ORIGINAL ARTICLE

Prevalence of Beta Thalassemia and Other Hemoglobinopathies - Carriers Status of Students in Educational Institutes of Central Punjab

SHABNAM BASHIR[1], YASMEEN EHSAN[2], HUSSAIN JAFRI[3]
[1]Project Director, [2]Project Coordinator, [3]Deputy Project Director, Punjab Thalassemia Prevention Program, F. J.Medical University, Lahore, Pakistan.
***Corresponding author**: Dr. Shabnam Bashir, Project Director, Punjab Thalassemia Prevention Program, F. J. Medical University, Lahore. E.mail:docshabnam2000@yahoo.com*

ABSTRACT
Background: Hemoglobinopathies are a group of genetic disorders characterized by abnormalities in qualitative or quantitative production of haemoglobin (Hb). Beta thalassemia is the most common inherited blood disorder found in Pakistan, other less common are HbE, HbD-Punjab and HbS. This study is part of awareness campaigns carried out among school and college students to educate them regarding these diseases especially beta thalassemia, its inheritance pattern and various prevention tools available.
Subjects and Methods: Awareness about the disease, its complications, treatment and prevention were given to the students. Free, voluntary carrier screening was done by collecting 3 ml of EDTA blood for complete blood count (CBC) and haemoglobin electrophoresis/HPLC. Genetic counselling and extended family screening was offered to students at risk.
Results: A total of 3057 students were screened during the study period. About 77 were found to be carriers of beta thalassemia, 12 of HbD, 7 of HbE, and 1 was compound heterozygous for HbE and beta thalassemia. The collective prevalence of carriers was 3.1%.
Conclusion: Awareness seminars help to provide information about the disease and its prevention. The collected data about the prevalence and distribution is helpful in devising prevention strategies to help affected individual to make informed choices.

Keywords: Beta thalassemia trait, hemoglobinopathies, voluntary carrier, screening

INTRODUCTION
Hemoglobinopathies and globin gene disorders are the autosomal recessive, single gene disorders characterized by abnormalities in qualitative or quantitative production of haemoglobin and about 1700 mutations are recognized so far.[1] Approximately 360 million people are carriers of clinically significant hemoglobinopathy gene and constitute about 5.2% of the world population. Among these, about 100 million are carriers of beta thalassemia with a carrier frequency of 1.5%. Each year about 0.3 million infants are born all over the world with a major hemoglobinopathy.[2] Beta thalassemia is the most common hemoglobinopathy found in Pakistan along with HbS, HbE and HbD-Punjab with a carrier frequency of approximately 5%.[3] Carrier screening is defined as a type of medical investigation to detect whether or not carrier status for a recessive disorder is present in a couple or a person. They may have not a priori risk of being a carrier based on their or their parents personal or family disease history.[4] The carriers or heterozygous of these disorders are symptom free. At risk couples (where both partners are carriers) have a 25% chance of having a child with beta thalassemia major in each pregnancy.[5] Awareness with premarital screening and genetic counselling is the main primary prevention strategy used in thalassemia endemic countries for reducing the burden of this deadly disease.[6] Screening for genetic disorders, like thalassemia, aims to reduce the burden of these disorders by identifying those who are at increased risk. This also enables them to receive information about their personal health and potential health of their offspring. Detection of asymptomatic carriers by reliable laboratory methods remains the corner stone of prevention of thalassemia. Some of the haematological parameters on CBC are used to identify these carriers and this is helpful in designing carrier screening programs. Measurements of MCV< 80fl and MCH < 26pg are helpful in identifying potential beta thalassemia

carriers and confirmed by HbA2 estimation.[7] The aim of study is to study prevalence of carrier state among the school and college students and create awareness about the disease. This will facilitate reproductive decision making in future parents.

