

HETEROZYGOUS BETA THALASSEMIA IN PARENTS OF CHILDREN WITH BETA THALASSEMIA MAJOR

Imran-ud-din Khattak¹, Sania Tanweer Khattak¹, Jehanzeb Khan²

¹Saidu Medical College, Swat and ²Postgraduate Medical Institute, Lady Reading Hospital Peshawar, Pakistan

ABSTRACT

Background: Beta thalassemia is the most common single gene disorder causing a major genetic health problem in the world. Beta thalassemia is common in Pakistan. This study was conducted to find out the pattern of transmission of beta thalassemia in the affected families.

Material and Methods: One hundred families having children with beta thalassemia major were analyzed at Postgraduate Medical Institute, Lady Reading Hospital Peshawar, Pakistan, for detection of beta thalassaemia trait. Screening was performed in both maternal and paternal parents and grandparents.

Results: Four hundred and fifteen (69.2%) cases of heterozygous beta thalassaemia were detected in the survey of 100 families (600 subjects), having beta thalassaemia major children. Altered red cell morphology such as hypochromia, microcytosis and aniso-poikilocytosis were seen in all these cases. Haemoglobin A₂ level ranged 4.0-6.9%, MCV of less than 77 fl and MCH of less than 26.4 pg were found in all the subjects with beta thalassaemia trait. The frequency of consanguineous marriages in parents was first cousin 72%, second cousin 5%, distant cousin 4% and un-related 19%.

Conclusion: Consanguinity was found to be present in most of the parents of patients with beta thalassaemia major. This can be minimized by health education, nation-wide screening and provision of genetic counseling to the affected families.

Key words: Beta thalassemia, Parents, Consanguinity.

INTRODUCTION

Thalassemias are a heterogeneous group of genetic heritable disorders of haemoglobin synthesis.¹ Beta thalassemia probably is the most common single gene disorder causing a major genetic health problem in the world.² Pakistan lies in the thalassemia belt and b-thalassemia is common here.³ If an average of 5.4% is taken as the national carrier rate,³⁻⁴ there would be approximately 5-6 million carriers in Pakistan. The average cost of treating one thalassaemic patient is Pakistani Rupees 10,000 per annum in addition to blood requirements which is not affordable with our limited resources.⁵ An alternative long term approach would be to reduce the number of these patients through parental screening and genetic counseling.⁶ Identification of b-thalassaemia carriers and provision of prenatal diagnosis of homozygous conception using oligonucleotide probes and restriction enzyme analysis,^{7,8} followed by termination of pregnancy will allow couples at risk to avoid having children with b-thalassemia.^{9,10}

Presence of hereditary haemoglobin disorders in Pakistan is well known, though the data is not so extensive.¹⁰

This study was conducted to find out the overall pattern of transmission of the disease in the affected families.

MATERIAL AND METHODS

A total of 100 families of known thalassemia major children were selected whose parents and grand parents (both maternal and paternal) were alive. They belonged to district Peshawar. All were thoroughly interviewed and clinically examined for jaundice, hepatosplenomegaly and lymphadenopathy. The relevant information regarding age, sex, ethnic origin (caste /tribe) and place of birth / inhabitation, family history and consanguinity were recorded. 7.0 ml of venous blood was collected from each of the subjects and distributed in the following manner:

- i. 2.0 ml blood was mixed with potassium EDTA to a final concentration of 1.5 mg/ml and used for blood counts, red cell indices and reticulocyte count.
- ii. 2.0 ml blood was mixed with potassium EDTA to a final concentration of 1.5 mg/ml and used for preparation of haemolysate for

haemoglobin electrophoresis and estimation of HbA₂ and HbF.

- iii. 3.0 ml blood was transferred into a sterile glass test tube and allowed to clot. Clear serum was separated into a sterile cryotube and preserved at -20°C for estimation of serum ferritin.

