CEP Clin Exp Pediatr Vol. 68, No. 6, 437–444, 2025
https://doi.org/10.3345/cep.2024.01284

Original article

Impact of Xmn1 polymorphism on hydroxyurea therapy in children with HbE-β non-transfusion dependent thalassemia: a cohort study

Saheli Roy, MD[1], Paramita Bhattacharya, PhD[2], Atanu Kumar Dutta, MD[3], Mrinal Kanti Das, MD[4]

[1]P.D.Hinduja Hospital Mumbai, Pediatrics IPGME&R, Kolkata, West Bengal, Mumbai, India; [2]Genetic Services Unit, Biotechnology Research Innovation Council-National Institute of Biomedical Genomics, PG Polyclinic Building, Kolkata, West Bengal, Kolkata, India; [3]Department of Biochemistry, AIIMS, Kalyani, West Bengal, Kalyani, India; [4]Department of Pediatrics, IPGME&R, Kolkata, West Bengal, Kolkata, India

Background: Fetal hemoglobin (HbF) inducers, among which hydroxyurea is the most extensively used, have shifted the paradigm toward the treatment of non-transfusion-dependent thalassemia (NTDT). Xmn1 polymorphism (rs7482144) is characterized by substitution (C>T) at -158 position of the γ-globin gene, which leads to CC, CT, or TT genotype. Recently, the role of the Xmn1 polymorphism as a modifier of hydroxyurea therapy has attracted immense research interest.

Purpose: This study aimed to estimate the prevalence of the Xmn1 polymorphism and determine its impact on the efficacy of hydroxyurea therapy in children with NTDT in Eastern India.

Methods: This observational ambispective cohort study involved the assessment of 50 patients with NTDT, of whom 28 qualified, who had been receiving hydroxyurea for less than a month. Relevant molecular analyses were performed, and data on the annual transfusion requirement (ATR), height, and HbF level before starting hydroxyurea treatment were derived from medical records. The same parameters were reassessed after 6 months of hydroxyurea therapy. Furthermore, patients were monitored for drug toxicity.

Results: All patients included in this study exhibited HbE-β-thalassemia, thus implying it to be one of the commonest NTDT genotypes in Eastern India. The prevalence rates of CC and CT were 43% and 57%, respectively, and none of the patients harbored the TT genotype. Toxicity developed in 22% of patients; however, it was not significantly associated with the Xmn1 polymorphism. Significant decrease in ATR and increase in height were observed following hydroxyurea therapy in both groups. Nevertheless, the change was more marked in CT genotype (median ATR drop: 33%, increase in median height: 3.7%, p_{CT}=0.001) than in CC genotype (median ATR drop: 28%, increase in median height: 2.8%, p_{CC}= 0.003).

Conclusion: The T allele of the Xmn1 polymorphism had a favorable effect on the efficacy of hydroxyurea in patients with HbE-β-NTDT.

Key words: HbE-B thalassemia, Fetal hemoglobin inducers, Hydroxyurea, Genetic polymorphism, *Xmn1* polymorphism

Key message

Question: Does the T allele of Xmn1 polymorphism favorably influence hydroxyurea efficacy in children of Eastern descent with fetal hemoglobin (HbE)-β non-transfusion dependent thalassemia (NTDT)?

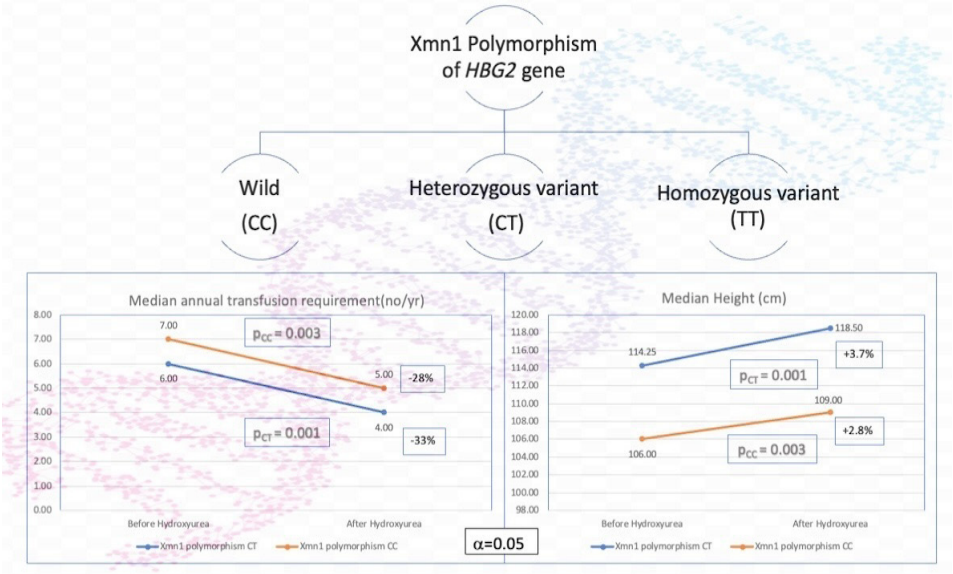
Finding: Decrease in transfusion requirement and increase in height following hydroxyurea therapy was noted in both groups, however, change in CT was more critical than that in CC genotype.

