

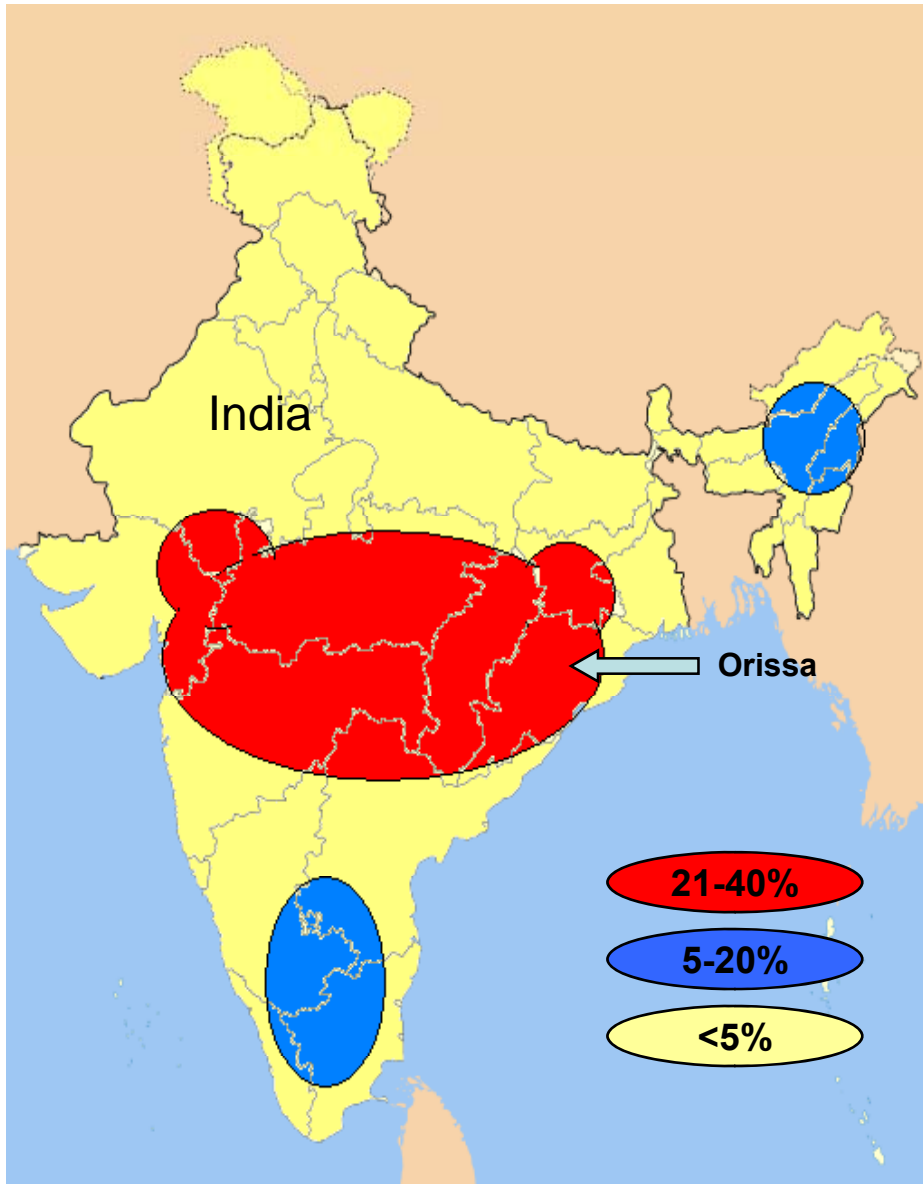


Epidemiology & Clinical aspects of Sickle Cell Disease in India



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Prevalence of Sickle Cell gene in India



India's more than one billion population. It has a complex caste system.

Practice of endogamy and consanguineous marriages are very common.

Sickle gene frequency is between 5% to 40%, distributed in 3 different geographic zones.

The State of Orissa where this study was undertaken falls into the High prevalence zone (21-40%).

Study Site

Place of Study: Sickle Cell Clinic and Molecular Biology Laboratory, V.S.S. Medical College, Burla , Orissa, India

The hospital caters to a population of about 40 million from western part of Orissa state and adjoining districts of Chhatisgarh state.

The area is highly endemic for *P.falciparum* malaria

Study Population: Tribal/aboriginals - 12%
Scheduled castes (socially deprived) - 26%
Backward castes (socially underprivileged) – 46%
Upper castes (socially privileged castes) - 16% .

Cross-sectional studies showed prevalence of sickle gene 21%, α -thalassemia 25%, β -thalassemia 5.0%, HbE & HbD <1% each (Unpublished observations)

Period of Study: May 1998 to April 2009

Study Subjects

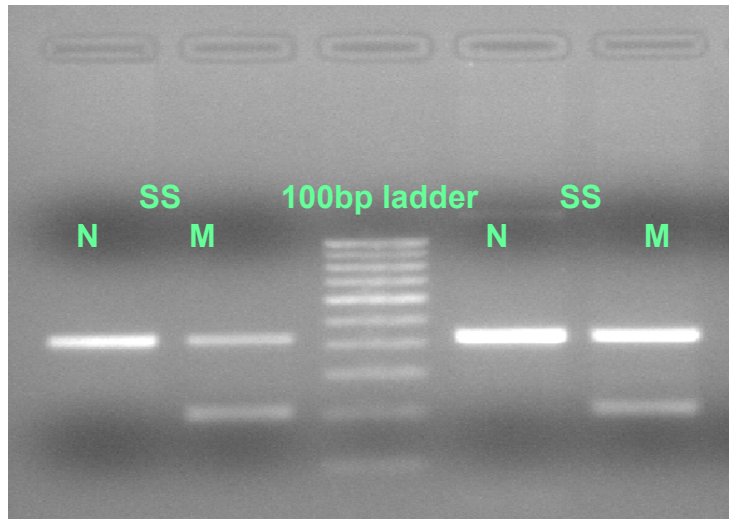
We are running a sickle cell clinic in the VSS Medical College Hospital, Burla, since last 11 years. The patients usually attend the clinic through.

