ORIGINAL ARTICLE

The Genetic Determinants for Long-term Response to Hydroxyurea Therapy in Indian β-Thalassemia Patients

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ABSTRACT

Introduction: Hydroxyurea has been the only drug useful in reducing transfusion requirements in a few β-thalassemia patients. However, there have not been many studies to evaluate the long-term efficacy and safety of this drug. It is also unclear whether genetic and epigenetic factors contribute to the variable response to hydroxyurea. **Materials and Methods:** A retrospective analysis of 56 transfusions dependent β-thalassemia intermedia and 43 β-thalassemia major patients on hydroxyurea therapy (15–20 mg/kg/day) followed for 4.5 ± 0.5 years was undertaken. β-thalassemia mutations, α-genotypes, Xmn-I polymorphisms, 3- single nucleotide polymorphism (SNPs) in the *BCL11A* gene, and 2-SNPs in the *HBS1L-MYB* intergenic regions were studied. **Results:** A total of 30 β-thalassemia intermedia patients were good-responders (maintain hemoglobin >7.5g/dL without transfusions), whereas 26 β-thalassemia intermedia and 43 β-thalassemia - major patients were non-responders. The age at first clinical presentation was significantly higher among the responders (84.2±56.0 months). The presence of Xmn-I (+/+) was significantly higher among the responders. None of the other genetic factors such as β-thalassemia mutations, α-thalassemia or polymorphisms in *BCL11A* (rs11886868, rs766432, and rs4671393), and *HBS1L-MYB* genes (rs9399137 and rs4895441) showed any correlation with response to therapy. **Conclusion:** Nearly 53.6% of β-thalassemia patients with late onset of symptoms (transfusion requirements after 4–5 years of age) showed a good long-term response to hydroxyurea therapy. Apart from the Xmn-I polymorphism, none of the other genetic determinants contributed to the variable response to hydroxyurea therapy in Indian patients.

Key words: *BCL11A gene*, fetal hemoglobin, *HBS1L-MYB* intergenic region, hydroxyurea, single nucleotide polymorphisms, Xmn-I polymorphism, α-thalassemia, β-thalassemia

INTRODUCTION

β-thalassemia is an autosomal recessive disorder of hemoglobin (Hb) production caused by a reduced amount (β^+) or absence (β^0) of β-globin chain synthesis resulting in a relative excess of unbound α-globin chains that precipitate in erythroid precursors leading to ineffective erythropoiesis.[1] The pathogenesis of β-thalassemia is a complex, multisystem process with different genetic and epigenetic markers influencing the phenotype of the disease.[2]

An estimated 1.5% of the global population are carriers of β-thalassemia, with about 60,000 symptomatic individuals born annually.[3] In India, the carrier rate for β-thalassemia varies from 1% to 17%[4,5] among certain high-risk communities with an average frequency of 3–4%[6,7] and an estimated birth of 8000–10,000 babies with severe forms

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of thalassemia each year. Few of them receive optimal treatment[6] while the majority die early as a consequence of severe anemia and iron overload.[5,8]

Hydroxyurea has been successfully used to induce fetal Hb (HbF) production and reduce the clinical severity of sickle cell disease (SCD) patients.[9-11] However, the response has been variable among β-thalassemia patients with β-thalassemia intermedia cases and few HbE-β-thalassemia being more responsive to the therapy with no further transfusion requirements than those with β-thalassemia major.[12-16]

As the response to hydroxyurea therapy in individual β-thalassemia patients is unpredictable, the question remains as to who will respond to this drug and for how long? Many genetic markers linked and unlinked to the β-globin gene have been shown to induce HbF production. The three quantitative trait loci present on chromosomes 6, 8 and the X-chromosome have shown some promise in having regulatory roles in the production of HbF and response to hydroxyurea in patients with sickle cell anemia.[17]

Furthermore, several other single nucleotide polymorphism (SNPs) located in intron 2 of the *BCL11A* gene on chromosome 2, which is a transcription factor responsible for γ to β-globin switch during erythroid differentiation[18,19] and the *HBS1L-MYB* intergenic region on chromosome 6, responsible for γ-globin regulation which also influences the γ to β-globin switch[20,21] have been shown to influence HbF level in adults, and thus, they could be associated with a milder phenotype and better response to hydroxyurea therapy.

