Open Access Original Article

Detection of Beta Thalassemia Trait in Siblings of Beta Thalassemia Major Patients in Karachi: A Step towards Beta Thalassemia Major Free Society

Ghazal Irfan[1], Maeesa Wadood[2] Sarah Azhar[3], Muhammad Khan[4] RuqayaNangrejo[5], Tooba Khan[6] , Yasir Rishi[7]

[1,3-7]*Baqai Medical University, Karachi*
[2]*Baqai Institute of Hematology, Karachi*

Abstract

Objective: To detect the β-Thalassemia trait in siblings of β-Thalassemia major (BTM) patients in Karachi, with the goal of identifying carriers and promoting preventive strategies.
Methodology: A cross-sectional study was conducted at Muhammadi Institute of Hematology in Karachi from 2019 to 2021. A total of 400 siblings of BTM patients were screened using complete blood count (CBC) and high-performance liquid chromatography (HPLC). The hematological parameters were analyzed using SPSS, and statistical comparisons between carriers and non-carriers were performed using an independent t-test ($p<0.05$).
Results: Out of 400 screened siblings, 168 (42%) were identified as carriers of the β-Thalassemia trait. Hematological analysis revealed that carriers exhibited significantly lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) but had higher red blood cell (RBC) counts compared to non-carriers. Hemoglobin analysis showed that HbA2 levels were significantly elevated in carriers, confirming the presence of the trait. Ethnicity-wise distribution indicated a higher prevalence among Balochi (36.3%) and Sindhi (29.8%) populations.
Conclusion: The study highlights a high prevalence of β-Thalassemia trait in siblings of BTM patients. Early detection through systematic screening and genetic counseling is essential to prevent further transmission of the disease. Nationwide preventive programs should be implemented to reduce the burden of β-Thalassemia major in Pakistan.
Keywords: Beta Thalassemia, Hemoglobinopathies, Carrier Screening, Genetic Counseling.

Address for Correspondence
Dr. Ghazal Irfan
dr.ghaxal@gmail.com

Introduction

Hemoglobin is a quaternary structure-based protein that is responsible for carrying oxygen within the red blood cells of the human. Hemoglobin consists of two chains of α and βglobin that combines together with a heme molecule to form what is known as hemoglobin.[1-2] Like all other proteins in the body, hemoglobin can also fall victim to various abnormalities which are known as hemoglobinopathies[3].A hereditary form of anemia with Mendelian recessive autosomal pattern of inheritance, Beta-thalassemia is a condition where there is either absence or lack of production of the beta chains in hemoglobin.[4, 5]

Due to the molecular heterogeneity, there are three forms of thalassemia which include thalassemia major, minor, and intermedia. Patients suffering from thalassemia major usually have severe anemia in the first two years of their life thus require regular transfusions.[6] Beta thalassemia major (BTM) is diagnosed very early in life and the patient usually has signs of hemolysis as well.[7] BTM patients are unfortunately completely dependent on blood transfusions throughout their life, unless they opt to undergo transplant of bone marrow. They also suffer from various complications of diseases as well from the treatment that they are receiving.[8-9] In Pakistan Beta thalassemia is a very common hemoglobinopathy with a gene pool of 9.8 million carriers throughout general population.[10] Pakistan is among the countries that has the highest rate of prevalence of transfusion dependent thalassemia major patients on the planet.[11] BTM is a disease which can be eradicated from the community if proper measures and health policies are established. However, this requires a high level of intervention into the population at the highest risk. Literature strongly supports that prevention programs have indeed led to a significant reduction in the disease rate. Beta-thalassemia minor is another form of beta thalassemia, in which the patient is usually asymptomatic and shows only mild anemia on CBC labs.[12] The thalassemia minor trait identifies the fact that the

Authorship Contribution: [1-3,6]Conceived and planned the idea of the study, [3]final approval of the version to be published, [4-5]Collecting the data, drafting the work or revising it critically for important intellectual content, [7]Literature review,

Funding Source: none
Conflict of Interest: none
Received: Oct 19, 2024
Accepted: Dec 30, 2024

individual has inherited only one defected gene from the parent, which means that the child is a carrier of the disease.[13] However these individuals are instrumental in the propagation of the gene as they can transmit the disease if married to a person with similar genetic makeup. Being more precise, if both partners carry beta thalassemia trait then there is 25% chance of having homozygous beta thalassemia offspring. There should be a wide spread study on beta-thalassemia throughout the country of Pakistan, however, due to a lack of resources and ill-fated policies these programs have not been properly initiated or effectively implemented. Considering this factor, the present study is designed to identify the presence of thalassemia minor in the siblings of Beta-thalassemia major patients. As a matter of fact, these children are a strong indicator of the presence of thalassemia gene in population. The objective of this study was to detect β-Thalassemia trait in siblings of BTM patients in Karachi.