- **on their own with usual symptoms of skeletal-muscular pain, anemia, icterus, chronic leg ulceration, growth retardation, etc,**
- **referred from other departments of the medical college hospital, from district and sub-divisional hospitals, and from private health care system.**
- **those found positive from the family survey of SCD patients.**
- **those detected during cross-sectional prevalence survey.**

Investigations

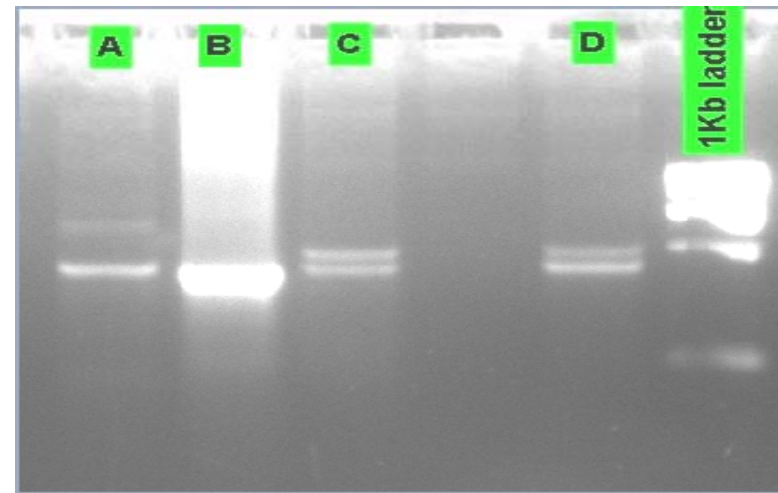
- **Sickling slide test & Hb electrophoresis (pH 8.6)**
- **Quantization of various Hb fractions by HPLC (Biorad , USA)**
- **Confirmation of sickle mutation Cd6 (GAG-> GTG) - ARMS-PCR.**
- **Other baseline investigations including CBC, renal function tests, liver function tests, radiological examination of shoulder and Hip, USG of abdomen, and other specific investigations as and when required.**
- **Cases found positive by slide test, electrophoresis and confirmed by ARMS-PCR were registered and followed up at the Sickle Cell Clinic at 3 months interval.**

ARMS PCR for sickle mutation



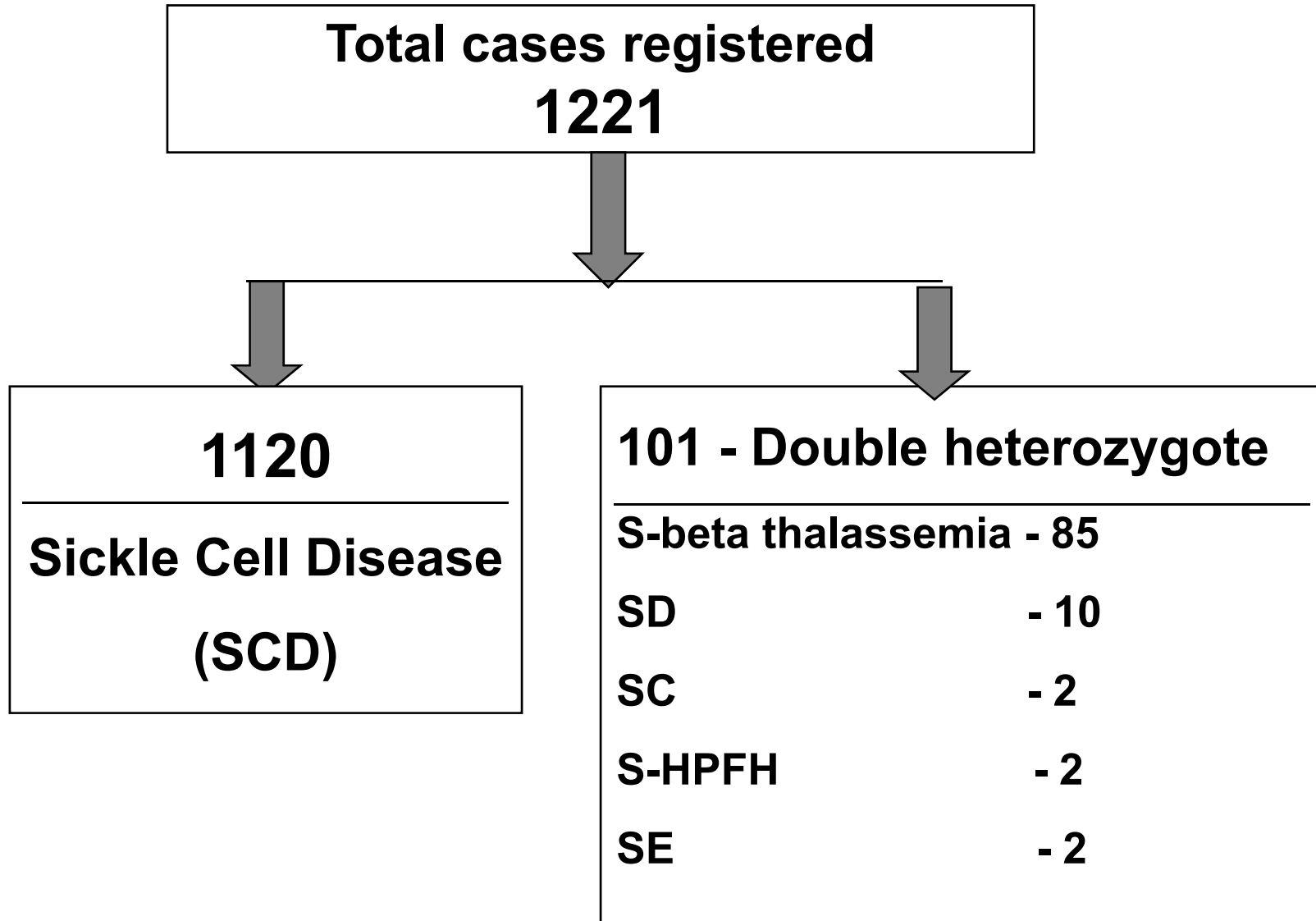
Control band ~400bp ; Normal/
Mutated band ~200bp