Here, we present an extended 4.5 ± 0.5 years follow-up of our earlier described cohort of β-thalassemia patients on hydroxyurea therapy to understand the long-term efficacy of the drug and an analysis of the genetic polymorphic markers situated on *BCL11A* and *HBS1L-MYB* genes, in addition to the Xmn-I polymorphism known to influence HbF synthesis, to evaluate their contribution to the response to hydroxyurea therapy in Indian β-thalassemia patients.

MATERIALS AND METHODS

A total of 99 β-thalassemia patients on hydroxyurea (Cytodrox, Cipla Ltd., Mumbai) therapy (15–20 mg/kg/day) were followed up for 4.5 ± 0.5 years. These include 56 β-thalassemia intermedia patients (age 3–44 years) (27 - males and 29 - females) who clinically presented between the ages of 3 and 17 years and most of whom became transfusion dependent thereafter and 43 β-thalassemia major patients (age 11-28 years) (23 - males and 20 - females) who presented within the 1st year of life with regular BT requirements thereafter. A 2 years' follow-up data of 79 patients on hydroxyurea therapy had been reported by us earlier.[15] However, this study evaluates whether hydroxyurea has the same efficacy for an extended period on the same patients as seen earlier.

As described earlier,[15] those patients whose age at clinical presentation was over 2 years and the Hb level at presentation was >6 g/dL with the frequency of transfusions being every 3–6 months initially were labeled as β-thalassemia intermedia. However, the majority of these patients had subsequently become transfusion dependent before starting hydroxyurea therapy.

After the approval by the Ethical Committee of our institute for this study, an informed consent in writing was taken from all the patients and parents of pediatric patients before enrolling them for hydroxyurea therapy.

The clinical history and anthropometric measurements were recorded in all cases before starting therapy. A monthly follow-up of these patients to evaluate the clinical and hematological changes was done after starting hydroxyurea therapy. The number of remaining capsules of hydroxyurea during each follow-up evaluated the therapeutic adherence. Hydroxyurea was stopped if a fall was observed in the neutrophil counts (<2.5 × 10^3/μl), platelet counts (<80 × 10^3/μl), and Hb level (<6 g/dL). Furthermore, monitored was the hair loss, gastrointestinal problems, rash and liver, and kidney functions. Hydroxyurea was administered only to those patients whose follow-up and therapeutic adherence was ensured. The patients were also on folic acid tablets (5 mg/day) and standard iron chelators deferiprone or deferasirox as recommended. A good response to the therapy was indicated by maintenance of the steady state Hb level above 7.5 g/dL without any further BT.[15]

Hematological Studies

Hematological analysis of the patients, their parents, and available siblings was done using blood collected in EDTA vacutainers. Sysmex K1000 hematology analyzer was used for a complete blood count, and standard methods were used for peripheral blood smear examination and reticulocyte count. Hb analysis was done on the Variant Hb Testing System (Biorad Laboratories, Inc., Hercules, CA, USA). Serum ferritin levels were measured by ELISA (Demeditec Diagnostics GmbH, Kiek, Germany).

Molecular Analysis

The β-thalassemia mutations were characterized as described earlier[15] by reverse dot blot hybridization, amplification refractory mutation system[22] or by automated DNA sequencing on the ABI prism-310 sequencer for uncharacterized mutations. Xmn-I polymorphism was studied by PCR and restriction enzyme digestion.[22] Multiplex PCR was used to detect 8 αgene deletions.[23] Three SNPs in the *BCL11A* gene (rs11886868, rs766432, and rs4671393) and 2 SNPs in the *HBS1L-MYB* intergenic region (rs9399137 and

rs4895441) was studied using PCR and DNA sequencing on the ABI-310 automated DNA sequencer using the BigDye terminator kit (Applied Biosystems, Foster City, California, USA).

Statistical Analysis

The hematological values before starting hydroxyurea therapy were compared with those after therapy using the Wilcoxon Signed-Rank Test and data were presented as mean±SD, and $P < 0.01$ was considered statistically significant. All statistical analysis was done using the SYSTAT 10 software.

