ORIGINAL ARTICLE

Haematological profile of adult sickle cell disease patients in North Maharashtra

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Abstract:

Background:

Sickle cell anaemia is a major genetic disease in India that presents major challenges to our health care systems.

Aim and Objective: The aim of this study was to evaluate the haematological profile of Sickle Cell Disease (SCD) patients in the steady state from tribal population of North Maharashtra.

Material & methods: Thirty six sickle cell disease patients in steady state, 43 sickle cell carriers and 43 normal healthy volunteers with age group18-40 years were recruited for the study. Subjects having history of vaso-occlussive crisis, blood transfusion and serious illness within last three months were excluded from the study.

Results: We found low levels of haemoglobin 7.86 ± 1.93gm/dl, Red Blood Cell count 2.93 ± 0.73 milli/cmm, as well as the Packed Cell Volume 27.18 ± 5.35% in the male and female SCD patients when compared with the carriers and normal subjects. Mean Corpuscular Volume 93.91 ± 6.9 was found to be higher whereas Mean Corpuscular Haemoglobin Concentration 28.71 ± 2.19 values were less ($P<0.05$) in the patients than the carriers and controls. Mean Corpuscular Haemoglobin 26.91 ± 2.07 levels of the patients were not statistically significant when compared with carriers and normal subjects.

Conclusion: Our results show, moderate to severe anemia and high foetal haemoglobin levels in the adult SCD patients. Sickle cell carriers and the normal control subjects showed mild to moderate anaemia. The occurrence of anaemia in these patients suggests that there is strong need to monitor these patients, to prevent triggering factors of vaso-occlussive crisis. The data so obtained would help in the management, prevention and control programme for SCD patients at the primary health care centers in India.

Keywords: Sickle cell Haemoglobin, Anaemia, Haematological values

Introduction:

Sickle Cell Disease (SCD) is a major genetic disease in India that presents major challenges to our health care systems[1]. SCD is particularly common among the people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries [2-4]. SCD is an autosomal recessive hemoglobinopathy, caused by the substitution of valine for glutamic acid at the 6th position of the β-(beta) polypeptide chain of hemoglobin. Under the deoxygenated conditions, Sickle Cell Haemoglobin (HbS) is polymerized, leading to the formation of long fibres inside the Red Blood Cells (RBC). The RBCs containing HbS will distort into elongated sickle shapes. These sickled and rigid RBCs have shortened lifespan and undergo intravascular and extravascularhemolysis. Furthermore, sickled RBCs can adhere to the vascular endothelium, ultimately blocking the normal blood flow through the vasculature [5-6].

In Maharashtra sickle cell gene is widely spread in all districts of Vidarbha region, North Maharashtra (Satpuda ranges) and some part of Marathwada region [7-10]. The prevalence of sickle cell disease is very high, amongst the Scheduled Tribes (ST) mainly in Bhill and Pawara tribal groups of North Maharashtra regions. It is also found in the population belongs to Scheduled Castes (SC) and Other Backward Classes (OBC) [11]. Despite the high prevalence of SCD in the tribal population of North Maharashtra, very few studies were carried out in adult SCD patients. Since these groups are living in geographically remote hilly areas, existing health facilities are inadequate and hence remain backward in all aspect of life including health and education. These tribal people are under the influence of superstitions, especially towards health problems. There is need to find most suitable and practically feasible parameters that could predict disease severity and aid in therapy. Since anaemia is a dominant feature of SCD, the purpose of this study is to evaluate haematological profile for early prediction of sickle cell crisis.

Material and Methods:

The present study was carried out in Shri Bhausaheb Hire Govt. Medical College, Dhule and District Hospital Nandurbar. Most of the patients in this study were from the tribal population of North Maharashtra (Dhule, Nandurbar and Jalgaon district). The study was approved by local ethical committee. The written informed consent from all participants was obtained prior to the study. A total of 122 subjects in the age group 18-40 participated in the study, which included 36 diagnosed sickle cell disease patients (SS), 43 sickle cell carriers (AS) and 43 healthy (AA) controls. The SS patients and AS carriers were confirmed by HPLC analysis. The control subjects were without

any haemoglobinopathies and were matched for age, sex and socio-economic aspects.