Methodology

This cross-sectional analytical study was conducted at the Muhammadi Institute of Hematology in Karachi from 2019 to 2021. In the study 400 siblings of BTM patients were included in the study. Written informed consent was obtained from all of the participants along with their parents. The study was initiated once ethical approval was achieved from Baqai Medical University, Karachi. Any participants that were under the age of 6 months or had a transfusion history in the last three months were excluded from the study.

A comprehensive history was obtained from all the patients concerning their personal and past medical history. Once completed, a general physical examination was done and the vitals were recorded. 5ml of venous blood was drawn and collected in EDTA tube that was commercially available. All aseptic guidelines and precautions were followed while collecting specimen. Blood samples were collected from all the participants to record the complete blood count (CBC), peripheral smear morphology, and high-performance liquid chromatography (HPLC). These tests were done to determine the red cell count, white blood cell count, platelet count, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and lastly the red cell distribution width using an automated cell analyzer. The laboratory results were shared and discussed with the participants along with their parents/guardians. Genetic counselling sessions were also carried out with the individuals along with their parents/guardians. This was done to create awareness regarding how to prevent the transmission of BTM in the future generations. Data was presented in the form of mean and standard deviation. Data was analyzed using statistical package of social sciences (SPSS). The mean differences of CBC and HPLC parameters between non-carrier groups and carrier groups were compared using the independent-test. The differences of various parameters between carriers and non-carriers were carried out bychi-square test. The P-value was set at 0.05.

Results

From the 400 siblings screened, 168 (42%) were found to be carriers of the beta thalassemia trait. On CBC interpretation, those who were carriers showed a significant reduction in MCV and MCH, whereas their RBC count was high and MCHC was normal. The HbA, HbF, and Hb A2 levels were also significantly different between the carrier group and non-carrier group.The study involved analyzing several characteristics among carriers and non-carriers of Beta Thalassemia trait. Regarding ethnicity, carriers were most prevalent among the Balochi (36.3%) and Sindhi (29.8%) populations. Conversely, the Urdu-speaking population had a notably lower percentage of carriers (6.0%) compared to non-carriers (14.2%), while the Pathan and Punjabi ethnicities showed more balanced distributions between carriers (13.7% and 14.3%, respectively) and non-carriers (16.8% and 21.1%, respectively). (Table I)

A detailed examination of red blood cell (RBC) morphology revealed significant differences between carriers and non-carriers. Among carriers, a striking 97.0% exhibited hypochromia and microcytosis, which were present in only 3.9% of non-carriers. Anisocytosis with mild hypochromia was observed in 3.9% of carriers, and various forms of anisocytosis coupled with hypochromia or normochromia were noted among smaller subsets of the carrier group, with percentages ranging from 3.4% to 19.8%. Normocytic normochromic RBCs were predominant in non-carriers (63.8%) but less common among carriers [Table I]. The mean RBC count was significantly higher in carriers (5.08 ± 0.14) compared to non-carriers (4.37 ± 0.27). Hemoglobin (Hb)

levels were markedly lower in carriers, averaging 9.0 ± 1.0 g/dL, while non-carriers had an average of 12.0 ± 0.00 g/dL. Hematocrit (HCT) levels were similar between the two groups, with carriers at 36.36 ± 1.67% and non-carriers at 36.31 ± 1.64%. The mean corpuscular volume (MCV) was significantly lower in carriers (67.0 ± 2.0 fL) than in non-carriers (81.0 ± 8.0 fL), as was the mean corpuscular hemoglobin (MCH), which was 20.51 ± 1.13 pg in carriers and 26.03 ± 3.05 pg in non-carriers. Mean corpuscular hemoglobin concentration (MCHC) showed a slight but statistically significant difference, with carriers at 31.57 ± 0.5 g/dL and non-carriers at 31.94 ± 1.05 g/dL.

