ORIGINAL ARTICLE

MOLECULAR CHARACTERIZATION OF BETA THALASSEMIA MAJOR IN PUNJAB, PAKISTAN

Muhammad Irtza Tanveer[1]✉, Sadaf Farzand[1], Areeba Manzoor[1], Ambereen Anwar[1], Saima Farhan[2], Saira Moin[2]

[1]Department of Pathology, Punjab Institute of Cardiology, Lahore-Pakistan
[2]Department of Hematology, Sundas Foundation, Lahore-Pakistan

Background: β-Thalassemia major is among the most prevalent hereditary blood disorders in Pakistan. A person develops β-Thalassemia major when they inherit two mutated alleles for the *HBB* gene. This study aimed to identify the profile of mutations that are found in the population of Punjab, Pakistan, to develop a mutation-targeted diagnostic panel for the detection and better control of the disease. **Methods**: The study was conducted at SUNMAC, Sundas Foundation, Lahore. A cross-sectional approach was adopted for this study, and a non-probability consecutive sampling technique was employed to recruit 133 patients between January 2023 and December 2024. Blood samples from the patients were collected and tested using Multiplex ARMS-PCR for common and rare *HBB* mutations in Punjab. **Results**: Our findings revealed that homozygosity was found in 79.6 % of the cases and compound heterozygosity in the remaining 20.4%. Among the 13 mutations involved in our study: IVSI-5 (G>C) (33.8%), Fr8-9 (+G) (28.6%), Fr41-42 (-CTTT) (15%), IVSI-1 (G>A) (7.1%). These four mutations were common and accounted for 77.5% of the cases. Less commonly encountered mutations involved Codon5 (-CT), Codon15 (A>T), Codon30 (G>C), Cap+1 (A>C), Del619, Fr16 (-C). Three haemoglobin variants- HbE Cd-26, HbS Cd-6, and HbC Cd-6 were also investigated in this study. **Conclusion**: The most frequently found mutation in our study population is IVSI-5 (G>C). Many additional mutations, such as IVSI-1 (G>A), Fr8-9 (+G), and Fr41-42 (-CTTT), contributed significantly to the disease burden.

Keywords: *HBB* mutations; β-Thalassemia major; Multiplex ARMS-PCR

Citation: Tanveer MI, Farzand S, Manzoor A, Anwar A, Farhan S, Moin S. Molecular Characterization of Beta Thalassemia Major in Punjab, Pakistan. J Ayub Med Coll Abbottabad 2025;37(3):334–8.

DOI: 10.55519/JAMC-03-14563

INTRODUCTION

β-Thalassemia -an inherited monogenic haematological disorder disrupts the formation of Haemoglobin, a protein vital for transporting oxygen in human red blood cells. β-Thalassemia is among the most prevalent haemoglobinopathies in South Asian, Middle Eastern, Mediterranean, and African populations.[1] Around 60,000 cases of β-Thalassemia are reported annually around the world. A study reports that the global incidence of β-Thalassemia is 18.28 per 100,000 individuals.[2] About 5-8% of the Pakistani population is a carrier of β-Thalassemia.[3] Around 5000 individuals with β-Thalassemia major are reported each year in Pakistan.[4]

This disease is inherited in an autosomal recessive pattern and results from the mutations in the *HBB* gene on chromosome 11, specifically at 11p15.4, resulting in ineffective erythropoiesis, diminished and defective haemoglobin production, varying degrees of anaemia, and in severe cases, a significant dependency on blood transfusions. The extent of anaemia in β-Thalassemia patients is typically dependent on the specific mutation, whether it leads to absent beta-globin production (β^0) or reduced beta-globin production (β^+). Two genes encode the β-globin chain, and depending upon the number of mutated genes carried by the patient, we can classify β-Thalassemia in three major types: A patient carrying one mutated gene will manifest features of β-Thalassemia minor, while a patient carrying two mutated β-globin genes will be diagnosed as β-Thalassemia intermedia or β-Thalassemia major. Laboratory diagnosis of β-Thalassemia requires a complete blood count[5], revealing hypochromic, microcytic anaemia, and hemoglobin electrophoresis, which shows abnormal haemoglobin variants. Molecular techniques such as ARMS-PCR and DNA sequencing are used to detect the exact mutations in the *HBB* gene. Prenatal detection can also be achieved through diagnostic techniques like amniocentesis or chorionic villus sampling (CVS).

