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Study of Haematological Profile of Thalassemia Patients With Reference To High Performance Liquid Chromatography

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ABSTRACT
Study of Haematological Profile of Thalassemia Patients With Reference To High Performance Liquid Chromatography
Objective: *Beta thalassemia and their interaction with structural HbS and HbE variants are major public health problem in India. This study was carried out to assess the usefulness of HPLC in diagnosis of thalassemia patients and its variants.*
Methodology: *209 thalassemia cases during 2.4 year period were investigated.CBC, peripheral smear, reticulocyte count, fetal fraction, sickling tests, HbF, HbA_2, HbA_0, HbE; HbS levels were estimated using BIORAD variant automated HPLC technique.*
Results: *Out of 209 cases, thalasssemia trait was the most common followed by thalassemia major, HbSβ thalassemia trait, HbEβ thalassemia trait, thalassemia intermedia, δβ thalassemia trait. Buddha community (34.93%) was the most common ethnic group. Consanguinity of 21.53% was observed. The various haemoglobin levels were studied in these groups.*
Conclusion: *HPLC was found to be simple, rapid and reliable method for detection of thalassemia. Consanguinity of 21.53% highlights the role of premarital counselling in thalassemia patients especially in Buddha and Muslim communities. This emphasises the need of community based targeted awareness, counselling and field work in these cases which is possible by using HPLC.*
Keywords: *Thalassemia, HPLC, HbS, HbE.*

Introduction

The term "thalassemia" is derived from the Greek, thalassa (sea) and haima (blood). Beta thalassemia syndromes are group of hereditary blood disorders characterised by reduced or absent beta globin chain synthesis. Most thalassemias are inherited as recessive traits [1]. In India the mean prevalence of beta thalassemia gene is 3.3% and 6000 to 8000 children are born every year with thalassemia major [2]. The β thalassemias and their interaction

with structural haemoglobin (Hb) variants like HbS and HbE are a major public health problem in India [3]. Automated cation-exchange high performance liquid chromatography (HPLC) has emerged as an excellent screening and diagnostic tool for diagnosing beta thalassemias [4].

Materials and Method

The main objectives of this study were:

1. To study clinical features, haematological parameters and HPLC findings in patients of thalassemia.
2. To generate data regarding ethnicity and consanguinity in these patients
3. To evaluate the role of family study.

The present study is a cross-sectional descriptive study conducted in Government Medical College, Aurangabad over 2.4 year duration. Inclusion criteria included patients with positive fetal fraction test, patients with clinical diagnosis/ suspicion of thalassemia on haematological workup and family members of these patients. The patients who received blood transfusion in the last 6 months were excluded from the study. A specific algorithm was followed in the study:

1. Haematological profile of cases was done. It included CBC (complete blood count), PS (peripheral smear), reticulocyte count, fetal fraction, and sickling test. BM (bone marrow) was done whenever required.
2. Using AUTOMATED BIORAD VARIANT HPLC, percentage levels of HbF, HbA_2, HbA_0, HbS, HbE were estimated to diagnose the cases. Repeat HPLC after complete vitamin B12 therapy/iron therapy were done in some cases. (Specific elution windows are defined for abnormal haemoglobins like HbS, HbD and HbE using the β thalassemia short program)
3. Study of family members whenever possible was done to confirm the diagnosis and to determine ethnic background and consanguinity.

Results

Table No 1: Cases grouped as:

Group	Gr-A Thal major (TM)	Gr-B Thal trait (TT)	Gr-C S beta thal trait (SβTT)	Gr-D E beta thal trait (EβTT)	Gr-Rare Thal Inter (TI)	Gr-Rare Delta beta thal trait (δβTT)	TOTAL
No of cases	35+1* Total=**36** (17.22%)	108+23* Total=**131** (62.68%)	29+1* Total=**30** (14.35%)	5+1* Total=**6** (2.87%)	2+1* Total=**3** (1.44%)	3 Total=**3** (1.44 %)	**182+27*** **=209**

Gr-Group.

Note: (Cases marked by (*) were provisionally diagnosed without family study)

Cases with (HbA_2 > 3.9%) were provisionally diagnosed as thalassemia trait [5, 6].

Out of 209 cases of thalassemia maximum cases were of thalassemia trait (TT) 131cases (62.68%) and the other group wise case distribution is as above. In 182 (87.08%) cases family study was performed and in 27 (12.92%) cases family study was not possible. Reasons for incomplete family studies were poor socio-economic status, distance, lack of awareness, remarriage/death, reluctance (especially in father), alcoholism and psychosocial reasons.

