RESEARCH ARTICLE

Spectrum of HBB gene variants and major endocrine complications in thalassemia patients of Pakistan

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Abstract

Objective: To determine the molecular characterisation of beta-thalassemia major patients, pattern of major endocrine complications and its association with haemoglobin subunit beta gene variants.

Method: The cross-sectional study was conducted from November 2021 to November 2022 after approval from the ethics review committee of Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan, and comprised of 88 patients with beta thalassemia major aged >8 years and having serum ferritin level >1000 µg/L. The subjects were analysed for haemoglobin subunit beta gene variants and major endocrine complications, like growth retardation, hypogonadism, hypothyroidism, hypoparathyroidism and diabetic abnormalities using an automatic chemistry analyser, fully automatic chemiluminescence immunoassay analyser, enzyme-linked immunosorbent assay and real-time polymerase chain reaction. Data was analysed using SPSS 25.

Results: Of the 88 subjects, 40(45.4%) were girls and 48(54.5%) were boys. The overall mean age was 12±2.81 years. Of the total, 55(62.5%) had growth retardation, 41(46.6%) were cases of hypogonadism, 16(18.1%) hypothyroidism, 5(5.7%) hypoparathyroidism, 3(3.4%) diabetes mellitus and 8 (9.1%) had impaired glucose tolerance. Also, 65(73.9%) patients confronted at least one endocrine complication. Endocrine complications were strongly associated with serum ferritin levels (p=0.000). The most common haemoglobin subunit beta gene variant identified was IVSI-5 (G>C) in 36(40.9%), and the least identified variant was cluster of differenctiation-CD26(G>A) 1(1.1%). The association between haemoglobin subunit beta gene variants with endocrine complications was statistically non-significant (p>0.05).

Conclusion: IVSI-5 (G>C) was found to be the most frequent haemoglobin subunit beta gene variant among beta-thalassemia major patients.

Keywords: Beta-thalassemia major, Endocrine complications, Growth retardation, Hypothyroidism, Parathyroidism, Diabetes mellitus. (JPMA 73: 2013; 2023) **DOI: https://doi.org/10.47391/JPMA.8599**

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Introduction

Thalassemia disorders represent a family of hereditary anaemias with an autosomal recessive pattern caused by the mutation in the gene cluster of haemoglobin (Hb), resulting in the impairment of globin chain synthesis.[1] A Hb molecule comprises four polypeptide chains, 2 alpha and 2 beta, attached to the heme molecule. The synthesis of defective Hb due to these faulty globin chains leads to the early lysis of red blood cells (RBCs).[2] Thalassemia disorder is categorised into alpha and beta thalassemia based on the defective alpha and beta polypeptide chains. Thalassemia major, thalassemia intermedia and thalassemia minor are the three clinical subtypes of beta thalassemia disease.[3] Beta thalassemia-major (BTM) patients face frequent haemolysis and ineffective erythropoiesis, leading to severe anaemia, thus requiring lifelong blood transfusions. Repeated transfusions lead to iron overload.[4] Intestinal iron absorption is also increased in thalassemia-major due to the hepcidin suppression by twisted gastrulation protein homolog-1 (TWSG1) and growth differentiation factor-15 (GDF-15), released from erythroblasts during ineffective erythropoiesis.[5] This excess iron amasses in tissues, especially the liver, heart and endocrine organs.[6]

Growth retardation, hypogonadism, hypoparathyroidism, hypothyroidism and glycaemic abnormalities are the major endocrine complications reported in iron-overloaded patients of BTM.[7] Patients with thalassemia illnesses have a frequent correlation between ferritin level and growth abnormalities. Reactive oxygen species (ROS) production is increased during iron overload, negatively affecting bone metabolisms and leading to growth retardation.[8] Iron accumulates in the pituitary gland of thalassemia patients causes the failure of the functioning of the hypothalamic-pituitary axis. It affects the serum levels of testosterone, luteinizing hormone (LH) and follicular stimulating hormones (FSH), impaired breast development in females, low spermatogenesis in males, and ultimately hypogonadism in both genders.[9] The excess iron in thyroid glands induces free radical damage and cellular-level lipid

