Panacea Journal of Medical Sciences 2024;14(3):654–658

Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Original Research Article

Antenatal screening for β thalassemia with mutation analysis in a tertiary care centre of Eastern India

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ARTICLE INFO

Article history:
Received 07-04-2024
Accepted 20-05-2024
Available online 21-12-2024

Keywords:
Reverse Hybridization Technique
Dot Blot Analysis
Antenatal Thalassemia β Screening

ABSTRACT

Introduction: β-thalassemia poses a major public health problem in India. Reverse hybridization StripAssay® method is reported to be rapid, simple, reproducible and less expensive.
Aim: The aim of the study was to detect occurrence of β-thalassemia carriers among the ante-natal mothers by Hb HPLC followed by detection of β-thalassemia mutations in antenatal mothers with β-thalassemia carrier state as well as borderline HbA2 value. The second step of this study was detection of β- thalassemia mutations in husbands of antenatal mothers who are β-thalassemia carriers as detected by Hb HPLC.
Materials and Methods: Total 734 antenatal mothers were recruited in the study after obtaining informed consent. All were screened by Hb HPLC and mutation analysis done as per study protocol by reverse hybridization StripAssay®.
Result and Conclusion: Among 734 antenatal mothers who were included in the study, 30 subjects (4.08%) were β- thalassemia trait (BTT) and 24 (3.27%) of them had borderline HbA2 value as per Hb HPLC done by Biorad D10^{TM}. Commonest mutant allele detected was IVS 1-5(G>C).

1. Introduction

β -thalassemia is an inherited autosomal recessive disorder in which more than 200 different mutations affecting different levels of β -globin gene expression result in a β-thalassemia phenotype.[1,2] Although a limited number of alleles account for the majority of β -thalassemia sub-types which result from point mutations or deletions/insertions of just one or two nucleotides.[3] The widely studied method of detecting the relative frequency of different β -thalassemia mutations and their association with β globin haplotypes are polymerase chain reaction-based methods for known mutations such as amplification refractory mutation analysis (ARMS), heteroduplex analysis, restriction enzyme and dot-blot analyses. All these methods are labour intense, time consuming and more liable to contamination compared to the reverse hybridization StripAssay® method which is characterized by rapid turnaround time, high reproducibility and less liable to contamination.[4]

2. Aims

This study was designed to detect occurrence of β-thalassemia carriers among the ante-natal mothers by Hb HPLC followed by detection of β-thalassemia mutations in antenatal mothers with β-thalassemia carrier state as well as borderline HbA_2 value. The second step of this study involved detection of β- thalassemia mutations in husbands of antenatal mothers who are β-thalassemia carriers as detected by Hb HPLC.

3. Materials and Methods

This is a hospital based cross-sectional study conducted from September 2021 to August 2022 in the Dept.

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https://doi.org/10.18231/j.pjms.2024.116

of Pathology, ESI-PGIMSR Manicktala, Kolkata, West Bengal after obtaining approval from Institutional Ethical Committee.

All antenatal mothers registered at the antenatal clinic of the said institute were included in the study for β thalassemia screening by Hb HPLC irrespective of gestational age. Hb HPLC was done by BIORAD D-10™ which is an automated HbA1c and HbF/HbA2 system that utilizes gold standard ion-exchange HPLC technology. Subjects having HbA_2 value 3.5%-07% were evaluated for β thalassemia mutation study by β-globin StripAssay® IME. Spouses of all antenatal mothers having HbA_2 value 04%-07% were evaluated for β thalassemia mutation study by β-globin StripAssay® IME.

Antenatal mothers were divided into two groups ,first one with HbA2 value 3.5-3.9% (borderline value- a total of 24 individuals) and second group comprises the antenatal mothers with HbA2 value 4.0-7.0% (beta-thalassemia carriers - a total of 30 individuals). Mutation study was done on a total of 54 individuals.Mutation study was done on spouses of the beta-thalassemia carrier mothers irrespective of their HbA2 value (a total of 19 individuals, note:- all the 30 husbands of beta-thalassemia carrier mothers were contacted, out of which only these 19 individuals gave consent to be included in the study.)

