International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Study of IVS 1-5 (G→C) Mutation in the Beta Thalassaemia Patients of a Tertiary Care Hospital of North East India

Monalisha Saikia Borah[1], Prasanta Kumar Bhattacharya[2], MauchumiSaikia Pathak[3]

[1]Assistant Professor & Head, Department of Bioscience, Asian Institute of Management & Technology, Guwahati, Assam, India
[2]Professor & Head, Department of General Medicine, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya, India
[3]Professor & Head, Department of Biochemistry, Silchar Medical College & Hospital, Silchar, Assam, India
Corresponding Author: **Dr. Monalisha Saikia Borah,** Assistant Professor & Head, Department of Bioscience, Asian Institute of Management & Technology, Guwahati, Assam, India

Abstract: ***Thalassaemia is one of the most common inherited genetic disorders prevalent worldwide. In India, certain communities were identified with high risk of beta thalassaemia. There are more than 200 different types of beta thalassaemia mutations worldwide. The most common beta thalassaemia mutation, IVS 1-5 (G→C) is being analyzed here to get information whether this common mutation is prevailing among the domicile of Northeast region of India or not. The study included characterization of IVS 1-5 (G→C) mutations by ARMS-PCR. Among the total 460 cases referred for Haemoglobin typing, the occurrence of IVS 1-5 (G→C) was observed among the 149 cases having beta thalassaemia. Among the 149 studied cases, 94 cases (63.09%) were positive for IVS 1-5 (G→C) mutation. The rest 55 were negative for IVS 1-5 (G→C). This preliminary information regarding the mutational pattern is important for establishing prenatal diagnosis programmes. The results showed that, IVS 1-5 (G→C) mutations is the most frequent mutation encountered among the beta thalassaemic samples of this Northeast region of India.***

Keywords: Haemoglobinopathies, Thalassaemia, ARMS-PCR, IVS 1-5 (G→C) mutation

1. Introduction

Thalassaemia and hemoglobinopathies are one of the most commonly inherited genetic disorders worldwide and even in India. Certain communities in India have a high predisposition to beta-thalassemia. To offer prenatal diagnosis and to prevent the birth of an affected child, mutation testing in clinically diagnosed beta-thalassemia patients/carriers is a prerequisite [1].Population screening has identified certain communities in India with high risk of beta-thalassemia, the prevalence of carrier status in some being as high as 17% [2].Thalassaemia has been recognized by the World Health Organization as an important inherited disorder which has an impact mainly on the populations of low income countries. The prevalence of variant haemoglobins varies considerably with geographic location and racial group. Four haemoglobin variants, Hb S, Hb C, Hb E, and Hb D each affects millions worldwide and they represent a major public health problem in many areas of the world including South East Asia[3]. HbE trait is the most frequent haemoglobin disorder in Southeast Asia, where its prevalence is estimated to be 30%. Although Hb E trait is associated with no morbidity, the offspring of individuals who carry this haemoglobin variant may exhibit Hb E- β-thalassaemia if the other parent has β-thalassaemia trait and contributes that gene. This combination is the most common cause of transfusion-dependent thalassaemia in areas of Southeast Asia [4]. A high incidence of haemoglobinopathies and thalassaemias are encountered and their combination is unique for the northeast region of India. In upper Assam of Northeast India, there is a high rate of occurrence of these haemoglobinopathies and thalassaemia [5].The overall prevalence of β-thalassaemia trait was 2.78 % and varied from 1.48 to 3.64 % in different states, while the prevalence of β-thalassaemia trait in 59 ethnic groups varied from 0 to 9.3 %. HbE trait was mainly seen in Dibrugarh in Assam (23.9 %) and Kolkata in West Bengal (3.92 %). In six ethnic groups from Assam, the prevalence of Hb E trait varied from 41.1 to 66.7 %. Few subjects with δβ- thalassaemia, HPFH, HbS trait, Hb D trait, Hb E homozygous and Hb E β-thalassaemia as well as Hb S homozygous and Hb S-β-thalassaemia (<1 %) were also identified [6]. Hb E trait (15.42%) was the most common variant identified in rural community of Darjeeling district, West Bengal in an antenatal screening followed by the prevalence of homozygous Hb E, Hb E beta thalassaemia, beta-thalassaemia trait and HbS-trait was 6.91%, 0.53%, 2.12% and 1.06% respectively with a rare single case of haemoglobin J Meerut [7].