Alpha Thalassemia by Multiplex PCR



LaneA - Normal alpha2 gene,
LaneB- Alpha4.2-alpha 4.2 double gene
deletion,
LaneC & D- Alpha 3.7 deletion

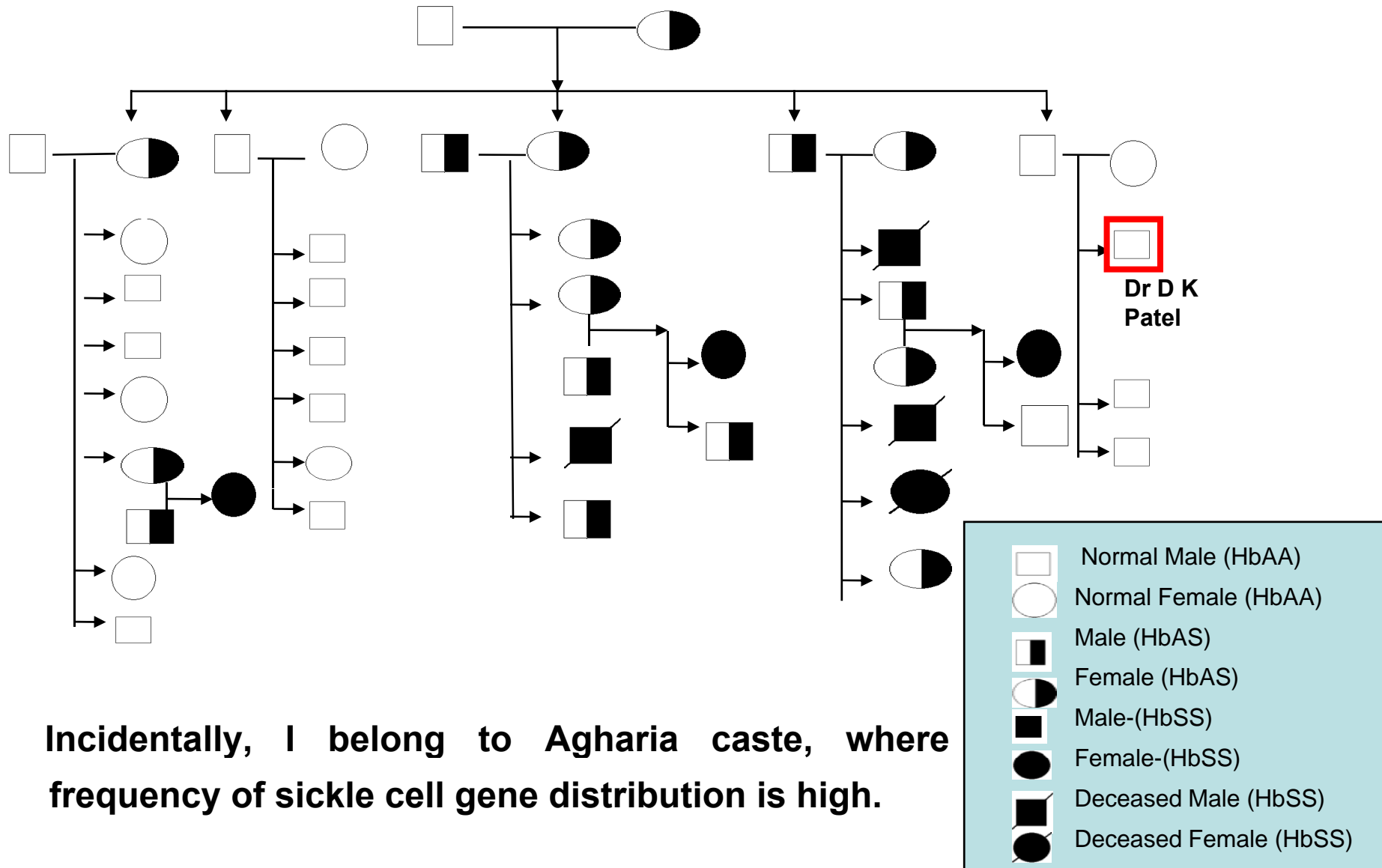
Enrollment of sickle cell hemoglobinopathy cases into Sickle Cell Clinic



Caste-wise distribution of sickle cell disease

Caste	No.	%	Total %
Non Tribal (Backward Caste)			
Kuilta	221	19.7	
Agharia	154	13.7	
Gouda	151	13.5	
Chasa, Teli, Dumal, others	252	22.5	69.5
Scheduled Caste	285	25.4	25.4
Scheduled Tribe	57	5.1	5.1
	1120	100	100

Family tree of Dr Dilip Kumar Patel, Orissa, India



Incidentally, I belong to Agharia caste, where frequency of sickle cell gene distribution is high.