Table I: Population characteristics and their comparison between carrier and non-carrier groups.

Population Characteristics		Study Groups		
		Carrier	Non-Carrier	P value
		Column % (#)	Column N %	
Gender	Male	61.9% (104)	59.9% (139)	0.687
	Female	38.1% (64)	40.1% (93)	
Ethnicity	Sindhi	29.8% (50)	23.7% (55)	0.004
	Balochi	36.3% (61)	24.1% (56)	
	Pathan	13.7% (23)	16.8% (39)	
	Punjabi	14.3% (24)	21.1% (49)	
	Urdu Speaking	6.0% (10)	14.2% (33)	
Age Groups	1-5	29.8% (50)	28.4% (66)	0.749
	6-10	39.3% (66)	37.9% (88)	
	11-15	28.0% (47)	28.4% (66)	
	16-20	3.0%(5)	5.2% (12)	
RBC Morphology	Anisocytosis		4.7% (11)	<0.005
	AnisocytosisMild hypochromia		3.9% (9)	
	Anisocytosis Normochromic		19.8% (46)	
	Hypochromia/ Microcytosis	97.0% (163)	3.9% (9)	
	Basophillic stippling	3.0% (5)		
	Normocytic Normochromic		63.8% (148)	

Red cell distribution width (RDW-SD) was also lower in carriers (36.38 ± 2.61) compared to non-carriers (37.72 ± 2.61), further distinguishing the two groups. White blood cell (WBC) counts were lower in carriers (8.4 ± 1.18 x 10^9/L) than in non-carriers (8.52 ± 2.37 x 10^9/L). Platelet (PLT) counts were nearly identical between carriers (287.6 ± 108.6 x 10^9/L) and non-carriers (287.4 ± 109.9 x 10^9/L), indicating no significant difference in this parameter. However, hemoglobin fractions differed markedly, with carriers having higher Hb A2 levels (5.6 ± 0.22%) and lower Hb F levels (0.16 ± 0.08%) compared to non-carriers, who had Hb A2 levels at 2.68 ± 0.27% and Hb F levels at 0.89 ± 0.47%. Hb A levels were also slightly lower in carriers (94.57 ± 0.23%) compared to non-carriers (96.56 ± 0.45%) [Table II].

Table II: Comparison of study parameters between the carrier and non-carrier groups.

Study Parameters	Study Groups		
	Carrier	Non-Carrier	P value
	Mean ± SD	Mean ± SD	
Age (Years)	8.23 ± 3.99	8.51 ± 4.14	0.501
RBC	5.08 ± 0.14	4.37 ± 0.27	<0.005
Hb	9.0± 1.0	12.0 ± 0.00	<0.005
HCT	36.36± 1.67	36.31± 1.64	0.995
MCV	67.0 ± 2.0	81.0 ± 8.0	<0.005
MCH	20.51± 1.13	26.03± 3.05	<0.005
MCHC	31.57± 0.5	31.94± 1.05	<0.005
RDW-SD	36.38± 2.61	37.72±2.61	<0.005
WBC	8.4 ± 1.18	8.52 ± 2.37	<0.005
PLT	287.6± 108.6	287.4± 109.9	0.988
Hb A2	5.6± 0.22	2.68 ± 0.27	<0.005
Hb F	0.16 ± 0.08	0.89 ± 0.47	<0.005
Hb A	94.57± 0.23	96.56± 0.45	<0.005