SUBJECTS AND METHODS

Screening of students was carried out from 2012 till 2017. Steps followed were: A preliminary meeting was arranged with the head of the institution and utility and objectives of the activity were highlighted. Detailed information about disease, its prevention and methodology adopted was discussed.[8] An awareness seminar was then arranged in the institution to provide detailed information to teachers and students. A presentation about the disease was given with genetic information of recessive inheritance, sign and symptoms of the disease with its possible complications and the available treatment options. A review of successful prevention programs all over the world was presented with special emphasis on significance of voluntary and mandatory carrier screening. The implication for being a carrier and the opportunity for the students to take up free services of carrier screening were discussed. Printed awareness material was also distributed to all attending participants. Voluntary carrier screening was offered to all students. The written consent of students above at the age of 18 years and of the parents of students below 18 years age was requested before sampling. All results obtained were sealed individually and sent back to focal representative of the institution. In case of positive carrier screen, the individual was directed to seek free services of genetic counselling from project genetic counsellors. The families of the carrier students were offered the services of extended carrier screening after genetic counselling at their doorstep. Three ml of EDTA blood sample was collected in vial and analysed sequentially. The haematology analyser (Sysmex) was used to determine peripheral cell count and red blood cell indices using standard procedure. The haematological parameters include red cell indices and absolute values followed by separation and measurement of Hb fractions by high performance liquid chromatography (HPLC)/ electrophoresis for the identification of thalassemia carriers.[9,10] Beta thalassemia carriers present with microcytic, hypochromic blood picture. The cut-off values of MCV and MCH used for indicating thalassemia were 76fl and 26pg respectively.[9,10] Family history of thalassemia, transfusion history in family, consanguineous marriage of parents and ethnicity were also documented as they provide useful information in approaching the laboratory diagnosis of beta thalassemia. Quantitative estimation of HbA2 was used for beta thalassemia carrier detection. The screening methods employed were cation exchange HPLC and capillary zone electrophoresis (CZE). The expected normal range for HbA2 being 2.4-3.5% in normal subjects, values between 3.6-7% were regarded as being typical for beta thalassemia carriers.[10] The values between 3.2- 3.5% were considered borderline for further investigation like iron profile, especially in young subjects and couples at risk. Regarding HbE, on Bio-Rad Variant II, it co elutes with HbA2 and CZE is done to confirm it. All haemoglobin variants were confirmed by alternative, second screening method.

RESULTS

Total 3057 students were screened for hemoglobinopathies, including 1552 males and 1505 females with mean age of 18 years. All educational institutions were located in Central Punjab (Table1).

Those having microcytic hypochromic picture were further tested for carrier status (Table 2). Among carriers, mean value for haemoglobin was 9.07g/dl, MCV=72.52 fl/red cell, MCH=23.6 pg/red cell and RBC count 4.93 $X10^6$/µl. On HPLC the mean HbA2 was found to be 5.04% and beta thalassemia prevalence was found to be 2.51%. Cut off limit of HbA2 for diagnosis of beta thalassemia trait was 3.5%.

There were 7 (0.22%) HbE carriers. All showed microcytic hypochromic blood picture and on HPLC characteristic marked increase in A2 (>8%). On HPLC, HbE co-elutes with HbA2. Even though a provisional diagnosis could be made with this high A2 but for confirmation a second screening method (CZE) was used to clearly separate HbE from A2 (Figure 1). HbD-Punjab was other most common hemoglobinopathy in Punjab. There were 12 carriers of HbD-Punjab and a homozygous of HbD. One case of compound heterozygous (HbE/Beta thalassemia) case was also diagnosed in a young son of an employee at one of the colleges.

Table 1: Distribution of study population and prevalence of various hemoglobinopathies