**Molecular epidemiology:
β-globin gene cluster haplotype in SCD patients (n=261)**

Haplotype	No. case	Percentage
Arab India (homozygous)	210	80.5
Arab India / Senegal	6	2.3
Arab/ Atypical (A1)	6	2.3
Others	39	14.9
Total	261	100%

**Molecular epidemiology:
Xmnl polymorphism in SCD patients (n=261)**

Xmnl (+/+): 85% cases

Xmnl (+/-): 05 % cases

Xmnl (-/-): 10% cases

Molecular epidemiology: α -thalassemia in SCD patients

69 of 261 (26.2%) SCD patients investigated were found to have α -thalassemia.

Genotype	No. cases	Percentage
Heterozygous 3.7 Del	61	23.3
Homozygous 3.7 Del	7	2.5
Heterozygous 4.2 Del	1	0.4
Total	69	26.2%

Fetal hemoglobin in SCD patients

In earlier studies HbF estimated by **alkali denaturation method** reported mean HbF level of 16.64%. We estimated HbF by HPLC (**Biorad, USA**) which is a more sensitive method.

Range: 3.9 – 46.5%

Mean HbF: 22.3± 6.9%

HbF	No case	% case
0 - <10	78	7.0
>=10 - <20	410	36.6
>=20 - <30	511	45.6
>=30 - <40	114	10.2
>=40	7	0.6
Total	1120	

Clinical profile of SCD cases (n= 1120)

<i>Presentation</i>	<i>% of total cases</i>
Mean Age	22.0 ± 11.6 yrs
Asymptomatic cases	6.7%
Painful crisis	86.6%
Mod & Severe Anemia (Hb<10g%)	64.2%
Hemolytic jaundice	54.4%
Infections	36%

<i>Presentation</i>	<i>% of total cases</i>
Splenomegaly	50.8%
Avascular necrosis	19.3%
Cholelithiasis	27%
Chronic renal failure	17%
CNS (Stroke)	2.6%
Leg Ulcer	1.9%
Priapism	0.3% (3 pts)
Mortality	4.7% (53 pts)

Painful crisis was the commonest presentation (86.6%), followed by anemia (64.2%) and hemolytic jaundice.

Painful crisis- Incidence

- **Commonest presentation - (86.6%)**
- **Average pain rate - 1.3 episodes/pt year but highly variable ranging from complete absence of painful crisis to >10 per year.**
- **Factors responsible for this variable expression are HbF, beta globin gene cluster haplotype, alpha thalassemia, and XmnI polymorphism.**
- **Earlier studies have failed to show protective effect of HbF on the frequency of painful crises. We re-evaluated these findings in a larger sample size, for a longer period of time, and using HPLC, a sensitive method of HbF estimation.**

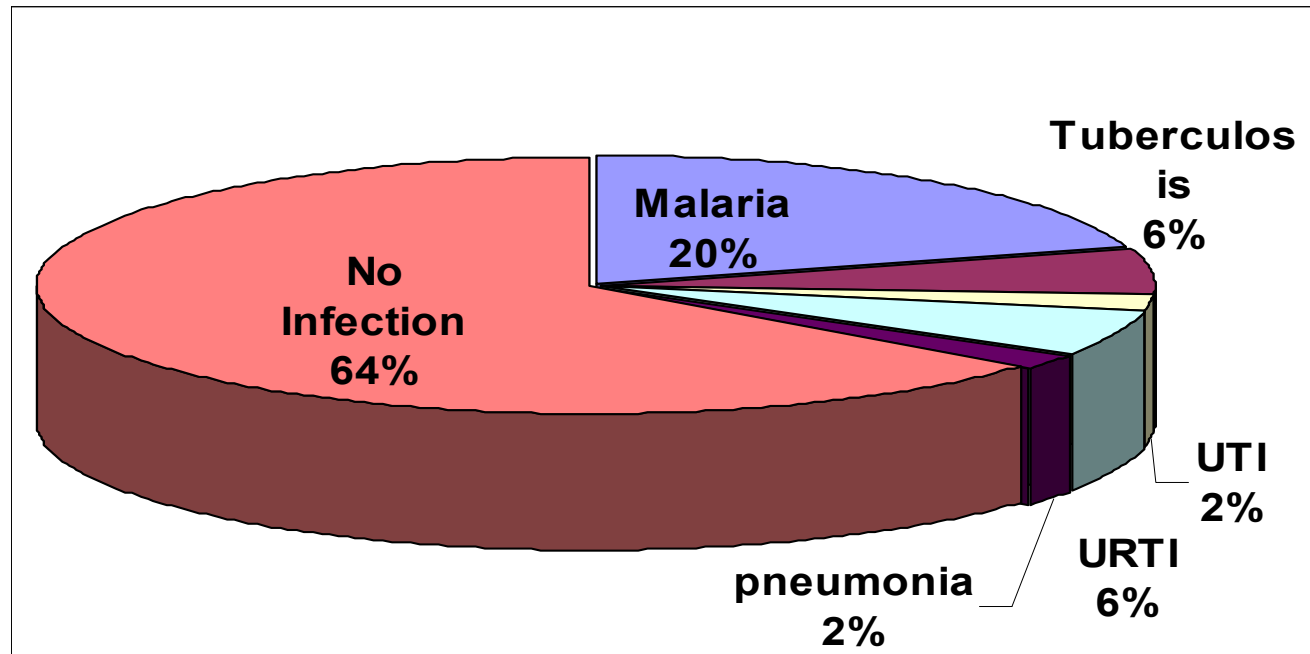
Painful crisis- Association with Age, Hb, HbF, Alpha thalassemia & XmnI polymorphism

- A statistically significant **negative correlation** was observed between frequency of painful crises and HbF concentration ($r = -0.28$; $P < 0.05$).
- Relative risk (RR) of painful crisis at HbF of $<15\%$, $<20\%$, and $<25\%$ was 0.72, 0.40 and 0.30 respectively, indicating decrease in episodes of painful crises with every increase in HbF **without a threshold level**.
- Age, Hb conc, alpha thalassemia & XmnI polymorphism did not influence the frequency of painful crises ($p > 0.05$).