Majority of β-thalassaemia are caused by point mutations [8]. Studies on the molecular genetics of thalassaemia in various ethnic groups have shown that each group tends to have its own set of common mutations. These mutations affect the gene expression by a variety of mechanisms [9]. Worldwide more than 200 different β- thalassaemia mutations have been identified, and among them, about 28 mutations have been documented in Indian patients. 6 mutations, 619 bp deletion at 3' end of β- globin gene, IVS1- 5(G→C), IVS1-1 (G→T), frame-shift mutations FS 8/9, codon 41/42and nonsense codon 15, account for 90-94% of the beta-mutations in India [10].The IVS1-5 mutation is the commonest mutation found in the Indian population and its prevalence (in homozygous state) varies from 22.8 to 81.4% in different regions of India[11]. In India, mutations of codon 5 and codons 47/48 were found exclusively in migrants from Pakistan and mutation of codon 88 was detected only in subjects from Punjab,

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Haryana and Uttar Pradesh [12].Seven β- thalassaemia mutations accounting for 89 % (71 of 80) of the alleles in Eastern Indian population have been identified and majority (67.5%) was due to IVS-1 mutation [13]. In South Western Maharashtra 93.66 % in 126 β-thalassaemia carrier subjects were either IVS I-5 (G→C), IVS I-1 (G→T), codon 8/9, codon 41/42, codon 15 (G→A), and 619 bp and 6.34 % remained uncharacterized. 65.07 % showed the most common (prevalent) type of mutation, IVS I-5 (G→C), followed by IVS I-1 (G→T) showed by 9.52 % subjects. 2.38 % subjects showed 619 bp deletion, codon 8/9 and codon 15 (G→A) mutations were present in 6.34% each. Only 3.96% subjects showed codon 41/42 [14].

2. Materials and Methods

Ethical Clearance was obtained from the Institutional Ethics Committee for this hospital based study.

The blood samples were collected from anaemic subjects attending / admitted to Gauhati Medical College and Hospital, who were suspected of having variant haemoglobin after clinical observation and were referred for Haemoglobin typing. Subjects who were given blood transfusion within a period of 3 months were excluded from this study. The Gauhati Medical College & Hospital is a tertiary care hospital of Northeast region of India and many patients from all the neighbouring states comes here for medical treatment. A total of 460 cases from different regions of Northeast Indian populations were screened for Haemoglobinopathies and thalassaemia within a period of two years. Clinical and family history was recorded in a Proforma and the blood samples were collected after taking written Informed consent. In case of minor the parents/ guardians were asked to sign the consent form.About 2.5 ml of venous blood was collected in vaccutainer coated with Ethylene Diamine Tetra acetic acid (EDTA) as an anticoagulant. The blood samples were analyzed for Complete blood count (CBC) using the automated haematology analyzer (pocH-100*i*, SYSMEX CORPORATION, KOBE JAPAN) [15], within 24 hours of blood collection. On the same day itself the blood samples were screened for haemoglobinopathies and thalassaemia and the characterization of the samples along with quantification of the different Hb components, i.e. Hb A, Hb A_2, Hb A_2/E, Hb F, etc were done by the fully automated ion exchange high performance liquid chromatography (HPLC) based Haemoglobin Testing System (D-10,Bio Rad, USA) [16].

The samples which were positive for beta thalassaemia major or minor or samples which were positive for compound Hb E –beta thalassaemia and compound HbS-beta thalassaemia, those samples were stored in -20^{o}C freezer for molecular analysis.
The genomic DNA was isolated using column based genomic DNA extraction kits.

ARMS-PCR (Amplification Refractory Mutation System – Polymerase Chain Reaction) was done to identify the beta thalassaemia mutation pattern IVS1-5 (G→C). Primer sets which were selected for ARMS analysis of mutations for beta thalassaemia are shown in Table 1 [17]. For all ARMS-PCR reactions Primer C: 5--CAA TGT ATC ATG CCT CTT TGC ACC -3- and Primer D: 5--GAG TCA AGG CTG AGA GAT GCA GGA- 3' was used as control primers which yield a product size of 861 bp.

