Corelation Between Clinical Profile and Quantity of Abnormal Hemoglobin in Sickle Cell Hemoglobinopathy

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ABSRACT

Introduction :-Despite the unicity of the genetic mutation that is responsible, clinical presentation of the sickle cell hemoglobinopathy was found to be different according to regions and patients. Haemoglobin F level has been a useful criterion in predicting the clinical severity of sickle cell disease.

Aims and objectives :- Study the clinical presentation of sickle cell haemoglobinopathy cases with special reference to "sickle cell crises" and correlate the severity of clinical features (including "sickle cell crises") with subtypes of hemoglobin quantified on High Performance Liquid Chromatography (HPLC).

Type of study :- Cross sectional analytical study.

Methodology:- Subjects of sickle cell hemoglobinopathy (having age 12 yrs and above of both gender after fulfilling inclusion and exclusion criteria) attending medicine and/or sickle cell OPD or admitted in medicine indoor wards were subjected to detailed history, clinical examination and laboratory investigations including HPLC. Correlation between various parameters (clinical and laboratory) and quantity of HbF and HbS was studied.

Results:-Maximum cases, 35 out of 60 (58.3%) were in 13 - 19 age group range. Mean age of onset of symptom was 8.04 years. Vaso-occlusive crisis was the most common manifestation followed by severe anemia, while leg ulcers and stroke were rare presentations. The frequency of vaso-occlusive crisis varied widely among patients with range of 0.3- 3 episodes per year, the average frequency being 0.6 episodes per patient per year. The most common site of pain in vaso-occlusive crisis was limbs (68.7%), hands (62.5%), knees (59.3%), abdomen (31.2%) and chest (25%). Fever was the most common precipitating factor for vaso-occlusive crisis (31.2%). The mean HbF level was 21.9%. The mean HbS level was 70.7%. HbF had positive co-relation with Hb% (r = 0.3), mean age of onset of first symptom (r = 0.5) and negative co-relation with frequency of vaso-occlusive crisis (r = -0.4) and WBC count (r = -0.4). HbS had positive co-relation with WBC count (r = 0.5) and frequency of vaso-occlusive crisis (r = 0.3) and negative co-relation with mean age of onset of first symptom (r = -0.4) and Hb% (r = -0.1).

Conclusion:- Clinical profile of sickle cell anemia is less severe in this region as compared to African countries. High levels of HbF has a definitive role in ameliorating the severity of sickle cell anemia.

INTRODUCTION

Sickle cell anemia alone is the most common heritable hematologic disease affecting humans. The prevalence of sickle cell disease in central India as per study of **Shukla** and **Solanki** is high in certain localities of Vidharbha region of Maharashtra.[1] Most of the patients with sickle cell disease suffer from hemolytic anaemia, intermittent episodes of vaso-occlusive crises in connective and musculoskeletal structures, produce painful ischaemia manifested by acute pain and tenderness, fever, tachycardia and anxiety. These recurrent episodes , called painful crisis , are the most comman clinical manifestation.

Despite the unicity of the genetic mutation that is responsible , clinical presentation of the sickle cell disease was found to be different according to regions and patients. Phenotypic heterogenicity of patients with sickle cell disease were linked to the difference between haplotypes of beta-globin gene. Five haplotypes were identified in the world – **Benin, Senegal, Bantu, Cameroon and Arabian-Indian**. Senegal and Arabian-Indian haplotype are associated

with moderate form of sickle cell disease.[2] Several biological and environmental factors are presumed to account for the morbidity pattern in patients with sickle cell disease; e.g. fetal hemoglobin is one of the biological factors thought to decrease morbidity in these patients. Haemoglobin F level has been a useful criterion in predicting the clinical severity of sickle cell disease. Many studies had shown the relationship between haemoglobin F value and clinical severity in sickle cell anaemia patients.[11]

In this study we used frequency of vaso-occlusive crisis, mean age of onset of symptom, antecedent blood transfusion, splenic size, acute chest syndrome, degree of anaemia, avascular necrosis, white blood cell count and leg ulcer as measures of clinical severity assessment. The clinical severity then correlated with quantity of abnormal haemoglobin i.e. HbF and HbS, determined by **high performance liquid chromatography.** Such study would be helpful in predicting the adverse outcomes in patients with sickle cell disease at earlier stages by detecting HbF and HbS levels and also would help to gear up different treatment modalities to raise or decrease proportions of particular abnormal haemoglobin levels to ameliorate the severity of sickle cell disease.