Meaning: T allele of Xmn1 polymorphism favorably influences hydroxyurea efficacy in children with HbE-β NTDT.

Introduction

Thalassemia syndromes are a group of genetic disorders characterized by quantitative hemoglobinopathy, eventually leading to chronic hemolytic anemia. β-thalassemia results from the decreased production of β-globin chains and is one of the most common single-gene disorders worldwide. This condition is inherited in an autosomal recessive manner, with an exceptionally high prevalence in Southeast Asian and Mediterranean populations. Compound heterozygosity of β-thalassemia with fetal hemo-

Corresponding author: Saheli Roy, MD. P.D.Hinduja Hospital Mumbai, Pediatrics IPGME&R, Kolkata, West Bengal, Mumbai, India
✉ Email: saheliroydr059@gmail.com, https://orcid.org/0000-0001-6337-1704
Received: 27 August 2024, Revised: 17 December 2024, Accepted: 20 December 2024



Graphical abstract. *Xmn1* polymorphism (rs7482144) is a genetic polymorphism characterized by substitution (C>T) at the -158 position of γ-globin gene leading to the following 3 genotypes: homozygous for major allele (CC), heterozygous (CT), and homozygous for minor allele (TT). This study cohort consisted of only CT and CC genotypes without any TT genotype. The figure shows line diagrams demonstrating comparison between CC and CT genotypes in terms of median annual transfusion requirement (ATR) and median height before and after hydroxyurea in patients of HbE-β non-transfusion-dependent thalassemia. A statistically significant decrease in ATR and increase in height was noted following hydroxyurea therapy in both groups. Still, change in CT (median ATR drop: 33%, increase in median height: 3.7%, p_{CT}=0.001) was more marked compared to that in CC genotype (median ATR drop: 28%, increase in median height: 2.8%, p_{CC}=0.003).

globin (HbE) leads to HbE-β-thalassemia, which is quite common in Eastern India. HbE-β-thalassemia is, thus, one of the predominant forms of symptomatic thalassemia apart from β-thalassemia major and intermedia in this part of the country.[1] Clinically, thalassemia can be categorized as the more severe transfusion-dependent thalassemia (TDT) and the relatively less severe non-transfusion-dependent thalassemia (NTDT). HbE-β-thalassemia has diverse clinical presentations ranging from TDT to NTDT. The global burden of TDT is considerable, and NTDT constitutes only a small indistinct proportion of patients.[2]

Blood transfusion, together with iron chelators, has long since been the mainstay of treatment for symptomatic thalassemia syndrome. Transfusion provides symptomatic relief and improves growth; however, patients often face an elevated risk of blood-borne infections and iron overload. Although the latter can be attenuated to some extent with iron chelators, the overall quality of life is affected. This issue has prompted a search for alternative treatment modalities, of which the HbF inducer has gained widespread attention. Hydroxyurea is the most commonly used and studied medicine in this group of drugs, especially in patients with NTDT, and its use has been recommended by the Thalassemia International Federation (TIF).[3] However, it remains an uncharted territory as knowledge gaps exist regarding its mechanism of action, efficacy, response modifiers, and safety profile.

The efficacy of hydroxyurea is considered to be influenced by certain modifiers, one of which is a single nucleotide polymorphism (SNP) known as *Xmn1* polymorphism (rs7482144). This polymorphism is characterized by substitution (C>T) at -158 position of globin gene 2 (HBG2) in the β-globin locus (HBB). This polymorphism is present in approximately 30% of the individuals in most populations.[4] The SNP database is a free public archive of a broad range of SNPs. A specific nonredundant number, known as the reference SNP (rs) number, is assigned to individual SNPs, which facilitates easy and uniform identification of a specific SNP from the database. Xmn1 polymorphism is designated by *rs7482144*, and it results in 3 genotypes: homozygous for major allele (CC), heterozygous (CT), and homozygous for minor allele (TT). The presence of the T allele (CT/TT) has been shown to facilitate an improved response to hydroxyurea treatment compared with the absence of this allele. This substitution results in the reduced binding of transcription factors that silence the γ-globin gene production,[5] thus increasing the HbF percentage. Therefore, in patients with the T allele, hydroxyurea plays a synergistic role in HbF induction and enhances the treatment outcome. However, hydroxyurea response in β-thalassemia is multifactorial and includes genetic factors (certain γ-globin gene mutations: IVSI_1,

CD30) as well as environmental factors such as the age of the first transfusion.[6] This interplay of multiple factors can explain the variations in the response to hydroxyurea therapy in patients with thalassemia.