Higher fetal hemoglobin concentration in patients with sickle cell disease in eastern India reduces frequency of painful crisis

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Infections in Sickle Cell Disease



36% of patients had some form of infection, the commonest being malaria (20%).

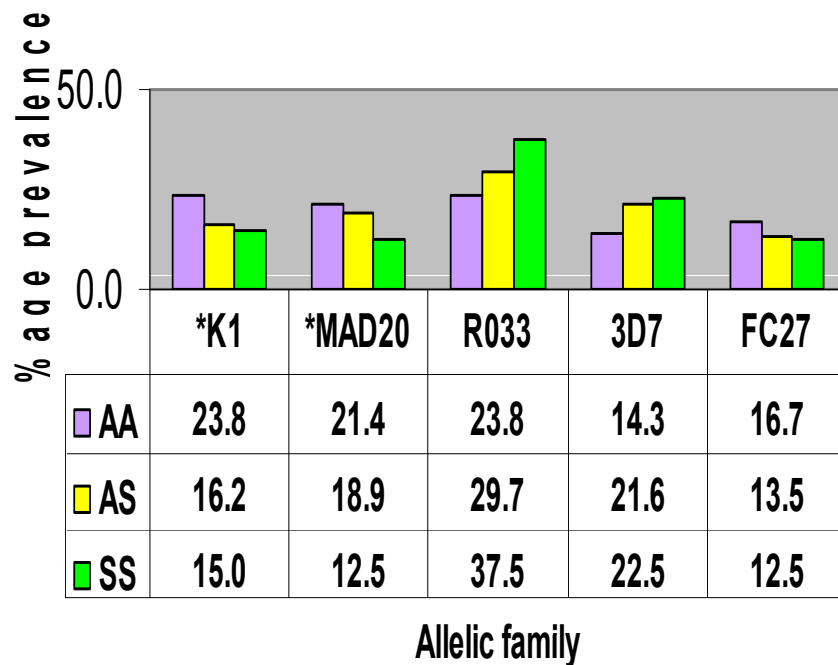
Malaria in Sickle Cell Disease

- **Malaria was the commonest infection found in SCD cases. The incidence rate was 6.8 per 100 pt year.**
- **Majority (92%) had *P.falciparum* infection, *P.vivax* and mixed infection constituted the rest.**
- **Malaria was the precipitating factor for painful crisis in 10.8% cases.**
- **Amongst the 53 SCD deaths, Falciparum malaria leading to multi organ dysfunction was the cause of death in 14(26%) cases**

Influence of sickle gene on the prevalence & multiplicity of *P.falciparum* infection in symptomatic malaria

•Cases : AA-25, AS-18, SS-17

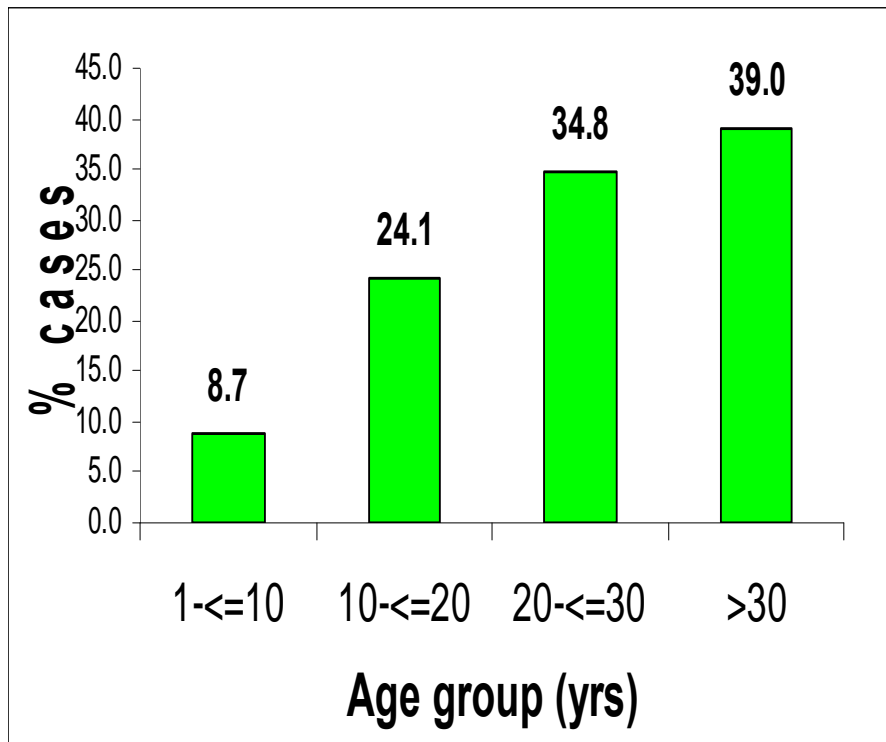
Prevalence of msp1 and 2 allelic families in Hb AA, AS and SS cases



- Method: Nested PCR.
- *MSP-1* (K1, MAD20 & R033 clones).
- *MSP-2* (3D7 & FC27 clones).
- Prevalence of K1 and MAD20 was significantly high in AA in comparison to AA & SS. The prevalence of other clones was similar in the three groups.
- Multiclonal infection was found in 84.6% of AA, 40% of AS, and 37.5% of SS cases.
- Multiplicity of infection (MOI) was 3.6 in AA, 2.0 in AS and 2.0 in SS cases (p=0.04).

