in 4 cases of presumed homozygous sickle cell (SS) disease among the Tharu community of Nepal. It is of interest because this is a group outside the typical sickle cell belts of central India and the North of Tamil Nadu and Kerala. Only 5 patients with sickle cell trait were included; these 5 patients were parents of those with SS disease. Cause of jaundice in case numbers 2 and 6 and of splenomegaly in case number 6 with sickle cell trait could not be ascertained. It may be due to some other pathology.

Pain in chest and abdomen could have been caused by capillary thromboses, leading to minor infarcts in these regions. Splenic infarcts followed by inflammation might have produced splenomegaly during early stages of sickle cell disease. Patients with SCD are prone to develop infections. Bacterial infections might have contributed to fever.

Mid-western region of Nepal is infested with Plasmodium vivax. High frequency of malaria in this region might have resulted in biological advantage for patients with HbS. It has been postulated that people with HbS gene might be more resistant to falciparum malaria[6]. In addition, HbS appeared to inhibit the multiplication of Plasmodium falciparum[7].

The mean hematological findings in homozygous and heterozygous sickle cell disease is non-significant. This could be due to additional nutritional and other causes in these cases.

High level of HbF was detected in serum of one of our patients (no.9). In another study, number of blood transfusions and hospitalization rates were much less in those sickle cell disease (SCD) patients which produced higher level of HbF (>12%) when compared with low HbF producers[8].

Another interesting finding was the detection of relatively low level of HbA (6.5%) in a patient with HbS β+ thalassemia (patient no.9). This patient had fever, arthralgia, pain in chest and abdomen and jaundice. He had palpable spleen 4 cm below left subcostal margin. He also had moderate anemia (Hb 8 gm/dl). This patient had more severe disease when compared with an earlier study involving 4 patients with HbA concentration >38% and very mild disease[9]. In another study, two types of S/Thal (with and without HbA) had distinctive haematological and clinical characteristics. Non-HbA type had lower haemoglobin levels, a more rapid hemolysis and more severe course when compared with HbA type[10]. Higher levels of HbA may be associated with a milder disease[11]. However, occasional patient with compound heterozygous state may not develop symptoms until the onset of puberty, early adult life or pregnancy[12]. Genes modifying the inflammatory response or expression of cytokines may alter the clinical severity of disease[12].

Conclusion

Persons with HbS gene are known to have the biological advantage against malaria. The subjects with HbS gene survived in malaria endemic regions.

Conflicts of interest : Nil

Ethical approval : This study was approved by the ethical committee of Nepalgunj medical college, Nepal. The study was performed according to the standards laid down by Helsinski declaration and later amendments. All individuals enrolled in this study gave their consent prior to their inclusion in this study.

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Clinical findings of patients with homozygous sickle cell disease (βglobin genotype SS).

Patient no.	Age in years	Gender	Pallor	Fever	Joint pain	Pain in chest and abdomen	Jaundice	Splenomegaly
1	21	F	+	+	+	+	-	np
3	23	F	+	+	+	-	+	4 cm
5	9	M	+	+	+	-	+	5 cm
8	15	F	+	+	+	-	-	np

np = non-palpable, M = male, F = female

Hematological findings in patients with homozygous sickle cell disease (βglobin genotype SS).

Patient No.	Total Hbgm/dl	Hct Cc%	RBC million /mm^3	MCV fl	MCH pg	MCHC cc%	RDW %	Reticulocyte index	Target cells %	Sickled cells %
1	8.4	25.7	3.56	72.2	23.6	32.7	19.7	1.4	6	8
3	4.7	16.9	1.99	84.9	23.6	27.8	19.8	1.4	-	9
5	7.4	24.1	4.44	54.3	16.7	30.7	16.0	1.8	-	-
8	10.5	36.7	5.20	65.4	26.2	35.2	14.7	0.8	5	2

+ Positive, - negative

Results of sickling test and hemoglobin electrophoresis in patients with homozygous sickle cell disease (βglobin genotype SS).

