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Clinico-Haematological Profile of Thalassemia Intermedia Patients

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ABSTRACT Clinico-haematological profile of 93 patients (60 males, 33 females) of thalassemia intermedia with age between 6 months to 46 years (mean 13.5) is being presented. They comprised of 76 homozygous and 17 heterozygous for β thalassemia. The mean haemoglobin at presentation was 7.4 g/dl. Anthropometric measurements in 62 children below 19 years of age revealed height less than 3rd percentile in 63.4% male and 57.1% female children while weight was less than 3rd percentile in 76% males and 81% female children. Fifty-three of 93 patients were followed up for a mean period of 3.7 years (range 1-14). Twenty-five of 53 (47%) patients had progression of thalassemic facies. Pathological fractures, osteoporotic changes, compression fracture, and gall stones were observed in 8,3,1 and 2 patients respectively. Fifty-four of 93 patients required infrequent blood transfusions ranging from 1-2 unit per year, while 34 patients never required blood transfusions. Serum ferritin levels were done in 16 cases which varied between 132-5600 ng/ml. Sixteen of 27 patients who were above 15 years of age or had clinical suspicion of endocrinopathies were found to have endocrine abnormalities like hypocalcemia (5 cases), hypothyroidism (3 cases), growth hormone deficiency (2 cases) and primary gonadal failure (2 cases). Twelve of 33 (36.4%) women had 19 pregnancies during the study. Ten of these had full term normal deliveries, while 5 were premature neonates. Two neonates died because of prematurity and/or infection. Four women had abortions. Nineteen patients were investigated for 5 common genetic mutations. Eleven of these patients showed compound heterozygous pattern while 7 cases were true homozygous with highest frequency of IVS1-nt-1 (G-T) mutations (44.7%). Blood transfusion policy, endocrine problems, pregnancy outcome, other therapeutic options and inheritance pattern for these patients have been reviewed.

INTRODUCTION

The thalassemia syndromes are a heterogeneous group of inherited disorders characterized by decreased either β or α globin chain synthesis. β homozygous thalassemia state presents with variable degree of anemia from early childhood and are generally transfusion dependent, a condition, clinically known as thalassemia major. On the other hand β heterozygous cases (thalassemia minor) are almost asymptomatic with normal or slightly reduced levels of haemoglobin (Hb). However an intermediate condition which may have either heterozygous or homozygous pattern of inheritance, requires minimal or no blood transfusion and has milder clinical course than thalassemia major but is severe enough as compared to thalassemia minor. It manifests generally after 2 years of age and does not require regular transfusion therapy (Rund et al. 1997). Only in few studies, patients with thalassemia intermedia have been evaluated for clinico–hematological profile or for molecular studies. Sixty-five to nearly 80% of these patients have homozygous inheritance based upon either hemoglobin F levels or genetic studies (Rund et al. 1997; Nadkarni et al. 2001). The mild clinical expression in thalassemia intermedia is related to a lesser imbalance between β and α globin chain synthesis as compared to patients with thalassemia major. This may result from (a) inheritance of mild beta thalassemia mutations (b) co-inheritance of alpha thalassemia (Weatherall et al. 2001; Kulozik et al. 1987) (c) genetic interactions which may result in increased synthesis of fetal haemoglobin (Cappillini et al. 1981) and association of *Xmn*I polymorphism in $^{G}\gamma$ region (Nadkarni et al. 2001).

This study presents the clinico-hematological profile in 93 cases with thalassemia intermedia which happens to be the largest study amongst Indian patients

MATERIALS AND METHODS

Patients with thalassemia presenting to the haematology clinic of department of Haema-

