Original Article

A Hospital Based Study Of Hb Variant and Beta Thalassaemia Mutational Pattern Characterization Among the People of Northeast Region of India

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ABSTRACT

Background: Haemoglobinopathies and thalassaemia are the most common genetic disorders prevalent worldwide. In North East India, Hb E, β- thalassaemia, Hb S and Compound HbE-β thalassaemia are the most prevalent Hb variants. *Aim:*Detection of Hb variants are done by High Performance Liquid Chromatography (HPLC) based Haemoglobin testing system. The beta thalassaemia mutation is being analyzed here to get first hand information on the type of beta thalassaemia mutation prevailing among the population of Northeast region of India.

Methods: The Hb variants were identified by HPLC based method and the beta thalassaemia mutations namely IVS 1-5 (G->C), IVS 1-1 (G->A), IVS 1-1 (G-T), Codon 8/9 and Codon 41/42 were characterized by ARMS-PCR.

Results: Among the 460 cases referred for Hb variant diagnosis, 313 (68.04%) were positive for Haemoglobinopathies or thalassaemias and the rest 147 (31.96%) did not have any type of haemoglobinopathies or thalassaemia. Total 149 cases were having either beta thalassaemia major, beta thalassaemia minor or compound heterozygous with beta thalassaemia. The mutational patterns were identified, in 105 samples (70.469%) and in the rest 44 samples (29.53%) the mutational patterns remained uncharacterized. Among the 105 samples, 63.09% were positive for IVS 1-5 (G->C) mutation and 7.38% were positive with Codon 41/42 mutation.

Conclusion: This preliminary information regarding the Hb variants occurrence and mutational pattern is important for establishing prenatal diagnosis programmes. The results showed that, Haemoglobinopathies and thalassaemia are common among the people of North east region of India and need counseling and awareness programme to reduce the risk of occurrence of this genetic disorder.

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Introduction

Haemoglobinopathies and thalassaemia are the most common haematological genetic disorders. Haemoglobinopathies are characterized by the production of structurally defective haemoglobin because of abnormalities in the formation of the globin moiety and thalassaemias are characterized by a reduced rate of normal haemoglobin due to decreased synthesis or absent of synthesis of the globin polypeptide chains. [1] With variable geographic distribution, haemoglobinopathies are the worldwide prevalent monogenic disorders.[2] In Southeast Asia and the Indian subcontinent, this has been considered as common disorders of blood posing a major genetic and public health problem.[3] 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. The structural alterations, referred in case of Haemoglobinopathies, are mostly due to the substitution of amino acid.[4] Haemoglobin E trait is the third most common haemoglobin disorder in the world and the most frequent in Southeast Asia, where its prevalence is estimated to be 30%. Although Haemoglobin E trait is associated with no morbidity, the offspring of individuals who carry this haemoglobin variant may exhibit haemoglobin 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.[5] 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.[6] 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 %. Hb E 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, Hb S 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. [7] Haemoglobin E (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 hemoglobin S-trait was 6.91%, 0.53%, 2.12% and 1.06% respectively with a rare single case of hemoglobin J Meerut.[8]

Majority of β-thalassaemias are caused by point mutations. [9] Studies on the molecular genetics of thalassaemia in various ethnic groups have shown that each group tends to have it is own set of common mutations. These mutations affect the gene expression by a variety of mechanisms.[10] More than 200 different β- thalassaemia mutations have been identified all over the world, and among them, about 28 mutations have been documented in Indian patients. 6 mutations, 619 bp deletion at 3' end of β- globin gene, IVS 1-5 (G->C), IVS 1-1 (G-T), Codon 8/9 and Codon 41/42 and nonsense codon 15, account for 90-94% of the beta-mutations in India.[11] The IVS-1-5 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, being the highest in Tamil Nadu in southeastern India. In the north-western part of India the 619 bp deletion mutation is the commonest beta-thalassaemia mutation observed in patients originating from Sindh, Gujarat or among the families migrated from Pakistan during partition of the country in 1947.[12] 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, Haryana and Uttar Pradesh.[13] 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. [14] 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 (+G), codon 41/42 (–TCTT), Codon 15 (G–>A), and 619 bp and 6.34 % remained uncharacterized. 65.07 % showed the most common 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 (+G) and codon 15 (G–>A) mutations were present in 6.34% each. Only 3.96 % subjects showed codon 41/42 (–TCTT).[15]

