Section: Blood Bank

Original Research Article

MUTATION ANALYSIS OF β-THALASSEMIA IN PATIENTS AT TERTIARY CARE: AN INITIAL PERSPECTIVE

Received : 10/01/2023
Received in revised form : 18/02/2023
Accepted : 14/03/2023

Keywords:
Mutations, B thalassemia, homozygous, traits, DNA sequencing, tertiary care.

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DOI: 10.47009/jamp.2023.5.2.187

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5(2); 882-886

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Abstract

Background: β-thalassemia is a heterogenetic clinical condition exhibiting a state ranging from non-transfusion dependent thalassemia towards the state of transfusion dependent thalassemia consequently challenging therapeutic decision of a Thalassemia traits vary in different individuals ranging from mild – severe thereby causing thalassemia major, thalassemia minor and thalassemia intermediate depending upon various genetic factors. This study was conducted with the aim to analyze the mutations of beta-thalassemia associated with patients in tertiary care. **Materials and Methods:** It is an observational retrospective study in which 50 patients were recruited on the basis of the thalassemia traits present in them. With an informed written consent of the participating patients 2ml of peripheral venous blood was drawn as per ICMR guidelines. CBC and clinical records were maintained. The 2ml sample was further investigated by capillary electrophoresis and cell counter parameters of blood phenotypes. Assessment of mutations present in the genes was done by using the DNA sequencing, amplification and refraction of polymerase chain reaction and end point polymerase chain reaction. Clinical implications - This study will play an essential role in highlighting the clinical knowledge related to the genetic modifier, pathophysiology, and other associated complications in relation to the monitoring of the patient. It will help the clinicians to identify the clinical scores of the patients in order to suggest a proper treatment protocol for them. **Result:** Total of 80 patients were referred to the tertiary hospital and among them 50 patients were tested positive for β-thalassemia. The mean age of the 50 participants was calculated out to be 34.5 ± 4.5. Mutational analysis reveals that all the patients were homozygous with HBB genetic mutations IVS 1:5 (G > C); c.92 + 5 [G > C] found in majority of the patients. **Conclusion:** The results of the study concluded that among different patients presented in a tertiary health care setting, all patients represented to have homozygous zygosity with most common gene mutations observed them to be IVS 1:5 (G > C); c.92 + 5 [G > C].

INTRODUCTION

β-thalassemia is a genetic hemoglobinopathy which occur due to defect in b globin locus present on chromosome 11. This locus is composed of five genes and constitute half of the oxygen carrying capacity of hemoglobin.[1] The locus is affected with more than300 mutations due to mutation in HB B gene of chromosome 11.[2,3] There is abnormal synthesis of hemoglobin beta chains resulting in severe anemic condition.[2]The incidence of B thalassemia has been observed to be 1/100,000 cases and severity is dependent upon the type of mutations present in the gene. Two beta chains are absent from the synthesis then the patient has the condition of thalassemia major also termed as Cooley's anemia and therefore patient requires blood transfusion throughout his life.[4]On the contrary, thalassemia minor is less severe and a symptomatic involving mild anemia and microcytosis thereby increasing the levels of HbA2.[5]Between both the condition of thalassemia major and thalassemia minor exists a

ISSN(O): 2687-5365; ISSN(P): 2753-6556

condition known as thalassemia intermediate which is totally dependent upon the severity of anemia.[3]

Mutation analysis of β-thalassemia in patients at tertiary care refers to the examination of the genetic mutations responsible for β-thalassemia in patients who seek treatment at a tertiary care facility.[6] This study is important for identifying the specific genetic mutations that cause the disorder in a particular population or region, which can inform the development of targeted screening and treatment programs.[7] Such studies typically involve analyzing DNA samples from affected individuals using techniques such as polymerase chain reaction (PCR) and sequencing to detect specific mutations.[8] The results of these analyses can provide valuable information on the prevalence of different mutations in a population and their clinical implications. Overall, mutation analysis of β-thalassemia is an important area of research that can help improve the diagnosis and treatment of this disorder, as well as inform efforts to prevent its transmission through genetic counseling and screening programs.[9,10]

Literature suggests that according to DNA analysis red blood cells are abnormal in all cases of β-thalassemia.[7] This analysis is conducted to evaluate the mutations present in the genes produced by beta and alpha globin.[8]In some cases, full family analysis is done for evaluating the status of carrier mutations in all the family members.[11]However, these mutations are specific in some geographical regions of the world with cases of thalassemia major most prominently seen in the Thailand. In Pakistan, it has been observed that there is no specific disease pattern and both the cases of thalassemia minor and major are observed.[12]In severe cases of thalassemia major, bone marrow transplants are usually suggested as the continuous blood transfusions sometimes exert an overload on the potential of iron.[13] Therefore, iron chelates are usually recommended for preventing the internal damage to the organs in case of overload of iron.[14] A study has been conducted which states that proper screening is essential to determine the patients of B thalassemia therefore, gene sequencing technology, electrophoresis, end point PCR and refractory amplification of polymerase chain reaction mutation system. This study was conducted to throw light on the analysis of mutations observed in B thalassemia patients of tertiary hospital.[15]