Patient no.	Sickling test	Hemoglobin electrophoresis				βglobin genotype	Name
		HbA2	HbS	HbF	HbA		
1	+	0.60	99.6	-	-	β^s/β^s	Homozygous sickle cell disease
3	+	1.72	98.3	-	-	β^s/β^s	- do -
5	+	1.90	98.1	-	-	β^s/β^s	- do -
8	+	5.80	94.2	-	-	β^s/β^s	- do -

+ Positive, - negative, results of hemoglobin electtophoresis have been expressed as percentage (%).

Table 4 : Clinical findings of patients with heterozygous sickle cell trait (βglobin genotype AS).

Patient no.	Age in years	Gender	Pallor	Fever	Joint pain	Pain in chest and abdomen	Jaundice	Splenomegaly
2	19	F	+	-	-	-	+	np
4	60	M	+	+	+	-	-	np
6	32	F	-	+	+	-	+	6 cm

7	12	M	+	+	+	+	-	np
10	64	M	-	-	-	-	-	np

np = non-palpable, M = male,F = female

Table 5 : Hematological findings in patients with heterozygous sickle cell trait

Patient No.	Total Hbgm/dl	Hct cc%	RBC million /mm^3	MCV fl	MCH pg	MCHC cc%	RDW %	Reticulocyte index	Target cells %	Sickled cells %
2	13.9	41.0	5.96	69.0	23.4	35.8	15.9	1.8	-	-
4	10.0	32.7	4.78	68.4	21.1	30.9	15.9	1.3	-	2.2
6	9.0	32.0	5.11	68.0	33.0	26.0	14.2	1.6	-	7
7	6.2	20.7	2.69	77.0	23.0	30.0	23.6	9.0	4	-
10	13.5	45.1	4.95	91.0	27.0	37.0	14.9	0.7	-	-

Table 6 Results of sickling test and hemoglobin electrophoresis in subjects with heterozygous sickle cell trait

Patient no.	Age in years	Sickling test	Hemoglobin electrophoresis				Name
			HbA2	HbS	HbF	HbA	
2	19	+	0.6	20.2	-	77.9	Sickle cell trait
4	60	+	2.9	3.8	-	93.0	- do -
6	32	+	1.1	25	-	74.0	- do -
7	12	+	0.8	24.3	-	75.0	- do -
10	64	+	5.0	33.2	0.8	52.0	- do -

+ positive, hemoglobin electrophoresis results have been expressed as percentage (%).

Table 7 Shows mean values among 3 groups of subjects.

Hematological parameters	(A) Homozygous sickle cell disease (n = 4)	(B) Heterozygous sickle cell trait (n = 5)	(C) HbS β+thalassemia (n = 1)
Total Hbgm/dl	7.75	10.52	8.0
Hct cc%	25.85	34.3	24.0
RBC million/mm^3	3.79	4.69	3.83
MCV fl	69.2	74.68	62.4
MCH pg	22.5	25.5	21.0
MCHC cc%	31.6	31.94	34.0
RDW%	17.55	16.9	14.0
Reticulocyte index	1.35	2.88	2.2
Target cells%	2.7	0.9	4.0
Sickled cells %	4.7	0.54	2.0

Table 8 Shows mean values of different hemoglobins in 3 groups of patients.

Groups of patients	Number of patients	Hemoglobin electrophoresis			
		HbA2	HbS	HbF	HbA
(A) Homozygous sickle cell disease (βglobin genotype SS)	4	2.2	97.5	-	-
(B) Heterozygous sickle cell trait (βglobin genotype AS)	5	2.8	16.4	-	60.38
(C) Double heterozygous HbS β+thalassemia	1	5.7	66.4	16.4	6.4

Hemoglobin electrophoresis results have been expressed as percentage (%).