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 or from subjects who were already diagnosed with Hb variants but who were free of blood transfusion or blood transfusion not given within a period of 3 months. The Gauhati Medical

College & Hospital of Assam is one of the largest tertiary care hospital of Northeast region of India and many patients from all the neighbouring states of Assam 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 2 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),[16] 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).[17]

The samples which were positive for beta thalassaemia major or minor or samples which were positive for Compound Hb E –beta thalassaemia and Compound S-beta thalassaemia, those samples were stored in -20°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 among the five studied mutations viz. IVS1-5 (G->C), IVS1-1 (G->T), CD8/9, CD 41/42 (-TCTT) and IVS 1-1(G->A). Primer sets which were selected for ARMS analysis of mutations for beta thalassaemia are shown in Table 1 and 2. [18,19] 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 an internal control which yield a product size of 861 bp.

Optimization of ARMS –PCR reaction was accomplished after several trials. A 25**µl** 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., 1991, with some modifications. Amplification is done with 1 cycle Initial denaturation at 93 °C for 5 mins, 25 cycles each of denaturation at 93°C for 1 minute and annealing at 66°C for 2 mins, 1 cycle each of extension at 66ºC for 3 minutes and final extension at 72°C for 5 minutes and finally a 10°C as holding temperature.

Table 1: Primer sequences used for the detection of the common beta thalassaemia mutations by ARMS – PCR.[18, 19]

MUTATION	OLIGONUCLEOTIDE SEQUENCE	SECOND PRIMER	PRODUCT SIZE (BASE PAIR)
IVS1-5 (G->C)	CTCCTTAAACCTGTCTTGTAACCTTGTTAG	B	285
IVSI-1 (G->T)	TTAAACCTGTCTTGTAACCTTGATACGAAA	B	281
Cd 8/9 (+G)	CCTTGCCCCACAGGGCAGTAACGGCACACC	B	225
Cd 41/42 (-TCTT)	GAGTGGACAGATCCCCAAAGGACTCAACCT	B	439
IVSI-1 (G->A)	TTAAACCTGTCTTGTAACCTTGATACCGAT	B	281

***Note:** Sequence of Primer B: ACCTCACCCTGTGGAGCCAC

Table 2: Primer sequences used for the detection of the normal DNA sequences by ARMS –PCR.[18, 19]

MUTATION	OLIGONUCLEOTIDE SEQUENCE	SECOND PRIMER	PRODUCT SIZE (BASE PAIR)
IVS1-5 (G->C)	CTCCTTAAACCTGTCTTGTAACCTTGTTAC	B	285
IVSI-1 (G->T)	GATGAAGTTGGTGGTGAGGCCCTGGGTAGG	A	455
Cd 8/9 (+G)	CCTTGCCCCACAGGGCAGTAACGGCACACT	B	225
Cd 41/42(-TCTT)	GAGTGGACAGATCCCCAAAGGACTCAAAGA	B	439
IVSI-1 (G->A)	TTAAACCTGTCTTGTAACCTTGATACCCAC	B	281

***Note:** Sequence of Primer A: CCCCTTCCTATGACATGAACTTAA
Sequence of Primer B: ACCTCACCCTGTGGAGCCAC

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 product size with that of the DNA ladder to detect the mutations accordingly.

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

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. The occurrence of the different type of Hb variants in the study group were Hb E heterozygous (110, 23.9%); Hb E homozygous (32, 6.9%); Beta thalassaemia minor (101, 21.9%); Beta thalassaemia major (10, 2.7%), Compound Hb E- beta thalassaemia (37, 8.04%); Hb S trait (14, 3.04%); Hb S disease (8, 1.7%)and Compound Hb S- beta thalassaemia (1, 0.22%) and No Haemoglobinopathies or thalassaemia(147, 31.96%) (Figure 1: Bar Diagram Showing Occurrence of Haemoglobinopathies and 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.