MATERIALS AND METHODS

This study was conducted at Department of Genetics, Maulana Azad Medical College, New Delhi, India. Duration of study was from Sept 2021 to Feb 2022. Total of 80 patients were referred to the tertiary hospital and among them 50 patients were tested positive for beta thalassemia. Inclusion criteria was patients tested positive for any of the stage of thalassemia. Mutational analysis was conducted for each of the recruited patients for determining the level of thalassemia i.e., minor, major and intermediate. All the patients were tested against the biochemical and clinical parameters and documented in pre-designed proforma. Study was conducted after getting approval from the ethical committee of the Institute. Informed consent was taken from all the patients prior to the conduction of study along with the demonstration of the study purpose to the patients.

Analysis of Data

Electrophoresis was done as the process of extracting the Hb levels from the capillary beds. From 2 ml of blood, samples of DNA were extracted by using DNA blood automatic machine Kit by using the kit manual. The extracted DNA genome was then stored at 4 o C until future use16.

Sequencing and Amplification

Thermal cycler was used for the purpose of amplification. The products of PCR were combined with gel of 2.5 % agarose and stained with ethidium bromide for visualizing it using transilluminating ultraviolet radiation. The sequencing was done by using big dye terminator sequencing kit for further highlighting the genetic mutations.[17]

Analysis of Gene Mutations

Gene mutations of globin B were particularized by PCR-ARMS for two sets of alleles required for the identification of eight different mutations such as c.92 + 5 (G > C), c.51 del C, c.27 dup G, c.126 _ 129 del CTTT, c.17_ 18 del CT, c.92 + 1 (G > A), c.79 G > A (Hb E), andc.48 G > A10. Genes of B thalassemia were individualized by direct sequencing of DNA through applied genetic analyzer biosystems (ABI). Sanger sequencing was used for the detection of c.92 + 5 (G > C) gene mutation. Analysis of the data was carried by using mutation surveyor and codon code aligner.[18]

RESULTS

Patients referred to the tertiary care hospital were analyzed for the traits of B thalassemia based on Hb levels. Following the mutational analysis of all the recruited patients, it has been observed that28 of the patients were having the HBB genetic mutation of IVS 1:5 (G > C); c.92 + 5 [G > C], 4 patients had gene mutation of Fr.41 /42 (-CTTT); c.126 _ 129 del CTT, 4 patients had gene mutations of IVS 1:1 (G > A); c.92 + 1 [G >A], Cd.16 (-C); c.51 del C, 2 patients had gene mutation of Cd./8/9 (+G); c.27 dup G, 2 patients had gene mutations of Cd.5 (-CT); c.17 _ 18 del CT, 8 patients had gene mutations of Cd.26 (G > A); c.79 G > A [Hb E], 1 patient had gene mutations of cd.15 (G > A) ; c.48 G > A.[19]Some patients reported to have thalassemia minor symptoms, some were reported to have thalassemia intermediate symptoms whereas few patients were having the symptoms of thalassemia major.[20] The hemoglobinopathies of these patients suggest that they have high levels of hemoglobin

ISSN(O): 2687-5365; ISSN(P): 2753-6556

along with decrease in the mean corpuscle level of hemoglobin and altered levels of Hb E (2.5 %), Hb F (3.5 %) and Hb D (6.7 %) calculated through capillary electrophoresis.[10]

Table 1: Gene mutations.

S. No	HBB gene mutations	Zygosity
1	Fr.41 /42 (-CTTT); c.126 _ 129 del CTT	Homozygous
2	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
3	Fr.41 /42 (-CTTT); c.126 _ 129 del CTT	Homozygous
4	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
5	IVS 1:1 (G > A); c.92 + 1 [G > A]	Homozygous
6	Cd.16 (-C); c.51 del C	Homozygous
7	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
8	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
9	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
10	Cd./8/9 (+G); c.27 dup G	Homozygous
11	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
12	Fr.41 /42 (-CTTT); c.126 _ 129 del CTT	Homozygous
13	Cd.5 (-CT); c.17 _ 18 del CT	Homozygous
14	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
15	Cd./8/9 (+G); c.27 dup G	Homozygous
16	IVS 1:1 (G > A); c.92 + 1 [G > A]	Homozygous
17	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
18	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
19	cd.15 (G > A); c.48 G > A	Homozygous
20	Fr.41 /42 (-CTTT); c.126 _ 129 del CTT	Homozygous
21	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
22	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
23	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
24	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
25	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
26	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
27	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
28	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
29	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
30	IVS 1:1 (G > A); c.92 + 1 [G > A]	Homozygous
31	cd.15 (G > A); c.48 G > A	Homozygous
32	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
33	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
34	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
35	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
36	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
37	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
38	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
39	Cd.26 (G > A); c.79 G > A [Hb E]	Homozygous
40	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
41	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
42	Cd.41 /42 (-CTTT); c.126 _ 129 del CTTT	Homozygous
43	Cd.41 /42 (-CTTT); c.126 _ 129 del CTTT	Homozygous
44	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
45	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
46	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
47	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
48	IVS 1:1 (G > A); c.92 + 1 [G > A]	Homozygous
49	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous
50	IVS 1:5 (G > C); c.92 + 5 [G > C]	Homozygous