Among all the HbF inducers, hydroxyurea is the most commonly used. It was approved by the U.S. Food & Drug Administration for use in children with sickle cell anemia in 2017[7] and also has been recommended in a selected group of patients with NTDT by the TIF.[3] However, considering its drug toxicity profile and benefit only in a subset of patients, targeted therapy is desirable. Hence, there is an unmet need to evaluate the efficacy of hydroxyurea and determine the modifiers of its response to reap the maximum benefit with minimum risk. Only a limited number of studies have been conducted in this regard globally, and such studies in Eastern India are few and far apart. Moreover, most available studies are focused on TDT, with minimal information on NTDT. This study was conducted to fill the gaps in the existing research. The objectives included: (1) estimating the prevalence of Xmn1 polymorphism in the study cohort; (2) assessing the efficacy of hydroxyurea therapy in patients with NTDT in Eastern India; and (3) determining the effect of Xmn1 polymorphism on the efficacy of hydroxyurea therapy in these patients. This study tested the hypothesis that the T allele of Xmn1 polymorphism had a favorable impact on the efficacy of hydroxyurea therapy in children with NTDT.

Methods

This observational analytical ambispective cohort study was conducted from March 2020 to July 2021 in the Department of Pediatrics, IPGME&R, a tertiary hospital in Kolkata, West Bengal, located in Eastern India, with approval from the Institutional Ethics Committee (IPGME &R/IEC/2020/014 dated January 28, 2020). Although the total study duration was 16 months, patients were enrolled only in the initial 10 months so that a minimum of 6-month follow-up could be ensured in all participants. All consecutive eligible children with NTDT who had been recently initiated on hydroxyurea therapy (<1 month ago) at the thalassemia control unit of the institution were included in this study after obtaining informed consent from guardians of all participants and informed assent from children aged 7–12 years.

1. Inclusion criteria

(1) Children with NTDT confirmed via hemoglobin high-performance liquid chromatography or molecular analysis; (2) Age group of 2–12 years; (3) Hydroxyurea therapy started within 1 month before inclusion in the study; (4) Adequate medical records available to support data collection.

NTDT was defined as thalassemia requiring infrequent transfusions, less than 8 per year. These patients usually present after 2 years of age and include β-thalassemia intermedia, mild, and moderate phenotypes of HbE-β-thalassemia and α-thalassemia intermedia (HbH disease).[3]

2. Exclusion criteria

(1) Patients who received blood transfusion within the last 4 weeks (to avoid compromising the molecular analysis); (2) Patients who were unwilling to undergo regular follow-up for 6 months after the therapy.

At the time of enrolment in the study, baseline data before commencing hydroxyurea therapy, including ATR (defined as the average number of transfusions over the year), height, and HbF levels, were obtained from their past medical records. The children were examined for any adverse clinical effects of the drug using laboratory tests such as complete blood count (CBC), liver function tests (LFTs), and renal function tests (RFTs). Furthermore, relevant molecular genetic analysis was performed free of charge at Genetics Service Unit, BRIC-National Institute of Biomedical Genomics, a collaborative genetic laboratory of the institute (methodological details have been described in the following subsection). The dose of hydroxyurea was adjusted to 10–15 mg/kg/day as a single dose. The children also received folic acid supplementation during the entire length of the study and were transfused when the hemoglobin level fell below the range of 5–6 g/dL. Children on hydroxyurea therapy were followed up prospectively in the outpatient department during the 6 months of treatment. During this time, patients were periodically evaluated for any adverse effects via monthly outpatient department visits and scheduled laboratory tests, such as CBC, LFTs, and RFTs, every 2 weeks for the first 3 months and then monthly for the remaining 3 months. This follow-up was supported by telephonic interviews when needed.

The drug was temporarily halted if any of the following effects were noted: (1) absolute neutrophil count <1,500/μL; (2) platelet count <1,00,000/μL; (3) more than a two-fold increase in serum hepatic transaminases from the baseline; (4) >50% rise in serum creatinine from the baseline.[8] After the normalization of parameters, the drug was restarted and the patient was closely monitored. However, the drug was permanently discontinued if the adverse effects reappeared. At the end of 6 months of therapy, the ATR was calculated by extrapolating data from 6 months postonset of hydroxyurea and the height of the children was recorded using a stadiometer. HbF (%)