AGE: In thalassemia major all the cases were in 0-10 years of age group (the average of presentation was 1.6 yrs). In thalassemia trait**,** maximum cases were in 21-30 years of age group, because this group was mostly comprised of parents of an affected child. In sickle beta thalassemia trait maximum cases were in 11-20 years of age group. In HbE-beta thalassemia trait maximum 4 cases were in 11-30 years of age group. In thalassemia intermedia 1 case was in 0-10 yrs age group, 2 cases in 11-20 yrs group and in delta

beta thalassemia trait 3 cases were in 21-30 yrs of age group.

SEX: Overall there were 101 males (48.33%) and 108 females (51.67%) in the present study.

ETHNIC BACKGROUND: Buddha (34.93 %) was the most common ethnic background among all groups followed by Muslims (16.27 %), Mahar and Banjara (9.09% each); Maratha (8.13%), less common were Koli, Mang and Sindhis.

Consanguinity

It was observed in 45 (21.53 %) cases. Maximum cases of consanguinity were seen in Muslims 20 (44.44%) followed by Buddha community 17 (37.78%).

CLINICAL PRESENTATION

Table No 2: Clinical Presentation of cases among all groups:

Clinical Features	Gr-A TM T=36		Gr-B TT T=131		Gr-C SβTT T=30		Gr-D EβTT T=6		Gr-Rare TI T=3		Gr-Rare δβTT T=3		Total =209
	N	%	n	%	N	%	n	%	n	%	n	%	
Pallor	36	100	58	44.27	25	83.33	6	100	2	66.67	2	66.7	129
Jaundice	8	22.22	1	0.76	11	36.66	1	16.66	2	66.67	1	33.3	24
VOC	-	-	-	-	7	23.33	-	-	-	-	-	-	7
Abdo pain	9	25	2	1.52	6	20	1	16.66	1	33.3	-	-	19
Leg ulcer	2	5.55	-	-	1	3.33	1	16.66	1	33.3	-	-	5

Majority of cases presented clinically as pallor (Hb<10gm %) in all the groups followed by jaundice, abdominal pain. Leg ulcer was not seen in thalassemia trait group and δβTT group. Vaso-occlusive crisis (VOC) was seen only in SβTT (23.33%). 71 cases (33.97%) cases had splenomegaly (abnormal enlargement of spleen confirmed by clinical examination). 44 cases (21.05 %) presented with hepatomegaly (abnormal enlargement of liver confirmed by clinical examination).

The thalassemia trait group was divided further-

Table No 3-

Thalassemia Trait Group	Parents of affected child	Non-Parent group
Total =131 cases	108	23

A specific algorithm was followed for the 23 cases of newly diagnosed thalassemia trait group. When HbA2 level of >3.9% was detected after HPLC test, it was confirmed by a repeat HPLC test. In all these cases an attempt to exclude other causes of increase HbA_2 was made by- CBC, PS, haemolytic fractions, BM examination, thyroid profile, HIV test, and repeat HPLC after complete treatment of megaloblastic anemia with vitamin B12 therapy was done whenever required. After the above procedure these cases were classified as thalassemia trait.

Exclusion of other causes of decreased HbA_2 from δβTT patients was done by-CBC; PS, haemolytic fractions, BM examination, iron profile and repeat HPLC test after 3 months of complete iron therapy were done whenever required. After the above procedure the cases were classified as δβTT patients based upon HbA_2 and HbF levels detected by HPLC.

Table No 4. Mean haematological parameters in all groups:

Parameters	Group-A TM	Group-B TT	Group-C Sβ thal trait	Group-D Eβ thal trait	Group-Rare TI	Group Rare δβTT
Hb (gm %)	5.48±1.45	10.39±1.46	7.97±1.98	8.03±1.13	7.97±0.84	10.5±1.9
RBC count (million/cu.mm)	3.24±1.02	4.43±0.72	4.03±1.03	4.23±0.80	3.7±0.26	4.97±0.50
PCV (%)	17.05±4.61	31.10±5.24	25.15±5.57	25.65±3.03	23.4±1.97	30.5±5.31
MCV (fl)	62.41±5.83	76.29±8.15	75.6±8.11	74.17±7.005	64.8±3.64	64.18±3.52
MCH (pg)	18.52±2.99	21.97±2.91	21.71±3.59	23.72±3.57	20±2.96	21.37±2.08
MCHC (gm %)	25.04±4.08	27.66±3.97	27.80±3.59	29.05±0.97	26.9±4.33	32.77±2.48
RDW (%)	17.98±1.88	14.15±1.23	16.11±1.96	16.57±2.73	15.07±1.07	15.57±1.35

*(All figures mentioned are mean ± SD).