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peroxidation, affecting thyroid functions and hypothyroidism.[10] Multi-blood transfusions in thalassemia-major patients cause iron deposition in the parathyroid gland that results in decreased parathyroid hormone (PTH), which in turn causes hypoparathyroidism.[11] Patients with thalassemia have a high risk of getting diabetes mellitus (DM). Iron accumulation in pancreatic beta cells damages these cells, and, thus, insulin resistance (IR) is moderated.[12] The gene mainly involved in beta-thalassemia disorder is the haemoglobin subunit beta (HBB) gene, which has more than 300 recognised mutations and 40 of them are the critical source of thalassemia disease.[13] The spectrum of HBB gene variants varies among the population of different ethnic groups. Molecular characterisation of beta-thalassemia patients in South-East Asia exhibited the most frequent mutation as IVS1+5G>C, trailed by cluster of differentiation-30 (CD-30) G>C. The rare mutations observed were FS8/9, IVS1+1G>T and CD-15.[14] In Bangladesh, nine variants of the HBB gene had been identified. The most prevalent were IVSI-5 G>C, IVSII-16 G>C, CD-26/HbE G>A and CD-2 T>C. While CD-1 T>A, CD-30 G>C, CD-2 C>A and IVS-II-81 C>T were the uncommon HBB gene variants.[15] A study on Arab, Fars, Lur, Bakhtiary and Kord ethnicities revealed IVSII-1 as the most repeatedly found mutation in Lur and Fars populations. Arab and Bakhtiary people had CD-36/37 mutation predominantly, and many Kord people had CD-8/9 mutation as well.[16]

The most common HBB gene variants observed in Pakistani thalassemia patients are IVS1-5 (G>C), Fr-8-9, Del-619bp, Fr-41-42, CD-5 (-CT), IVS1-1 (G>T), CD-15 (G-A) and CD-30 (G>C). Different areas represent variations in HBB gene variants within Pakistan. The most frequent HBB gene variants noted in Khyber Pakhtunkhwa (KP) province were Fr-8-9 (+G), Fr-41-42 (-TTCT) and CD-5 (-CT), while in Karachi IVS1-5 (G>C), IVS1-1 (G>T) and Fr-8-9 were commonly detected variants.[17,18] Variable genetic defects influence the rate of iron overload and the development of endocrine complications. For example, patients with the IVS-11-745 HBB gene variant expressed a higher frequency of DM. There is data scarcity about the association of HBB gene variants with the manifestation of endocrine complications persists, but a study on thalassemia patients in Egypt indicated the possibility of a relationship between HBB gene variants with endocrine complications.[19] The current study was planned to determine the molecular characterisation of BTM patients, pattern of major endocrine complications and its association with HBB gene variants.

Patients and Methods

The cross-sectional study was conducted from November 2021 to November 2022 after approval from the ethics review committee of Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, Pakistan. Convenient sampling technique was used to select the study subjects. Informed verbal consent was taken from the patients and parents. The sample size was calculated by using the formula:[20] n=Z2P(1−P)/d2 where n is the sample size, Z was taken as 95%, P (expected prevalence)[19] was used as 0.07%, and d the precision taken as 2%. The calculated sample size came to be 66. For the purpose of increasing accuracy and reliability, the calculated sample size was inflated by >20%, so the total study subjects examined were 88.

Those included were BTM patients aged >8 years having serum ferritin level >1000 µg/L. Those with a family history of hypogonadism, hypothyroidism, hypoparathyroidism, growth retardation, or DM were excluded.

The study was carried out in three phases. During the first phase, diagnosed thalassemia-major patients were identified and re-confirmed by history and medical record. In the second phase, growth retardation was examined by observing height-to-weight chart according to age and gender of the patients.[21] The gonadal status was observed by tanner scoring.[22,23] In the third phase, venous blood sample was drawn in vacutainers containing gel and ethylenediaminetetraacetic acid (EDTA) for laboratory investigations that were conducted at the Pathology and Molecular Biology and Genetics Laboratories of LUMHS. Serum calcium, serum phosphate, glucose tolerance test (GTT) and serum ferritin were analysed using an auto-chemistry analyser (Roche Modular-501). Free thyroxine (T4), thyroid stimulating hormone (TSH), estradiol, testosterone, FSH and LH were analysed using a fully automatic chemiluminescence immunoassay (Abbot Allinity). insulin-like growth factor-1 (IGF-1), growth hormone (GH) and intact parathyroid hormone (iPTH) were performed using enzyme-linked immunosorbent assay (ELISA) technique. For HBB gene variants, deoxyribonucleic acid (DNA) (QiAamp DNA Qiagen Mini-Kit with complete Meltpro® HBB Test, Pakistan) was used.