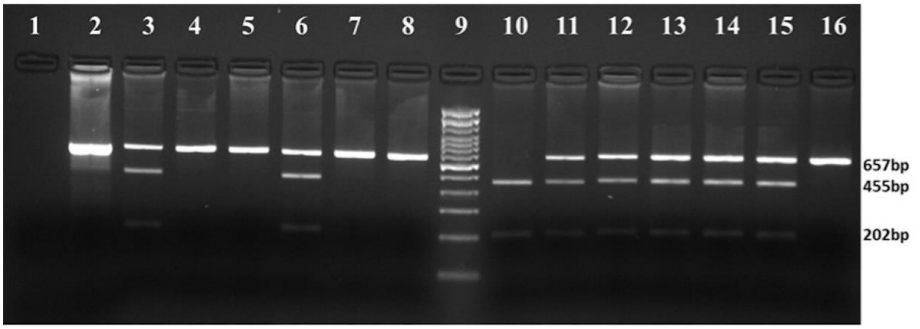


Fig. 1. Polymerase chain reaction RFLP to detect Xmn1 polymorphism. Ethidium bromide-stained photograph of an agarose gel showing lane 1: no template control, lane 2: negative control undigested, lane 3–16: digested products, lane 3, 6, 11, 12, 13, 14, 15: CT, lane 4, 5, 7, 8, 16: CC, lane 10: TT, lane 9: 100-bp ladder.

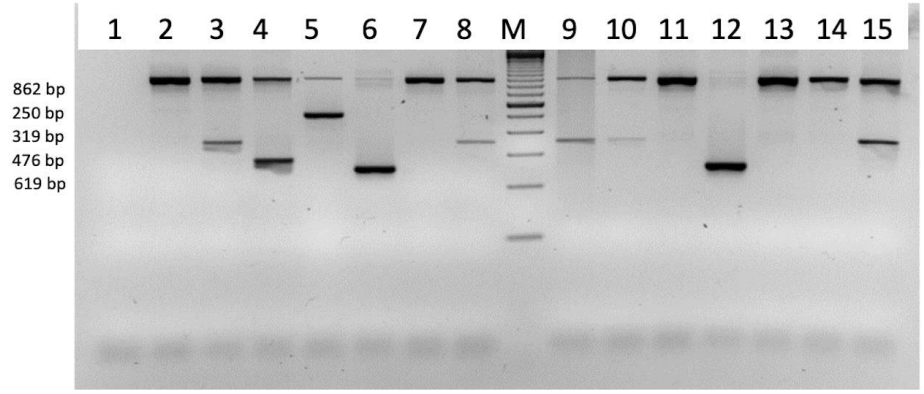


Fig. 2. Multiplex amplification refractory mutation system-polymerase chain reaction for 4 common mutations of β-thalassemia. Ethidium bromide-stained photograph of an agarose gel showing lane 1: no template control, lane 2: negative control 862 bp, lane 3: positive control for IVS1-5(G>C) 319 bp, lane 4: positive control for Cd 41/42 (-CTTT) 476 bp, lane 5: positive control for Cd 8/9(+G) 250 bp, lane 6: positive control for 619-bp deletion heterozygous state, lane 7, 11, 13, 14: no common HBB mutation, lane 8, 9, 10, 15: IVS1-5(G>C) mutation, lane 12: 619-bp deletion heterozygous state, lane M: 100 bp ladder.

was estimated using the cation exchange method with a Bio-Rad Variant II machine, which was the same as the one used for the estimation of pretreatment HbF values to facilitate comparison. HbF was estimated at any timepoint only in patients with a minimum gap of 2 months from the last transfusion. The investigator was blinded to the results of genetic polymorphism until the completion of final data collection, whereas the analyst was blinded to the study hypothesis. These measures were adopted to eliminate interviewer and analyst bias in this study.

3. Molecular methods

Polymerase chain reaction (PCR)-restriction fragment length polymorphism was performed to detect *Xmn1* polymorphism (Fig. 1), and HbE mutation and β-thalassemia genotypes were detected using the amplification refractory mutation system PCR (ARMS-PCR). The common β-thalassemia genotypes were: IVS1-5 (G>C), Cd 8/9 (+G), Cd 41/42 (-CTTT), and 619-bp deletion (Fig. 2). Moreover, β-thalassemia mutations that could not be identified using ARMS-PCR were determined using Sanger sequencing. The primer sequences used for these tests have been provided in the supplementary material.

4. Statistical analysis

Data were entered in a Microsoft Excel spreadsheet. Categorical variables were expressed as the number and percentage of patients and compared across the groups using Pearson chi-square test for the independence of attributes or the Fisher exact test as appropriate. Quantitative variables were expressed as mean, median, and standard deviation and compared across the groups using the Mann-Whitney *U* test. Comparison over time was performed using the Wilcoxon signed-rank test. The statistical software IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA) was used for analysis. An α-level of 5% was considered, i.e., if a *P* value was <0.05, it was considered statistically significant.