Thalassemia major patients had low haemoglobin levels (5.48±1.45) as compared to the other groups. In thalassemia trait and δβTT patients the haemoglobin levels were above 10gm%. RDW (red cell distribution width) was found to be high in thalassemia major patients (17.98±1.88) as compared to other groups.

Table No 5. Average haemoglobin levels by HPLC in all groups:

	Group-A TM	Group-B TT	Group–C Sβ thal trait	Group–D Eβ thal trait	Group-Rare TI	Group-Rare δβTT
HbF (%)	92.35±4.83	0.69±0.84	17.57±8.39	19±12.83	43.47±0.40	14.47±1.97
HbA_0 (%)	4.86±4.02	83.58±2.22	6.44±6.68	26.43±15.96	47.9±0.52	77.47±2.66
HbA_2 (%)	3.46±1.21	5.57±0.63	5.27±0.83		4.53±1.44	1.97±0.50
HbS (%)	-	-	70.08±8.76	-	-	-
HbE (%)	-	-	-	48.43±10.68	-	-

In thalassemia major group the average HbF levels were high; it was (92.35±4.83).Elevated HbF levels of (43.47±0.40) were encountered in thalassemia intermedia patients. The cut off value of HbA_2 >3.9%, was used to diagnose thalassemia trait, after exclusion of the other causes of increase HbA_2 [5, 6]. In thalassemia trait patients the average HbA2 level was found to be (5.57±0.60).

Discussion

Thalassemia trait constituted the commonest disorder with 131 cases (62.68 %) in the present study. Most of the cases in this group were parents of an affected child. In, thalassemia major group 100% of cases presented in <10years of age with average age of presentation being 1.6 years which was consistent with Patel J et al study (90.65 % <10 years) [7] . In EβTT patient's average age was 20.33 years, SβTT 17.7 years and in thalassemia intermedia was 11 years in the present study.

Buddha was the most common caste affected 73 (34.93%), Muslims (16.27 %), Mahar and Banjara (9.09% each) in our study. Ambekar SS et al, also found the Navbudha 55 cases (33.5%) most common ethnicity affected similar to our study followed by Maratha 51 (30.5%) and Muslims 22 (13.2%) [8]. Shah SJ et al study stated that 2 out of 2 cases (100%) of HbEβ thalassemia patients were Muslims and in the present study 3 out of 6 cases (50%) of HbEβ thalassemia patients were Muslims [9].

Consanguinity was seen in 45 cases out of 209 cases (21.53%). This may be due to common practice of consanguineous marriages in Muslim (44.44%) and Buddha (37.78%) communities. Baig et al (2006) observed that very high (>81%) consanguinity and low literacy rate were the risk factors for high incidence of β-thalassemia in South Punjab, because of this there was a need of thalassemia prevention program in this part of Pakistan [10].

Shah SJ et al reported all 35 cases with pallor (100%), 31 cases with splenomegaly (88.6%), 25 cases with hepatomegaly (71.4%) and 8 cases with icterus (22.9%) [9]. While in our study, pallor was the commonest presentation seen in 129 cases (61.72%) followed by jaundice seen in 24 (11.48%), splenomegaly in 71 (33.97%), hepat-

omegaly in 44 (21.05%). Vaso-occlusive crises seen in only Sβ thalassemia trait patients in 7 cases.

Studies by Rao.S et al [6], Patel.J et al [7] observed low haemoglobin levels in β-thalassemia major <7g%. Similar was the finding in our study, thalassemia major group showed haemoglobin levels of (5.48±1.45) as compared to the other groups. In Rao.S et al study thalasssemia trait and δβTT patients the haemoglobin levels were >10 gm%, similar was the findings in these groups in our study. In the present study the HbF level in thalassemia major group was high >90% (92.38±4.83), as compared to the others studies like Gonzalez-Redondo JM et al [11], Rao.S et al [6] Patel J et al [7]. C.Vani and S.Mamta [12] where HbF level was quite variable <90%. This may be due to, the reason, that the cases included in the present study did not receive any blood transfusion. Fucharoen S et al study HbA2 level of (5.5 ± 1.26) was noted [13]. The average HbA_2 level in thalassemia trait group in the present study matched with HbA_2 level in Rao S. et al study and it was (5.5 ± 0.6). In C.Vani and S.Mamta HbA2 level was 5.4% [12].