Borderline HbA_2 value was defined as 3.5% - 3.9% & β-thalassemia carrier was defined as 04%-07%.[5–7]

All subjects were recruited in the study after taking written informed consent.

Unwilling subjects, persons with history of blood transfusion within last 12 weeks and subjects having hyperthyroidism, thyrotoxicosis or undergoing anti-retroviral treatment for HIV were excluded from the study.

From each subject 02 mL of blood was collected each in an EDTA and a clot enhancer vacutainer. Initial evaluation for the following parameters was done:

Hemoglobin level, RBC indices (PCV, MCV, MCH, MCHC, RDW-CV), & Mentzer index, Hb HPLC.

Targeted mutation analysis by β-globin StripAssay®IME (CE & IVD approved) in cases meeting criteria discussed above (Table 1).

Table 1: Twenty-two β-globin mutations covered by the StripAssay®IME

Position	Sequence Alteration	β thalassemia type
cap+1	A>C	β^+
codon 5	-CT	β
codon 6	A>T(HbS)	-
codon 8	-AA	β^0
codon 8/9	+G	β^0
codon 15	TGG>TAG	β^0
codon 16	-C	β^0
codon 22	7bp del	β^0
codon 30	G>C	β^0
IVS 1.1	G>A	β^0
IVS 1.1	G>T	β^0
IVS 1.5	G>C	β^+
IVS 1.6	T>C	β^+
IVS 1.110	G>A	β^+
IVS 1-25	25bp del	β^0
codon 36/37	-T	β^0
codon 39	C>T	β^0
codon 41/42	-TTCT	β^0
codon 44	-C	β^0
IVS 2.1	G>A	β^0
IVS 2.745	C>G	β^+
619bp del		β^0

Statistical analysis was done by MS Excel 2021

4. Result

In the present study total 734 antenatal mothers were included as per inclusion criteria. It was observed in this study, that only 31.48% of antenatal females had registered themselves in the antenatal clinic on or before 12 weeks of gestation. Among them 30 subjects (4.08%) were β thalassemia trait (BTT) and 24 (3.27%) of them had borderline HbA_2 value as per Hb HPLC done by Biorad D10™. Only 6 BTT out of 30 (20%) were aware of their carrier status beforehand. Husbands of all 30 BTT pregnant women were contacted. Only 19 (63.33%) of those spouses turned up and were undergone Hb HPLC.

Table 2: Clinical and laboratory data of 54 antenatal mothers

Parameters	Mean (SD)
Age (Years)	26.89 (± 5.87)
Gestational age (weeks)	15.15(±4.59)
Laboratory Results	
Hb (g/dL)	10.59 (±1.43)
RBC	4.68 (±0.89)
Hct (%)	34.46 (±3.92)
MCV (fL)	75.4 (±12.9)
MCH (pg)	23.2 (±4.4)
MCHC	30.56 (±1.26)
Hb HPLC	
HbF (%)	1.12 (±0.85)
HbA2(%)	4.64(±1.02)

Among 24 cases with borderline HbA_2 values, 21 had MCH > 27 pg and MCV >80 fl in 22 cases.

RDW-CV <14% in 04 cases and Mentzer index >13 in all cases.

The result of mutation study of all the 30 cases of β thalassemia traits are summarized in Table 3.

Amongst husbands of 19 BTTs, though no β-globin chain mutation could be identified in any of these cases by the β-globin StripAssay® IME utilized in the present study, the possibility of presence of other mutations cannot

Table 3: Result of mutation study of 30 cases of β thalassemia traits

Mutation (Heterozygous state)	Number of Subjects
IVS 1-5 (G>C)	26
Double heterozygous for IVS 1-5 (G>C) and codon6 (A>T)	1
Codon 41/42[-TTCT]	1
Codon 30[G>C]	1
Codon 15 (TGG>TAG)	1

be ruled out which are not covered in the StripAssay® used in the current study.