RESULTS

A total of 30 of the 56 β-thalassemia intermedia patients (53.6%) responded to hydroxyurea therapy and became transfusion independent (good-responders) with Hb levels being maintained >7.5 g/dL. 26 β-thalassemia intermedia patients and 43 β-thalassemia major patients did not respond well to hydroxyurea therapy and eventually stopped hydroxyurea therapy after 6 months and continued to be transfusion dependent (non-responders). The mean age at presentation was 84.2 ± 56.0 months among the responders and 28.3 ± 27.3 months among the non-responders [Figure 1]. This difference was statistically significant ($P < 0.001$). 23.3% of the patients (7 out of 30) who responded to hydroxyurea therapy were splenectomized.

One of the responders (aged 20 years) became transfusion dependent again after 3 years on hydroxyurea therapy and another responder (aged 18 years), who had uncontrollable diabetes, died due to septicemia. Four patients stopped coming to our center for follow-up as they found an alternative center close to their place of residence, and hence we lost their follow-up. Six β-thalassemia intermedia patients and 13 β-thalassemia major patients had shown a 50% reduction in their transfusion requirements (partial-responders) after 6 months of starting hydroxyurea therapy. However, after 22 months on therapy, these partial-responders could not maintain the required Hb levels and gradually became transfusion dependent showing no beneficial effect of hydroxyurea therapy and hence were considered as non-responders to hydroxyurea therapy which was discontinued later.[15] Responders also reported a general well-being in their physical condition with improvement in the day-to-day quality of life.

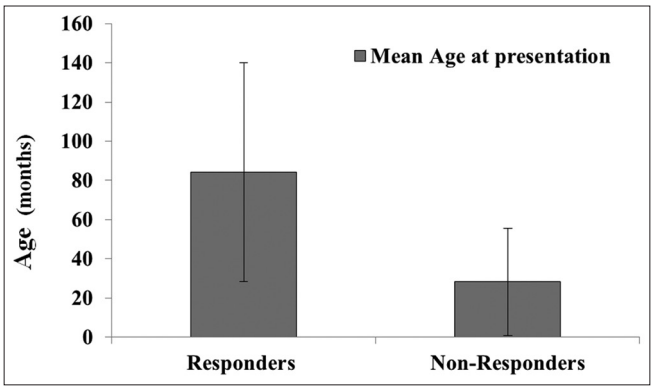

Figure 1: Mean age at presentation of the disease among responders and non-responders

The hematological parameters of the patients before and 4.5 ± 0.5 years after hydroxyurea therapy are given in Table 1. The responders showed a statistically significant increase in Hb, mean corpuscular volume, and HbF after hydroxyurea therapy. A decrease in the serum ferritin was seen in both the groups; however, the difference was not statistically significant. Repeated episodes of neutropenia were seen in 14 patients after which hydroxyurea was discontinued, and the patients were categorized as non-responders. Besides these, the drug was well tolerated in the other patients.

Molecular characterization of the β-thalassemia mutations showed that 9 responders were homozygous for the IVS1-5(G→C) mutation and 5 were homozygous for the IVS1-1(G→T) mutation. Other mutations seen in responders were Cap site+1(A→C), −88(C→T), codon 15(G→A), codon 16(C→T), codon 30(G→C), codon 41/42(-CTTT), IVSII-837(T→G), and the 619 bp deletion. Two patients were compound heterozygous for β and δβ-thalassemia and one was homozygous for δβ-thalassemia. The most common Indian mutation IVS1-5(G→C) was present in the homozygous condition in 17 non-responders. The other mutations seen among the non-responders were IVS1-5(G→T), codon 8/9(+G), IVS 1-1(G→T), codon 41/42(-CTTT), codon 30(G→C), codon 5(-CT), codon 16(-C), Cap site +1(A→C), codon 15(G→A), −87(C→G), codon 55(-A), -88(C→T), and the 619 bp deletion. The percentage of β^0/β^0 and β^+/β^+ thalassemia mutations among the responders and non-responders was 40% and 36.7% and 31.9% and 31.9%, respectively. One patient was heterozygous for the IVS1-5(G→C) mutation with associated α-gene triplication and two patients were compound heterozygous for β- and δβ-thalassemia. No correlation was found between different β-thalassemia genotypes and response to hydroxyurea therapy.