Discussion

The results of the study showed that a significant difference was observed between carriers and non-carriers in terms of red blood cell morphology, hemoglobin and red blood cells. As mentioned previously thalassemia is the most widely prevalent disorder related to hemoglobin in the world. The management of thalassemia has improved considerably now, due to the availability of safe blood transfusion, better regimens of iron chelation therapy, the management of complications and good supporting care. This has allowed children to live a near normal life.[14] The only definitive treatment modality available for treating thalassemia is hematopoietic stem cell transplantation.[15] BTM is a widely prevalent genetic disorder in our country, with

figures suggesting that there are almost 100,000 people with BTM. What's more alarming is that there are 7000-9000 children annually born with this disease.[16-17] Even if there are management options available, its duration place a huge financial and psychological burden on the family and the health care system of our country. The purpose of this study was to identify carriers in sibling with BTM. The carrier screening strategy is preventative in nature and has led to a reduction in the disease in many countries. A similar program spanning in to two decades in Sardinia led to a reduction of thalassemia major births from 1:4000 to 1:250.[18] A reduction of up to 16% in birth rate of homozygous thalassemia patients has been observed due to the efforts of different global preventive strategies.[19] In the 400 siblings that were studied, 168 (42%) of the subjects were found to be carriers of beta thalassemia.[20] The frequency of beta thalassemia was reported as 5-7% in Pakistan; however, our findings shows that the prevalence is more profound in index families as compare to the general population. In our study, we also studied the red blood cell count and the respected indices that help in the diagnosis of beta thalassemia trait.

Our study showed that the HbA2 level in carriers of beta thalassemia trait was 5.59±0.215, while the hallmark of detecting the carrier state of beta thalassemia is more than 3.5%. This is in line with studies conducted by Qazi et al who went onto report a HbA2 level of 5.8% and Ragb et al who reported a HbA2 level of 5.56 21-22. In our study the mean corpuscular volume of beta thalassemia in the carrier was 66.91±1.51, whereas the mean corpuscular hemoglobin was 20.51±1.12. This finding is in line with another study by Sharma et al who conducted a similar study to our screening for carriers in siblings of beta thalassemia major patients also reported an MCV of 62.08±11 and MCH 21.35±4.17. Our study clearly showed that there is significant difference in CBC and hemoglobin parameters between carrier groups and non-carrier groups. RBC, Hb, MCV, MCH, HbA2, HbF, and HbA all showed significant difference between the carrier group and non-carrier group. RBC, Hb, MCV, and MCHC were all significantly lower than the non-carrier groups, while HbA2 was significantly higher. Beta thalassemia trait is more prevalent in siblings of BTM patients. Therefore, what required is the targeted screening and effective genetic counseling. A nation-wide intervention needs to be initiated to halt the spread of disease in the nation.

Conclusion

The results of this study showed that the prevalence rate of beta thalassemia trait was higher in the siblings of beta-thalassemia major patients and therefore should be screened and identified at an earlier rate to prevent further propagation of gene. Beta-thalassemia is a concerning condition in Pakistan, timely intervention, screening, and counseling needs to be carried out in carriers with the aid of further studies.

References

1. Ahmed MH, Ghatge MS, Safo MK. Hemoglobin: structure, function and allostery. In: Vertebrate and invertebrate respiratory proteins, lipoproteins and other body fluid proteins. 2020;345-82. https://doi.org/10.1007/978-3-030-41769-7_14
2. Marengo-Rowe AJ. Structure-function relations of human hemoglobins. Baylor Univ Med Cent Proc. 2006 Jul 1;19(3):239-45. https://doi.org/10.1080/08998280.2006.11928171
3. Khandros E, Kwiatkowski JL. Hemoglobinopathies. In: Lanzkowsky's Manual of Pediatric Hematology and Oncology. Academic Press; 2022;161-92. https://doi.org/10.1016/B978-0-12-821671-2.00013-1
4. Karponi G, Zogas N. Gene therapy for beta-thalassemia: updated perspectives. Appl Clin Genet. 2019;12:167-71. https://doi.org/10.2147/TACG.S178546
5. Weatherall DJ. Thalassaemia: the long road from bedside to genome. Nat Rev Genet. 2004;5(8):625-31. https://doi.org/10.1038/nrg1406
6. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010;5(1):1-5. https://doi.org/10.1186/1750-1172-5-11
7. Memish ZA, Owaidah TM, Saeedi MY. Marked regional variations in the prevalence of sickle cell disease and β-thalassemia in Saudi Arabia: findings from the premarital screening and genetic counseling program. J Epidemiol Glob Health.2011;1(1):61-8. https://doi.org/10.1016/j.jegh.2011.06.002
8. Al Hawsawi ZM, Sairafy MH, Tarawah AM, Zolaly MA, Al Hegaily AR. Experience with combination therapy of deferiprone and desferrioxamine in β-thalassemia major patients with iron overload at Maternity and Children Hospital, Al Madinah Al Munawarah, Saudi Arabia. J Taibah Univ Med Sci. 2010;5(1):27-35. https://doi.org/10.1016/S1658-3612(10)70121-9
9. Porter JB. Concepts and goals in the management of transfusional iron overload. Am J Hematol. 2007 Dec;82(S12):1136-9. https://doi.org/10.1002/ajh.21100
10. Ehsan H, Wahab A, Anwer F, Iftikhar R, Yousaf MN. Prevalence of transfusion transmissible infections in beta-thalassemia major patients in Pakistan: a systematic review. Cureus. 2020;12(8). https://doi.org/10.7759/cureus.10070
11. Jalali S, Hassan M, Mahboob S, Jabeen F. Prevalence of β-thalassemic patients associated with consanguinity and anti-