5. Discussion

This study was conducted at the Department of Pathology of ESI-PGIMSR Manicktala, Kolkata, West Bengal from September 2021 to August 2022. In this time-period total 734 pregnant women had reported to the department for antenatal screening of β thalassemia.

The patients who had agreed to be included in this study had undergone different evaluations like CBC, Hb HPLC, Serum iron profile. β-globin mutation analysis was done by β-globin StripAssay® IME (Vienna Lab Diagnostics GmbH) in relevant cases.

5.1. Demography

From the data generated it was inferred that the occurrence of β- thalassemia carrier in antenatal mothers is 4.08%.This corroborated with previous studies citing the prevalence of β-thalassemia carriers among Indian antenatal mothers which had been found to be ranging from 2.78% to 8.45%.[8–10] Similarly one study conducted in neighboring districts of West Bengal (Burdwan, Undivided Midnapur and 24-Parganas North) found the prevalence to be 4.61%.[11]

As observed in the present study, the occurrence of mothers with borderline HbA_2 (3.5%-3.9%) was 3.27% which was substantially lower than the finding by Giambona et al.[12] In the later study in Italian population, prevalence of borderline HbA_2 values were found to be 16.75 % out of one screening program where borderline HbA_2 values has been defined as between 3.1 to 3.9%.[12] This observation is more than that of reported by Mosca et al, where the prevalence of borderline HbA_2 level (3.3%-3.7%) were found to be 2.2% and 3% respectively in two different centers in Italy.[13] Probably this is due to the difference in the range of HbA_2 values considered for defining the borderline cases. In a study conducted in Malaysia, Rosnah et al found the prevalence to be 3.4% when borderline HbA_2 level defined between 3.0%-3.9%.[14]

It was seen in our study that in some cases husbands of BTT mothers did not turn up for blood sample collection which prevented identification of at-risk couple. In this study only 63.33% of husbands of BTT mothers had turned up, which is marginally higher to the earlier studies conducted on pregnant females in Karnataka and West Bengal where only 50%-60.8% of the BTT women had managed to bring their partners for undergoing screening test.[15,16]

5.2. Gestational age at presentation

It was observed in this study, that only 31.48% of antenatal females had registered themselves in the antenatal clinic on or before 12 weeks of gestation. However , this is significantly better than the finding by Colah et al in a study conducted in Western India among the general population , where only 15% to 20% of the females registered at the antenatal clinic in the first trimester.[17] This was probably due to the fact that this institute only caters to the ESI beneficiaries who have easy access to standard healthcare service.

Only 6 BTT out of 30 (20%) were aware of their carrier status beforehand, which reflects the importance of mandatory β-thalassemia screening at pre-marital level.

5.3. Complete blood count (CBC)

Among the BTT 70% (21 out of 30) patients were anemic (considering Hb<11gm % in first trimester and Hb<10.5gm % in second trimester).[18] According to reported literature[19,20] β-thalassemia carriers had low MCV and MCH values. 80 femtolitre and 27 picogram served the purpose of accepted cut-offs for MCV and MCH respectively which hold strong in this study as well. All 30 of BTT had MCH value < 27 pg& 28 BTT out of 30 had MCV value < 80 fl. But in a study conducted by Cao and Kan, 13.5% of β-thalassemia carriers had normal to borderline MCV and MCH values.[20]

Lee et al[21] suggested that RDW-CV should not be used as a stand-alone parameter to screen the BTT and to differentiate them from iron deficiency anemia(IDA) due to its low reliability. This should only be used as an adjunct to other parameters, this issue also stands strong according to this study. Not a single β-thalassemia carrier out of 30 cases had RDW-CV <14. This discordance may be explained by associated iron deficiency anaemia in antenatal mothers. This observation warrants a separate study.

The present study could not justify the effectiveness and reliability of Mentzer index as a tool for differentiating β-thalassemia carriers from IDA, as had also been observed by Vehapoglu et al.[22]

In this study, among the 30 BTT, 17 cases were with Mentzer index value <13, but on the other hand, there are 13 BTT with Mentzer index value >13.