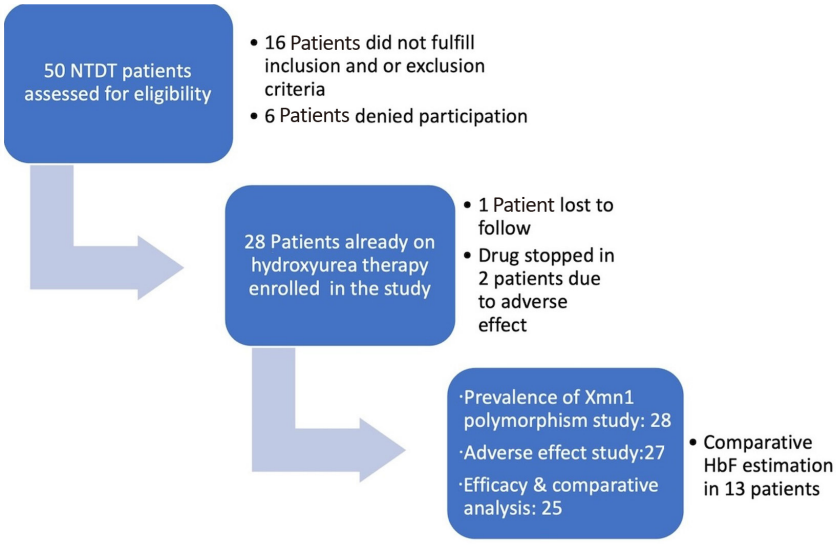


Fig. 3. Flow diagram of the study. Fifty patients with non–transfusion-dependent thalassemia (NTDT) were assessed for eligibility. Of these, 16 did not fulfill the inclusion criteria while 6 denied consent for participation. The remaining 28 patients who were already on hydroxyurea for less than a month were enrolled into the study. Of these 28 patients, one was lost to follow-up, while the drug had to be discontinued in 2 due to adverse effects. Prevalence study of Xmn1 polymorphism was done in all 28 patients, adverse effects were studied in 27 patients, and estimation of hydroxyurea efficacy and its comparative analysis between the genotypes was done in 25 patients. Although baseline fetal hemoglobin (HbF) was available for all patients, comparative HbF study pre- and posttherapy could be done in only 13 patients who had a gap of at least 2 months from last transfusion at time of testing.

Results

1. Study flow and patient characteristics

A total of 50 patients with NTDT were evaluated for eligibility, of which 16 patients did not fulfill the inclusion and/or exclusion criteria and 6 refused to participate. The remaining 28 patients who were already receiving hydroxyurea therapy for <1 month were enrolled in the study; of these, 1 patient was lost to follow-up and the drug had to be discontinued in 2 patients because of adverse effects. The prevalence of Xmn1 polymorphism was estimated in all 28 patients, the adverse effect of hydroxyurea therapy was studied in 27 patients, and the efficacy of this therapy and its comparative analysis between the genotypes was examined in 25 patients. Although baseline HbF was estimated in all patients, comparative HbF study pre- and posttherapy could be performed in only 13 patients who had a gap of at least 2 months from the last transfusion at the time of HbF assay (Fig. 3). The baseline characteristics of the study population are summarized in Table 1.

Table 1. Table depicting the baseline characteristics of the study population (N=28)

Baseline characteristics of the study population	Value
Sex	
Male	16 (57)
Female	12 (43)
Age of study population (yr)	8.0±2.6
Age of onset of transfusion (yr)	4.0±1.8
Baseline transfusion requirement (number/yr)	6.5±3.6
Stature	
Normal	7 (25)
Moderate stunting	14 (50)
Severe stunting	7 (25)
HbF of the study population (%)	24.3±12.2
Positive family history for similar disease	13 (46)

Values are presented as number (%) or median±standard deviation.
HbF, fetal hemoglobin.

2. Genotypic characteristics of the study population

All patients in the study population exhibited HbE-β-thalassemia, and the commonest genotype was IVS1-5 (G>C), which was seen in 21 patients (75%). Thus, majority of the population harbored IVS1-5 (G>C) and HbE mutation in the compound heterozygous state. The prevalence rates of CC and CT genotypes of Xmn1 polymorphism were 12 (43%) and 16 (57%), respectively, with none having the TT genotype.

3. Hydroxyurea exposure and adverse effects

Hydroxyurea was administered at a median dose of 10 +1.40 mg/kg/day (10–15 mg/kg/day) for a total duration of 6 months. Drug toxicity developed in 6 patients (22%), and permanent discontinuation was required only in 2 patients (7%). Myelosuppression was the most common

adverse effect observed, followed by transaminitis and elevated creatinine levels (Fig. 4). A statistically significant association was not found between Xmn1 polymorphism and drug toxicity (P=0.357) in this study.

4. Overall efficacy of hydroxyurea therapy

In 25 patients who completed 6 months of hydroxyurea therapy, the median ATR was significantly lower (P<0.001) and the median height was significantly higher (P<0.001) at the end of the therapy. HbF was compared over time in 13 patients, and a statistically significant increase in its level was noted at the end of the treatment (P=0.023).