Inclusion Criteria:

Patients included in this study were in their steady state, no history of crises in the last 3 months, and without any symptoms related to SCD or other diseases which could affect the hematological findings;

Exclusion criteria: History of blood transfusion in the last 3 months, previous history of surgery, or were suffering from any other diseases and the patients those were not willing to participate in the study.

Collection of blood:

Under all aseptic precautions, 2-3 ml of blood was drawn from ante-cubital vein by clean vein-puncture with a sterile plastic syringe and blood sample was collected in an EDTA (anticoagulant) tube. Quantification of Foetal Haemoglobin (HbF) was done on Bio-Rad variant system using High Performance Liquid Chromatography (HPLC).

Following haematological parameters were measured on BC-5000 auto haematology analyzer. Haemoglobin (Hb), Red Blood Cell count (RBC), Pack Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC).

Statistical analysis:

Student 't' test (unpaired) was used for statistical comparison of the means. Results were expressed as Mean ± SD with significance level at 0.05.

Result:

Various haematological parameters in male and female patients / subjects are shown in the table-1. Hb concentrations, RBC counts and PCV were lower in SCD patients in both the sexes. The MCV was comparatively high in female SCD patients as compared to males. MCH and MCHC were found to be low in both male and female patients. HbF concentration was found to be higher in female SCD patients than male SCD patients. However, the difference was not statistically significant.

In sickle cell carriers and normal healthy subjects, Hb concentration, RBC count and PCV were lower in both the sexes however, in females it was found to be significantly lower ($P<0.05$) as compared to male counterparts.

Hb concentration, RBC count and PCV was significantly low ($P<0.05$) in SCD patients as compared to sickle cell carriers, and the normal controls (Table-2). However, the MCV was significantly high ($P<0.05$) and MCHC was significantly low ($P<0.05$) in SCD patients as compared to sickle cell carriers, and the normal healthy controls. We found low Hb concentration, RBC count and PCV in sickle cell patients as well as carriers as compared to the normal control subjects of both the sexes.

Table 1: Gender Related Values of Haematological Parameters of Sickle Cell Patients (SS), Sickle Cell Carriers (AS) and Control Subjects (AA)

Phenotype	SS		AS		AA	
Gender	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
n	17	19	23	20	22	21
Hb (gms/dl)	8.34 ± 1.91	7.58 ± 1.84	11.91 ± 1.45	*10.06 ± 1.96	12.60 ± 1.31	10.81 ± 1.42
RBC	3.11 ± 0.79	2.77 ± 0.63	4.54 ± 0.46	*3.9 ± 0.59	4.90 ± 0.56	4.07 ± 0.65
PCV (%)	27.53 ± 4.83	26.17± 5.27	36.58± 2.63	*31.69 ±	39.74 ± 2.06	33.90 ± 3.46
MCV μm^3	90.44 ± 8.39	94.97 ± 4.75	81.06 ± 6.8	82.00 ± 7.4	81.91 ± 8.38	84.20 ± 8.17
MCH (pg/dl)	27.06 ± 1.94	27.35 ± 2.24	26.29 ± 2.53	25.81 ± 3.24	25.81 ± 1.96	26.75 ± 2.35
MCHC (g/dl)	30.06 ± 2.24	28.81 ± 2.11	32.55 ± 3.13	31.56 ± 3.72	31.68 ± 2.34	31.86 ± 2.07
HbF(%)	18.04 ± 6.89	19.34 ± 6.49	0.85 ± 0.36	1.75 ± 1.32	-	-

The figures are Mean ± SD; Hb = Haemoglobin in gram/dL; RBC = Red Blood Cell count in millions/cubic milimeter; PCV = Packed cell volume; MCV = Mean corpuscular volume in cubic micrometer; MCH =Mean corpuscular haemoglobin in picogram/dL; MCHC = Mean corpuscular haemoglobin concentration in gram/dL; HbF = Foetal haemoglobin in percentage of total Hb.