MATERIAL & METHODS

Study Setting:

A cross-sectional analytical study was conducted at tertiary care teaching hospital in central India. Study participants comprise of cases of sickle cell anemia, attending general medicine OPD, sickle cell OPD and Indoor wards of Department of Medicine, with age > 12 year, irrespective of their gender, origin, caste and ethnic background. The present study is carried out from January 2007 to June 2008.

Cases who had received blood transfusion within three months prior to the day of inclusion and cases who were and/or are on Hydroxyurea therapy were excluded. Total number of sickle cell anemia cases studied were 60.

After screening consecutive cases of sickle cell anemia, as per above criteria, and after detailed history and complete clinical examination, 3ml of venous blood was withdrawn, mixed with EDTA and sent to pathology laboratory for HPLC. Other laboratory and radiological investigations were also done.

Statistical analysis: Statistical analysis was done with the help of Microsoft excel 2003 version software using Student 't' test. Probability value of $p<0.05$ were considered significant while $p<0.01$ taken as highly significant.

RESULTS

The study includes 60 cases of sickle cell anemia, all above 12 years of age.Age range was from 13 to 50 years with majority belonging to 13 — 19 years age group range (58.33%). 33 (55%) cases were from Mahar community, 11 (18.33%) cases were from Kunbi community, 04 (06.67%) cases from muslim community.

Table no. 1 shows the presentation of patients in present study.

The mean age of onset of first symptom was 8.04 years in this study. Maximum patients (50%) became symptomatic between 5-10 years age group. 16 (26.66%) patients had first symptom before the age of 5 years and 14 (23.33%) patients had first symptom after the age of 10 years.

The study revealed that 28 (46.66%) patients had frequency of VOC less than or equal to one episode per year, while 27 (45%) had greater than one episode of VOC per year. Out of 60 patients 5 patients never had an episode of VOC. Out of 60 patients in this study, 32 patients presented as vaso-occlusive crisis (VOC). Among them limbs were the most common site of pain (68.75%), followed by hands (62.5%), knees (59.37%), abdomen (31.25%) and chest (25%). Only 3 patients (9.37%) complained of backache. Among the 32 vaso-occlusive crisis (VOC) cases in present study, fever was found as precipitating factor in 10 patients (31.25%) followed by exposure to cold (25%), exhaustion and severe physical activity (21.87%) and dehydration (06.25%). In 5 patients no precipitating cause was identified.

Table no. 2 shows the comparison between frequency of vaso-occlusive crisis and level HbF and HbS.

Sickle cell anemia patients having acute chest syndrome (ACS) had mean HbF level of 15.14% as compared to 22.53% in patients not having acute chest syndrome ($p=0.02$). But there was no statistically significant difference in HbS levels between these groups ($p= 0.58$).

Sickle cell anemia patients having avascular necrosis (AVN) of femur head had mean HbF level of 18.09%

as compared to 23.30% in patients not having avascular necrosis of femur head (p=0.82). But there was statistical significant difference in HbS levels between these groups (p=0.02); Patients having avascular necrosis of femur head had mean HbS level of 74.46% as compared to 69.45% in patients not having avascular necrosis of femur head.

Patients who had received blood transfusion before three months prior to the day of inclusion had mean HbF level of 21.52% as compared to 23.46% in patients who had not received blood transfusion (p> 0.05). Mean HbS level was 70.80% in patients who had received blood transfusion before three months prior to the day of inclusion as compared to 65.87% in patients who had not received blood transfusion (p> 0.05).

Sickle cell anemia patients having leg ulcers had mean HbF levels of 7.60% as compared to 21% in patients not having leg ulcers (p=0.007). Mean HbS level was 87% in patients who had leg ulcers as compared to 70.79% in patients who did not have leg ulcer (p=0.005).

Sickle cell anemia patients having mean age of onset of symptom between 5-10 years had mean HbF levels of 19.39% as compared to 30.18% in patients having mean age of onset of symptoms after 10 years age (p< 0.0001). Mean HbS level was 73.04% in patients having mean age of onset of symptoms between 5-10 years as compared to 63.40% in patients having mean age of onset of symptoms above 10 years

(p< 0.0001). Sickle cell anemia patients having HB% less than or equal to 6 gms/dl had mean HbF levels of 18.86% as compared to 23.55% in patients having HB% greater than 6 gms/dl (p=0.01). Mean HbS level was 72.87% in patients having HB% less than or equal to 6 gms/dl as compared to 69.67% in patients having HB% greater than 6 gms/dl (p=0.13). Sickle cell anemia patients having autosplenectomy had mean HbF levels of 18.60% as compared to 23.41% in patients having splenomegaly (p=0.01). Mean HbS level was 75.34% in patients having autosplenectomy as compared to 68.77% in patients having splenomegaly (p=0.002).

