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Can Trimodal Distribution of HbS Levels in Sickle Cell Traits Be Used To Predict the Associated Alpha-Thalassemia For Screening Cases in Central India?

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ABSTRACT

***Background*:** Until now, trimodal distribution of HbS has been seen by six different studies in the world when associated with alpha-thalassemia with confirmation by corresponding alpha-genotyping studies. The RBC indices reduce as alpha-globin genes reduce in sickle cell trait (SCT) patients, which decreases the extent of intravascular sickling and thus betters the clinical course of the patients. This is a pioneer study conducted on Central Indian poor population to use the already proven six studies to screen associated alpha-thalassemia in SCT patients thus, circumventing the much costlier alpha-genotyping studies. Moreover, it aimed to study the haematological parameters in such cases.

***Methods*:** The study was performed at RHDMC, IGGMC, Nagpur, India from 2003 to 2012. The sample population was suspected cases of haemolytic anaemia. CBC and RBC indices were obtained by a cell analyzer. The sickle solubility test positively screened cases were confirmed by agar-gel haemoglobin electrophoresis at pH 8.6. Finally, quantitative assessment of haemoglobin variants was performed by HPLC.

***Results*:** Out of total 5819 cases over ten years, 933 cases were sickle heterozygotes. Overall, 180/933 subjects were predicted to be homozygous alpha-thalassemia and 338/933 were heterozygous alpha-thalassemia, based on trimodal distribution of HbS.

***Conclusion*:** Genotyping is costlier for majority of the poor non-affording patients in Indian government set-ups, so this study is suitable to screen for associated alpha-thalassemia in SCT patients.

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Introduction

Haemoglobin consists of alpha and beta gene clusters on chromosomes, 16 and 11 respectively (1). The two genetic disorders associated with Hb synthesis are: syndromes related with absent or reduced globin chain production called thalassemia and the syndromes due to structural alterations in the Hb molecule, which produce abnormal hemoglobinopathies (2). Thalassaemias are hereditary hemoglobinopathies seen worldwide. They are classified into two types based on the type of reduction in globin chains as α-thalassemia and β-thalassemia (3). α-thalassemia is very common

inherited single gene disorder in the world (4). As compared to α+, the α0 alleles are less common in India. In India, α-thalassaemia is mainly endemic in tribal areas, which includes the Central Indian population (5).

When the amino acid glutamate is replaced by valine at β6 position, sickle haemoglobin (HbS) is formed. The sickle cell disease then affects the endemic areas due to consanguineous marriages and marriages within the same affected lower castes of Central India like scheduled castes and scheduled tribes (6). The haemoglobinopathies are seen mainly in the malaria endemic areas as it is known to provide increased survival benefits against malaria in such cases (7, 8).

SCT is a mild disease but can be passed on to their children. In Central India, SCT is occasionally associated with α-thalassemia causing alteration in RBC indices; reduce the intra-vascular sickling by decreasing the formation of HbS. The latter occurs because fewer alpha chains are available to combine with defective βs chains.

No such previous Central Indian study has been published until date. This article presents a new viewpoint because trimodal distribution of HbS levels in SCT cases with RBC indices are indeed useful in predicting the associated alpha-genotype for population screening without need for the costlier alpha-genotyping studies in poor countries. This is the difference between our study and other six studies worldwide, which did confirmatory alpha-genotyping to back their similar respective studies (9-17).

Materials and methods

With approval of Ethics Committee and written consent from patients, the study was performed over ten years from 2003 to 2012. Five-ml anti-coagulated EDTA blood samples were sent to haematology section from admitted cases of haemolytic anaemia. The sample population were thus the suspected cases of haemolytic anaemia referred from OPD, sickle camps and family members of the confirmed cases. CBC and RBC parameters were measured using Sysmex XX-21 cell analyzer. Iron deficiency was ruled out by normal serum ferritin levels in the 933 SCT cases studied.