Table 2: Comparative Haematological Parameters in Sickle Cell Patients (SS), Sickle Cell Carriers (AS) and Control Subjects (AA)

Parameters	SS (n=36)	AS (n=43)	P value (SS vs AS)	AA (n=43)	P value (SS vs AA)
Hb (g/dl)	7.86 ± 1.93	11.05 ± 1.94	P<0.05	11.23 ± 1.59	P<0.05
RBC	2.93 ± 0.73	4.24 ± 0.62	P < 0.05	4.27 ± 0.72	P < 0.05
PCV (%)	27.18 ± 5.35	34.31 ± 3.97	P < 0.05	34.72 ± 3.93	P < 0.05
MCV (μm^3)	93.91 ± 6.19	81.50 ± 7.11	P < 0.05	82.25 ± 8.14	P < 0.05
MCH (pg/dl)	26.91 ± 2.07	26.07 ± 2.89	P=0.39	26.47 ± 2.34	P=1.99
MCHC (g/dl)	28.71 ± 2.19	32.09 ± 3.45	P < 0.05	32.28 ± 2.18	P < 0.05
HbF (%)	18.73 ± 6.72	0.85 ± 0.36	P < 0.05	--	P < 0.05

*The figures are Mean ± SD; *P<0.05. Hb = Haemoglobin in gram/dL; RBC = Red Blood Cell count in millions/cubic milimeter; PCV = Packed cell volume; MCV = Mean corpuscular volume in cubic micrometer; MCH =Mean corpuscular haemoglobin in picogram/dL; MCHC = Mean corpuscular haemoglobin concentration in gram/dL; HbF = Foetal haemoglobin in percentage of total Hb*

Discussion:

The sickle cell disease patients experience enormous clinical complications than the sickle cell carriers, who generally lead a normal life. The present study is a systematic and honest attempt to report the hematological profile of sickle cell disease patients, sickle cell carriers and the normal healthy subjects from the tribal population of North Maharashtra.

We found lower levels of haemoglobin, RBC count and PCV in the male and the female patients of sickle cell disease. Although the hemoglobin concentration, RBC counts and PCV was low in female SCD patients as compared to male SCD patients, it was statistically not significant (P = 0.42). Increased erythropoesis due to androgens in males, and blood loss in females during menstruation may be responsible for higher levels of hemoglobin and erythrocyte count in males. The results obtained in this study were comparable with previous Indian studies of Khan et al and Shrikhande et al [12-13].

Increased rate of hemolysis during oxygenation and deoxygenation process associated with recurrent infections in sickle cell anemic patients could account for these decreased values. There could also a blunted response to erythropoietin secretion in sickle cell anemia. The rate of its increased secretion may not be proportional to the degree of anemia. This may be due to right shifted hemoglobin dissociation curve seen in sickle cell disease [14-15].

The mean MCV was high in both the sexes of SCD patients, whereas MCHC values were low in our study which was comparable to other studies [7,16-17]. No gender related difference was seen in MCV, MCH and MCHC values. In the SCD patients, vitamin B_{12} and folic acid are maintained in a critically balanced state. Increased demand in erythropoesis due to chronic hemolysis or pregnancy in females causes deficiency state and leads to macrocytosis [18]. Low MCV values reported in some studies may be due to confounding factors such as co-existing iron deficiency anemia and other unknown factors such as α-thalassemia which is frequent and often associated to SCD [19-20].