Sickle cell anemia patients having WBC count less than 10,000 per cubic mm had mean HbF levels of 24.11% as compared to 16.35% in patients with WBC count greater than or equal to 10,000 per cubic mm (p <0.0001). Mean HbS level was 68% in patients having WBC count less than 10,000 per cubic mm as compared to 77.12% in patients with WBC count greater than or equal to 10,000 per cubic mm (p <0.0001).

Table no 4 shows the co-relation of HbF with clinical parameters.

Table no 5 shows the co-relation of HbS with clinical parameters.

Table No. 1

Presentation of patients in presentstudy

Presentation	Cases (n=60)
VOC (joint pains, etc)	32 (53.33%)
Severe Anemia (generalized weakness)	21 (35%)
AVN (head of femur)	16 (26.66%)
Infections	10 (16.66%)
Acute chest syndrome(ACS)	04 (06.66%)
Leg ulcer	02 (03.33%)
Stroke	00 (00%)
Asymptomatic	23 (38.33%)

*The total percentage exceeds 100% because many cases had more than one symptom. VOC- vaso-occlusive crisis; AVN- avascular necrosis; ACS- acute chest syndrome.

Table No. 2

Frequency of VOC (average number of episodes per year)	HbF	HbS
⩽ 1 VOC (n = 28) Range 0.3-1	26.31 ± 4.08%	67.60 ± 5.29%
>1 VOC (n = 27) Range 1.2-3	16.59 ± 5.46 %	74.61 ± 8.66 %
Level of significance	p = <0.0001	p = <0.001

Frequency of vaso-occlusive crisis (VOC) compared with HbF and HbS levels

Table No. 3

Co-relation between clinical parameters and HbF

levels

Clinical parameter	Co-relation (r) with HbF	Significance (p)
Hb%	0.335	.009
WBC count	-0.456	<0.001
Mean age of onset of symptoms	0.523	<0.001
Frequency of VOC	-0.466	<0.001

Table No. 4

Clinical parameter	Co-relation with HbS	Significance (p)
Hb%	-0.151	0.248
WBC count	0.508	<0.001
Mean age of onset of symptoms	-0.475	<0.001
Frequency of VOC	0.344	0.007

Co-relation between clinical parameters and HbS levels

DISCUSSION

This study may be useful in designing therapeutic trials. Exclusion of children with relatively mild manifestation of disease will allow a larger treatment effect to be easily observed, thus reducing the number of subjects in the study and preventing low risk children from receiving high risk therapy in the study.

In the present study male population out numbered female population with male to female ratio of 1.22:1. The higher number of males can be explained by the sex ratio in our region.[3,4] **Shrikhande AV** et al [5] also reported similar male to female ratio in sickle cell anemia patients in this region. In present study mean HbF was 21.91± 7.27%, being slightly higher in females as compared to males. These values are consistent with the findings observed by Shrikande AV et al.[5] It is already quoted that HbF production was genetically determined and **Arab-Indian** haplotype is associated with higher HbF levels as compared to **Camroon** haplotype. This explains the low value of HbF in Nigerian population by **Kotila TR** et al.[6]

S Diop et al [7] reported mean age of onset of first symptom in sickle cell anemia was 9.8 years in **Senegal. Orah SP** et al [8] found that it was 1.6 years in USA. Mean age of onset was 8.04 years in present study. As stated earlier, **Senegal** and **Arab-Indian** haplotype are associated with moderate form of sickle cell disease as compared to **Benin, Bantu** and **Cameroon** haplotypes. High HbF level in RBCs protects them from sickling, the phenomenon responsible for clinical manifestation of the hemoglobinopathy. So manifestations of sickle cell anemia are delayed due to high HbF levels.[9]

S Diop et al [7] and **Powars DR** et al [10] observed VOC as the most common manifestation in sickle cell anemia patients. This finding was constant in present study. Incidence of ACS, leg ulcer was significantly lower in present study and in study done by **S Diop** et al [7] in **Senegal** as compared to the observation made by **Powars DR** et al in **USA**. VOC are the most common manifestation of sickle cell disease but it's frequency vary from person to person depending on various environmental and genetic factors. Some cases with very low frequency of VOC or no VOC are commonly seen in regions associated with **Senegal** and **Arab-Indian** haplotype.