Gall stone: Prevalence & association with age, sex, HbF & bilirubin

Prevalence of cholelithiasis with age



- Diagnostic Method: USG

- Earlier studies before routine USG showed prevalence of 5-10%.

- Overall prevalence was **27%** & it increased progressively with age. It was high as compared to normal population (5.6%).

- No correlation with sex & HbF conc. However serum bilirubin was high (8.1mg%) in comparison to the normal (2.8mg%).

Splenomegaly: prevalence & influence of Age, HbF, alpha thalassemia & malaria

- Persistent and gross splenic enlargement are peculiarities of **Indian** Sickle Cell Disease patients.
- Prevalence by **USG**– **50.8% cases** highest in **11-30 age group**
- Gross splenomegaly (longitudinal diameter >15cm) was found in **13.4%**.
- The frequency was high in those with **HbF beyond 25%**,
- Cases with HbF **below 15%** had **smaller spleens** (chi2 = 21.4, P < 0.05).
- **No influence of heterozygous alpha thalassemia on spleen size.**
- **Malaria** infection was strongly associated with **persistent splenomegaly** (p=0.01).

Splenomegaly in Sickle Cell Disease : **Traditional method of treatment**



Area over the enlarged spleen is burnt with a hot iron rod.

Avascular necrosis

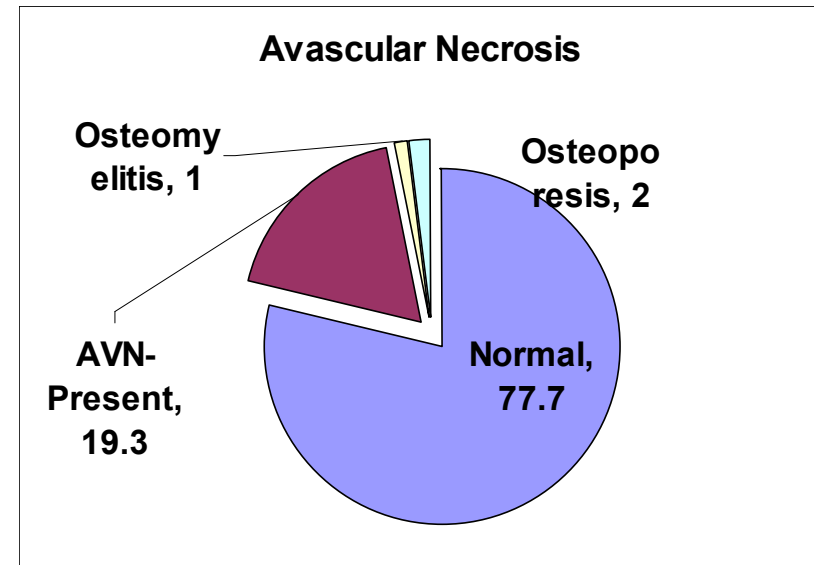
- **Wide geographical variation: USA: 4-19% , Jamaica: 10-15% & Saudi Arabia 27-42 % India: 5.75%**
- **Risk factors that has been found to be associated with of hip necrosis in the Cooperative Sickle Cell Study were high hematocrit, low MCV & low aspartate amino transferase & presence of alpha thalassemia.**
- **But in Eastern Saudi Arabia alpha thalassemia had no effect on high prevalence of hip necrosis.**

Reference

1. Serjeant GR, Serjeant BE. Sickle Cell Disease. 3rd ed. Oxford University press, 2001 : 261.
2. Kar BC et al., Lancet. 1986 ;2(8517):1198-1201.
3. Tanaka, K. R.et al. Blood. 11:998-1007, 1956.
4. Padmos MA et al. *Br J Hematol.* 1991;79:93–8.

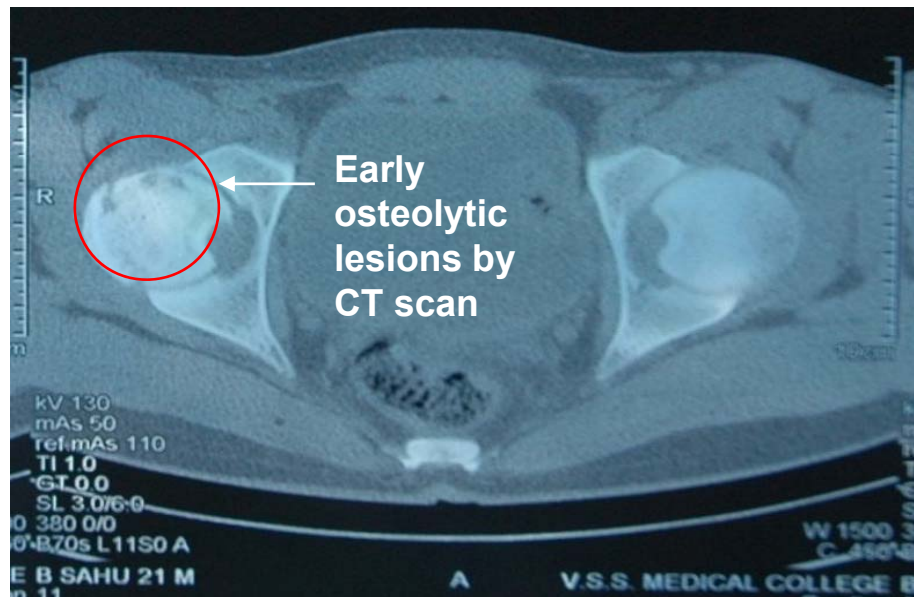
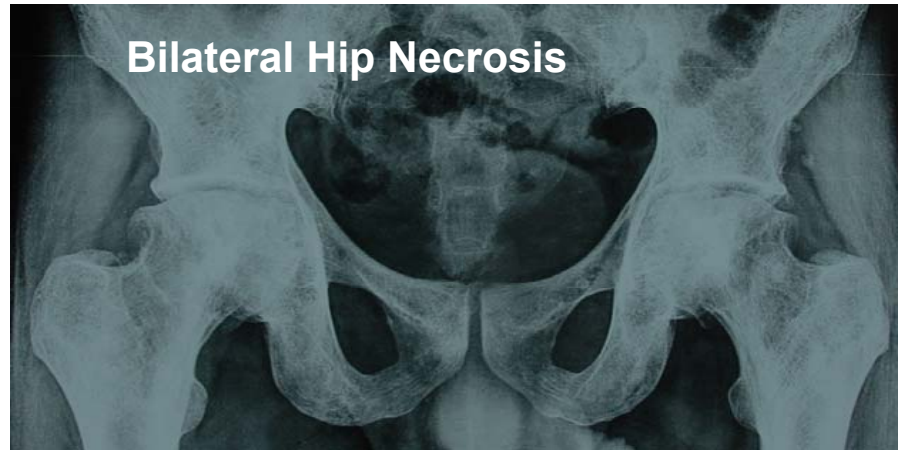
Avascular necrosis : Association with HbF, alpha thalassemia, painful crisis & tuberculosis (n=475)