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tology at AIIMS from year 1989 to 2001 were included in the present study. All these patients were referred for evaluation of anemia. Clinical criteria like age at onset of disease, sex, presence of pallor, jaundice, haemolytic facies, failure to thrive and/or failure to gain weight and requirement of blood transfusions were recorded. History regarding caste, origin, consanguinity was also taken. Physical examination included various anthropometric measurements such as height, weight, haemolytic facies, jaundice, presence of lymphnodes, liver and spleen size were recorded. All other possible causes of anemia and/or splenomegaly like iron, B12, folic acid deficiency and other genetic disorders like hereditary spherocytosis, G6PD deficiency and other haemoglobinopathies were excluded. The diagnosis of thalassemia intermedia was based upon the clinical presentation, haematological investigations and family studies. Ninety-three patients of thalassemia intermedia were seen and 63 of them were followed up from 1 year to 14 years (mean 3.7) to study the clinical course of the disease. All these patients were managed with folic acid, vitamins and other supportive care. Blood transfusions were given only when their haemoglobin dropped significantly either due to stress of infection or any other causes. Twelve women who had 19 pregnancies were given blood transfusion to maintain haemoglobin levels between 8-10 g/dl and were monitored closely to study the outcome of pregnancy.

Investigations like haemoglobin levels, complete blood counts with peripheral blood smear examination for red cell morphology were done by standard methods (Dacie et al. 2001). Estimation of HbA2 and HbF was done by column chromatography and Singer's alkali denaturation method respectively (Dacie et al. 2001). Hb electrophoresis was performed in all the cases on agarose gel at pH 8.6. High performance liquid chromatography (HPLC, Bio-Rad Variant) was performed wherever required. These studies were also carried out in parents of these patients. Other studies for enzymopathies (G6PD, PK), paroxysmal nocturnal haemoglobinuria, hereditary spherocytosis and serum iron studies were undertaken to exclude other causes of anemia. Skiagram of chest and bones, abdominal ultrasound were done wherever indicated. Various biochemical tests such as serum calcium, serum phosphate, 24 hour urinary calcium and phosphate and thyroid function tests for T3,T4, TSH and growth hormone, tests for gonadal functions like FSH, LH, Prolactin, glucose tolerance test and ACTH stimulation tests were done in 27 cases to evaluate the presence of endocrine abnormalities. Nuclear studies were carried out in 15 cases for evaluation of hypersplenism.(International Committee for Standardization Haematology 1980).

RESULTS

There were 93 patients (60 male, 33 female) with mean age of presentation 13.5 years (range 6 months – 46 years). Majority of patients presented between 5-15 years of age. The haemoglobin levels in these 93 patients at presentation varied between 2.2-11.3 g/dl with mean of 7.4 g/dl. Five of 93 patients had Hb level below 5.0 g/dl. Such a low level of Hb was attributed to hypersplenism in 3 cases and superimposed infection and vitamin B12 or folic acid deficiency in one case each.

The peripheral blood smear examination showed microcytic and hypochromic red cells with mild to moderate degree of anisocytosis, poikilocytosis, few broken red cells and nucleated red cells. Based on the levels of HbF, HbA2, clinical features and family studies 76 of 93 (81.7%) patients were found to have β homozygous thalassemia. These patients had mean HbF of 49.6% (range18.3-98.5) and a mean HbA2 of 2.4% (range 1.1-8.4). However, 17 patients were characterized to be β heterozygous thalassemia, had high HbA2 (mean 4.2%), with a range of 3.6-7.4 (Table 1). HbF levels were low in these patients (mean 3.3%, range 0.2-9.8). The mean age at presentation was higher in heterozygous thalassemia (21 years) as compared to homozygous state (mean 11.5 years). Presence of jaundice was seen more frequently in heterozygous thalasemia (71%) as compared with homozygous (42%). Seventy-one (93%) patients (56 homozygous thalassemia and 15 patients of heterozygous thalassemia) showed enlargement of spleen (range 3-20 cms). Twenty–seven of them had a spleen size ≥ 6 cm. Hepatomegaly was seen in 86 of 93 (92.5%) patients (75.3% homozygous, 17.2% heterozygous). Ten of these patients (11.6%) had liver size ≥ 6 cm. There was no difference in haemoglobin levels between these two groups. Fifty-three of 93 (57%) patients were followed for progression of clinical features, while 27 cases who had clinical suspicion of any