Institution	Total	β thalassemia trait	HbE Carrier	HbD carrier	Others
Agriculture University Faisalabad	149	6			
Fatima Memorial Hospital, Lahore	203	5		1	1*
Fatima Memorial Hospital, Lahore	102	1		1	
Herbanspura School	28	1			
Governor House school	110	4			
Govt. College Faisalabad	287	8		2	
Govt. Islamia College	100	5			
PG College, Chiniot	100	1			
Medina Teaching University, FSD	200	3		1	
PGC, Lahore	69	2			
SMDC, Lahore	48	0			
Sahiwal Medical College	60	2	1		1**
Continental Medical College	24				
Continental Medical College	48				
Shifa Medical College	29				
University of Education	100	3		1	
CIMS Kalma Chowk	80	3	1	1	
CIMS Samanabad	80	1		2	
Women University Faisalabad	78	3			
Central Model School	107	5			
Ayesha PG college	153	4			
Govt. Degree College	113	1			
MAO College	94	4		1	
Dial Singh college	71		1		
Govt. Girls school	90	2	2		
Govt. Islamia	51	5	1	1	
Hailey College	121	5	1	1	
Imperial College	362	3			
Total	**3057**	**77 (2.51%)**	**7 (0.22%)**	**12 (0.39%)**	**2 (0.065%)**

* Homozygous for D Punjab
**HbE/beta

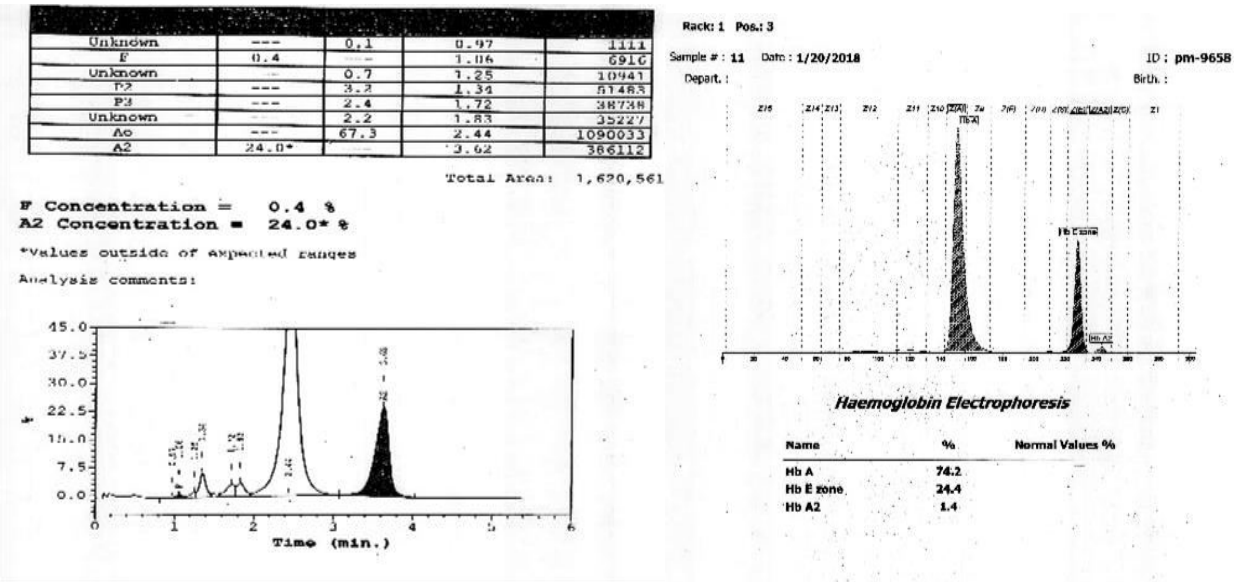

Figure 1: HbE-markedly increased (A2>8%) on HPLC further confirmed on capillary zone electrophoresis (CZE) as A2 clearly separates from HbE.