Table 2 shows the analysis of 3 SNPs in the *BCL11A* gene (rs11886868, rs766432, and rs4671393), 2 SNPs in the *HBS1L-MYB* intergenic region (rs9399137 and rs4895441), the Xmn-I polymorphism (rs7482144) and α-genotypes among the two groups. Except for the Xmn-I polymorphism, no other genetic marker showed any statistically significant correlation between the responders and non-responders to hydroxyurea therapy. However, associated α-thalassemia was more common among the responders.

Table 1: Hematological parameters of β-thalassemia patients before and after hydroxyurea therapy

Parameters	Responders (n=30)		Non-Responders (n=69)	
	Before starting HU	After HU (4.5±0.5 years)	Before starting HU	After HU
Hb (g/dL)	7.5±1.5	8.9±1.4**	7.7±1.3	7.3±1.0
MCV (fL)	73.1±7.9	78.4±9.8*	81.1±6.9	83.2±7.8
MCH (pg)	23.5±3.2	25.0±2.9	27.9±3.4	27.5±2.4
MCHC (g/dL)	31.8±2.4	32.0±1.8	34.3±2.8	32.9±1.8
HbF (%)	49.5±35.3	83.0±27.7**	8.8±14.7	21.1±17.2**
HbF/F-cells (pg)	16.8±6.2	22.7±6.0**	7.3±4.5	12.2±5.6**
Serum ferritin (ng)	3943.8±4311.6	2814.7±3724.9	6165.7±3296.6	4744.5±2633.1*

Values are Mean±SD, *=*P*<0.01, **=*P*<0.001

DISCUSSION

Hydroxyurea is the only hope for a few β-thalassemia patients, besides bone marrow transplant. The exact mechanism of action of hydroxyurea though unclear, it is believed to act through many different ways; one of them being the induction of HbF synthesis thereby reducing the amount of unbound α-globin chains which, in turn, reduces membrane damage, hemolysis and helps to maintain a stable Hb level.

Many studies have shown a good response to hydroxyurea therapy in a few sets of β-thalassemia patients;[12-15] however, an extended study was required to evaluate the long-term efficacy of this drug on the transfusion requirements of the patients. Here, we carried out an extended follow-up (4.5 ± 0.5 years) of β-thalassemia patients on hydroxyurea therapy and compared the clinical outcome with that seen after the initial 2 years' follow-up of 79 of these patients on hydroxyurea therapy. We had reported earlier that 58% of β-thalassemia intermedia patients became transfusion independent whereas 16% of β-thalassemia intermedia patients showed a 50% reduction in transfusion requirements after hydroxyurea therapy. Among the β-thalassemia major patients, only 32% had shown a 50% reduction in their transfusion requirements after hydroxyurea therapy.[15] Further, in this study, we also tried to correlate the response to hydroxyurea therapy with some of the well-established molecular markers of HbF induction or those that could reduce clinical severity of the disease in terms of transfusion requirements to understand their role in predicting the response to hydroxyurea therapy.

A decrease in the efficacy of hydroxyurea therapy after a long-term follow-up (45–66 months) was shown in patients who initially had a good response.[24] This was further supported by Rigano *et al*. 2010 who observed desensitization to hydroxyurea therapy after a long-term follow-up in both *in vivo* treated patients who were good responders in the short-term follow-up and *in vitro* in erythroid treated cultures.[25] This may lead to the hypothesis that long-term toxic effect of hydroxyurea on bone marrow stem cells may lead to drug-related bone marrow failure leading to reduced hematopoietic stem cell proliferation and differentiation. Our studies are in agreement with these results as we also found a decrease in efficacy of hydroxyurea therapy in our patients who on a short-term follow-up of 22 months had earlier shown a 50% reduction in transfusion requirements.[15] These patients were gradually unable to maintain stable Hb for >30 days and hence went back on their regular transfusion regimen and stopped hydroxyurea therapy. However, those patients who were good responders to the therapy maintained stable Hb (>7.5 g/dL) and did not require transfusions for >4 years proving the long-term efficacy of hydroxyurea.