Table 1: Thalassemia Intermedia: Results of 93 patients

	Age at presentation (Years)	*Hb (g/dl)*	*HbA_2 (%)*	*HbF (%)*
Homozygous Thalassemia (n=76)	11.5 (0.6-38)	7.4 (2.2-11.3)	2.4 (1.1-8.4)	49.6 (18.3-98.5)
Heterozygous Thalassemia (n=17)	21 (5-46)	7.5 (3.0-11.5)	4.2 (3.6-7.4)	3.3 (0.2-9.8)

endocrinopathy or above 14 years of age were investigated for presence of endocrinopathies. The mean follow up period was 3.7 years (range 1 year-14 years)

Majority of patients in both the groups required infrequent blood transfusions i.e. 1-2 unit/year. However, the increase in mean haemoglobin level (7.8 gm/dl, range 4.2-12.2) was observed during follow up with supportive treatment like folic acid and vitamin B_{12}. Four patients in both the groups required frequent blood transfusions either under the situation of stress or secondary to hypersplenism. Twelve of 15 suspected cases of hypersplenism (10 homozygous and 2 heterozygous) were confirmed on nuclear studies. Four of these 12 patients underwent splenectomy and had improvement in their haemoglobin levels. Two patients in homozygous group required frequent blood transfusions, one female patient during her pregnancy while another patient became transfusion dependent from 5½ years of age possibly secondary to hypersplenism. Two patients in heterozygous group also showed an increased requirement of blood at 20 and 38 years of age. Both these patients were found to have features of hypersplenism. Thirty-five of 93 (37.6%) patients in both groups (31 homozygous and 4 heterozygous) never required blood transfusion in their life. Serum ferritin levels were measured in 16 cases and it varied between 132 to 5600 ng/ml with mean of 1647 ng/ml.

Height and weight were recorded in all the cases using standard methods. In 62 children who were less than 19 years, height and weight were plotted on growth curves according to Tanner's classification(Tanner et al 1976). Twenty-six of 41 (63.4%) male and 12 of 21 (57.1%) female children had height less than 3rd percentile while weight was less than 3rd percentile in 31 of 41 (76%) male children and 17 of 21 (81%) female children. Only 6 of 62 (9.7%) children (5 male and one female) had both weight and height within normal range for their age. Four of these children had both weight and height above 50th percentile.

Twenty-five of 53 (47%) patients had progression of thalassemic faceis with prominence of maxillary and frontal bone during the study, though these children were able to maintain their haemoglobin between 7.4-7.5 g/dl with or without blood transfusions. Three patients were found to have osteoporotic changes on radiological examination. Pathological fractures were observed in eight children between 2-7 years of age affecting right and left tibia, right fibula, right femur, right radius and ulna, right clevicle, left foot and phalanges of left foot. Their haemoglobin levels ranged from 6 to 8 g/dl. Compression of T_{12} vertebrae was seen in one case. Gallstones secondary to chronic haemolysis were seen in two patients on abdominal ultrasound which was done on clinical suspicion.

Twelve of 33 (36.4%) female patients were in the reproductive age group and had a total of 19 pregnancies during the study. They were advised to maintain their haemoglobin between 9-10 g/dl by repeated blood transfusions. Ten of them had full term normal delivery and other 5 were premature deliveries. Of these five babies three were alive and two died during the neonatal period because of the prematurity. Four spontaneous abortions between 10-18 weeks of gestation were observed in three patients with their haemoglobin levels ranged between 6.3 to 8.7 g/dl. All these patients showed increased requirement of transfusion during their gestational period.

Twenty-seven of 93 patients were investigated for various endocrine abnormalities. Sixteen of 27 (59%) patients were found to have evidence for one or more endocrine abnormalities. Five of 27 (18.5%) patients had hypocalcemia while 3 of 27 (11.1%) patients had hypothyroidism. Growth hormone deficiency, hypopara-thyroidism, primary gonadal failure were observed in two cases each. Two patients were also found to have impaired glucose tolerance tests suggestive of diabetes mellitus.

Nineteen (18 homozygous and 1 heterozy-

gous) patients were investigated to determine the molecular defects by amplification refractory mutation system (ARMS) – PCR technique (Newten et al 1989) for five common mutations namely IVS1-nt-1(G-T), IVS1-nt-5 (G-C), 619 bp deletion, codon 8/9 (+G) and codon 41/42 (-CTTT). Parents of these patients were also investigated for these five mutations. Seven of 18 patients were true homozygous, showing same mutation on both the chromosomes [IVS1-nt-1 (G-T) in 6 and IVS1-nt-5(G-C) in 1 patient]. While 11 patients had compound heterozygous mutations. Seven of 12 patients also showed 158 (C-T) substitution in $^{G}\gamma$ promotor region for *Xmn*I polymorphism. Six of them were homozygous (+/+) , one was heterozygous while 5 patients were negative for this mutations.