Globally, more than 200 mutations associated with β-Thalassemia have been identified. Studies show that eight *HBB* mutations are commonly found across all the provinces of Pakistan[6], while some mutations are limited to the population of specific provinces. Punjab, one of the provinces of Pakistan, bears a heavy burden of β-Thalassemia Major patients due to consanguineous marriages and limited public awareness about the disease. Literature suggests that 70% of the reported β-

Thalassemia cases in Pakistan are seen in consanguineous marriages.[7] A study conducted in 2006 reported the highest frequency and prevalence of the IVSI-5 (G>C) mutation among populations in various cities of Punjab, Pakistan.[8]

Our study aimed to evaluate several common and rare mutations in β-Thalassemia Major patients in Punjab using Molecular testing Techniques. The research was conducted at the Sundas Foundation, Lahore, one of the largest Thalassemia centers in Punjab, which receives patients for blood transfusions from both urban and rural areas throughout Punjab. Understanding the mutation spectrum of the *HBB* gene in Punjab, Pakistan, is crucial for enhancing targeted diagnostics, therapeutic advancements, genetic counselling, and designing effective prevention programs.

MATERIAL AND METHODS

This cross-sectional study was conducted at SUNMAC (Sundas Molecular Analysis Center), Sundas Foundation, Lahore. The duration of the study was January 1, 2023, to December 31, 2024. Our sample size was 133, calculated at a 95% confidence level and a 5% margin of error, based on the expected percentage. Ethical approval was obtained from the Institutional Review Board of Sundas Foundation, Lahore. All eligible 133 patients who fulfilled the inclusion criteria of our study were recruited using a non-probability consecutive sampling technique. Informed written consent was taken from the Guardians of all the patients. The eligibility was restricted to the patients residing only in Punjab with a confirmed diagnosis of β-Thalassemia major, based on haemoglobin electrophoresis, HbA_2>3.5%, and requiring periodic transfusion. Only one member of the affected families was included in the study to limit the chance of sample bias. The exclusion included patients with other types of transfusion-dependent anaemias.

Five mL of the venous blood from each patient was drawn in an EDTA container and subjected to Multiplex ARMS-PCR for the detection of various β-Thalassemia mutations. Chromosomal DNA was acquired from leukocytes using a DNA extraction kit (Qiagen DNA extraction kit). Primers for 17 *HBB* mutations: IVSI-1 (G>A), IVS2-1 (G>A), IVSI-25 (G>A), IVSI-5 (G>C), Fr8-9 (+G), Fr16 (-C), Fr41-42 (-CTTT), Codon5 (-CT), Codon15 (A>T), Codon30 (G>C), Codon39 (C>T), Del619, -88(C→T), HbE Cd-26, HbS Cd-6, HbC Cd-6, HbD (Punjab) were designed and used. Mutations were screened using multiplex ARMS-PCR, followed by visualization and identification through Gel Electrophoresis. All data were recorded and assessed descriptively using SPSS version 25.0.

RESULTS

Our study consisted of 133 patients diagnosed with β-Thalassemia major in Punjab, Pakistan, including 71 males (53.4%) and 62 females (46.4%). Of these 133 patients, 106 were homozygous for the mutation, while compound heterozygosity was observed in 27 patients.

A total of 266 chromosomes were screened for mutations specific to β-Thalassemia Major in Punjab. In total, 13 different mutations and 3 haemoglobin variants were detected. Eight frequent mutations (IVSI-5 (G>C), IVSI-1 (G>A), Fr8-9 (+G), Fr41-42 (-CTTT), Codon5 (-CT), Codon30 (G>C), Codon15 (A>T), Del 619) accounted for 94% of the mutations while 6% comprised uncommon mutations.