Mtvarelidze *et al*. showed a remarkable response to hydroxyurea therapy in 3 out of 6 β-thalassemia patients with regular BT requirements. These patients remained transfusion-free for 5 years on hydroxyurea therapy.[26]

El-Beshlawy *et al*. showed a follow-up (35.4 ± 19.2 months) of 100 patients on hydroxyurea therapy. 33% of the patients became transfusion-free with a statistically significant increase in Hb, HbF, and a significant decrease in serum ferritin after hydroxyurea therapy and 46% were partial responders. No correlation could be established with respect to age, sex, or genotype toward the prediction of response to hydroxyurea therapy.[27] Our study also showed a reduction in serum ferritin levels in both the groups after hydroxyurea therapy. This is in agreement with our earlier report where hydroxyurea has been shown to be a potential iron chelator in an animal model and transfusion-dependent β-thalassemia patients.[28,29]

Many genetic markers such as the Xmn-I polymorphism, polymorphisms on the *BCL11A* gene and the *HBS1L* and *MYB* intergenic region, β-globin gene mutations, and α-thalassemia have been suggested as predictive markers of high HbF and hence better response to hydroxyurea therapy. However, they were not completely dependable for determining the response to hydroxyurea therapy as none of the marker except the

Table 2: Presence of genetic modifiers in the responders and non-responders of hydroxyurea therapy

Genotype	Responders 30 (%)	Non-responders 69 (%)	*P* value
BCL11A rs11886868 (Favorable allele – C)			
C/C	26.6	30.4	*P*>0.01
T/C	50.0	55.3	*P*>0.01
T/T	23.4	14.3	*P*>0.01
BCL11A rs4671393 (Favorable allele – A)			
A/A	3.3	6.1	*P*>0.01
G/A	30.0	18.5	*P*>0.01
G/G	66.7	75.4	*P*>0.01
BCL11A rs766432 (Favorable allele – C)			
C/C	3.3	4.3	*P*>0.01
C/A	23.3	23.2	*P*>0.01
A/A	73.4	69.6	*P*>0.01
HBS1L-MYB rs4895441 (Favorable allele – G)			
G/G	3.3	0	*P*>0.01
A/G	20.0	37.9	*P*>0.01
A/A	76.7	62.1	*P*>0.01
HBS1L-MYB rs9399137 (Favorable allele – C)			
C/C	3.3	0	*P*>0.01
T/C	16.7	35.8	*P*>0.01
T/T	80.0	61.2	*P*>0.01
β-globin - Xmn I rs7482144 (Favorable allele – T)			
T/T	73.3	20.3	*P* <0.001
T/C	16.7	37.7	*P*>0.01
C/C	10.0	42.0	*P* <0.001
α-genotype			
αα/αα	60.0	76.8	*P*>0.01
α-gene deletion			
$-\alpha^{3.7}/\alpha\alpha$	30.0	17.5	*P*>0.01
$-\alpha^{3.7}/-\alpha^{3.7}$	10.0	4.3	*P*>0.01
α-gene triplication	0	1.4	*P*>0.01
β-genotype			
β^+/β^+	36.7	31.9	*P*>0.01
β^+/β^0	13.4	34.8	*P*>0.01
β^0/β^0	40.0	31.9	*P*>0.01
$\beta^0/\delta\beta^0$	6.6	0	*P*>0.01
$\delta\beta^0/\delta\beta^0$	3.3	0	*P*>0.01
$\beta^+/\alpha\alpha\alpha$	0	1.4	*P*>0.01

Xmn-I showed a statistically significant difference between the two groups. Thus, the quest to understand the predictive markers for hydroxyurea therapy continues.

