

Premarital genetic screening for beta thalassemia carrier status of indexed families using HbA2 electrophoresis

Aneeqa Nosheen,¹ Inamullah,² Habib Ahmad,³ Iftikhar Qayum,⁴ Noaman Siddiqui,⁵ Fida Muhammad Abbasi,⁶ Aziz ud Din,⁷ Muhammad Sajjad Iqbal⁸

Abstract

Objectives: To devise a strategy for prevention of beta thalassemia in newborns through reliable screening of indexed families.

Methods: The cross-sectional study was conducted over six months in 2011 and comprised blood samples collected from subjects belonging to different ethnic groups from families of beta thalassemia major children registered with the Abbotonion Medical Association Blood Care Centre, Abbottabad, in Pakistan's Khyber Pakhtunkhwa province. Electrophoretic separation of human haemoglobin like A, F, S and C was done and then haemoglobin in the gel was immobilised in a fixative solution and the gel was dried to a film. Haemoglobin pattern was visualised by staining the film with a protein-specific stain. The pattern was quantified by densitometry.

Results: Of the 98 samples, 57(58.2%) had β -thalassemia trait with elevated haemoglobin alpha 2 level, and 41(41.8%) had normal level. Out of the 57 carriers, 33(57.89%) were males and 24(42.10%) were females. Mean age of carriers was 11.65 ± 6.25 years compared to 10.93 ± 7.75 in normal patients. Mean haemoglobin alpha 2 level of carriers was $5.2 \pm 0.56\%$ compared to $2.34 \pm 0.57\%$ in normal subjects.

Conclusion: Carrying out mass screening programmes throughout Pakistan for the detection of thalassemia carriers and providing them the benefit of marriage counselling may decrease the incidence of thalassemia Major.

Keywords: Thalassemia, Newborns, Indexed families. (JPMA 65: 1047; 2015)

Introduction

Thalassemia is one of the major monogenetic single-gene disorders globally and it was the first disease studied by using techniques of molecular biology.^{1,2} The term thalassemia comes from the Greek word "thalas" which means the sea, and "emia" that stands for blood.^{3,4} The composite word came into use because this type of anaemia was originally described only in countries bordering the Mediterranean Sea. Thalassemia was not recognised as a clinical entity until 1925, when a syndrome was described as occurring early in life that was associated with splenomegaly and bony deformities.⁴ There are approximately 240 million people worldwide who are heterozygous for β -thalassemia and approximately 200,000 affected homozygotes are born annually.¹ They are mainly distributed in the regions previously endemic for malaria, including Mediterranean, Middle East, and parts of Africa, India, Southeast Asia and southern China.⁵ Indian subcontinent and southeast Asia have the highest prevalence of β -thalassemia and comprise the so-called thalassemia belt.^{1,2}

β -thalassemia is the most common genetic disorder in Pakistan.⁶ Pakistan has a population of approximately 180 million people and β -thalassemia has an overall carrier frequency of more than 5% in Pakistan and there are approximately nine million carriers of β -thalassemia in the country.⁷ Approximately 40,000 cases of transfusion-dependent children with thalassemia major are presently registered and each year nearly 5000 affected children are born nationwide.

Like many other developing Asian countries, β -thalassemia poses an increasing burden for healthcare services in Pakistan and it is not possible to provide blood transfusion and iron chelation therapy to all patients with limited available national resources. Bone marrow transplantation is extremely expensive and unaffordable for most Pakistani patients. In countries like Pakistan, prevention is the least expensive and most effective means to deal with β -thalassemia. The best chance of preventing thalassemia major from occurring is to detect the carriers at a pre-marital stage and prevent them from getting married by giving them proper marriage counselling. It is thus worthwhile to carry out screening programmes for detection of thalassemia carriers and to provide them the benefit of marriage counselling to decrease the incidence of fresh thalassemia major cases. There have previously been few studies on β -thalassemia

.....
^{1-3,6-8}Department of Genetics, Hazara University, Mansehra, ⁴Rehman Medical College, Khyber Pakhtunkhwa, Peshawar, ⁵Pathology Department, Ayub Medical College Abbottabad, Pakistan.

Correspondence: Inamullah. Email: drinamullah34@gmail.com

mutations in various regions and ethnic groups of Pakistan. The current study was planned to investigate the β -thalassemia carrier status in pre-marital couples and siblings of thalassemia major patients of Hazara region of Pakistan for pre-marital counselling.