IVSI-5 (G>C) was found in 90 alleles, constituting 33.8% of the results followed by Fr 8-9(+G) constituting 28.6% of total present in 76 alleles; Fr41-42 (-CTTT): 40 alleles (15%); IVSI-1 (G>A): 19 alleles (7.1%); Codon5 (-CT):10 alleles (3.8%); Codon30 (G>C): 9 alleles (3.4%); Del619: 4 alleles (1.5%); Codon15 (A>T): 2 alleles (0.8%). Less common mutation included: HbE Cd-26: 7 alleles (2.6%); Cap+1 (A>C): 3 alleles (1.1%); HbS Cd-6: 2 alleles (0.8%); HbC Cd-6: 2 alleles (0.8%) and Fr16 (-C): 2 alleles (0.8%). Our results defining the predominant β-Thalassemia mutations in Punjab are summarized in tabular form as below:

Table-1: Various β-Thalassemia mutations along with their Human Genome Variation Society (HGVS) Nomenclature and impact on β-Globin

Mutation	HGVS Nomenclature	Phenotype
IVSI-5 (G>C)	*HBB* c.92+5G>C	β^+
Fr8-9 (+G)	*HBB* c.27dupG	β^0
Fr41-42 (-CTTT)	*HBB* c.126_129delCTTT	β^0
IVSI-1 (G>A)	*HBB* c.92+1G>A	β^0
Codon5 (-CT)	*HBB* c.17_18delCT	β^0
Codon30 (G>C)	*HBB* c.91G>C	β^+
HbE Cd-26	*HBB* c.79G>A	Hb variant
Del619	*HBB* c.315+1_+619del	β^0
Cap+1 (A>C)	*HBB* c.-50A>C	β^+
Codon15 (A>T)	*HBB* c.46G>A	β^0
HbS Cd-6	*HBB* c.20A>T	Hb variant
HbC Cd-6	*HBB* c.19G>A	Hb variant
Fr16 (-C)	*HBB* c.50delC	β^0

Table-2: Allelic frequency and prevalence of various β-Thalassemia mutations.

Mutation	Allelic Frequency(n)	Prevalence (%)
IVSI-5 (G>C)	90	33.8
Fr8-9 (+G)	76	28.6
Fr41-42 (-CTTT)	40	15.0
IVSI-1 (G>A)	19	7.1
Codon5 (-CT)	10	3.8
Codon30 (G>C)	9	3.4
HbE Cd-26	7	2.6
Del619	4	1.5
Cap+1 (A>C)	3	1.1
Codon15 (A>T)	2	0.8
HbS Cd-6	2	0.8
HbC Cd-6	2	0.8
Fr16 (-C)	2	0.8
Total	266	100

DISCUSSION

This research focused on identifying and analysing the mutation profile of β-Thalassemia major in patients from Punjab, Pakistan**,** and evaluating the impact of different genotypes on disease severity. Our findings confirmed the diversity of β-Thalassemia mutations in this region. The predominance of specific mutations is reflective of both regional patterns and high rates of consanguinity.

The data obtained revealed that males (n=71, 53.4%) and females (n=62, 46.4%) were approximately in equal ratio (1:1). Given that β-Thalassemia major follows an autosomal recessive inheritance pattern, the finding supports the fact that both genders have an equal risk of getting β-Thalassemia Major, as in autosomal recessive disorders.

The study involved 79.6% homozygous individuals and 20.4% compound heterozygous individuals. The homozygous individuals typically exhibit a complete or near complete absence of beta globin and present with the classical β-Thalassemia phenotype. The presence of two different mutated genes, i.e., compound heterozygosity, results in a variable phenotype depending on the nature of the mutations involved. For example, the combination of a severe mutation (β^0) with a mild mutation (β+) allows residual beta globin to manifest milder clinical complications. This finding reinforces the role of consanguineous marriages, leading to the highest percentage of homozygous cases in β-Thalassemia Major.[9] It underscores the importance of carrier screening and genetic counselling, particularly within families carrying the genetic risk of β-Thalassemia Major.