Complete blood count (CBC) and red cell indices were measured on Micros Cobas-16 Haematology auto-analyzer within two hour of sample collection. The equipment was calibrated daily by using low normal and high commercial controls obtained from Cobas. Quality control was performed by running the same normal specimen after every 25 test specimens. Estimation of HbA₂ was done spectrophotometrically after elution of the haemoglobin bands separated on cellulose acetate strips in Tris/EDTA/borate buffer at pH 8.6.¹¹ Haemoglobin F was measured by modified Betk method⁴ based on its resistance to denaturation at alkaline pH. Serum ferritin was estimated by ELISA method in all the cases having microcytic hypochromic blood picture with low MCV (< 77 fl). Reticulocyte count was performed by brilliant cresyl blue supra-vital staining. The criteria used for the diagnosis of b-thalassaemia trait were Haemoglobin A₂ level of ³ 4.0% supported by MCV < 77fl, MCH < 26 pg, altered red cell morphology and normal or raised serum ferritin level.

RESULTS

A total of 415 cases (69.2%) of heterozygous beta thalassaemia were detected in survey of 100 families of the known beta thalassaemia major children. Ninety six families (96%) out of 100 were pathans and only 4 families belonged to miscellaneous group. Three hundred and ninety nine (96.1%) out of 415 subjects with heterozygous beta thalassaemia were pathans and 16 (3.9%) belonged to miscellaneous group. (Table-1)

Altered red cell morphology such as hypochromia, microcytosis and aniso-poikilocytosis were seen in all the cases. Haemoglobin A₂ level ranged 4.0-6.9%, MCV of less than 77 fl and MCH

of 26.4 pg or less in all subjects of beta thalassaemia trait. (Table-2)

The frequency of consanguineous marriages in parents were first cousin 72%, second cousin 5%, distant cousin 4% and not relative 19%. (Table 3)

DISCUSSION

The occurrence of hereditary haemoglobin disorders in Pakistan has been known for a long time although the data is limited.¹² The actual magnitude of these hereditary disorders in Pakistan has been masked by infections and nutritional deficiencies. If these acquired diseases are reduced successfully, hereditary disorders including haemoglobinopathies would become important national problem.¹³ The prevalence of \hat{a} -thalassaemia carrier state found in the relatives of beta thalassaemia major children is more than 50%.¹⁴ The latest study performed on the siblings of beta thalassaemia major children showed that the incidence of beta thalassaemia trait among the siblings was 58% with a male to female ratio of 0.9:1.¹⁵ Consanguineous marriages are quite common in Pakistan, especially in pathans. This social practice may have compounded the problems.¹⁶ All the beta thalassaemia heterozygotes showed some degree of hypochromia with MCH ranging between 15.4-26.4 pg (median 20.33). Similarly 100% (415/415) of the carriers had MCV value of less than 77 fl. This finding is in concurrence with early study at Armed Forces Institute of Pathology and with the major work done earlier by Italians where MCV value of 77 fl or less was used as the main parameter for preliminary screening for thalassaemia trait.¹⁷ (Table-4) The estimation of MCV and MCH by electronic haematology analyzer appears to be a cost effective method of screening for beta thalassaemia heterozygotes.

Haemoglobin A₂ level of 4% or more appears to be highly significant level for the diagnosis of heterozygous beta thalassaemia in the present study. This was supported by low MCV, low MCH and abnormal red cell morphology.

Table-1: Distribution pattern of heterozygous beta thalassaemia in population groups.

Ethnic Group	Number of subjects	Families	Percentage
Pathans	399	96	96.1%
Miscellaneous	16	4	3.9%
Total	415	100	100%

Table-2: Haematological data in heterozygous beta thalassemia and healthy subjects.

Haematological Parameter	Beta Thalassemia minor (n = 415)	Healthy Population (n = 185)	p-value
Hb (g/dl)	12.08 ± 1.34 (6.1 – 15.5)	14.5 ± 0.73 (12.5 – 15.5)	HS
TRBC (x 10 ¹² /L)	5.94 ± 0.64 (3.95 – 7.39)	5.01 ± 0.50 (4.05 – 6.65)	HS
PCV (L/L)	0.358 ± 4.21 (0.211 – 0.476)	0.400 ± 0.023 (0.370 – 0.460)	HS
MCV (fl)	61.73 ± 4.55 (48.3 – 75.6)	80.2 ± 6.50 (78 – 91)	HS
MCH (pg)	20.33 ± 1.76 (15.4 – 26.4)	29.2 ± 2.101 (27.2 – 29.9)	HS
MCHC (g/dl)	33.12 ± 1.45 (27.7 – 38.0)	33.5 ± 1.5 (30.7 – 36.2)	S
HbF (%)	0.89 ± 0.15 (0.6 – 1.4)	0.9 ± 0.22 (0.6 – 1.3)	NS
HbA ₂ (%)	5.36 ± 0.57 (4.0 – 6.9)	3.1 ± 0.27 (2.5 – 3.4)	HS
Reticulocytes (%)	1.6 ± 0.45 (0.8 – 4.0)	1.8 ± 0.66 (0.8 – 2.5)	S
Serum ferritin (ng/ml)	43.0 ± 18.5 (2 – 104)	74.0 ± 30.5 (6 – 136)	HS