Subjects and Methods

The cross-sectional study was conducted over six months in 2011 and comprised blood samples collected from subjects belonging to different ethnic groups from families of beta thalassemia major children registered with the Abbottonian Medical Association Blood Care Centre, Abbottabad, in Pakistan's Khyber Pakhtunkhwa province. The samples were collected using ethylenediaminetetraacetic acid (EDTA) tubes from both genders of pre-marital age group. Informed consent was obtained from all the subjects before filling out the questionnaire.

Paragon Haemoglobin (Hb) electrophoresis kit was used for the electrophoretic separation of Hb to screen for clinically important Hb variants. The kit was used in conjunction with the Paragon Acid Hb (Acid Hb) electrophoresis kit to confirm the identification of Hb variants. Alkaline Hb electrophoresis on agarose gel was used as a screening procedure for Hb A, F, S and C. Electrophoresis is based upon the fact that Hb, when placed in an electrical field, will migrate towards one of the electrodes. After electrophoresis, the Hb in the gel was immobilised in a fixative solution and the gel was dried to a film. The Hb pattern was visualised by staining the film with a protein-specific stain. The pattern was either visually interpreted or quantified by densitometry. The specimen of choice for the routine investigation of Hb is a red blood cell haemolysate. That is why blood samples should be collected in the EDTA, sodium oxalate or

heparin anticoagulants.

Data was stored and analysed using SPSS 15. Mean + standard deviation was calculated for age and haemoglobin alpha 2 level (HbA2) levels of all the subjects.

Results

Of the 98 subjects, 57(58.2%) were male and 41(41.8%) were female. Mean age of males was 10.85 ± 6.83 years and that of females was 12.04 ± 7.02 years (overall range: 0.5-25 years). Mean HbA2 of males was 3.98 ± 1.56 and of females it was 4.02 ± 1.48 . Overall, 57(58.2%) had β -thalassemia trait with elevated HbA2 level, and 41(41.8%) had normal level. Patients with consanguineous parents were 55(56.1%) with mean age of 11.49 ± 7.66 years and those having marriage with distant relatives were 29(29.6%) with mean age of 11.76 ± 5.2 years. The mean HbA2% of consanguineous marriage was 3.83 ± 1.60 and for distant relatives 4.41 ± 1.39 . The mean age of carriers was 11.65 ± 6.25 years and of normal patients it was 10.93 ± 7.75 years. The mean for HbA2% of carriers was 5.2 ± 0.56 and of normal subjects it was 2.34 ± 0.57 .

Out of the 57 carriers, 33(57.89%) were males and 24(42.10%) were females.

The males and females were categorised in three groups according to their age groups i.e. 1-10 years, 11-20 years, and 21-25 years. Of the 25 subjects with carrier status from 1 to 10 years of age, 15(60%) were males with mean HbA2% of 5.12 ± 0.57 and 10 females with mean HbA2% of 5.02 ± 0.48 . Of the 25 carriers from 11 to 20 years of age, 14(56%) were males with mean HbA2% of 5.35 ± 0.56 and 11 were females with mean HbA2% of 5.32 ± 0.58 . Of the 7 carriers from 21 to 25 years of age, 4(57%) were males with mean HbA2% of 5.05 ± 0.79 and 3(43%) were females