In our study, we identified several concurrent β-Thalassemia mutations, with IVSI-5 (G>C) (splice site mutation at intron 1, position +5) emerging as the predominant mutation in the region, succeeded by Fr8-9 (+G) (frameshift mutation involving insertion between codon 8 and 9), Fr41-42 (-CTTT) (frame shift mutation involving the deletion of 4 bases between codon 41 and 42) and IVS1-1(G>A) (splice site mutation at intron 1, position +1). These findings are consistent with the study done in 2012, reporting a peak of IVSI-5 (G>**C)** in Sindhi, Balochi, and Pathan populations.[10] The study conducted in 2017 identified that Fr8-9 (+G) is the most prevalent mutation in the Punjabi population.[11] In contrast, our findings indicated a shift in mutation patterns with IVSI-5 (G>C) emerging as the predominant mutation currently observed. The change of mutation pattern is attributed to the changes in population dynamics and genetic drift such as increased intermarriages in an ethnic group carrying IVSI-5 (G>C); carrier screening efforts that played a significant role in reducing marriages among Fr8-9 (+G) carriers; prenatal diagnosis may have helped in prevention of births with Fr8-9 (+G); better diagnosis of IVSI-5 (G>C) due to advanced molecular techniques e.g. ARMS-PCR and gene sequencing. This finding supports the effectiveness of a mutation-targeted diagnostic panel for early detection, especially in premarital and prenatal screening programs.

A study conducted at the national level by Usman *et al.* revealed that the IVSI-5 (G>C) was the most dominant *HBB* mutation among all the major ethnic groups in Pakistan.[12] A study by Khateeb *et al.* also demonstrated that IVSI-5 (G>C) followed by Fr 8-9 (+G) are the most prevalent mutations in the Sindh province of Pakistan.[13] Similar findings have been observed that IVSI-5 (G>C) followed by Fr 8-9 (+G) were the leading β-Thalassemia mutations in Northern Pakistan.[14] Contrasting trends have been observed in Khyber Pakhtunkhwa (KPK), where the high frequencies of Fr 8-9 (+G) and Codon 15 (G>A) were noted.[15] This variation in the mutation spectrum across various regions of Pakistan may be attributed to ethnic background and consanguineous and endogamous marriage practices which contribute to the maintenance of region-specific mutation clusters.

Among the β-Thalassemia mutations identified in our study, a novel mutation Cap+1 (A>C) (cap site mutation at transcription start site) was also reported at a very low frequency. Cap+1 (A>C) is a point mutation at the +1 position of the *HBB* gene's 5′UTR (5′ untranslated region), adjacent to the transcription start site, which affects the initiation of transcription.[16] This mutation occurs commonly in northern India, which serves as the hotspot of this mutation.[17] This result may be explained by the effects of migration and ancestral links.

Similar trends have been reported in Northern India where IVSI-1 (G>A), Codon15 (G >A) and Fr41-42 (-CTTT) represent the leading Beta thalassemia mutations.[18] In contrast, Fr41-42 (–TTCT) has been reported as the highly prevalent HBB mutation in Bangladesh.[19] This indicates that the mutation landscape of South Asian countries is closely aligned with that of Pakistan. Conversely, Mediterranean countries such as Turkey, Egypt and Spain demonstrate a markedly different mutation spectrum of β-Thalassemia major. IVSI-110 (G>A), IVSI-6 (T>C), IVSII-1 (G>A), IVSII-745 (C>G) have been reported as the most frequent mutations in Turkey,[20] IVSI-110 (G>A), IVSI-6 (T>C), and IVSI-1(G>A) were predominant mutation in Egypt,[21] IVSI-1(G>A), Codon39 (C>T), and IVSI-110 (G>A) emerged as the most common mutations across Spain.[22] The Mediterranean regions exhibit a distinctly

different molecular profile of β-Thalassemia major due to diverse population backgrounds.

Our results demonstrated the limited distribution of Codon5 (-CT), Codon30 (G>C), Codon15 (A>T), Del619, Fr16(-C) in the region. The findings reflect the necessity of including these mutations in the extended mutation screening panels targeting diverse ethnic backgrounds.

During our studies, three haemoglobin variants, i.e., HbE, HbS, and HbC, were found in 4.2% of cases. All these variants are produced as a result of a missense mutation, specifically HbE at codon 26 of exon 1 of *HBB*, while HbS and HbC are at codon 6 of exon 1 of *HBB*. These variants tend to produce a mild effect when co-inherited with β^{+}-mutated genes but lead to severe clinical manifestations in case of homozygous or β^{0} compound heterozygous states. HbE variant is the most prevalent Haemoglobin variant identified during our studies, this observation aligns with the findings by Mansoor *et al.* which stated that HbE is the most common haemoglobin variant in Pakistan.[23] HbD (Punjab) -the mutation historically specific to the region of Punjab,[24] has been reported in many prior studies; no case of HbD (Punjab**)** was noted in our study cohort. This might reflect changes in population genetics, or could simply be attributed to the relatively small sample size and the random distribution of mutations.