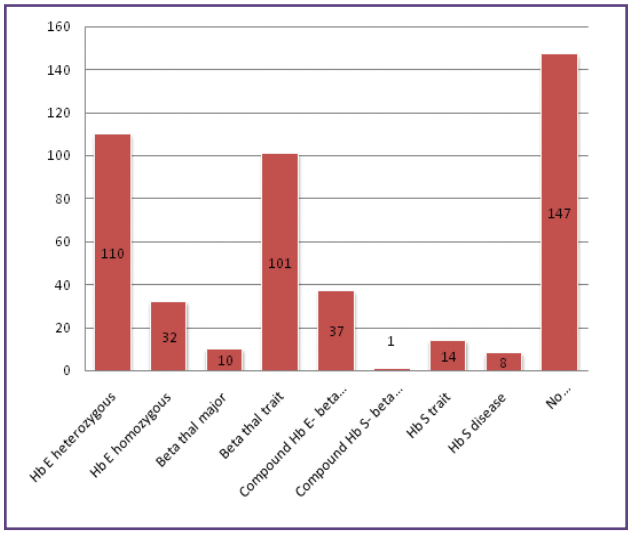

Fig. 1: Bar Diagram Showing Occurrence of Haemoglobinopathies and Thalassaemia

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 of the amplified products. So out of the 149 cases studied for beta thalassaemia mutational pattern, in 105 samples (70.469%), the mutational patterns were identified. Among which 63.09% were positive for IVS 1-5 (G->C) mutation [Figure 2] and 7.38% were positive with Codon 41/42 mutation [Figure 3]. In the rest 44 samples (29.53%) the mutational pattern remained uncharacterized. The results showed that, IVS 1-5 (G->C) mutations is the most frequent mutation compared with other mutations studied among the beta thalassaemic samples of this Northeast region of India.

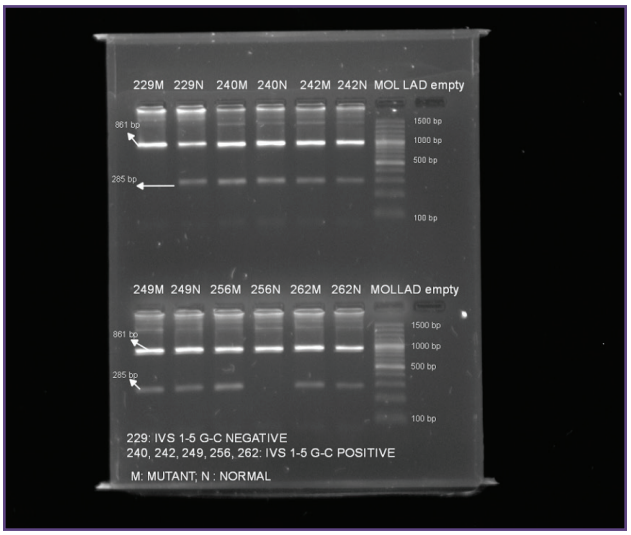

Fig. 2: Gel picture showing IVS 1-5 G->C Beta Thalassaemia Mutation

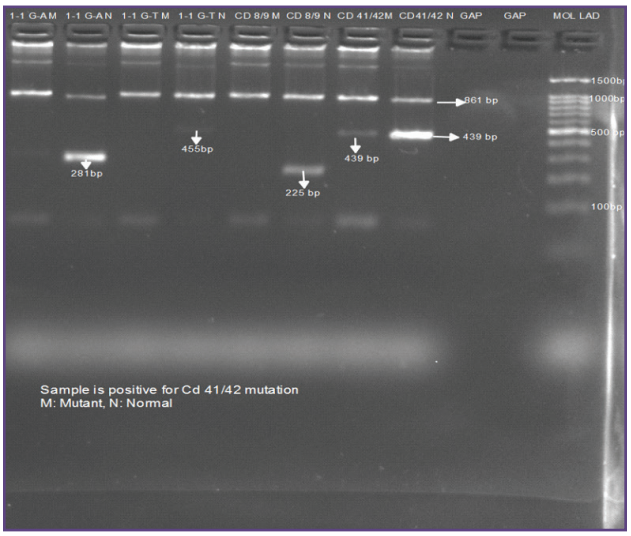

Fig. 3: Gel Picture Showing Sample Positive For Cd 41/42 Beta Thalassaemia Mutation

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 Hb S, Compound Hb E- beta thalassaemia and beta thalassaemia is encountered among the people of Northeast region of India.