Sickle solubility test was used as a screening test. Sickle solubility test positive cases confirmed by Agar gel HbEp at alkaline pH 8.6. The agar gel electrophoresis gave variety of diagnosis, e.g. HbSS, HbAS, HbS/D, HbS/E, HbS/β. Quantitative estimation of haemoglobin variants like HbA2, HbS, HbF, and HbA was done by High Performance Liquid Chromatography (HPLC-Bio-Rad-Variant ™) on these cases.

Patient blood samples were considered as sickle positive with positive sickle solubility test with corresponding 'AS' pattern on haemoglobin electrophoresis on agar gel. HPLC was applied to screen all the 'AS' pattern cases for typing of the haemoglobin variants. Only those cases were segregated as SCT cases with HbS percentage upto 40%. RBC indices on CBC strip along with HPLC chromatogram was used for analysis of these SCT cases. Mean values, standard deviations, and frequency distributions were used the haematological study. Student's t-test was used to compare the means of groups using GraphPad software. $P<0.05$ was considered as statistically significant case.

Results

Out of total 5819 cases from 2003 to 2012, 933 cases were sickle heterozygotes (AS) characterised by HbA >HbS, with HbS upto 40% on HPLC.

Histogram and normal probability plot were plotted by the computer. The histogram showed

Table 1
Trimodal distribution of HbS levels in SCT cases in Central India with varying corresponding RBC indices to predict the co-existent alpha-genotype

HbS	Mean MCV ±SD	Mean MCH ±SD	Mean MCHC ±SD	Alpha-thalassemia genotype suspected	No. of cases (%)
34.1-40%	84.33± 1.94	26.33± 3.53	31.02± 3.38	SCT+Normal α-globin genotype (αα /αα)	415 cases (44.80%)
28.1-34%	77.87± 12.46	24.4 ± 7.01	30.54± 4.08	SCT+Heterozygous a- thalassaemia (-α /αα)	338 cases (36.23%)
10 - 28%	75.6 ± 13.90	23.02± 6.3	28.98± 5.01	SCT+Homozygous a- thalassaemia (-α /-α)	180 cases (19.29%)
				Total cases	933 cases

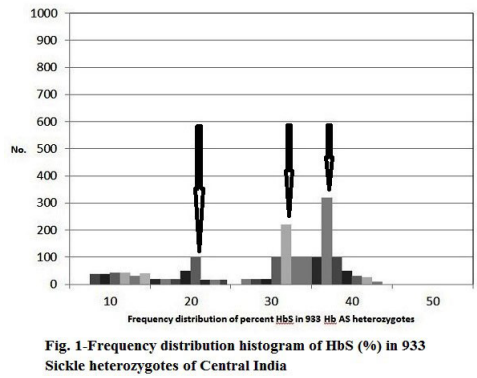

Fig. 1-Frequency distribution histogram of HbS (%) in 933 Sickle heterozygotes of Central India

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Frequency distribution histogram of HbS (%) in 933 Sickle heterozygotes of Central India

a trimodal distribution of HbS (Fig. 1). The peaks were named as peak A, B and C respectively. The mean HbS, standard deviation and range for each peak was calculated. When the three peaks were separated, the ranges obtained for HbS were 34.1-40, 28.1-34 and 10 - 28% in A, B and C category, respectively. By this way we determined the HbS levels of 28% and 34% for different alpha thalassemia genotypes.

Table 1 shows that 180 out of total 933 SCT cases were predicted to be α-thalassemia (-α/-α) and 338/933 were heterozygous α-thalassemia (-α/αα), based on the trimodal distribution of HbS. The maximum patients were sickle heterozygotes without suspected α-thalassemia. In sickle heterozygotes with α-thalassemia, the HbS percentage goes on decreasing from normal α-globin genotype followed by α-thalassemia heterozygotes and then α-thalassemia homozygotes, in that order.