These findings highlight the significance of comprehensive molecular screening not only for common *HBB* mutations but also for haemoglobin variants that can greatly affect the phenotype when co-inherited with β-Thalassemia mutations. Inclusion of such variants in diagnostic panels may enhance the accuracy of disease diagnosis and patient management.

Nowadays, prevention of thalassemia is being addressed through population-specific molecular screening for carrier status, genetic counselling, and awareness of the disease at the urban and rural level is one of the major goals to prevent thalassemia. Our observations highlight the significance of population-specific molecular data for guiding β-Thalassemia carrier control programs.

CONCLUSIONS

This research underlines the molecular assessment of β-Thalassemia major in Punjab, Pakistan, identifying the prevalent and rare mutations. The findings suggest that IVSI-5 (G>C) succeeded by Fr8-9 (+G), Fr41-42 (-CTTT) and IVSI-1 (G>A) are broadly distributed mutations in our population. The findings highlight the presence of several less common and rare variants such as HbE, HbS, and HbC. The findings underscore the need for reinforcing region-specific diagnostic panels and establishing an accessible mutation database in Punjab that can better support clinicians and genetic counsellors in diagnosis, management, and genetic counselling of affected individuals with β-Thalassemia Major.

AUTHORS' CONTRIBUTION

MIT: Conceptualization of study design, data analysis, write-up. SF: Data interpretation. AM: Literature search. AA, SF: Proof reading. SM: Data collection.

REFERENCES

1. Zhang S, Chen Z, Chen M, Huang H. Current status and trends in thalassemia burden across South, East and Southeast Asia, 1990–2021 a systematic analysis for the global burden of disease study 2021. BMC Public Health 2024;24(1):3472.
2. Tuo Y, Li Y, Li Y, Ma J, Yang X, Wu S, *et al*. Global, regional, and national burden of thalassemia, 1990–2021: a systematic analysis for the global burden of disease study 2021. EClinicalMedicine 2024;72:102619.
3. Anwar MU, Bozdar M, Syeda SS, Shah SH. Beta Thalassemia Mutation Analysis in Fetal Samples for Optimal Mutation Screening Strategy. Pak Armed Forces Med J 2023;73(5):1396.
4. Khaliq S. Thalassemia in Pakistan. Hemoglobin 2022;46(1):12–4.
5. Miri-Aliabad G. Beta Thalassemia: An Overview of Epidemiology, Clinical Features, Diagnosis, and Management. Avicenna J Clin Med 2025;31(4):255–63.
6. Tariq A, Khurshid S, Sajjad M. Evaluation of the high resolution melting approach for detection of β-thalassemia gene mutations. Hemoglobin 2021;45(1):20–4.
7. Hassni MA, Din M, Babar KM, Lehri SA, Hasni MT, Raza MH, *et al*. Frequency of Different Types of Mutations in β-Thalassemia Major Patients Presenting in Dr. Akber Niazi Teaching Hospital Islamabad. Pak-Euro J Med Life Sci 2024;7(2):338a-h.
8. Baig SM, Azhar A, Hassan H, Baig JM, Kiyani A, Hameed U, *et al.* Spectrum of beta-thalassemia mutations in various regions of Punjab and Islamabad, Pakistan: establishment of prenatal diagnosis. Haematologica 2006;91(1):13–5.
9. Aziz A, Tehreem H, Aslam R, Javed MU, Ali MS, Riaz A, *et al.* Consanguineous marriages and thalassemia major in Pakistan: A cross-sectional study on awareness and prevalence. Am J Med Sci Pharm Res 2024;6(7):49–61.
10. Waqas M, Ahmed N, Malik HS, Qamar U, Javed H, Hussain A. Relationship of frequency of IVS 1-5 (GC), Fr 8-9 (+ G), Fr 41-42 and Cd-5 mutations of β-thalassemia traits with red cell indices. J Islamabad Med Dent Coll 2017;6(4):196–202.
11. Hassan K, Rasheed M, Asif N, Anwar T, Tahir M. Spectrum of mutations of beta thalassemia. J Islamabad Med Dent Coll 2017;6(4):196–202.
12. Usman M, Moinuddin M, Ghani R, Usman S. Screening of Five Common Beta Thalassemia Mutations in the Pakistani Population: A basis for prenatal diagnosis. Sultan Qaboos Univ Med J. 2009 Dec;9(3):305-310.
13. Khateeb B, Moatter T, Shaghil AM, Haroon S, Kakepoto GN. (2000). Genetic diversity of betathalassemia mutations in Pakistani population. Journal of Pakistan Medical Association, 50(9), 293-296
14. Haq EU, Naeem A, Awan UA, Khan SN, Zohaib M, Uddin G. High prevalence and molecular spectrum of β-thalassemia trait in Haripur and Abbottabad districts of Pakistan: implications for regional carrier screening programs. Egyptian Journal of Medical Human Genetics. 2025 Oct 15;26(1):167.
15. Jalil T, Yousafzai Y M, Rashid I, Ahmed S, Ali A, Fatima S, *et al*. Mutational Analysis of Beta Thalassaemia by Multiplex