DISCUSSION

Thalassaemia is a global public health problem, but its burden is overwhelming in developing world, particularly in Asian countries where fewer resources are available for dealing with the problem.[11] Pakistan is a country which is currently fighting the ever-increasing burden of beta thalassemia major. Available statistics, largely derived from prevalence of carriers and birth rate, indicate that the country is not successful in decelerating the disease incidence let alone prevention. Haemoglobinopathies do not have a definite medical care, thus the World Health Organization (WHO) has recommended a prevention and control program including enlightenment of the public, screening for asymptomatic carriers, antenatal detection and genetic counselling.[12] Carrier are then informed and genetic counselling is imparted so that they understand their options and reproductive risks.[1] Punjab Thalassemia prevention program (PTPP) is the only Government run facility providing free of cost services for thalassemia and other hemoglobinopathies carrier screening as well as prenatal diagnosis by chorionic villous sampling and molecular diagnosis. This study was carried out as part of educational institutions awareness campaigns during which voluntary carrier screening is offered to the students. In a previous study carried out in a high school in Antakya, Turkey, 83.7% of the students stated that they wanted to be educated about hemoglobinopathies in the school and 89.1% wanted to know if they were carriers.[13] Prevalence for beta thalassemia in young population represented in institutes of Central Punjab in present study is 2.5%. Similar studies had been conducted in other provinces of Pakistan.[14,15] Prevalence of beta thalassemia trait among the students of schools, colleges and universities of Nawabshah city was reported to be 4.9%.[15] Prevalence may even vary in Southern and Northern Punjab. Detection of carriers in this particular study is fruitful in establishing the true prevalence and even more beneficial to the carriers detected. The ultimate objective is to create awareness regarding the disease pattern so that the informed decision making among future generation is propagated. In a questionnaire-based cross-sectional study conducted in six randomly chosen non-medical universities to assess the students' knowledge of β-thalassemia and premarital screening, and their attitude towards such a program showed poor background knowledge but there was a positive attitude towards premarital screening.[16] Such awareness activities are also helpful to address myths, false notions, beliefs and practices in a relatively

Table 2: Assessment of Haematological Parameters (N=3057)

Diagnosis	No. of Cases	Hb (g/dl)	MCV (fl)	RBC (X10^6/µl)	MCH (pg)
Non-carrier	2958	13.1-17.5	69-107	4.26-6.68	27.1-33.4
β thal Trait	77	9.8-13.5	58-87	4.50-7.52	16.4-24
HbE carrier	07	8.8-11.2	64.4-70.5	6.10	15.1-16.5
HbD carrier	12	11.1-11.2	74.6-35.7	3.80-4.93	23.3-29.2
Homozygous D	01	12.9	73.9	5.05	25.5
Hb β+HbE	01	3.9	79.7	1.53	25.5

conservative society. Imparting knowledge to young potential parents highlights the need of a mass awareness campaign and subsequent implementation of a premarital screening program.[16] Such studies also emphasize the importance of screening and awareness in rural areas of the country as well.

The other hemoglobinopathies that are identified in this study are HbD and HbE hemoglobinopathy. HbD-Punjab happens due to a point mutation in position 121 codon (GAA→CAA) with the substitution of glutamine for glutamic acid in the beta globin chain.[17] It is considered to be a benign hemoglobinopathy and of little clinical significance. Its basic significance is with HbS compound heterozygous state. In compound HbD/HbS state there is severe sickling which may resemble sickle cell disease.[18] On the other hand, Haemoglobin E (HbE) is rather a common β-haemoglobin variant throughout Asia. It occurs due to a point mutation at position 26 of beta globin gene which results in the substitution of lysine for glutamic acid. It is a variant, which is produced at a slightly reduced rate and results in a mild form of β thalassemia, its coinheritance with β thalassemia, haemoglobin E β thalassemia, however is mostly severe form of β thalassemia.[19] Recognition of these potential compound heterozygous states are important as far as their diagnosis, clinical outcome and implications for taking options for prenatal diagnosis is concerned.

CONCLUSION

Carriers state of Beta thalassemia is a major hemoglobinopathy seen among students of various educational institutions of Punjab along with few other clinically relevant haemoglobin variants. It is therefore imperative to create awareness about these inherited haemoglobin disorders among general public especially the young population who are prospective parents. Imparting awareness regarding thalassemia and its rather straight forward prevention remains a key to success of thalassemia control program.