5. Efficacy of hydroxyurea therapy in relation to Xmn1 polymorphism

The response was analyzed in the 2 groups of patients with CC and CT genotypes at the end of the study period in terms of ATR, height, and HbF (%) and compared across time before and after the study. The results of the analysis are summarized in Table 2. A statistically significant decrease in transfusion requirement and increase in height were observed after hydroxyurea therapy in both groups. Nonetheless, the change in CT genotype (median ATR drop: 33%, increase in median height: 3.7%, p_{CT}=0.001) carried more weightage than that in the CC genotype (median ATR drop: 28%, increase in median height: 2.8%, p_{CC}=0.003) (ref: graphical abstract). In contrast, a statistically significant association was not found between *Xmn1* polymorphism and the change in HbF level following hydroxyurea therapy in this study (p_{CT}=0.139, p_{CC}=0.068).

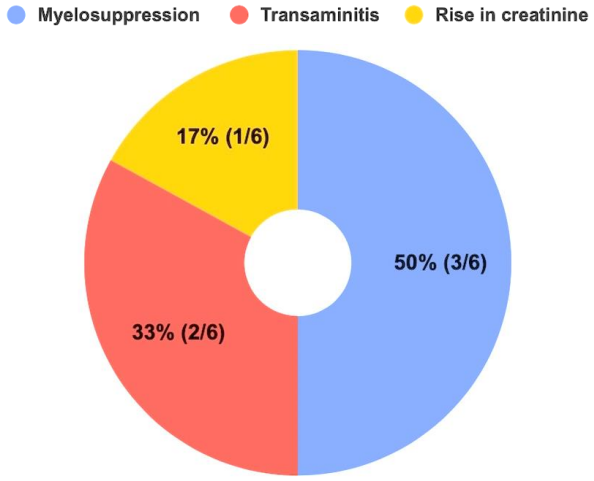


Fig. 4. Drug toxicity profile of hydroxyurea. Doughnut chart showing the toxicity profile of hydroxyurea in the study population. Toxicity developed in 6 patients in total. Most common toxicity encountered was myelosuppression (n=3, 50%), followed by transaminitis (n=2, 33%) and elevation in creatinine level (n=1, 17%).

Discussion

Since the recognition of thalassemia as a distinct entity, blood transfusion has been the cornerstone of treatment.; however, the advent of HbF inducers has transformed the scenario. Hydroxyurea is the most used drug in this category.[3] Unfortunately, it is not free of adverse effects; hence, it is essential to strike a balance between the risks and benefits of using this medicine. Certain modifiers of hydroxyurea response have been studied in the past, but this study attempted to explore whether *Xmn1* polymorphism plays a role in modifying the response to hydroxyurea, especially in the less-explored NTDT population. The ultimate objective was to guide the selection of candidates for hydroxyurea therapy.

In this study cohort, all patients were compound heterozygous for HbE-β-thalassemia, indicating that it might be the commonest form of NTDT in Eastern India. Another study previously performed at the same location, i.e., Kolkata, West Bengal, has reported similar findings.[1] In our study population, the commonest β-thalassemia genotype was IVSI-5 (G>C), which was encountered in 75% of the patients. This result has been corroborated by the findings of 2 other studies, which also reported IVSI-5 (G>C) to be the commonest *HBB* gene mutation, with prevalence rates of approximately 33% and 44%.[9,10]

At the end of 6 months of hydroxyurea therapy, the

Table 2. Table depicting a comparison of various parameters before and after hydroxyurea therapy in relation to *Xmn1* polymorphism

Parameter	Before hydroxyurea						After hydroxyurea						Comparison	
	CT			CC			CT			CC			CT	CC
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	*P* value	
ATR (number/yr)	7.13	6.00	3.22	7.50	7.00	4.27	3.36	4.00 (-33%)	1.86	4.18	5.00 (-28%)	2.79	**0.001**	**0.003**
Height (cm)	112.66	114.25	15.59	108.29	106.00	15.12	115.86	118.50 (+3.7%)	16.35	116.59	109.00 (+2.8%)	11.93	**0.001**	**0.003**
HbF (%)	24.67	23.40	13.95	26.50	24.90	7.54	31.81	32.00	11.05	35.38	37.10	12.01	0.139	0.068

Annual transfusion requirement (ATR), height and fetal hemoglobin (HbF) level were compared in the study population before and after hydroxyurea therapy using the Wilcoxon Signed-rank test (SPSS ver. 22) among different genotypes of Xmn1 polymorphism. There was a statistically significant decrease in ATR and an increase in height in both CT and CC genotypes following therapy. Still, the change in CT (median ATR drop: 33%, increase in median height: 3.7%, P=0.001) was more critical than that in CC (median ATR drop: 28%, increase in median height: 2.8%, P=0.003). Change in HbF level was, however, statistically insignificant in both the genotypes. SD, standard deviation.
Boldface indicates a statistically significant difference with P<0.05.

