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Original Article

Thalassemia carrier screening in siblings of thalassemia major patients by HbA_2 estimation

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ABSTRACT

Objective: Thalassemia carrier screening in siblings of thalassemia major patients by HbA_2 estimation. **Methods:** This prospective, cross-sectional study was conducted at thalassemia welfare society of JK Lon Hospital, Kota. Siblings of thalassemia major patients registered at our hospital were investigated for chemically bonded ceramics and HbA_2 estimation by high performance liquid chromatography (HPLC) method. **Results:** A total of 121 cases were screened for carrier by HPLC method for HbA_2 estimation. Total 59 (48.76%) cases had HbA_2 level ≥3.5%, and considered as carrier while 62 (51.24%) cases were non-carrier. Mean HbA_2 value in carrier was 5.24% ± 1.14% and in non-carrier was 2.69% ± 0.51%. **Conclusion**: It was concluded that β thalassemia carriers are more prevalent in siblings of thalassemia major than the normal population.

Key words: *HbA_2 estimation, Siblings, Thalassemia carrier screening*

Thalassemia refers to genetic disorders in globin chain production. 3% of the world's population carries genes for β thalassemia [1]. It is the most common monogenic disorder in India. The prevalence of thalassemia trait varies from 1.0-14.9% in various regions of India. Incidence varies in different communities, religions, and ethnic group. It is estimated that more than 25 million people in India are carriers of the β-thalassemia gene and 8000 children are born every year with thalassemia major. Only 10–15% of these children receive optimal treatment. Community control of hemoglobinopathies relies mainly on outreach educational programs and genetic counseling with antenatal diagnosis [2].

Accurate and timely detection of various hemoglobin variants including β-thalassemia trait can prevent the occurrence of more serious disorder like thalassemia major in newborn [3]. There is definite need for carrier surveillance in our country. The various available options to screen carriers are (1) screening of school going children, (2) screening of high-risk community, (3) prenatal screening, (4) extended family screening, i.e. screening of relatives, if there is a thalassemia major child in family, and (5) routine antenatal screening in early pregnancy between 10 and 12 weeks [4]. There is a high prevalence of thalassemia in Kota and vicinity. Therefore, we performed this study to determine the prevalence of thalassemia carrier in siblings of thalassemic children.

MATERIALS AND METHODS

This prospective, cross-sectional study was conducted in the Department of Paediatrics, J. K. Lon Mother and Child Hospital, Government Medical College, Kota. The study was conducted between December 2013 and November 2014 after getting approval from the institutional ethics committee. Children aged below 18 years who were siblings of thalassemia major patients, registered at thalassemia welfare society at J.K. Lon Hospital, Kota were included in the study. Children with a history of recent blood transfusion in last 3 month**s** and children with any acute febrile illness were excluded from the study.

Children were recruited after taking informed consent from the parents or legal guardians. For all patients, a careful history was taken for any illness or blood transfusion. Family history was taken for consanguineous marriage, hemolytic anemia, and other significant illnesses. General physical examination (including vitals and anthropometry) and systemic examination were performed. After taking written consent, 5 ml of venous blood was collected and sent for complete blood count and high performance liquid chromatography (HPLC).

Complete blood count was performed by automated cell counter to get hemoglobin, red cell count, hematocrit, red cell distribution width (RDW), and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), leukocyte, and platelet counts. Cation exchange HPLC has been applied to identify hemoglobin variants using Bio Rad variant system. It identifies and quantifies hemoglobin by their retention time (RT) (the time at which they elute) and computing the area under the peak. In "Bio Rad Variant", hemoglobins are separated graphically and quantified spectrophotometrically utilizing sophisticated computer software. Before sample application, the instrument has to be primed and calibrated

followed by use of control to check reproducibility. The RT is stated in relation to that of control for HbA_2.

Data obtained was tabulated using version 17 of the Statistical Package for Social Sciences (SPSS, published SPSS Inc.). Chi-square test and Student's test were used to identify differences between groups.

RESULTS

A total of 133 cases were assessed for inclusion in the study; out of them, 12 cases were excluded and finally, 121 cases were screened for carrier of thalassemia by HbA_2 estimation. Out of 121 cases, 5 cases were <6 months old, 42 aged 6-59 months, 27 aged 5-10 years, and 47 cases were in 10-18 years age group. Out of 121 cases, 72 were male and 49 were female. Total 59 (48.76%) cases had HbA_2 level ≥3.5% (labeled as carrier), while 62 (51.24%) cases were non-carrier. Mean HbA_2 value was significantly higher ($P < 0.0001$) in carriers (5.24 ± 1.14%) than in non-carriers (2.69 ± 0.51%). Mean HbF level was 2.02 ± 0.41% in carriers and 3.63 ± 0.76% in non-carriers but difference was not significant ($P = 0.18$).

About 2 cases had a history of blood transfusion in the past; out of them, one case was carrier. Out of 121 cases, 1 case was diagnosed as thalassemia major (HbF 68%). Two cases had HbS level 36.2% and 37.8% without any clinical manifestation (sickle cell trait) and normal HbA_2 level (2.9 and 2.3). HbA level was between 85 to 98.6% in rest of the cases. The comparison of hematological parameters between carriers and non-carriers is given in Table 1.