In Rao S.et al study HbS level(71.7±5.8) and HbF was (18.3±8.4); in C.Vani and S.Mamta study HbS – 65.6% HbF-19.2% were seen ;while in the present study the HbS level was (70.08±8.76) and HbF level of (17.57±8.39) level was found. Thus overall HbS levels were > 60% and HbF levels were >15%. The average HbA_2 level in present study was (5.27±0.83). In 29 out of 30 cases, family HPLC study supported the diagnosis of double heterozygous SβTT.

Average HbE variant levels, in the present study was (48.43 ±10.68) and HbF level was (19 ±12.83). In Rao S et al study HbE was (52.3 ± 17.6) and HbF (21.7 ± 12.2); in Jha BM et al HbE variant level of 57.58 % and HbF 25.98 % were observed [14]. Thus in all the studies average HbF levels were approximately > 20% and average HbE variant levels were >40%. In 5 cases the diagnosis was supported by family HPLC study. In present study, on comparing Sβ group and Eβ group, it was seen that the level of abnormal haemoglobin (HbS/HbE) was more in Sβ group than Eβ group (HbS-70.08±8.76 and HbE-48.43±10.48). The level of HbF was more in Eβ group than Sβ group (19±12.83 in Eβ and 17.57±8.39 in Sβ). Similar was the observation in C.Vani and S.Mamta study [12]

According to Sachdev R et al study in patients of thalasemia intermedia the HbF levels are raised with a variable reduction in HbA_0 [5]. In the present study the cases of thalassemia intermedia were classified on the basis of: a) Clinical presentation of these patients being at a later age than thalassemia major. The average age of presentation was 11 years in our study. b) These patients required infrequent blood transfusion (1-2units/ year). c) Average HbF levels by HPLC were (43.47±0.40).

However, distinction of various genotypes of thalassemia intermedia is possible by molecular genetic studies. Hence, molecular studies are essential for definitive diagnosis of thalassemia intermedia. Among δβ thalassemia trait, Rao S et al reported HbF (13.3 ± 4.3), HbA2 - 2.8, while in our study HbF level was found to be (14.47 ± 1.97) and HbA2 (1.97±0.50) levels were observed. Family study was done in all the cases.

Conclusion

To conclude we found commonest disorder of all the groups was thalassemia trait which constituted 62.68% of the study, because family study was meticulously done in the present study. All the cases of thalassemia major group presented in first decade. In Sβ thalassemia group maximum cases were detected in second decade. This reflects that Sβ thalassemia cases are asymptomatic for longer period of time. Similar findings were seen in Eβ thalassemia, thalassemia intermedia, and δβ thalassemia trait group. Thus, it can be considered that thalassemia major manifests in early childhood while other groups present at later age.

Consanguinity was seen in 21.53% cases. Thus, it implies that the consanguineous marriage increases the frequency of thalassemia, highlighting the

role of premarital counselling. Buddha (34.93%) and Muslims (16.27%) were the most common ethnic groups affected. This emphasises the need to formulate community based studies and to implement strategies to increase the awareness about thalassemia in such communities. Pallor was the commonest presentation seen in 129 cases (61.72%) followed by jaundice in 24 cases (11.48%) and abdominal pain in 19 cases (9.09%). Vaso-occlusive crises were seen in only Sβ thalassemia group. Maximum cases of splenomegaly and hepatomegaly were seen thalassemia major group followed by Sβ thalassemia group.

In thalassemia major group, the HPLC levels of HbF was high >90% (92.38±4.83), in thalassemia trait group, HbA2 level was (5.5 ± 0.6). On comparing Sβ thalassemia group and Eβ thalassemia group, the percentage of abnormal haemoglobin (HbS/HbE) was more in Sβ thalassemia group than Eβ thalassemia group. The HbF level was more in HbEβ thalassemia group than HbSβ thalassemia group.

Family HPLC screening is useful for the diagnosis of difficult cases especially in double heterozygous states. HPLC screening helps to identify thalassemia carrier couples. It alerts them of their risk and options to avoid birth of a transfusion dependent β-thalassemia child. After identification of a carrier couple by HPLC test, an option for such a couple is prenatal diagnostic tests [15]. Prenatal diagnostic tests in an anxious couple of an affected child or child expired due to thalassemia; offers a positive impact during next pregnancy. This forms the basis of thalassemia prevention programmes. In present study it was not possible to differentiate $\beta^0\beta^0$, $\beta^+\beta^+$, $\beta^0\beta$, $\beta^+\beta$, $\beta^0\beta^+$ and other genotypes. Genetic study is essential for these cases.

Hence, this study emphasises the need of community based targeted study and field work. So, health care resources can be planned accordingly, to reduce the burden of thalassemia in India.

Conflict of interest: None.

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