Data was analysed using SPSS 25. Frequency of different HBB gene variants and endocrine complications were expressed as frequencies and percentages. Association of endocrine complications with HBB gene variants, blood transfusion and demographic variables were also assessed. Pearson correlation coefficient was calculated between overall endocrine complications and individual HBB gene variants. Logistic regression analysis was done to analyse the association of HBB gene variants with growth retardation, hypogonadism, hypothyroidism, hypoparathyroidism and DM abnormalities separately. $P<0.05$ was taken as significant.

Results

Of the 88 subjects, 40(45.4%) were girls and 48(54.5%) were boys. The overall mean age was 12±2.81 years. The most common HBB gene variant identified was IVSI-5 (G>C) in 36(40.9%), and the least identified variant was CD-26 (G>A) 1(1.1%) (Table 1).

Of the total, 55(62.5%) had growth retardation, 41(46.6%) were cases of hypogonadism, 16(18.1%) hypothyroidism, 5(5.7%) hypoparathyroidism, 3(3.4%) DM and 8(9.1%) had impaired glucose tolerance (IGT) (Table 2). Also, 65(73.9%) patients confronted at least one endocrine complication.

Blood transfusion had no significant association with endocrine complications (p>0.05). Endocrine complications were strongly associated with serum ferritin level, male gender and rural residence (Table 3). The association between HBB gene variants with endocrine complications was non-significant (p>0.05).

Logistic regression analysis showed a very weak correlation (r=0.16) of overall endocrine complications with individual HBB gene variants.

Table-1: Molecular characterisation of beta thalassemia-major patients.

HBB Gene Variant	n (%)
IVSI-5(G>C)	36 (40.9)
CD8/9(+G)	13 (14.8)
CD30(G>C)	8 (9.1)
CD15(G>A)	7 (8)
IVSI-1 (G>T)	7 (8)
CD16(-C)	5 (5.7)
Wild type	3 (3.4)
CD5(-CT)	3 (3.4)
Del619bp	3 (3.4)
CD41/42(-TTCT)	2 (2.3)
CD26(G>A)	1 (1.1)
Total	88 (100)

HBB: Haemoglobin subunit beta, CD: Cluster of differentiation.

Table-2: Pattern of major endocrine complications among patients of beta thalassemia-major.

	Category of Endocrine Disorder	Status	n (%)
Growth Status	Growth Retardation	Yes	55 (62.5)
		No	33 (37.5)
Gonadal Status	Hypogonadism	Yes	41 (46.6)
		No	47 (53.4)
Thyroid Status	Hypothyroidism	Yes	16 (18.1)
		No	72 (81.8)
Parathyroid Status	Hypoparathyroidism	Yes	5 (5.7)
		No	83 (94.3)
Diabetic Status	Diabetes Mellitus	Yes	3 (3.4)
	Impaired Glucose	Yes	8 (9.1)
	No Diabetes	No	77 (87.5)

Table-3: Association of endocrine complications with demographic variables and blood transfusion.

Variables	Category	Endocrine Complications		p-value
		Yes [n (%)]	**No** [n (%)]	
Gender	Female	24 (60)	16 (40)	<0.008
	Male	41 (85.4)	7 (14.6)	
Residence	Urban	17 (60.7)	11 (39.3)	<0.056
	Rural	48 (80)	12 (20)	
Socioeconomic	Poor	48 (78.7)	13 (21.3)	<0.123
status	Middle	17 (63)	10 (37)	
Consanguineous	Cousin Marriage	53 (73.6)	19 (26.4)	<0.9
Marriage	Out of Family marriage	12 (75)	4 (25)	
Monthly Blood	1-2	49 (72.1)	19 (27.9)	<0.505
Transfusions	Three and above	16 (80)	4 (20)	
	6-10	2 (100)	-	
Serum Ferritin	up to 2000	20 (47.6)	22 (52.4)	<0.001
	2001 and above	45 (97.82)	1 (2.18)	