DISCUSSION

Heterogenity of β thalassemia gene is well known. Over 200 mutations relating to beta thalassemia syndrome have been detected so far (Weatherall et al. 2001). Several mutations result in to absence or very little formation of β chains and are termed as major mutations resulting in to thalassemia major. Other mutations may result in mild phenotypic presentation. Several authors (Rund et al. 1997; Nadkarni et al. 2001; Weatherall et al. 2001;Aksoy et al. 1978; Thein et al. 1988). have tried to study the role of genetic factors in predicting the clinical severity in patients with β thalassemia. However, the exact pathogenesis of genetic interaction in thalassemia intermedia is still not well understood.

Twenty-eight different β thalassemia mutations have been identified among Indians so far(Colah et al. 1998). Molecular studies were performed in 19 patients and their families in the present study. Our patients have been investigated for only 5 common mutations and *Xmn*I polymorphism which is known to ameliorate the clinical severity if it is present. Six of 12 patients analyzed for 158 (C – T) substitution in $^{G}\gamma$ promoter region were homozygous (+/+) while one was heterozygous (+/–). Five patients were negative for *Xmn*I polymorphism. There was no difference in HbF levels between patients who were negative or positive for *XmnI* polymorphism. However, some authors have observed that presence of *Xmn*I polymorphism increases the HbF production and thereby being associated with less severe phenotype (Rund et al. 1997; Nadkarni et al. 2001; Efremov et al 1994). These observations are supported by two siblings in the present study who were homozygous for IVS1-nt-1 (G-T). Both the parents of these children were also positive for same mutation. However, one child 11 years old was homozygous for *Xmn*I polymorphism (+/+)and never received blood transfusion. Her brother 12 years old was negative for this polymorphism and required infrequent blood transfusion thereby suggesting that the presence of *Xmn*I polymorphism in homozygous state ameliorates the severity of the disease. The commonest mutation seen in our patient was IVS1-nt-1 (G-T) with a frequency of 44.7% followed by IVS1-nt-5 (G-C) (18.4%). Pattern of mutations in thalassemia intermedia are different in different regions of the world (Table 2) (Rund et al. 1997; Nadkarni et al. 2001; Madan et al. 1998; Garewal et al. 1994; Varawalla et al. 1991; Arora et al. 2001).One patient with heterozygous thalassemia showed 619 bp deletion in only one chromosome. This patient had required frequent blood transfusions. His mother was also tested for 5 common mutations and was found to be negative. However his father carried the same deletion in heterozygous form but was asymptomatic. Another patient who had 619 bp deletion and IVS1-nt-(GT) mutation was able to maintain his haemoglobin levels between 9.1-9.8 g/dl without any blood transfusion.

There are only few studies on clinical course, mutaions and on long term outcome in patients with thalassemia intermedia (Rund et al. 1997; Nadkarni et al. 2001; Weatherall et al. 2001). Seventy-six of 93 (81.7%) patients in the present study were homozygous with Hb F levels ranging from 18.3 to 98.5% (mean of 49.6). They presented with mild clinical course. Forty-three of 76 (56.6%) patients required infrequent transfusions. However, two patients in the same group became transfusion dependent one at the age of 5½ years because of hypersplenism and another lady at 27 years required 20 units of blood transfusions during her pregnancy. Thirty-one of 76 patients aged between 6 months to 31 years never required blood transfusion and were given only supportive treatment in the form of vitamin B12 and folic acid. Hb levels in these patients were in the range of 5.0-11.1 g/dl (mean 9.5) with high HbF levels (> 40%). High HbF levels are known to ameliorate the severity of disease and therefore, these patients may present later in life. Contrary to