Table 1: Comparison of hematological parameters between carrier and noncarrier

Mean	Carrier	Noncarrier	t/χ^2	*P*
Hb (g %)	9.89±2.08	9.43±2.42	-	0.21
RDW	14.86±4.00	15.07±5.61	0.22	0.82
HCT	30.79±5.00	29.24±7.13	1.4	0.15
RBC	5.26±1.00	3.75±0.92	8.8	<0.001
MCH	21.35±4.17	25.60±4.37	5.3	<0.001
MCV (fl)	62.08±11	79.7±11.78	8.4	<0.001
MCHC	31.55±3.17	31.65±2.38	0.19	0.84
TLC	8.17±2.56	8.45±4.12	0.48	0.63
Platelet count	261.23±100.11	236.80±145.91	1.1	0.29

RDW: Red cell distribution width, HCT: Hematocrit, RBC: Red blood cell, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, TLC: Total leukocyte count, MCV: Mean corpuscular volume

Table 2: Comparison of incidence of carrier in sibling of thalassemia major with others

Parameters	Present study	Ahmed et al. [11]	Mohanty et al. [12]	S.M. Baig et al. [13]	Mishra et al. [14]
Year	2014	2012	2008	2008	2013
No. of cases	121	591	25	27	33
No. of carrier	59	183	10	12	25
%	48.76	30.86	40	44.4	73.33

DISCUSSION

Preventive screening programs to identify carriers are being used by many countries where thalassemia is a common disease. Our study was done on the concept of cascade screening (extended family screening). We included the siblings (1st degree relative) of thalassemia major (index case) for screening. In our study, a total of 121 cases were screened for carrier of thalassemia by HPLC method for HbA_2 estimation, and total 59 (48.76%) cases were found to be carrier. The WHO reports that 5% of the population is carrier of different hemoglobinopathies [5]. According to ICMR data, the carrier frequency of hemoglobinopathies is reported between 3 and 17% in the different population [6]. The results of this study showed a higher prevalence of beta thalassemia trait among siblings of thalassemia patients than 5% in general population.

The previous studies conducted in Gujarat and West Bengal revealed the prevalence of hemoglobinopathies to be 37.5 and 20.47%, respectively [7,8]. In a study conducted in Maharashtra, the prevalence of beta thalassemia trait was 17% [9]. Antenatal screening was done in Mumbai and Behrampur (Orissa) shows the prevalence of carrier in pregnant mother was 1.99% and 8%, respectively [8,10]. Most of the previous studies did screening of general population and pregnant women, and only a few studies have been done in family, relative and siblings of thalassemia cases. As shown in Table 2, the prevalence is higher than the studies done by Ahmed et al [11] and Mohanty et al [12] and results are similar with Baig et al [13]. The differences in the results can be due to the selection of only siblings, geographic condition and number of cases and genetic heterogenicity of thalassemia gene.

In our study, HbA_2 level was between 3.5 and 7.2% in carriers and the mean HbA_2 was significantly higher ($P < 0.001$) in carriers than in non-carriers. Mean HbA_2 in the study done by Mohanty et al [12] Mirebehbahani et al [15] and Sujata et al [16] were 4.99, 5.18 and 4.53 respectively. Mean RDW was lower in carrier. RDW measures the coefficient of variation and is higher in iron deficiency anemia but not in the thalassemia where a uniform microcytic red cell population is seen with a normal RDW.

The mean red blood cell (RBC) count in carrier of hemoglobinopathies was increased and considered a useful diagnostic adjunct because the thalassemia has microcytic anemia with an increase in the RBC number, whereas other causes of microcytic anemia including iron deficiency anemia and anemia of chronic disease are typically associated with a decrease in the RBC number that is proportional to the degree of decrease in Hb concentration [8]. MCV is the key indicator for diagnosis and screening. Thalassemic individuals have a reduced MCV, and one study has suggested that an MCV of <72 is maximally sensitive and specific for the presumptive diagnosis of thalassemia [10,17]. A low MCH and low MCV are the clues for the diagnosis of thalassemia [9].

It is denoted that thalassemia carrier has characteristics hematological parameters with mild or no anemia with microcytic hypochromic RBCs, increased red cell counts and normal RDW

and hemoglobinopathies can be suspected on the basis of these parameters. We recommend screening to detect carriers in siblings of thalassemia major patients, and counseling them to screen the forecoming life partener for thalassemia carrier before marriage to prevent thalassemia major in their next generation. This study was limited by its small sample size and study subjects. A wide-scale population-based study with a larger sample size is needed to assess the exact prevalence of hemoglobinopathies and carriers in this part of the country.

CONCLUSION

It is concluded that β thalassemia carriers are more prevalent in siblings of thalassemia major than normal population. Hemoglobinopathies can be suspected on the basis of hematological parameters such as reduced MCV, reduced MCH, and elevated RBC count disproportionate to hemoglobin level.

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