ARMS-PCR in Khyber Pakhtunkhwa, Pakistan. J Ayub Med Coll Abbottabad 2019;31(1):98–103.
16. Haddad LA. Protein-Coding Genes. In: Human Genome Structure, Function and Clinical Considerations. Cham: Springer International Publishing 2021; p.93–138.
17. Garewal, Gurjeewan & Das, Reena & Awasthi, Anshu & Ahluwalia, Jasmina & Marwaha, *et al*. The clinical significance of the spectrum of interactions of CAP+1 (A→C), a silent β-globin gene mutation, with other β-thalassemia mutations and globin gene modifiers in north Indians. European journal of haematology. 2007; 79: 417-421.
18. Kuppusamy D, Ramachandran A, Nanda N, Kumar CG, Kar R. Characterisation of β-thalassemia mutations in a tertiary care referral hospital in southern India-A descriptive study. Indian J Hematol Blood Transfus 2024;28(3):1–7.
19. Chatterjee T, Chakravarty A, Chakravarty S, Chowdhury MA, Sultana R. Mutation spectrum of β-thalassemia and other hemoglobinopathies in Chittagong, Southeast Bangladesh. Hemoglobin. 2015 Nov 2;39(6):389-392.
20. Karaer DK, Ergün MA, RUHİ HI, Öztürk J, Kara H, Çakmak DR, Aydoğmuş T, PERÇİN FE. Development of a new real-time PCR screening kit for HbS and common beta-thalassemia mutations observed in Turkey. Turkish Journal of Medical Sciences. 2017;47(3):973-978.
21. El-Shanshory MR, Hagag AA, Shebl SS, Badria IM, Abd Elhameed AH, Abd El-Bar ES, *et al*. Spectrum of beta globin gene mutations in Egyptian children with β-thalassemia. Mediterranean journal of hematology and infectious diseases. 2014 Nov 1;6(1):e2014071.
22. Sánchez-Martínez LJ, Hernández CL, Rodríguez JN, Dugoujon JM, Novelletto A, Ropero P, Pereira L, *et al*.. Genetic variation patterns of β-thalassemia in Western Andalusia (Spain) reveal a structure of specific mutations within the Iberian Peninsula. Annals of Human Biology. 2021 Jul 4;48(5):406-417.
23. Mansoor N, Meraj F, Shaikh A, Jabbar N. Spectrum of hemoglobinopathies with hematological and biochemical profile: A five year experience from a tertiary care hospital. Pakistan Journal of Medical Sciences. 2022 Nov;38(8):2143.
24. Gupta V, Aggarwal P. Profile of hemoglobin D (HbD) disease in Eastern Uttar Pradesh: a single-center experience. Cureus. 2022 Oct 27;14(10).

Submitted: June 18, 2025	*Revised: December 14, 2025*	*Accepted: December 14, 2025*

Address for Correspondence:

Muhammad Irtza Tanveer, House No. 08, Street No. 08, Iqbal Road, Ghoray Shah, Lahore-Pakistan
Cell: +92 332 817 4199
Email: irtazatanveer316@gmail.com