- **Diagnostic method:** Plain X ray & CT.
- **AVN - 19.3%** (All except 1 had **femoral head necrosis**)
- **Osteoporosis - 2%; Osteomyelitis 1%.**

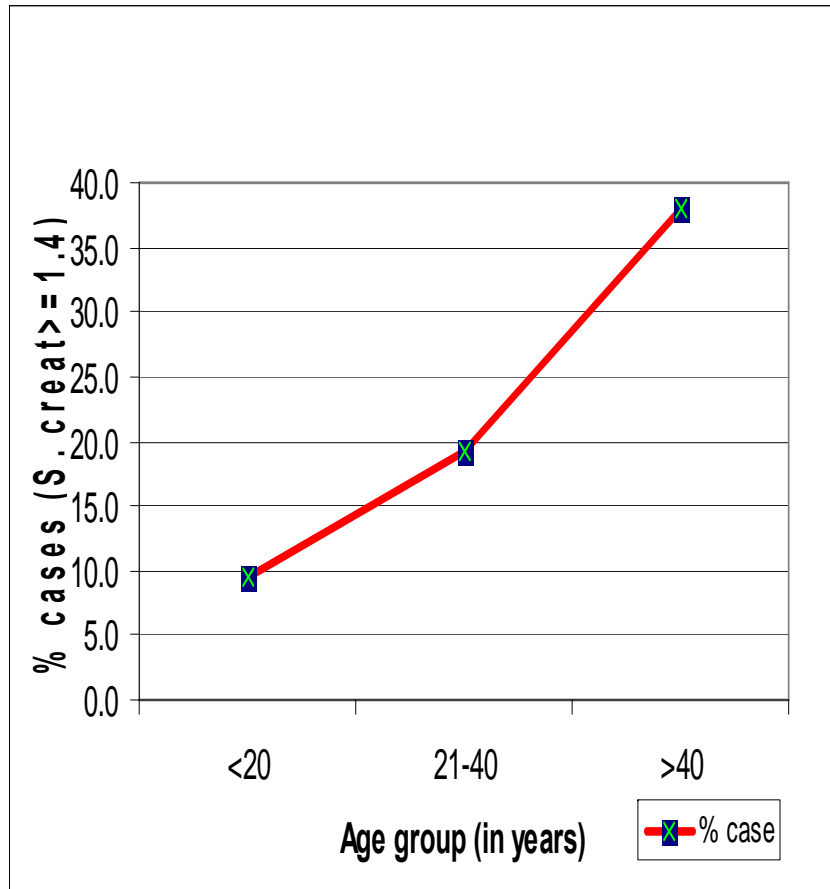


- No association was found between AVN and **alpha thalassemia & HbF concentration.**
- All these cases of AVN were investigated for tubercular infection.
- **11 of 87 (12.6%) had evidence of tuberculosis, and improved with anti tuberculous therapy.**

Avascular necrosis



Chronic Renal Failure



- **Diagnostic criteria:** Steady state serum creatinine > 1.4 mg/dl.
- Prevalence **17%** progressively increased with age, **40% above age of 40 yrs.**
- **Renal failure** as cause of death in **14%**.
- **Histopathological** study in 14 cases revealed enlarged & congested glomeruli in 21% cases, focal glomerulosclerosis in 28% cases & membrano proliferative changes in 21% cases.

Leg Ulcer, Priapism & Stroke

Leg Ulcer

- Prevalence - **21 pts (1.9%)**: (18 male;3 female); Mean age: **29 ±12 yrs.**
- The incidence was **not influenced by Hb or HbF levels.**
- **Course**: In all of them ulcer **healed with conservative treatment.**

Priapism

- **Priapism** was extremely rare - **3 cases** only.
- Mean age of first attack : **26.7 ± 9.9 years.**
- Treatment unsatisfactory even after shunt operation.

Central Nervous system

- CNS complications were rare.
- Only 6 patients had ischemic stroke while 2 had hemorrhagic stroke.