median ATR was significantly lower (P<0.001) and median height (P<0.001) and median HbF (%) were significantly higher (P=0.023) in our study population. The positive response to hydroxyurea noted in this study agrees with the observations of Keikhaei et al.[11] The study found a significant decrease in the transfusion rate (P=0.001) and a significant increase in HbF (%) (P=0.03) in patients with thalassemia intermedia treated using hydroxyurea. Approximately 22% of our patients developed drug toxicity, and permanent discontinuation was needed in 7% of the patients. A previous study from Rajasthan, India, also reported adverse effects in approximately 14% of the patients, with a clinically significant problem in only 4%.[8] The commonest adverse effect in our cohort was myelosuppression, which is indeed a potentially hazardous condition. However, all adverse effects were reversed after discontinuation of the therapy. Interestingly, adverse effects were noted at a lower dose (median dose: 10 mg/kg/day) in our study than at a higher dose (20 mg/kg/day) in the abovementioned study,[8] which could be attributed to genetic polymorphisms in drug-metabolizing enzymes.[12]

On analyzing the response to hydroxyurea therapy among different genotypes of Xmn1 polymorphism, a statistically significant decrease in median ATR and an increase in median height were observed following hydroxyurea therapy in both groups. Nonetheless, the change in CT (median ATR drop: 33%, increase in median height: 3.7%, p_{CT}=0.001) was more critical than that in the CC (median ATR drop: 28%, increase in median height: 2.8%, p_{CC}=0.003) genotype. Thus, patients with the T allele of Xmn1 polymorphism indeed had an edge over those without it. This observation confirmed the findings of some previous investigations in India and abroad.[13-15] Notably, a statistically significant association was not found between the *Xmn1* genotypes and either HbF (%) increase or adverse effects in our cohort. This finding helps us to draw an inference that although Xmn1 polymorphism modifies the clinical response to hydroxyurea, it does not apply to its biochemical response or adverse effect profile.

Most similar studies conducted till date have focused on either on β-TDT[14,15] and β-NTDT[5,15] patients or on HbE-βTDT patients.[13,16] Only one study was conducted by Italia et al.[16] on 11 patients with HbE-β-TDT and 2 patients with HbE-β-NTDT in Mumbai, India. Thus, our study is the first of its kind in Eastern India to evaluate the impact of Xmn1 polymorphism on the efficacy of hydroxyurea in a relatively larger group of patients with non-transfusion-dependent HbE-β-thalassemia.

This study, like most others, has certain limitations, which could be summarized as follows: (1) Small sample size with a short follow-up period, which was inadequate to evaluate the long-term efficacy. (2) Inability to determine the effectiveness of higher permissible dosage of the drug. (3) The lack of the TT genotype of Xmn1 polymorphism in the study population. (4) The presence of a certain selection bias, for instance, the fact that only patients with moderate HbE-β-NTDT who required transfusions attended the hospital and were enrolled and not the milder ones as they often do not seek medical attention. (5) Other parameters likely to influence the response to hydroxyurea therapy in these patients were not determined. Nevertheless, considering the lower prevalence of NTDT compared with TDT, we believe that our study has added substantially to the prevailing knowledge on the rational use of hydroxyurea in patients with HbE-β-NTDT in Eastern India and has emphasized the possible role of Xmn1 polymorphism as a modifier of hydroxyurea response.

Hydroxyurea is an effective adjunctive treatment for patients with NTDT. However, due to its potential adverse effects, cautious and targeted use of this drug is warranted, for which Xmn1 polymorphism can function as a guide. As patients possessing the T allele of this polymorphism exhibited a slightly better response to hydroxyurea, the benefits of using this drug, especially in these patients, outweigh the risks. Hence, Xmn1 polymorphism analysis can guide us in carefully selecting patients with HbE-β-NTDT for hydroxyurea therapy. However, hydroxyurea therapy showed a beneficial response in both groups, with relatively better results in those with the CT genotype, and the absence of the T allele did not substantially aggravate the risk of drug toxicity. Therefore, in case of non-availability of data on Xmn1 polymorphism, all patients with HbE-β-NTDT, particularly those in Eastern India, deserve a trial of hydroxyurea while closely monitoring for possible adverse effects.

In conclusion, Xmn1 polymorphism study can aid in selecting patients with HbE-β-NTDT for hydroxyurea therapy by rationalizing the benefit–risk assessment. However, further investigations with a larger sample size and multicentric studies encompassing all other modifiers are required to substantiate the results of this study.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

Funding: Genetics Service Unit (GSU) Grant: The project for "Establishment of Genetics Service Unit": Memo No. 795/BT(Estt)/1P-2/2016 dated 27/10/2016; funding for the molecular tests carried out in this study.