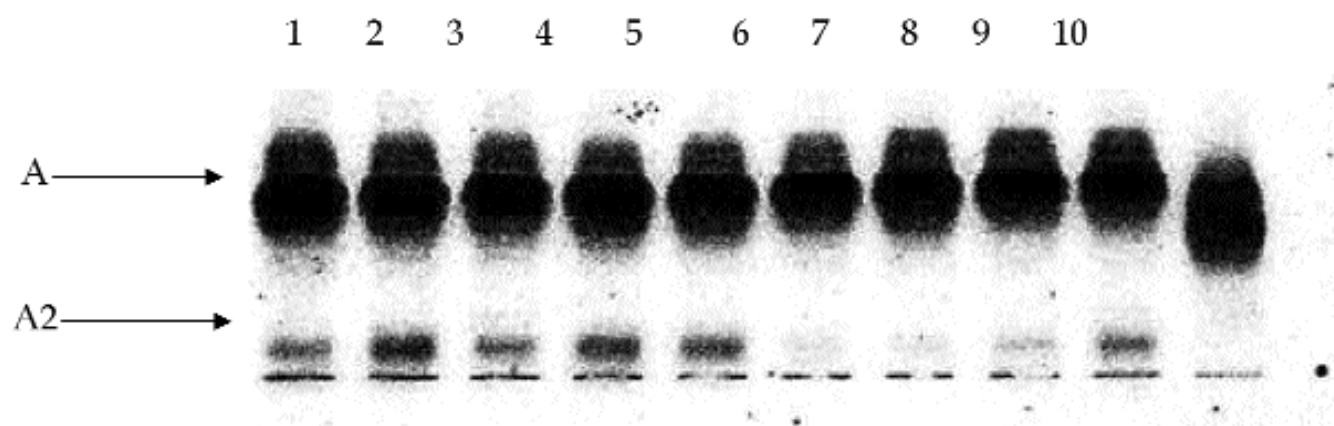


Figure: Lane 1 is positive control and 10 is negative control; 2, 3, 4, 5 and 9 are showing bands for raised haemoglobin alpha 2 (HbA2) level; 6, 7 and 8 are normal.

with mean HbA2% of 5.20 ± 0.62 .

Discussion

The overall occurrence of beta-thalassemia trait (BTT, carrier frequency) in the indexed families was in the range of 58.2% with elevated HbA2 level. Furthermore, in males the carrier frequency was 57.89%, and in females it was 42.10%. It was concluded that the carrier status of thalassemia among those families having thalassemia major children was very high.

Implementation of mandatory national premarital screening programme, and screening young and unmarried women for detection of carriers have strikingly reduced the incidence of infants born with thalassemia major in several countries worldwide.⁸ For example in Cyprus, Italy, USA, and recently UK and other parts of Europe and Africa, proper implementation of measures regarding thalassemia caused a marked reduction in the incidence and prevalence of the disease. A notable example is Cyprus where the incidence has dropped by 96%. Also premarital screening to identify carrier couples and subsequently provision of counselling in Iran has resulted in a 70% reduction in the annual birth rate of affected infants and a large amount of medical expenses.^{9,10}

In Pakistan, where these measures are still a long way off, there seems little choice but to embark on a research-oriented strategy, aimed at persuading and educating parents and other family members to allow screening for carriers as part of subsidised research projects so that the cost of screening tests may become affordable for poor

patients as well.

Conclusion

Guided marriage counselling that is scientifically backed by authentic screening and supplemented by molecular genetic diagnosis is recommended for the prevention of thalassemia.

References

1. Cao A, Saba L, Galanello R, Rosatelli M. Molecular diagnosis and carrier screening for B-thalassemia. *J Am Med Assoc* 2007; 278: 1273-7.
2. Weatherall DJ, Clegg JB. The β -thalassemia syndromes. 4th ed. Oxford: Blackwell Scientific Publication; 2001, 635-87.
3. Weatherall DJ, Clegg JB. The biology of the thalassemias. The thalassemia syndromes. 4th ed. Oxford: Blackwell Scientific Publication; 2001, 121-284.
4. Weatherall DJ. Molecular biology at the bedside. *Br Med J* 1986; 292: 1505-8.
5. Weatherall DJ. Phenotype-genotype relationships in monogenic disease. *Nat Rev Genet* 2001; 2: 245-55.
6. Colah R, Nadkarni A, Gorakshakar A, Phanasgaonkar S, Surve R, Subramaniam PG, et al. Impact of beta globin gene mutations on the clinical phenotype of beta thalassemia in India. *Blood Cells Mol* 2004; 33: 153-7.
7. Hafeez M, Aslam M, Ali A, Rashid Y, Jafri H. Regional and ethnic distribution of Beta thalassemia mutations and effect of consanguinity in patients referred for prenatal diagnosis. *J Coll Physicians Surg Pak* 2007; 17: 144-7.
8. Zlotogora J. Population programs for the detection of couples at risk for severe monogenic genetic diseases. *Hum Genet* 2009; 126: 247-53.
9. Christianson A, Streetly A, Darr A. Lessons from thalassaemia screening in Iran. *BMJ* 2004; 329: 1115-7.
10. Karimi M, Jamalian N, Yarmohammadi H, Askarnejad A, Afrasiabi A, Hashemi A. Premarital screening for beta-thalassaemia in Southern Iran: options for improving the programme. *J Med Screen* 2007; 14: 62-6.