In above table, as we move down in the three distinct groups, the reduced alpha chains caused reduction in RBC indices; reducing the clinical severity of the patients. The latter occurred due to reduced alpha chains available to combine with the defective βs chains.

Discussion

Generally, the α-chain is present in excess. However, when α-thalassemia coexists with SCT, the amount of α-chain available becomes rate limiting. When the amount of α chain is rate limiting, the affinity of non-α chains for the α-chain is βA > δ > βS.

The positively charged α-chain combines preferentially with the negatively charged βA chain, rather than with the positively charged βS chain, with a consequent reduction in the HbS percent. The δ-chain is also positively charged, so that the α-chain is likely to combine preferentially with βA, rather than with δ (9, 10). When the amount of α-chain is rate-limiting, combination with the δ-chain is favoured over combination with βS (Table 2). The alpha-genotyping studies are very costly and not easily available in most laboratories in Central India, we infer that the demonstra-

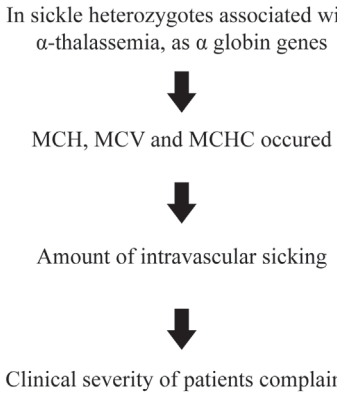

Fig. 2
Results of lowered ßs gene expression in SCT with alpha thalassemia

tion of trimodal distribution of HbS levels is a suitable method for screening for α-thalassemia in population studies in moderately equipped labs and provides an opportunity for parental counselling during pre-natal diagnosis.

In India, compared to other areas, sickle cell disease patients have higher hemoglobin, lower reticulocyte counts, and high levels of HbF with frequent alpha thalassemia association. Clinically, splenomegaly, anemia, bone pain crises are common while leg ulcers, priapism are rare in Indian sickle cell patients. Association of alpha thalassemia with SCT cases has better clinical outcome of the latter in Central India. When we compare our own study by Indian authors in Central India to the other similar studies done stated in Table 3, (11-17), it is clear that the mean HbS in our three groups is similar to an American study (11,12) on South Indian population and in East Saudi Arabia in (17). In these three mentioned studies, the mean HbS values were lower than the other studies because of the common Arab-India sickle cell haplotype of Indians and the East Arabs.

This retrospective study of 5819 patients in India with suspected haemolytic anaemia was performed to determine whether HbS levels can predict alpha thalassemia genotype. This work was performed by CBC based RBC indices and HbS quantitation by HPLC. We demonstrated a trimodal distribution of HbS which along with RBC indices provides a suitable method for screening for other thalassemia in patients with sickle cell-thalassemia. If confirmed, this would preclude the need for expensive genetic analysis for the diagnosis of alpha thalassemia and provide some certainty for the cause of unexplained microcytosis.

Table 2
Comparison of mean HbS values (%) in sickle cell heterozygotes (AS) with different number of active α-genes

Various studies in sickle cell heterozygotes patients (11-17)	α-Chain gene arrangement			Reference number
	αα/αα	-α/αα	-α/-α	
Georgia,USA-1977 (Huisman THJ et al)	41.2	35.5	28.1	13,14
California,USA-1978 (Embury SH et al)	40.0	35.2	29.3	15
Ontorio,Canada-1981 (Wong SC et al)	40.4	35.1	28.6	16
South India-1979 (Brittenham G et al)	36.8	31.6	24.8	11,12
East Saudi Arabia-1986 (El-Hazmi MAF)	40.0	31.5	23.0	17
Central India-2013 (Warpe BM et al)	36.5	30.5	20.5	*Present study*

Conclusion

Genotyping is costlier for majority of the poor non-affording patients in Indian government setups, so this study is suitable to screen for associated alpha-thalassemia in SCT patients.

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Conflict of interest

The authors do not have any competing interests.

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