Roy *et al*. demonstrated a significant association of rs2071348 on the *HBBP1* gene and rs4895441 on the *HBS1L-MYB* gene and Xmn-I polymorphism with high HbF levels in 31 β-thalassemia patients.[30] Neishaburg *et al*. studied the influence of polymorphisms in the *BCL11A* gene and at the polymorphic palindromic sequence of the locus control region (LCR) 5'HS4 in 100 patients. Only the polymorphism at the LCR 5'HS4 was found to

be statistically significant in determining the patient's phenotypic variability.[31]

Banan *et al*. studied the SNPs in the *BCL11A* gene (rs766432 and rs4671393) and the *HBS1L-MYB* gene (rs9399137, and rs4895441) in 89 transfusions dependent Iranian β-thalassemia patients to predict the responders to hydroxyurea therapy. Of the 89 patients, 41 were good responders whereas 26 patients were medium responders and 22 were non-responders. These responders showed a significant correlation with the presence of the Xmn-I polymorphism and the presence of C and A alleles in the *BCL11A* SNPs (rs766432 and rs4671393).[32] Our study showed no statistical correlation with these SNPs in the *BCL11A* gene and SNPs in the *HBS1L-MYB* intergenic regions and the responders to hydroxyurea therapy.

Genetic modifiers of HbF levels have also been evaluated in SCD patients. Lettre *et al*. demonstrated a 20% variation in HbF levels in SCD patients due to the polymorphisms in the *BCL11A, HBS1L-MYB,* and γ-globin gene (HBG2) and these SNPs were also associated with pain crisis rate in SCD.[33] Sheehan *et al*. showed 3 polymorphisms in the *BCL11A* gene (rs1427407, rs7557939, and rs11886868) associated with a significant increase in the baseline Hb and HbF as compared to the wildtype and these *BCL11A* gene polymorphisms and *HBS1L-MYB* intergenic region polymorphisms (rs28384513 and rs9399137) showed no influence on the degree of HbF increase in the 94 pediatric SCD patients on hydroxyurea therapy besides influencing the baseline HbF.[34] Similarly, Green *et al*. showed that SNPs in the *BCL11A* and *HBS1L* genes were not responsible for the increase in HbF, rather a polymorphism in the HBE loci was associated with the hydroxyurea-induced HbF increment in SCD patients.[35]

The cAMP and cGMP induces HbF in primary erythroid cells by downregulating the *BCL11A* gene thus providing the therapeutic scope for induction of HbF in these patients.[36] Basak *et al*. reported a rare microdeletion of 2p15–p16.1 in the *BCL11A* gene resulting in the persistence of HbF in adult life. The patients with this deletion also showed an autism spectrum disorder and developmental delay suggesting the role of *BCL11A* gene in silencing HbF in adult life and neurodevelopment.[37]

Alebouyeh *et al*. demonstrated an increase in Hb concentration and a reduction or independence in transfusion requirements after hydroxyurea therapy in transfusion-dependent Iranian patients; however, the response was attributed to the presence of Xmn-I (T) polymorphism and the less severe β-thalassemia mutation IVS2-1(G→A).[14] Karimi *et al*. showed that 78% of the regularly transfused β-thalassemia intermedia patients (age at presentation >2 years) showed a good response to hydroxyurea therapy (transfusion-free).[38] Dixit *et al*. demonstrated a good response to hydroxyurea therapy in thalassemia intermedia patients (45.9%) who started transfusion after 5 years of age and were on occasional transfusion or a regular transfusion regimen before hydroxyurea therapy.[39] El-Beshlawy *et al*. showed that the mean age at diagnosis of β-thalassemia intermedia patients was 4.11 ± 1.68 years of which 25 patients were frequently transfused before hydroxyurea therapy whereas after hydroxyurea therapy only 2 were regularly transfused, and the rest became transfusion independent.[27] Our results are in agreement with these studies, and a statistically significant difference was observed between the age at presentation among the responders and non-responders to hydroxyurea therapy [Figure 1]. We, therefore, suggest that the best indicator to start hydroxyurea in β-thalassemia patients is the age at presentation and those patients presenting after 2 years of age are more likely to respond even if they are transfusion dependent.

Thus, response to hydroxyurea therapy in β-thalassemia patients remains a mystery as there is no genetic marker which can accurately predict the response to the drug. However, our study suggests a good response to hydroxyurea therapy in those β-thalassemia patients who presented late with severe anemia and subsequently required transfusion therapy. Long-term efficacy can be ensured if the patient is a good responder to hydroxyurea therapy, not requiring transfusion and maintaining a stable Hb above 7.5 g/dL. None of the genetic determinants studied were useful in predicting the variable response to hydroxyurea therapy in Indian β-thalassemia patients.