Table 2: Frequency (%) of mutations in β thalassemia patients

	Data from various studies						
Mutations	*1*	*2*	*3*	*4*	*5a*	*5b*	*6*
IVS1-5(G-C)	22.8	23.0	38.3	28.5	60	52.1	18.4
619 bp del	34.8	17.0	19.2	27.5	10	14.6	2
FS8/9(+G)	13.0	12.0	16.4	12.3	5	6.3	3
IVS 1-1(G-T)	19.6	23.0	10	16.0	8.75	8.4	44.7
FS 41/42 (-CTTT)	9.8	12.0	9.7	7.9	7.5	4.1	1
CD15 (G-A)	-	-	2.3	-	2.5	4.1	-
CD30 (G-C)	-	-	0.9	0.6	2.5	0	-
CD16(-C)	-	0.7	0.6	1.7	0	2.1	-
Cap site +1 (A-C)	-	6.0	0.3	0.4	1.25	2.1	-
Others	-	6.0	-	0.4	0	4.1	-
Uncharacterized studies	-	0.7	2	-	-	-	-
IVS 2 position 1(G-A)	-	-	0.3	-	-	-	-
-88CT	-	-	0.2	-	0	2.1	-
Total Chromosomes	92	156	1404	916	80	50	38

1. Madan et al. 1998
2. Garewal et al. 1994
3. Varawala et al. 1991
4. Arora et al. 2001
5. Nadkarni et al. 2001 (5a. Thalassemia intermedia, 5b. Thalassemia major)
6. Present study

this some authors feel that increased level of HbF could be due to stress of anemia and high HbF levels may not be true indicator of mild disease (Rund et al. 1997). On the other hand Tamagnini et al. (1983) has described 14 cases of homozygous thalassemia with low HbF levels (< 20%) who presented with mild clinical course. Thus several authors have concluded that HbF levels does not correlate with Hb levels (Thein et al. 1988; Efremov et al. 1994). Seventeen of 93 (18.3%) patients with heterozygous thalassemia required occasional blood transfusion except two patients who received multiple transfusions secondary to hypersplenism. All these patients had high HbA2 (>3.6%) levels with mean HbF of 3.3%.

Five of 93 patients presented with haemoglobin levels less than 5 g/dl. Low haemoglobin in 3 of them was secondary to hypersplenism while in one patient each, it was secondary to superimposed infection and vitamin B12 or folic acid deficiency. Our observation support the previous studies (Weatherall et al. 2001; Tamagnini et al. 1983). Three of these patients were homozygous as HbF levels varied from 54.2-90%. While two patients were heterozygous with HbA2 levels 3.6% and 4.2%. Thus children with very low hemoglobin levels needs to be differentiated from thalassemia major. The study of clinical course, investigations for other contributory factors along with the identification of mutational defects become essential to differentiate between thalassemia intermedia and major.

Anthropometric measurements such as height and weight were plotted according to

Table 3: Pregnancy outcome (%) in β thalassemia patients

	1a	*1b*	*2*	*3a*	*3b*	*4*
FTND	73.9	85.2	80	41.3	72.7	52.6
Preterm	13.0	11.1	-	24.1	13.6	26.3
Abortions	8.7	3.7	20	20.6	9.1	20.1
Still birth	4.3	0	-	6.9	4.5	-
Neonatal death	-	-	-	6.9	0	10.5
Hb levels (g/dl)	>10	-	>10	<7.3	>10	-
Total no. of pregnancies	46	27	25	29	22	19

1 Scordis et al. 1998 (1a. Thalassemia major, 1b. Thalassemia intermedia)
2. Susan et al. 1998
3. Afifi et al. 1974 (3a. Hb <7.3 g/dl, 3b. Hb >10 g/dl)
4. Present study

Table 4: Prevalence (%) of endocrinopathies in β thalassemia patients

	Data from various studies					
	1	*2*	*3*	*4*	*5*	*6*
Delayed/failure puberty	50	-	-	-	36	-
Primary amenorrhoea	-	-	-	-	6.9	-
Secondary amenorrhoea	23	12.5	50	-	13.8	-
Hypogonadism/gondal failure	1.6	42	30	90.9	4.0	7.4
Diabetes mellitus / impaired GTT	4.9	32	18.8/24.7	7.9	24	7.4
Growth hormone deficiency	-	3.0	-	51.0	-	7.4
Hypothyroidism	6.2	4.0	9	2.5	5.7	11.1
Hypoparathyroidism	3.6	4.0	5	-	-	7.4
Short stature < 3rd percentile	-	32	34	*56	7**	61.3
Total number of patients	1861	226	117	84	50	93