Discussion

In the current study, the most frequently found HBB gene variant was IVSI-5(G>C), followed by CD-8/9(+G), CD-30(G>C), CD-15(G>A), IVSI-1 (G>T), CD-16(-C), wild type (WT), CD-5(-CT), Del-619bp, CD-41/42(-TTCT) and CD-26(G>A). The findings were not too dissimilar with earlier studies done in India and Pakistan.[24,25] A study further expressed that IVS-I-110 and IVS-II-1 were the most frequent HBB gene variants in Arab population, while IVSI-1 and IVS-II-745 mutations were the most prevalent HBB gene variants in Syrian, Jordanian and Egyptian populations. The Saudi Arabians were the only people to have codon 39, codon 6, Cap +1 and IVS-I-5, whereas the people of Lebanon had codon 39 mutation.[26]

The major endocrine complications studied in Sri Lanka were hypogonadism, followed by growth retardation, hypothyroidism, DM and hypoparathyroidism.[27] The current study showed growth retardation, hypogonadism, hypothyroidism, hypoparathyroidism, DM and IGT in thalassemia-major patients. Comparable findings were found in Iranian thalassemia patients in a study which did not address growth retardation. The present study found that 73.9% BTM patients, 65(73.9%) had at least one endocrine complication. The Iranian study reported a corresponding value of 86.8%.[28] A study on 50 thalassemia patients in India showed thalassemia children having short stature, delayed puberty, hypothyroidism and DM.[29]

As for as the association of HBB gene variants with major endocrine complications, the present study found no significant association. A study done in Egypt presented contrasting findings.[30]

The current study has limitations as the subject needed long-term exploration, but owing to time and budget constraints, the time duration was limited even with a reasonable sample size. A comparison group of healthy controls would have added more value to it. The study tried to overcome this limitation by using a regression model.

The relationship of HBB gene variants with major endocrine complications may be found if the same study could be extended with a large sample size.

Conclusion

The most frequently found HBB gene variant in BTM patients was IVSI-5(G>C), followed by CD-8/9(+G) and CD-30(G>C), while the least common variant detected was CD-26(G>A). Majority of patients had at least one endocrine complication. There was a significant association between high serum ferritin with endocrine complications. There was no significant association of HBB gene variants with the major endocrine complications.