In this study, the molecular basis of beta thalassaemia have been investigated among individuals from the Northeast region of India and 2 different β- thalassaemia mutations among the five studied were detected in 70.47% of the individuals investigated. Out of the studied cases, the IVS 1-5 (G->C) was the most common beta thalassaemia mutation encountered among the investigated individuals accounting for 63.089% and the beta thalassaemia mutation Codon 41/42 was detected in 7.38% of the individuals. The study correlates with previous study by Varawalla et.al.,[19] where the most common mutation identified among Asian Indians were IVS 1-5 (G->C). Also in that study the prevalence rate of IVS 1-5 (G->C) mutation and codon 41/42 mutation encountered in individuals from Bangladesh and Bengal were 60% and 20% respectively which are the neighbouring region of the Northeast India and in this study also the mutations IVS 1-5 (G->C) and codon 41/42 mutations detected in the study group are 63.089% and 7.38% respectively which slightly coincides with the previous study of Varawallla et.al., (1991).

According to a study by Panigrahi I., et.al.,[20] 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 followed by 11% prevalence of codon 41/42 mutation. Sinha S. et.al.,[21] 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 % and codon 41/42 prevalence rate was recorded as 6.1%. This present study on beta thalassaemia mutation patterns is relevant with the other beta thalassaemia profiling studies which were conducted by other researchers.

Verma I.C. et.al.,[13] characterized the mutations in the beta-thalassaemia gene and analyzed their regional distribution in Indi a 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. Mutations at codon 8/9 and codon 41/42 were distributed in all regions of India with frequency varying from 3% to 15% and the pattern of the mutations identified is similar with the findings of this research work.

Vaz F.E.E. et.al.,[22] analyzed beta thalassaemia mutations among the Indian population referred to their diagnostic centre, by ARMS PCR and the state- wide and community-wide distribution patterns of mutations indicated that IVS 1-5 (G->C) is the most common beta thalassaemia allele in the Indian population. Comparatively, in this research work also the most common beta thalassaemia mutation identified is IVS 1-5 (G->C) among the individuals of Northeast India.

On the other hand, codon 8/9, IVS1-1 (G->A) and IVS 1-1 (G->T) mutations were not found in all the beta thalassaemic samples investigated in this study. However, this does not means that these mutations were not present in Northeast Indian individuals, taken into consideration the small number of samples which, of course, were not presenting the real number of the thalassaemic patients in Northeast India. Moreover, the results showed that, IVS-1-5 (G->C) and codon 41/42 mutations were the higher frequent compared with other mutations studied in the thalassaemic samples.

Conclusion

The study revealed that, haemoglobinopathies and thalassaemiais widespread among the people of the Northeast region of India. Identification of mutational pattern among the five beta thalassaemia mutations IV1-5 (G->C), Codon 41/42, IVS 1-1(G->T), IVS1-1(G->A) and Codon 8/9 were done and the molecular analysis revealed that out of the 149 beta thalassaemia cases studied for mutational pattern, IVS 1-5 (G->C) mutations is the most frequent mutation compared with other mutations studied among the beta thalassaemic samples of this Northeast region of India.

The mutations codon 8/9, IVS1-1 (G->A) and IVS 1-1 (G->T) were not found in the beta thalassaemic samples investigated in this study. However, this does not implies that these mutations were not present in Northeast Indian individuals, because the number of samples in this study was small, which, of course, were not presenting the real number of the thalassaemic patients in Northeast India. More extensive study is required to determine the exact prevalence rate of the mutational pattern.

It may be concluded that in this region of the country, the rate of occurrence of these genetic disease is high. Northeast region of India is a hotspot zone for the variant Hb E. 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.

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Competing Interests
There is no competing interest.