1. Italian working group 1995
2. Kattamis et al. 1995
3. De Sanctis et al. 1996
4. Gulati et al. 2000
5. De Sanctis et al. 1998
6. Present study

* <5th percentile in >8 years
** female patients

Tanner's classification. Thirty-eight of 62 (61.3%) children had their height less than 3rd percentile which was higher as compared with studies even on patients with thalassemia major (Table 4) (De sanctis et al. 1996; Kattamis et al. 1995; Gulati et al. (2000). These children with thalassemia major received regular blood transfusions and haemoglobin levels were maintained above 10 g/dl. Gulati and his colleagues, 2000 have observed 54% of children above 8 years of age with thalassemia major had their height below 5th percentile. The Hb levels were maintained >10 g/dl but they were poorly chelated because of economic constraints. No significant difference was observed between the percentage of children in male and female group who had their weight and height less than 3rd percentile. Thus the retardation of anthropometric parameters depend on the haemoglobin levels rather than genetic mutations for thalassemia major or intermedia. Skeletal deformities and bone diseases have been described earlier in thalassemia intermedia by different workers (Gratwick et al. 1978). They described bone and joint pains, osteopenia and presence of microfractures. In our series apart from bony pains osteoporotic changes, pathological fractures and vertebral compressions were observed in 3,8 and 1 patient respectively. Prevalence of fractures and skeletal abnormalities could be secondary to undernutrition which was evident as weight was less than 3rd percentile in 77.4% of children and/or erythroid hyperplasia with subsequent osteomalacia. Iron overload leading to parathyroid endocrinopathies appears less likely as the mean serum ferritin levels were below 2000 ng/ml.

Patients with thalassemia intermedia have normal puberty and may have normal sexual development (Scordis et al. 1998). In the present study 12 women had 19 pregnancy during which anemia became severe and required blood transfusions during the gestational period. Skordis and his colleagues 1998, did not observe the worsening of anemia and over 85% of their cases delivered term babies. Three of 27 babies were pre term. However, in our study only 10 of 19 pregnancies resulted in normal full term babies with high prevalence of preterm (26.3%) and abortions (20.1%) which was attributed to chronic anemia, thereby suggesting that it is essential to maintain haemoglobin levels more than 10 g/dl during pregnancy to decrease the reproductive wastage (Table 3) (Scordis et al. 1998; Susan et al. 1998; Savona et al. 1994; Afifi et al. 1974). Lialios et al. (2000) effectively treated a pregnant patient of thalassemia intermedia with recombinant human erythropoietin (rHuEpo) therapy in combination with iron and folic acid as an alternative to blood transfusion therapy. However, this combination therapy need to be evaluated in larger series.

Presence of various endocrine disorders in present study have been compared with other studies (De Sanctis et al. 1996; Kattamis et al. 1995; Gulati et al. 2000; De Sanctis et al. 1998; Italian Working Group Study 1995) in patients with thalassemia major and intermedia (Table 4).

Prevalence of various disorders such as diabetes mellitus, hypogonadism, hypothyroidism, and growth hormone deficiencies are variable, however, these disorders are infrequent in thalassemia intermedia.

It is quite likely that thalassemia interemedia is underdiagnosed in our country as there are over 35 million carrier of β thalassemia. (Choudhry et al. 2000). There is need to conduct larger studies to determine the prevalence of thalassemia intermedia and to determine the effective form of therapy which should not result in growth retardation, high prevalence of fractures, bone disorders and foetal losses etc. Blood transfusion and chelation therapy has its own risk of blood transmitted infections and toxicity of chelation therapy besides its high cost. Alternative form of therapy such as hydroxyurea either alone or in combination with erythropoietin or other agents which could ameliorate the course of disease by increasing the HbF and Hb levels need to be evaluated to determine the effective form of therapy (Kattamis et al. 2001).

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