In SCD patients mean HbF level (18.73%) was higher in both the sexes and was comparable to few earlier Indian studies [13] however, some Indian studies reported low levels of HbF [21]. As the quality of life is dependent on the symptoms and the impact of an illness on an individual, timely and appropriate treatment is very much necessary for the patients with SCD because life-threatening complications are known to develop rapidly. Anemia, hepato-splenomegaly and vaso-occlusive crisis in the form of bone and joint pains and infections are such complications reported to be very common in SCD patients.

Haplotype studies suggest that sickle cell anaemia (SCA) in Indians is linked to the Arab-Indian haplotype having high levels of HbF. This has a mild clinical presentation which goes unnoticed, sometimes throughout the life. HbF is a blessing in disguise; actually it retards the pathogenic polymerization of sickled erythrocytes when it is present. SCD in association with higher HbF (>10%) levels tends to have less anaemia and milder clinical manifestations [22-24]. However, the phenotype of SCD varies significantly among different population groups of India and there is limited regional data. Therefore, it is rather difficult to say that higher levels of HbF in SCD will protect patients from the severity of the complications. The severity of SCD varies greatly between individuals, since not all patients have identical pleiotropic genes (secondary effectors genes). Some carriers may have mutated genes that can either ameliorate or exacerbate the phenotype [5]. Therefore, in view of these contradictory reports, and the present observations in the patients, it is rather difficult to comment conclusively on the role of HbF and SCD complications. Further research in this area and direction in future may give clearer picture.

The present study shows low levels of haemoglobin, RBC count, PCV and altered blood indices in the carriers than the normal healthy controls. This indicates that carriers were having mild to moderate anaemia even in the asymptomatic state. This latent state in the carriers could precipitate complications in specific situations. Surprisingly, the control group females in the present study also showed mild to moderate anemia. It appears that due to poverty, the overall nutritional status of the subjects is below average. There is need to provide balanced diet with additional supplements of vitamins to remain physically fit in geographically adverse hilly areas.

According to several studies, quality of life for sickle cell carriers is relatively normal without significant health problems [21, 25]. However, few other studies reported frequent complaints of fatigue, generalised weakness, breathlessness, joint pain, repeated abortions etc [9-10] in sickle cell carriers. The present results on the hematological profile most probably appears due to lack of balanced nutritional dietary intake or due to poverty and/or blood loss in females during menstruation or repeated abortions could be other possible reasons responsible for low haemoglobin concentration, RBC count and PCV in sickle cell carrier females.

Conclusion:

The results of this study shows moderate to severe anemia and high foetal haemoglobin levels in the adult SCD patients. Sickle cell carriers and the normal control subjects showed mild to moderate anaemia. Regular health check-ups and

monitoring of haematological profile may help to guide the clinician to prevent vaso-occlusive crisis and further complications. The data so obtained would help in the management, prevention and control programme for SCD patients at the primary health centers in India. Further, large cohort studies are needed to determine the association between clinical complications of the disease and changes in the hematological parameters along with foetal haemoglobin levels in the adult SCD patients.