Table 1: Primer sequences used for the detection of the common beta thalassaemia mutation by ARMS – PCR

Mutation	Oligonucleotide Sequence	Second Primer	Product Size (Base Pair)
IVS1-5 (G→C), Mutant	CTCCTTAAACCTGTCT TGTAACCTTGTTAG	B	285
IVS1-5 (G→C), Normal	CTCCTTAAACCTGTCT TGTAACCTTGTTAC	B	285

*Note: Sequence of Primer B: ACCTCACCCTGTGGAGCCAC

Optimization of ARMS –PCR reaction was accomplished after several trials. A 25µl PCR reaction mix was prepared by adding Deionized water, 10X buffer containing 15mM $MgCl_2$, dNTPs, Internal Control Primers (Forward & Reverse), Mutant or Normal Primer, Common reverse primer for Mutant or Normal, Taq DNA Polymerase and the DNA sample.

To detect the mutations, ARMS-PCR programme was adopted according to Varawalla et.al. [17], with some modifications. Amplification is done with 1 cycle Initial denaturation at 93^{o}C for 5 mins, 25 cycles each of denaturation at 93^{o}C for 1 minute and annealing at 66^{o}C for 2 mins, 1 cycle each of extension at 66^{o}C for 3 minutes and final extension at 72^{o}C for 5 minutes and finally a 10^{o}C as holding temperature.

The ARMS-PCR products and the ladder marker are resolved by electrophoresis. DNA bands are visualized using Gel Documentation system and the pattern of bands obtained on the gel are observed by comparing both the mutant and normal set according to the DNA product size with that of the DNA ladder to detect the mutation.

The data generated from the study after investigations were computed and analyzed using Microsoft Excel.

3. Results

Information available for individual samples after HPLC indicates that out of the total 460 subjects, 313 (68.04%) were positive for haemoglobinopathies or thalassaemias and the rest 147 (31.96%) did not have any type of haemoglobinopathies or thalassaemia. Molecular study was carried out in the samples which were positive for beta thalassaemia minor, beta thalassaemia major, compound Hb E- beta thalassaemia and compound Hb S-beta thalassaemia. Molecular analysis revealed that out of the 149 beta thalassaemia cases studied for mutational pattern, IVS 1-5 (G→C) was the most common mutation identified among them.

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In all successful ARMS-PCR reactions, the internal control product of 861 bp molecular weight was observed, which was considered as a mandatory sign of successful reaction upon gel electrophoresis. So out of the 149 cases studied for beta thalassaemia mutational pattern, in 94 samples (63.09%), the mutational pattern IVS 1-5 (G→C) was detected (Figure: 1). The rest 55 samples (36.91%) were negative for the IVS 1-5 (G→C) beta thalassaemia mutation.

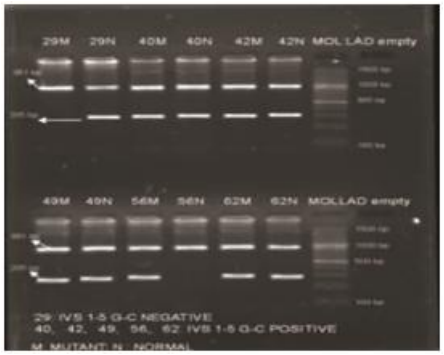


Figure 1: Gel picture showing IVS 1-5 (G→C) mutation

ARMS-PCR products of IVS-1-5(G→C) β-thalassaemia mutation on 2% agarose. Sample 40, 42, 49 and 52 are Heterozygous genotype; Sample 29 is negative for IVS 1-5 (G→C) mutation; Sample 56 is Homozygous genotype. Mol Lad: ladder marker DNA 100bp. N: Normal; M: Mutant. Empty: Nothing was loaded.

The results showed that, IVS 1-5 (G→C) mutations is the most frequent mutation among the studied beta thalassaemic samples of this region of India.

4. Discussion

The haemoglobinopathies and thalassaemia are genetic disorders and are prevalent worldwide. The most frequently observed haemoglobin variants in different parts of the world are Hb E, Hb S, Hb D, Hb C etc. Haemoglobin E is one of the most common haemoglobin variant prevalent especially in the South East Asian countries. The Northeast region of India along with Assam is a hot-spot zone for homozygous and heterozygous Hb E. Also beta thalassaemia is encountered among the domicile of Northeast region of India.