5.4. β-globin mutation analysis with β-globin StripAssay® IME

In this study, β- globin chain mutations were identified by the reverse hybridization based assay by β-globin StripAssay® IME (Vienna Lab Diagnostics GmbH). Soliman et al and Elmezayen et al have shown the effectiveness of βglobin StripAssay® MED (Mediterranean) by Vienna Lab Diagnostics GmbH ,which is only a variant of the StripAssay® used in this study and based on the principles of Reverse Dot Blot analysis, as a fast, easy-to-perform and reliable method for mutation analysis of β thalassemia patients in Egypt.[4,23] This strip is specific for mutations found in the geographical region around the Mediterranean sea and was also used successfully in Iraq by Eissa et al and Jalal et al.[24,25] Shalaan et al also found this method to be fit for genetic counselling and antenatal diagnosis.[26] It is as reliable as other molecular techniques as observed by Jaripour et al.[27]

In the current study, all the 30 cases were antenatal mothers with HbA_2 level 4.2%-6.9% by Hb HPLC. Out of the 30 BTT, in 26 (86.67%) subjects had only one type of β-globin mutation, IVS 1-5 (G>C). This mutation has been found to be the commonest β-globin mutation in different studies conducted in India but observed percentage in the current study is much higher than the findings of most of the studies ,where its prevalence varied from 46% to 60.3% in different regions of India.[28–30] It was even higher than the finding by Gazi et al from neighboring Bangladesh using DNA direct sequencing method where the prevalence was 81.4%.[31]

Four other β-thalassemia mutations were detected in our study, including one case of double heterozygous state which had one mutant allele for each of IVS 1-5 G>C and codon 6(A>T) HbS. Other mutations detected in this study are heterozygous state for codon 41/42 [-TTCT], codon30[G>C] & codon 15[TGG>TAG],

Rangan et al. used the term borderline to HbA2 levels 3.0-4.0% and detected β-globin mutations by PCR-based allele specific amplification refractory mutation system in 32% individuals in a study conducted in Northern India.[32] However, in this study , no β-globin chain mutation either could be identified in cases with borderline HbA_2 level of 3.5%-3.9% or in the individuals (husbands of BTT mothers) with normal RBC indices and normal HbA_2 levels.

Mutations like CAP+1 A>C, IVS 1.6 T>C which are associated with borderline or even normal HbA_2 values can be detected by utilizing the StripAssay® used in this study. However, presence of other β- thalassemia alleles (poly A (T>C)), β promoter mutations like -92(HBBc. -142 C>T), coinheritance of δ and β thalassemia, KLF1 gene mutations and triplication of α-globin gene could not be excluded.

Among 24 cases with borderline HbA_2 values, 21 had MCH > 27 pg and MCV >80 fl in 22 cases. RDW-CV <14% in 04 cases and Mentzer index >13 in all cases.

Amongst husbands of 19 BTTs, though no β-globin chain mutation could be identified in any of these cases by the β-globin StripAssay® IME utilized in the present study, the possibility of presence of other mutations cannot be ruled out which are not covered in the StripAssay® used in the current study.

6. Conclusion

Reverse hybridization StripAssay® provides a very rapid, accurate and less labor-intensive method for detection of β -thalassemia mutations. The most frequent mutation found in our study was IVS1-5(G>C). The most important limitation of the recent study is small sample size and lesser duration. Also, in this StripAssay® only a limited number of common mutations can be detected. Any mutations outside of the scope of the StripAssay® will not be detected and this method is also unable to detect mutations in the regulatory region of the b globin gene.

7. Future Scope

In a resource poor and densely populated country like ours, detection of β thalassemia mutations by this method will be a great boon not only for antenatal detection of cases but also in other scenarios in which the patients are already on regular transfusion program and therefore Hb HPLC is not an option.

8. Conflict of Interest

None of the authors has any conflict of interest to declare.

9. Source of Funding

None.

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Cite this article: Datta P, Dutta B, Abdullah M, Chakraborty J. Antenatal screening for β thalassemia with mutation analysis in a tertiary care centre of Eastern India. *Panacea J Med Sci* 2024;14(3):654-658.