Conflict of interest: None to declare

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References

1. Balgir RS. The burden of hemoglobinopathies in India and the challenges ahead. *Curr Sci* 2000; 79(11): 1536-1547.
2. Desai DV, Dhanani H. Sickle cell disease: History and origin. *Int J Hematol* 2004; 1(2): 51-56.
3. Serjeant GR. The geography of sickle cell disease: Opportunities for understanding its diversity. *Annals of Saudi Medicine* 1994; 14(3): 237–246.
4. Serjeant GR. Sickle-cell disease. *Lancet* 1997; 350(9079): 725–730.
5. Stuart MJ, Nagel RL. Sickle cell disease. *Lancet* 2004; 364: 1343-1360.
6. Ataga KI, Orringer EP. Hypercoagulability in sickle cell disease: a curious paradox. *Am J Med*. 2003; 115: 721–728.
7. Shukla RM, Solanki BR. Sickle cell trait in Central India. *Lancet* 1985; 1: 297-298.
8. Kamble M, Chaturvedi P. Epidemiology of sickle cell disease in a rural hospital of central India. *Indian Pediatr* 2000; 37: 391-396.
9. Chikhlikar K, Wilkinson A. A Study of Red Cell Parameters in Patients of Sickle Cell Trait. *J Dental and Med Sci* 2014; 13(3): 46-50.
10. Kate SL, Langejwar DP. Epidemiology of Sickle Cell Disorder in the State of Maharashtra. *Int J Human Genetics* 2002; 2(3): 161-167.
11. Kate SL. Health problems of tribal population groups from the state of Maharashtra. *Ind J Med Sci* 2001; 55: 99-108.
12. Khan Y, Thakur AS, Mehta R, *et al*. Hematological profile of sickle cell disease: A hospital based at CIMS, Bilaspur, Chhattishgarh. *Int J Appl Biol Pharm Technol* 2010; 1: 717–721.
13. Shrikhande AV, Dani AA, Tijare JR, Agrawal AK. Hematological profile of sickle cell disease in central India. *Ind J Hematol Blood Transfus* 23 (3-4): 92-98.

14. Kar BC, Satapathy RK, Kulozik M, Sirr S, Serjeant BE, Serjeant GR. Sickle cell disease in Orissa state, India. *Lancet* 1986; 328: 1198–1201.
15. Akinsegun Akinbam, Adedoyin Dosunmu, Adewumi Adediran, Olajumoke Oshinaike, Phillip Adebola and Olanrewaju Arogundade. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Research Notes* 2012; 5:396. DOI: 10.1186/1756-0500-5-396.
16. Serjeant GR, Serjeant BE. Sickle Cell Disease 3rd edn. *New York, NY: Oxford University Press;* 2001, 113-115pp.
17. Mohanty D, Mukherjee MB, Colah RB, Wadia M, Ghosh K, Chottray GP, et al. Iron deficiency anaemia in sickle cell disorders in India. *Indian J Med Res*. 2008; 127(4): 366-369.
18. Hayes RJ, Beckford M, Grandison Y, Mason K, Serjeant BE, Serjeant GR. The haematology of steady state homozygous sickle cell disease: frequency distributions, variation with age and sex, longitudinal observations. *Br J Haematol* 1985; 59: 369–382.
19. Figueiredo MS, Kerbauy J, Goncalves MS, Arruda VR, Saad ST, Sonati MF, et al. Effect of α-thalassemia and β-globin gene cluster haplotypes on the hematological and clinical features of sickle cell anemia in Brazil. *Am J Haematol* 1996; 53: 72–77.
20. Falusi AG, Latunji PO. Effects of alpha thalassemia and haemoglobin F (HbF) level on the clinical severity of sickle cell anaemia. *Eur J Haematol* 1994; 52: 13–15.
21. Goyal JP, Raghunath SV, Shah VB. Hematological profile of sickle cell disease from South Gujarat, India. *Hematology Report* 2012; 4(2): 8. DOI: 10.4081/hr.2012.e8.
22. Serjeant GR. The Natural History of Sickle Cell Disease. *Cold Spring Harb Perspect Med* 2013; DOI: 10.1101/cshperspect.a011783 originally published online June 28, 2013.
23. Tewari S, Rees D. Morbidity pattern of sickle cell disease in India: A single centre perspective. *Indian J Med Res* 2013; 138: 288-290.
24. Jain D, Italia K, Sarathi V, Ghoshand K, Colah R. Sickle Cell Anemia from Central India: A Retrospective Analysis. *Indian Pediatr* 2012; 49: 911-913.

25. Balgir RS, Mishra RK, Murmu B. Clinical and Hematological Profile of Hemoglobinopathies in Two Tribal Communities of Sundargarh District in Orissa, *India Int J Hum Genet* 2003; 3(4): 209-216.

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