In this study, the molecular bases of beta thalassaemia have been investigated among individuals from the Northeast region of India. IVS 1-5 (G→C) beta thalassaemia mutation was encountered among the investigated individuals accounting for 63.089%. The study correlates with previous study by Varawalla et.al. [17], where the most common mutation identified among Asian Indians were IVS 1-5 (G→C).

According to a study by Panigrahi I., et.al. [18], IVS 1-5 (G→C) is the most common mutation in the Indian population and in the Eastern region of India a high frequency of IVS 1-5 (G→C) (72%) is being reported. Sinha S. et.al, [19], studied the β- thalassaemia mutations in India at state and regional levels and reported that the prevalence of IVS 1-5 (G→C) varied from 44.8% in the North to 71.4% in the East region of India and in the study the alleles from Northeast region (n=461) were included in the all India analysis where the prevalence rate of IVS 1-5 (G→C) was recorded to be 54.7 %. This present study on beta thalassaemia mutation patterns is relevant with the other beta thalassaemia profiling studies which were conducted by previous researchers.

Verma I.C. et.al., [12], characterized the mutations in the beta-thalassaemia gene and analyzed their regional distribution in India and found out that among the Indians who were not migrant from Pakistan, the predominant mutation was IVS 1-5 (G→C), varying from 85% in the southern states and 66-70% in the eastern states to 47-60% in the northern states and the pattern of the mutation identified is similar with the findings of this research work.

5. Conclusion

The study revealed that, haemoglobinopathies and thalassaemia was widespread among the people of the Northeast region of India.

In the present study, identification of IV1-5 (G→C) mutation was done. The molecular analysis revealed that among the 149 beta thalassaemia cases studied for mutational pattern, IVS 1-5 (G→C) mutation is the most frequent mutation observed among 63.09% of the beta thalassaemic samples of this Northeast region of India.

It may be concluded that in this region of the country, the rate of occurrence of these genetic disease is high. The occurrence of this inherited disease can be curbed by implementing awareness programs, by imparting genetic counseling, by carrier screening, and by screening high risk couples of beta thalassaemia. Reduction of the rate of occurrence of such genetic disease is very much important because patients with genetic disease like beta thalassaemia major are burden for the family and society. Properly designed community- based studies are required as a health priority to curb genetic diseases. Mutation patters of different communities may help in the quick identification of beta thalassaemia mutations for prenatal diagnosis.

Molecular analysis like profiling of the beta thalassaemia mutations at state and at regional levels are very much necessary for genetic education, screening and for genetic counseling.

Mutational pattern study may help in successfully establishing a program of genetic counseling and may help in prenatal diagnosis of beta thalassaemia in order to reduce the burden of this disease in the society.

It is very much important to identify the common beta thalassaemia mutation of a region as this may help in successfully establishing a genetic counseling program and may help in carrying out prenatal diagnosis.

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6. Future Scope

This is a preliminary study and there are certain limitations as it is a hospital based study, but in future, this type of study can be done in communities of Northeast region to getan exact rate of prevalence of these genetic diseases and in determining the common beta thalassaemia mutations of this region.

7. Competing Interests

There is no competing interest.

Acknowledgement

The authors are thankful to Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India for the financial support.

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Authors' Profile



Dr. Monalisha Saikia Borah, M.Sc., Ph.D. Specialized in Medical Biotechnology. Worked as Senior Research Fellow in DBT Project in Department of Biochemistry of Gauhati Medical College & Hospital, Assam and presently working as the Assistant Professor & Head of Department of Bioscience, Asian Institute of Management & Technology, Guwahati, Assam.



Dr. Prasanta Kr. Bhattacharya has done his MD in Medicine and did his PhD. He is presently the Professor & Head of General Medicine of NEIGRIHMS, Shillong, Meghalaya. He has many publications in reputed International and National Journals.



Dr. Mauchumi Saikia Pathak is MD in Biochemistry and presently she is the Professor & Head of Department of Biochemistry of Silchar Medical College & Hospital, Silchar, Assam. She has published many research papers in International and National journals.

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