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References

1. Ayyash H, Sirdah M. Hematological and biochemical evaluation of β-thalassemia major (βTM) patients in Gaza Strip: A cross-sectional study. Int J Health Sci. 2018; 12:18-24.
2. Alkhtaua KJT, Al-Sammarraie HKI. TSH Correlation between thyroid-stimulating hormone (TSH) in Middle-aged with major beta-thalassemia. Int J Med Biol Med Sci. 2019; 2:01-8.
3. Aszhari F, Rustam Z, Subroto F, Semendawai A. Classification of thalassemia data using random forest algorithm. J Phys Conf Ser. 2020; 1490: 012050. DOI 10.1088/1742-6596/1490/1/012050
4. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol. 2020; 105:692-703. doi: 10.1111/ejh.13512.
5. Rahmani R, Naseri P, Safaroghli-Azar A, Tarighi S, Hojjati MT. Investigation of correlation between H63D and C282Y mutations in HFE gene and serum Ferritin level in beta-thalassemia major patients. Transfus Clin Biol. 2019; 26:249-52. doi: 10.1016/j.tracli.2019.05.003.
6. Fianza PI, Rahmawati A, Widihastha SH, Afifah S, Ghozali M, Indrajaya A, et al. Iron overload in transfusion-dependent Indonesian thalassemic patients. Anaemia. 2021; 4:1581831. doi: 10.1155/2021/5581831.
7. Tunç S, Sarbay H. Management of Endocrine Complications Affecting Survival and Quality of Life in Children and Adolescents with Thalassemia Major: A Single Center Experience. Cyprus J Med Sci. 2022; 7:120-8. DOI: 10.4274/cjms.2021.3362
8. Rathaur VK, Imran A, Pathania M. Growth pattern in thalassemic children and their correlation with serum ferritin. J Family Med Prim Care. 2020; 9:1166-69. doi: 10.4103/jfmpc.jfmpc_951_19.
9. Oehme NHB, Roelants M, Særvold Bruserud I, Madsen A, Eide GE, Bjerknes R, et al. Reference data for testicular volume measured with ultrasound and pubic hair in Norwegian boys are comparable with Northern European populations. Acta Paediatr. 2020; 109:1612-9. doi: 10.1111/apa.15159.
10. Rehman H, Masood J, Sheikh S, Mehboob Q. Frequency of hypothyroidism in patients of beta thalassemia major. APMC. 2019; 13:4-6.
11. Shawkat AJ, Jwaid AH. Clinical complications of beta-thalassemia major. IJPS. 2019; 28:1-8. DOI: https://doi.org/10.31351/vol28iss2pp1-8
12. Olivatto GM, Teixeira CRdS, Sisdelli MG, Zanetti ML, Silveira RCdCP, Gonçalves CV. Characterization of thalassemia major and diabetes mellitus patients at a reference center in Brazil. Hematol Transfus Cell Ther. 2019;41:139-44. doi: 10.1016/j.htct.2018.08.005.
13. Kountouris P, Kousiappa I, Papasavva T, Christopoulos G, Pavlou E, Petrou M, et al. The molecular spectrum and distribution of haemoglobinopathies in Cyprus: a 20-year retrospective study. Sci Rep. 2016; 6:1-10. doi: 10.1038/srep26371.
14. Mustafa I, Firdous N, Shebl FM, Shi Z, Saeed M, Zahir Z, et al. Genetic epidemiology of beta-thalassemia in the Maldives: 23 years of a beta-thalassemia screening program. Gene. 2020; 741:144544. doi: 10.1016/j.gene.2020.144544.
15. Sultana G, Begum R, Akhter H, Shamim Z, Rahim M, Chubey G. The complete Spectrum of beta (β) thalassemia mutations in Bangladeshi population. Austin Biomark Diagn. 2016; 3:1024-27.
16. Joola P, Andashti B, Hosseini SA, Zadeh SMM, Bahrami N. The frequency of beta-thalassemia mutations among carriers in Dezful city, southwest Iran. Iran J Public Health. 2020; 49:2438-40. doi: 10.18502/ijph.v49i12.4839.
17. Ansari SH, Shamsi TS, Bohray M, Khan MT, Farzana T, Perveen K, et al. Molecular epidemiology of β-thalassemia in Pakistan: far reaching implications. Blood. 2011; 118:5309-9.
18. Jalil T, Yousafzai YM, 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. 2019; 31:98-103.
19. Hassan T, Zakaria M, Fathy M, Arafa M, El Gebaly S, Emam A, et al. Association between genotype and disease complications in Egyptian patients with beta thalassemia: A Cross-sectional study. Sci Rep. 2018; 8:17730. doi: 10.1038/s41598-018-36175-9.
20. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterol Hepatol Bed Bench. 2013; 6:14-7.
21. Langtree I: Average Height to Weight Chart-Babies to Teenagers. [Online] [Cited 2017 November 30]. Available from: URL: https://www.disabled-world.com/calculators-charts/height-weight-teens.php
22. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970; 45:13-23. doi: 10.1136/adc.45.239.13.
23. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969; 44:291 doi: 10.1136/adc.44.235.291.
24. Shrivastava M, Bathri R, Chatterjee N. Mutational analysis of thalassemia in transfusion-dependent beta-thalassemia patients from central India. Asian J Transfus Sci. 2019; 13:105-9. doi: 10.4103/ajts.AJTS_115_18.
25. 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; 18:193-7. doi: 10.4103/0971-6866.100762.
26. Aqueel R, Anjum I. Beta Thalassemia: A Not-So-Rare Genetic Disorder In Pakistan. J Nat Appl Sci. 2019; 1:1-7.
27. Karunaratna A, Ranasingha JGS, Mudiyanse RM. Endocrine complications of beta–thalassemia major patients-cross-sectional study. Int J Blood Transfus Immunohematol. 2020; 10:1-7. doi:10.5348/100051Z02AK2020AK
28. Bordbar M, Bozorgi H, Saki F, Haghpanah S, Karimi M, Bazrafshan A, et al. Prevalence of endocrine disorders and their associated factors in transfusion-dependent thalassemia patients: a historical cohort study in Southern Iran. J Endocrinol Inves. 2019; 42:1467-76. doi: 10.1007/s40618-019-01072-z.
29. Dixit N, Shaw CK, Varshney GA, Kumar R, Saini PA, Verma P. Endocrinal complications in children and adolescents with thalassemia major in central India: an observational study. Indian J Pediatr. 2022; 89:983-88. doi: 10.1007/s12098-021-03883-6.
30. Al-Akhras A, Badr M, El-Safy U, Kohne E, Hassan T, Abdelrahman H, et al. Impact of genotype on endocrinal complications in β-thalassemia patients. Biomed Rep. 2016; 4:728-36. doi: 10.3892/br.2016.646.