HCV -antibody positivity - a cross sectional study. Pak J Zool. 2011;43:29-36.
12. Arora S, Rana D, Kolte S, Dawson L, Dhawan I. Validation of new indices for differentiation between iron deficiency anemia and beta thalessemia trait, a study in pregnant females. Int J Sci Rep. 2018;4(2):26. https://doi.org/10.18203/issn.2454-2156.IntJSciRep20180394
13. Khan MI, Khan HN, Usman M. Beta thalassemia trait: Diagnostic importance of haematological indices in detecting beta thalassemia trait patients. Prof Med J. 2018;25(4):545-50. https://doi.org/10.29309/TPMJ/2018.25.04.343
14. Dubey AP, Parakh A, Dublish S. Current trends in the management of beta thalassemia. Indian J Pediatr. 2008;75(7):739-43. https://doi.org/10.1007/s12098-008-0140-4
15. Wang X, Zhang X, Yu U, Wang C, Yang C, Li Y, et al. Co-Transplantation of Haploidentical Stem Cells and a Dose of Unrelated Cord Blood in Pediatric Patients with Thalassemia Major. Cell Transplant. 2021;30:0963689721994808. https://doi.org/10.1177/0963689721994808
16. Ansari SH, Shamsi TS, Ashraf M, Farzana T, Bohray M, Perveen K, et al. Molecular epidemiology of β-thalassemia in Pakistan: Far reaching implications. Indian J Hum Genet. 2012;18(2):193. https://doi.org/10.4103/0971-6866.100762
17. Black ML, Sinha S, Agarwal S, Colah R, Das R, Bellgard M, et al. A descriptive profile of β-thalassaemia mutations in India, Pakistan and Sri Lanka. J Community Genet. 2010;1(3):149-57. https://doi.org/10.1007/s12687-010-0026-9
18. Cao A, Rosatelli MC, Galanello R. Control of β-thalassaemia by carrier screening, genetic counselling and prenatal diagnosis: the Sardinian experience. In: Ciba Foundation Symposium 197-Variation in the Human Genome: Variation in the Human Genome. Chichester, UK: John Wiley & Sons, Ltd; 2007 Sep. p. 137-55. https://doi.org/10.1002/9780470514887.ch8
19. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008 Jun;86(6):480-7. https://doi.org/10.2471/BLT.06.036673
20. Khalid A, Butt AM, Shahid R, Hoor A. Thalassemia: Current Situation in Pakistan. Lahore Garrison Univ J Life Sci. 2020;4(4):309-18. https://doi.org/10.54692/lgujls.2020.0404126
21. Qazi RA. Screening for beta thalassemia trait. J Rawalpindi Med Coll. 2014 Jun 30;18(1):158-60.
22. Elrahim MA, Ibrahim RA, Ragb SM, El Fotoh W. Screening of β-thalassemia carriers in high school students in Shebin El-Kom, Menoufia Governorate. Menoufia Med J. 2021;34(1):237-42. https://doi.org/10.4103/mmj.mmj_358_19
23. Sharma G, Sharma D, Gulati RK. Thalassemia carrier screening in siblings of thalassemia major patients by HbA2 estimation. Indian J Child Health. 2016;3(3):258-60. https://doi.org/10.32677/IJCH.2016.v03.i03.020