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References
1. Das R. Disorders of Haemoglobin Structure and Synthesis. In: Saxena R, Pati HR, Mahapatra M, editors. deGruchy's Clinical Haematology in Medical Practice, 6thed. Wiley India Pvt. Ltd.; 2013. 120-145.
2. Krafft A, Breymann C. Haemoglobinopathy in Pregnancy: Diagnosis and Treatment. Current Medicinal Chemistry. 2004; 11 (21):2903-2909.
3. Fucharoen S, Winichagoon P. Haemoglobinopathies in Southeast Asia: molecular biology and clinical medicine. Haemoglobin. 1997; 21: 299-319.
4. Lukens JN. The abnormal hemoglobins: General Principles. In Lee GR, Foerster J, Lukens JN, Paraskevas F, Greer JP and Rodgera GM editors. Wintrobe's Clinical Hematology, 10th ed.Maryland, USA: William & Wilkins publisher; 1999. 1329-1345.
5. Goldbloom RB. Screening for Haemoglobinopathies in Canada. In: Canadian Task Force on the Periodic Health Examination. Canadian Guide to Clinical Preventive Health Care. Ottawa: Health Canada.
6. Baruah MK, Saikia M, Baruah A. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India- High performance liquid chromatography studies in 9000 patients. Indian J PatholMicrobiol.2014;57:236-43.
7. Mohanty D, Colah RB, Gorakshakar AC, et.al. Prevalence of beta thalassaemia and other Haemoglobinopathies in six cities in India- a multi centre study. J. Community Genetic. 2013; 4: 33-42.
8. Ghosh N, Chakrabarti I, Chakraborty M, Goswami BK. A community based pilot study on prevalence of haemoglobinopathies among the antenatal women in a rural area of Darjeeling district, West Bengal. Int J Med Public Health. 2013; 3:107-10.
9. Rund D, Rachmilewitz E. Beta-thalassemia. New Eng. J. Med. 2005; 353(11): 1135-46.
10. Owen TM, Chan KD, Dietz L, Zehnder JL, Schrijver I. Comprehensive and Efficient HBB Mutation Analysis for Detection of Hemoglobinopathies in a Pan-Ethnic Population. Am J ClinPathol. 2010; 133:700-707.
11. Balgir RS. The Burden of haemoglobinopatheies in India and the challenges ahead. Current Science. 2010; 79 (11): 1536-1547.
12. Grow K, Vashist M, Abrol P, Sharma S, Yadav R. Beta thalassaemia in India- current status and the challenges ahead. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(4): 28-33.
13. Verma IC, Saxena R, Thomas E, Jain PK. Regional distribution of beta thalassaemia mutations in India. Human Genetics. 1997; 100 (1): 109-13.
14. Dastidar DG, Dutta RN, Gupta P, Old JM. Detection of beta thalassaemia mutation in eastern Indian population by polymerase chain reaction. Indian J Med Res. 1994; 100: 111-114.
15. Satpute SB, Bankar MP, Momin AA. The prevalence of β-thalassaemia mutations in south western Maharashtra. Indian Journal of Clinical Biochemistry. 2012; 27(4); 389-393.
16. Instruction Manual.pocH-100i, SYSMEX Corporation, Kobe, Japan, Asia Pacific Edition B.
17. Instruction Manual. D-10 Dual Program, Bio-Rad Laboratories, United States.

18. Ithanet Electronic Infrastructure for Thalassaemia Research Network. Protocol for Amplification Refractory Mutation System (ARMS). Available from http://portal.ithanet.eu.
19. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of beta thalassaemia mutations on the Indian subcontinent- the basis for prenatal diagnosis. Br.J. Hematol. 1991; 78:242-247.
20. Panigrahi I, Marwaha RK. Mutational spectrum of thalassaemias in India. Indian Journal of Human Genetics. 2007; 13(1): 36-37.
21. Sinha S, Black ML, Agarwal S, Colah R. et. al. Profiling beta thalassaemia mutations in India at state and regional levels- implications for genetic education, screening and counseling programmes. The HUGO Journal.2009; 3:51-62.
22. Vaz FEE, Mahadik CBT, Banerjee MK, Gangal SG. Distribution of beta thalassaemia mutations in Indian population referred to a diagnostic center. Hemoglobin. 2000, 24(3): 184-194.