Author contribution: Conceptualization: MKD; Data curation: SR,PB; Formal analysis: SR, MKD, PB; Funding acquisition: PB; Methodology: MKD, PB, AKD; Project administration: PB, MKD; Visualization: SR, PB, AKD; Writing -original draft: SR; Writing - review & editing: MKD, PB, AKD

ORCID:
Saheli Roy https://orcid.org/0000-0001-6337-1704
Paramita Bhattacharya https://orcid.org/0000-0002-7915-4250
Atanu Kumar Dutta http://orcid.org/0000-0002-8413-3454
Mrinal Kanti Das https://orcid.org/0000-0002-3558-7556

References

1. Mandal PK, Nataraj KS, Baul SN, Ghosh MK, Dolai TK. Efficacy of dichlorophenolindophenol (DCIP) as screening test for Hb E: revisited. Indian J Hematol Blood Transfus 2020;36:535-41.
2. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010;115:4331-6.
3. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V. Guidelines for the management of non transfusion dependent thalassaemia (NTDT). In: Weatherall D, editor. Nicosia (Cyprus): Thalassaemia International Federation; 2013.
4. Thein SL, Menzel S, Lathrop M, Garner C. Control of fetal hemoglobin: new insights emerging from genomics and clinical implications. Hum Mol Genet 2009;18(R2):R216-23.
5. Motovali-Bashi M, Ghasemi T. Role of XmnIγG polymorphism in hydroxyurea treatment and fetal haemoglobin level at Isfahanian intermediate β-thalassemia patients. Iran Biomed J 2015;19:177-82.
6. Ansari SH, Hussain Z, Zohaib M, Parveen S, Kaleem B, Qamar H, et al. A pragmatic scoring tool to predict hydroxyurea response among β-thalassemia major patients in Pakistan. J Pediatr Hematol Oncol 2022;44:e77-83.
7. U.S. Food & Drug Administration. FDA approves hydroxyurea for treatment of pediatric patients with sickle cell anemia [Internet]. Silver Spring (MD): U.S. Food & Drug Administration; 2017 [cited 2024 Dec 15]. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-hydroxyurea-treatment-pediatric-patients-sickle-cell-ane mia.
8. Suthar K, Sharma P, Verma M, Goyal VK. Efficacy of high dose hydroxyurea in transfusion-dependent thalassemic children: a quasi-experimental study. Int J Contemp Pediatr 2017;4:1514-8.
9. Sharma N, Das R, Kaur J, Ahluwalia J, Trehan A, Bansal D, et al. Evaluation of the genetic basis of phenotypic heterogeneity in north Indian patients with thalassemia major. Eur J Haematol 2010;84:531-7.
10. Moghaddam EM, Bahrami S, Naderi M, Bazi A, Karimipoor M. Xmn1-158γG variant in β-thalassemia intermediate patients in the south-east of Iran. Int J Hematol Oncol Stem Cell Res 2017;11:165-71.
11. Keikhaei B, Yousefi H, Bahadoram M. Clinical and haematological effects of hydroxyurea in β-Thalassemia intermedia patients. J Clin Diagn Res 2015;9:1-3.
12. Yahouédéhou SCMA, Carvalho MOS, Oliveira RM, Santiago RP, da Guarda CC, Carvalho SP, et al. Sickle cell anemia patients in use of hydroxyurea: association between polymorphisms in genes encoding metabolizing drug enzymes and laboratory parameters. Dis Markers 2018;2018:6105691.
13. Ghosh D, Panja A, Saha D, Banerjee U, Datta AK, Basu A. Drug repurposing: hydroxyurea therapy improves the transfusion-free interval in HbE/Beta-thalassemia-major patients with the XmnI polymorphism. Genet Test Mol Biomarkers 2021;25:563-70.
14. Banan M, Bayat H, Azarkeivan A, Mohammadparast S, Kamali K, Farashi S, et al. The XmnI and BCL11A single nucleotide polymorphisms may help predict hydroxyurea response in Iranian β-thalassemia patients. Hemoglobin 2012;36:371-80.
15. Italia KY, Jijina F, Merchant R, Panjwani S, Nadkarni AH, Sawant PM, et al. Response to hydroxyurea in beta thalassemia major and intermedia: Experience in western India. Clin Chim Acta 2009;407:10-5.
16. Italia KY, Jijina F, Merchant R, Panjwani S, Nadkarni AH, Sawant PM, et al. Effect of hydroxyurea on the transfusion requirements in patients with severe HbE-beta thalassaemia: a genotypic and phenotypic study. J Clin Pathol 2010;63:147-50.

How to cite this article: Roy S, Bhattacharya P, Dutta AK, Das MK. Impact of Xmn1 polymorphism on hydroxyurea therapy in children with HbE-β non-transfusion dependent thalassemia: a cohort study. Clin Exp Pediatr 2025;68:437-44.