Enosolease ME et al [11] studied that VOC was more common at lower levels of fetal hemoglobin particularly when lower than 12% (p=0.01). There was a negative correlation between fetal haemoglobin & vaso-occlusive crisis (r= -0.561) which was statistically significant (p=0.001). We found a statistically significant negative correlation between HbF and frequency of VOC (r=-0.466, p=<0.001). There was statistically significant positive correlation between HbS and frequency of VOC (r=0.344, p=<0.007). Similar observation was made by **Al-Hagger M** et al [12] in **Egyptian** sickle cell anemia patients. But the observations in the study done by **Powars DR** et al [10] were contradictory to present study (no statistically significant correlation between frequency of VOC and HbF levels was found in their study. r =-0.379; p=0.66). It could be explained on the basis that study carried out by **Powars DR** et al [10] in **USA** had very low HbF levels and so overall protective effect was small . There may be some threshold level of HbF needed above which it ameliorates the severity of sickle cell disease.[13]

Omoti CE [14] found that there was statistically

significant difference between HbF level in patients having ACS (HbF-1.12%) as compared to patients not having ACS (HbF-2.45%) (p <0.0001). Similarly,in present study we found statistical significant difference between these two groups regarding HbF level (p=0.02). Raised HbF levels in RBCs decreases the amount of sickle HB and prevents in situ sickling in lung and thus ACS. But **Powars DR** et al [10] stated that there was no linear relationship between ACS and HbF levels (r = -0.093, p= 0.49). It can be explained by the fact that though raised HbF ameliorates the clinical severity of sickle cell anemia, there may be some treshold level of HbF above which it exerts this effect.

Diop S et al [7] stated that the mean age of onset of symptom was 9.8 years in **Senegal**. In present study the mean age of onset of symptom was 8.04 years. There was a statistically significant difference between HbF levels of sickle cell anemia patients having the mean age of onset of symptom between 5-10 years and those having mean age of onset of symptom greater than 10 years (p= 0.0001).

DJ Odenheimer et al [15] studied that there was statistical significant positive co-relation between HB% and HbF level (r=0.51, p< 0.001). In present study we also found statistically significant positive co-relation between HB% and HbF level (r= 0.335, p= 0.009).But **Hedo CC** et al [16] stated that HbF level failed to manifest significant co-relation with degree of anemia. This may be due to very low HbF level among **Nigerian** patients of sickle cell disease.

Powars DR et al [10] in their prospective study observed that incidence of AVN was 15% and there was no statistical significant relationship between AVN and HbF% (r = -0.035, p=0.25). In present study, incidence of AVN was 26.66%. and there was no statistical significant difference between HbF level among the patients not having AVN and patients having AVN (p=0.82%). But we found that HbS had statistical significant difference between these two groups (p= 0.02). Patients with higher HbF levels are protected from arterial vasculopathy and subsequent major organ destruction as compared to patients having low HbF level. These patients are healthier but in time will show the deleterious effect of HbS as AVN and retinopathy usually begin after age 30 and sickle nephropathy after age of 40 years. [17]

Olantuji PO et al [18] studied that WBC count had a statistical significant negative co-relation with HbF level (p=<0.05). In present study there was statistically significant negative co-relation between WBC count and HbF level (r = -0.456 , p=<0.001) and statistically significant positive co-relation between WBC count and HbS level (r= 0.508, P<0.001). Raised WBC exert their deleterious effect by releasing various inflammatory cytokines WBC also has adverse effect on vascular endothelium**,** which is related in part to abnormal adhesion.[19,20]

Kotila TR et al [6] in their study observed that patients with higher HbF levels are more likely to retain their spleen longer than their counterpart with lower values. Though Western researchers reported autosplenectomy as a frequent phenomenon in sickle cell anemia patients, persistence of spleen is more commonly encountered in Indian scenario. **Subhedar** [21] reported splenomegaly in 58.66% of cases. **Rita Sarkar** et al [22] found that 65% sickle cell anemia patients had splenomegaly. In present study, 56.66% cases had splenomegaly. The mean HbF level in patients having autosplenectomy was lower as compared to patients having splenomegaly (p=0.01). There was statistical significant difference in HbS level between these groups (p=0.002). In sickle cell anemia patients recurrent infarction in the spleen leads to destruction of the organ i.e. autosplenectomy and functional hyposplenism. If episodes of infarction are minimal and there is chronic hemolysis, we can get splenomegaly in such patients.

CONCLUSIONS

1. Vaso-occlusive crisis is observed to be the most common presentation in sickle cell anemia patients. Stroke and leg ulcers are uncommon manifestation in this region.
2. HbF levels are higher in sickle cell anemia patients in this region.
3. High level of HbF has a definitive role in ameliorating the severity of sickle cell anemia.

STUDY LIMITATIONS:

The study population in present study was small. Large number of patients of sickle cell anemia are needed to confirm results of present study. It was a cross sectional analytical study and there was no follow up of cases of sickle cell disease. Prospective study is necessary to calculate the various incidence rates and for proper

evaluation of clinical profile.

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