Leg Ulcer in Sickle Cell Disease



Rare presentation of SCD

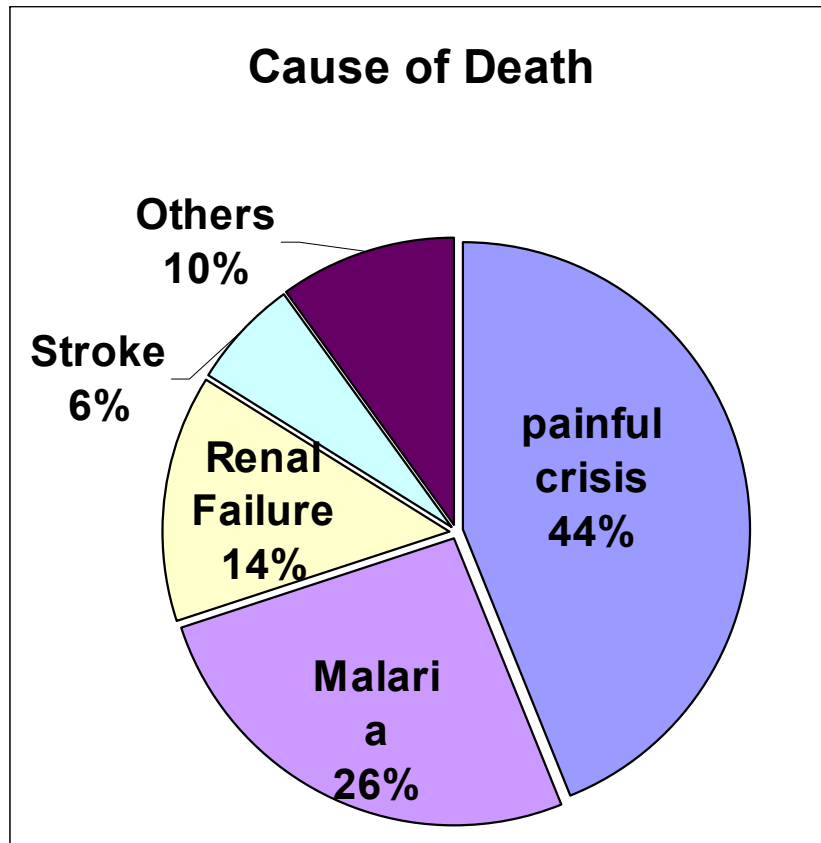


Budd chiari syndrome with Hepatic vein thrombosis.



Cavernous Sinus thrombosis

Mortality in Sickle Cell Disease



- **Fifty three** (Male-33, female-20) of 1120 cases (**4.7%**) died in hospital.

- **2.6 deaths per 100 pt year.**

- Median age at death: **25 (14 – 59) yr.**

- **Painful crisis - 44% (ACS-24%; multiorgan dysfunction -10%).**

- **Malaria- 14/53(26%)**

Hydroxyurea in Sickle Cell Disease

In view of high frequency of painful crisis in significant number of cases & other morbidities hydroxyurea was started.

No cases enrolled : 302

Dose: 10mg/Kg body wt, increased to 20mg/Kg if inadequate response.

Indication for Hydroxyurea

	No cases
Painful crisis >3/year	198
Anemia needing blood transfusion	80
Chronic organ damage	
AVN	21
CRF	32
ACS	6

Hydroxyurea in Sickle Cell Disease (Contd.....)

Period followup	No cases
>=6month-<1year	147
>=1 year-<2year	56
>=2 year-<3 year	63
>=3 year	36
Total	302

Outcome:

- Painful crisis reduced by 82%.
- Patients became transfusion independent.
- Restoration of normal renal function- 25%

Toxicity:

- Transient cytopenia 4%
- Hyperpigmentation- 0.6%
- Hypersensitivity & pruritus- 2%

Is Indian Sickle Cell Disease

Benign in comparison to other populations?

	India	CSSCD/Jamaica
No painful crisis	13.4%	39%
Pain rate	1.3	0.8
AVN	18.3%	4-19%
Cholelithiasis	27%	31%
Hb	8.2g%	8g%
Mortality	4.7%(2.4 / 100 pt year)	2.6%(0.5 / 100 pt year)
Leg Ulcer	1.9%	10-75%
Priapism	0.3%	2-6%
HbF	22.3± 6.9% (3.9 – 46.5%)	6.11 ± 4.21 % (0.4- 33.2)

Reference:

- Platt OS et al. N Engl J Med. 1991;325:11–16.
- Leikin SL et al. *Pediatrics*. 1989 Sep;84(3):500–508.
- Sergeant GR et al Sickle Cell Disease. Oxford University Press, 3rd ed, 2001: 240-280.

Sickle Cell Disease: Twins



Twin 1: Recurrent VOC on Hydroxyurea

Twin 2: Mildly symptomatic (Folic acid)



Both had repeated episodes of hemolytic anemia.

Now on Hydroxyurea and Transfusion Independent.

Summary & Conclusion

- Prevalence of sickle cell gene is high in India & both tribal and non-tribal populations are affected.
- **80.5%** have Asian Indian haplotype & **26.2%** have alpha thalassemia.
- Mean HbF level (HPLC) is **22.3± 6.9%** (Range: 3.9 – 46.5%)
- **6.7 %** patients are asymptomatic.
- The commonest symptom is painful crisis (**86.6%**). Higher HbF has been found to reduce the frequency of painful crisis.
- Malaria is the commonest infection, is a precipitating factor for painful crisis & responsible for **26%** of all deaths.
- Persistent splenic enlargement is common & related to high HbF & malaria.

Summary & Conclusion

- AVN is found in 19.3%. In 12.6% cases AVN was associated with tuberculosis.
- CRF is prevalent in 17% cases & increased progressively with age.
- Leg ulcer, Priapism & Stroke are uncommon.
- Mortality was 2.6 per 100 pt year. The commonest cause was painful crisis (44%) followed by malaria (26%).
- In spite of Asian haplotype & high HbF level the disease is not benign. Endemic malaria & Tuberculosis are important cause of high morbidity & mortality.
- Hydroxyurea significantly reduces the frequency of painful crisis & need for blood transfusion in Indian patients.

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