REFERENCES

1. Old J, Harteveld C. Carrier screening for the hemoglobinopathies: past, present and future. OBM Genetics 2017; 1(3): 1-28.
2. Hossain MS, Raheem E, Sultana TA, Ferdous S, Nahar N, Islam S, et al. Thalassemia in South Asia: clinical lessons learnt from Bangladesh. Orphanet Journal of Rare Diseases 2017; 12:93: doi.org/ 10.1186/s13023-017-1643-z.
3. Nosheen A, Inamullah, Ahmad H, Qayum I, Siddiqui N, Abbasi FM, et al. Premarital genetic screening for beta thalassemia carrier status of indexed families using HbA2 electrophoresis. J Pak Med Assoc 2015; 65(10):1047-9.
4. Henneman L, Borry P, Chokoshvili D, Cornel M C, van EL CG, Forzano F, et al. Responsible implementation of expanded carrier screening. Eur J Hum Genet 2016; 24(6): e1-e12. doi: 10.1038/ejhg.2015.271.
5. Saffi M, Howard N. Exploring the Effectiveness of Mandatory Premarital Screening and Genetic Counselling Programmes for β-Thalassemia in the Middle East: A Scoping Review. Public Health Genomics 2015; 18:193-203.
6. Waheed F, Fisher C, Awofeso A , Stanley D. Carrier screening for beta thalassemia in the Maldives: perceptions of parents of affected children who did not take part in screening and its consequences. J. Community Genet 2016; 7(3): 243-53.
7. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N, et al. Guidelines for

screening, diagnosis and management of hemoglobinopathies. Indian J Human Genet 2014; 20(2):101-19.
8. Lena-Russo D, Badens C, Aubinaud M, Merono F, Paolasso C, Martini N, et al. Outcome of a school screening program for carriers of haemoglobin disease. J Med Screen 2002; 9(2): 67-9.
9. Sur D, Chakravorty R. Prevalence of Hemoglobinopathies and Thalassemia Carriers in Women of Reproductive Age Group Especially the Prospective Mothers: A Single Center Study at West Bengal. J Hematol 2016; 5(3): 99-102.
10. Roth IL, Lachover B, Koran G, Levin C, Luci Z and Koran A. Detection of β-Thalassemia Carriers by Red Cell Parameters Obtained from Automatic Counters using Mathematical Formulas. Mediterr J Hematol Infect Dis 2018; 10(1); e2018008. doi:10.4084/MJHID.2018.008.
11. Brancaleoni V, Di Pierro E, Motta I, Cappelline MD. Laboratory diagnosis of thalassemia. Int J Lab Hematol 2016; 38(1): 32-40.
12. Salma RA, Saleh AK. Effectiveness of premarital screening program for thalassemia and sickle cell disorders in Ras Al Khaimah, United Arab Emirates. J Genet Med 2016;13(1): 26-30.
13. Savas N, Turhan E, Inandi T, Kaya H. Hemoglobinopathy awareness among high school students in Antakya (Antioch), Turkey. Int J Hematol 2010; 91(3): 413-8.
14. Qazi RA, Shams R, Hassan H, Asif N. Screening for Beta Thalassemia Trait. Journal of Rawalpindi Medical College (JRMC) 2014;18(1):158-60
15. Mirza A, Ghani A, Pal A, Sami A, Hannan S, Ashraf Z, et al. Thalassemia and premarital screening: potential for implementation of a screening program among young people in Pakistan. Haemoglobin 2013; 37(2):160-70.
16. Karimzaei T, Masoudi Q, Shahrakipour M, Navidiyan A, Jamalzae AA, Zoraqi Bamri A. Knowledge, Attitude and Practice of Carrier Thalassemia Marriage Volunteer in Prevention of Major Thalassemia. Glob J Health Sci 2015; 7(5): 364-70.
17. Fucharoen S, Changtrakun Y, Surapot S, Fucharoen G, Sanchaisuriya K. MOLECULAR CHARACTERIZATION OF HbD-Punjab [β121(GH4) Glu→Gln] IN THAILAND. Hemoglobin 2002; 26(3): 261–9.
18. Adekile A, Mullah-Ali A, Akar NA. Does Elevated Hemoglobin F Modulate the Phenotype in HbSD-Los Angeles? Acta Haematol 2010;123:135–39.
19. Orkin SH, Kazazian HH, Antonarakis SE, Ostrer H, Sabra C, Goff SC, et al. Abnormal RNA processing due to the exon mutation of β^E-globin gene. Nature 1982; 300: 768–9.