Key: HS = Highly significant (P < 0.001), S = Significant (P < 0.05), NS= Non-significant (P > 0.05).
n = Number of subjects. Figures in parentheses show the range of values.

Table-3: Frequency of consanguineous marriages in 100 families.

Relationship	No. of families	Percentage
First cousin marriage	72	72%
Second cousin marriage	5	5%
Distant cousin marriage	4	4%
Not relative marriage	19	19%
Total	100	100%

Cousin marriages were found to be present in 77% of the parents of subjects in this study. This can be minimized by nation-wide screening, health education and establishing special care centers where facilities for diagnosis and genetic counseling are available.

CONCLUSION

Consanguinity was found to be present in most of the parents of patients with beta thalassemia major. This can be minimized by health education, nation-wide screening and

Table-4: Haematological data in heterozygous beta thalassaemia patients in different studies.

Study	Race	Sex	No	Hb (g/dl)	RBC ($10^{12}/L$)	MCH (pg)	MCV (fl)
Weatherall and Clegg (1981)	British	M	32	11.8 + 1.5 (8.7-14.7)	5.6 + 0.6 (4.6-6.6)	21.5 + 1.3 (18.6-25.6)	70.5 + 4.2 (63.1-77.1)
		F	51	10.8 + 0.9 (8.4-12.5)	5.1 + 0.5 (4.3-6.7)	21.8 + 1.4 (18.8-25.1)	70.3 + 4.8 (63.0-82.1)
Dincol et al (1979)	Turkish	M	64	11.6 + 1.5 (7.8-1.7)	5.2 + 0.7 (3.9-6.7)	22 + 2 (18-25)	74 + 5 (66-82)
		F	81	10.3 + 1.2 (7.8-13.0)	4.7 + 0.6 (3.8-6.3)	21 + 2 (18-24)	74 + 4 (66-81)
Galanello et al (1979)	Sardinian	M	43	13.3 + 0.8 (11.4-15.3)	6.1 + 0.5 (5.0-7.4)	22 + 2 (14-25)	66 + 4 (59-85)
		F	107	11.8 + 0.9 (9.1-14.0)	5.4 + 0.4 (4.0-6.7)	22 + 2 (18-26)	66 + 4 (55-78)
Khattak and Saleem (1992)	Pakistani	M	14	13.6 + 1.04 (11.6-15.1)	6.7 + 0.67 (5.1-7.58)	20.3 + 1.35 (17.9-22.7)	63.4 + 5.1 (58-78)
		F	13	11.6 + 1.56 (9.1-13.8)	5.24 + 0.75 (4.15-6.64)	22.35 + 2.23 (18.6-25.3)	71.77 + 6.46 (62-82)
Anjum (1999)	Pakistani	M	26	9.54 + 1.46 (5.7-13.4)	4.77 + 0.98 (2.5-7.5)	21.15 + 4.67 (10.1-31.0)	90.75 + 11.25 (51-120)
		F	32	9.98 + 1.54 (4.2-13.9)	4.82 + 0.85 (3.0-6.6)	21.78 + 4.68 (18.4-26.0)	87.35 + 13.5 (51-125)
Present study (2000)	Pakistani	M	210	13.02 + 1.22 (9.12-15.5)	6.3 + 0.66 (4.01-7.39)	20.5 + 1.57 (17.1-25.0)	62.1 + 4.43 (51.0-71.6)
		F	205	11.07 + 0.78 (6.1-13.6)	5.63 + 0.62 (3.95-6.75)	20.16 + 1.76 (15.4-26.4)	61.35 + 4.45 (48.3-75.6)

provision of genetic counseling to the affected families.

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Address for Correspondence:

Dr. Imran-ud-din Khattak
Assistant Professor Pathology
Saidu Medical College
Swat, NWFP, Pakistan
Cell: 03018885001