DISCUSSION

β-thalassemia has been considered as a heterozygous disorder that can be inherited resulting from abnormal synthesis of hemoglobin on the B chain. β-thalassemia is most commonly found in people of Mediterranean, Middle Eastern, and South Asian descent, but it can occur in any ethnic group. Symptoms can include anemia, fatigue, bone deformities, and complications such as heart and liver disease.[15]It is essential to analyze and identify the carriers at the prenatal stage for planning its management in order to avoid any sort of miscarriages. The incidence of β-thalassemia mutations is not dependent upon the gender as the male to female ratio in different studies has been observed to be 1:1.[16]However, the variation in thalassemia is dependent upon several other factors such as ethnic diversity, inter racial marriages, regional differences, migration patterns, and designs of the study as observed by literature.[17] These all factors play an essential role in contributing towards the heterogeneity of mutations in β-thalassemia between and within populations. Treatment for thalassemia may include blood transfusions, iron chelation therapy (to remove excess iron from the

ISSN(O): 2687-5365; ISSN(P): 2753-6556

body), and folic acid supplements.[18] In severe cases, bone marrow transplant may be necessary. It is important for individuals with thalassemia to receive regular medical care and monitoring to manage their symptoms and prevent complications.[20] Genetic counseling is also recommended for individuals and families affected by thalassemia to better understand the condition and options for family planning.[21]

From the 50 patients recruited in the study, several mutations had been observed in them which varies from individuals to individuals. From total patients referred to the tertiary hospital, 70 % patients were diagnosed positively with thalassemia. Among them 55 % of the patients were diagnosed with Hb E traits.[22]The prevalence of various types of hemoglobinopathies specifically β-thalassemia, HbE, and Hb Dtraitsare the most common, followed by other types of hemoglobinopathies. The author suggests that this prevalence may be due to changing lifestyles, environmental factors, and genetic factors, as well as co-inheritance with other hemoglobinopathies such as HbE, HbD, and α-thalassemia. The author also notes that a smaller number of individuals with β-thalassemia major have heterozygosity/homozygosity and require blood transfusion.[23]

A study has been carried out which suggests that it is important to understand the prevalence and factors contributing to different types of hemoglobinopathies in order to improve prevention, diagnosis, and treatment strategies. The prevalence of co-inheritance of various types of hemoglobinopathies in Pakistan are HbE/β-thalassemia and HbD/β-thalassemia.[24] The author notes that these cases are less frequent and may have varying degrees of severity depending on genetic and environmental factors. The author also suggests that detected one case each of HbD/β-thalassemia and HbE/β-thalassemia, as well as several cases of HbE trait with mild heterozygous state. These patients may only require transfusion in severe conditions due to co-inheritance of the disease.[25]

The most common mutation found was IVS 1:5 (G > C); c.92 + 5 [G > C], followed by mutations of Cd.26 (G > A); c.79 G > A [Hb E].[10]These findings are consistent with other studies conducted in different geographical regions of Pakistan. However, the studies conducted in other populations, such as Thailand and China, found different mutations to be more prevalent, such as cd26 (A-G) HbE and cd41/42 (-TTCT) in Thailand and cd41/42 (-TTCT) and IVS-2 654 (C-T) in China.[15] This study also mentions other less common mutations found in the study, including c.92 + 5 (G > C), c.51 del C, c.27 dup G, c.126 _ 129 del CTTT, c.17_ 18 del CT, c.92 + 1 (G > A), c.79 G > A (Hb E), and c.48 G > A.[16]These findings highlight the genetic diversity of beta-thalassemia mutations and the need for population-specific screening and management strategies.[25]

CONCLUSION

The study conducted that out of 80 referral cases, 50 were affected by β-thalassemia. Among them, β-thalassemia carriers were the most common (55 %), followed by HbE trait and β-thalassemia major with heterozygous and homozygous conditions. The mutation IVS 1:5 (G > C); c.92 + 5 [G > C] exhibited the highest incidence in tertiary care hospital. The variation in incidence of these mutations is dependent on ethnic diversity, migration, genetic factors, and other lifestyle factors. The study recommends the need for mass screening, prenatal diagnostic techniques, genetic counselling, transfusion programs, and clinical management to be made available to affected populations before adopting assisted reproductive and preimplantation technologies in Pakistan.

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ISSN(O): 2687-5365; ISSN(P): 2753-6556