CONCLUSION

This study shows that the long-term use of hydroxyurea in β-thalassemia patients is safe. However, no genetic marker, except Xmn-I polymorphism to some extent, can predict the response to hydroxyurea therapy. Our study also proved that the late onset of the disease showed a better response to hydroxyurea therapy.

DISCLOSURES

Authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

KG and RC designed the research study. CS and KG clinically evaluated the patients, KI performed the hematological and molecular analysis, KI and AN analyzed the data. KI wrote the paper, and RC and KG critically reviewed the paper.

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REFERENCES

1. Weatherall DJ, Clegg JB. The Thalassemia Syndromes. 4th ed. Oxford, UK: Blackwell Scientific Publications, Oxford University Press; 2001. p. 100.
2. Rachmilewitz EA, Schrier SL. Pathophyisology of thalassemia. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, editors. Disorders of Hemoglobin: Genetics, Pathophysiology and Clinical Management. Cambridge: Cambridge University Press; 2001. p. 233-51.
3. Vichinsky EP. Changing patterns of thalassemia worldwide. Ann N Y Acad Sci 2005;1054:18-24.
4. Sukumaran PK. Abnormal haemoglobins in India. In: Sen NN, Basu AK, editors. Trends in Haematology. JB. Chatterjea Momorial Volume. Calcutta, India: Sree Saraswaty Press; 1975. p. 241-50.
5. Agarwal MB. The burden of haemoglobinopathies in India-time to wake up? J Assoc Physicians India 2005;53:1017-8.
6. Madan N, Sharma S, Sood SK, Colah R, Bhatia HM. Frequency of β-thalassemia trait and other hemoglobinopathies in Northern and Western India. Indian J Hum Genet 2010;16:16-25.
7. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, *et al.* Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. J Community Genet 2013;4:33-42.
8. Choudhry VP, Upadhyay A. Thalassemia screening and control programme. In: Ghosh K, Colah R, editors. Control and Management of Thalassemia and other Hemoglobinopathies in the Indian Subcontinent-Synoptic Views. Mumbai: National Institute of Immunohaematology; 2008. p. 36-44.
9. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, *et al.* Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the multicenter study of hydroxyurea in sickle cell anemia. N Engl J Med 1995;332:1317-22.
10. Bakanay SM, Dainer E, Clair B, Adekile A, Daitch L, Wells L, *et al.* Mortality in sickle cell patients on hydroxyurea therapy. Blood 2005;105:545-7.
11. Italia K, Jain D, Gattani S, Jijina F, Nadkarni A, Sawant P, *et al.* Hydroxyurea in sickle cell disease-a study of clinico-pharmacological efficacy in the Indian haplotype. Blood Cells Mol Dis 2009;42:25-31.
12. Arruda VR, Lima CS, Saad ST, Costa FF. Successful use of hydroxyurea in thalassaemia major. N Engl J Med 1997;336:964.
13. Loukopoulos D, Voskaridou E, Stamoulakatou A, Papassotiriou Y, Kalotychou V, Loutradi A, *et al.* Hydroxyurea therapy in thalassemia. Ann N Y Acad Sci 1998;850:120-8.
14. Alebouyeh M, Moussavi F, Haddad-Deylami H, Vossough P. Hydroxyurea in the treatment of major beta-thalassemia and importance of genetic screening. Ann Hematol 2004;83:430-3.
15. Italia KY, Jijina FJ, 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 FF, 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.
17. Ma Q, Wyszynski DF, Farrell JJ, Kutlar A, Farrer LA, Baldwin CT, *et al.* Fetal hemoglobin in sickle cell anemia: Genetic determinants of response to hydroxyurea. Pharmacogenomics J 2007;7:386-94.
18. Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, *et al.* Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 2008;322:1839-42.
19. Sankaran VG, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, *et al.* Developmental and species-divergent globin switching are driven by BCL11A. Nature 2009;460:1093-7.
20. Jiang J, Best S, Menzel S, Silver N, Lai MI, Surdulescu GL, *et al.* CMYB is involved in the regulation of fetal hemoglobin production in adults. Blood 2006;108:1077-83.
21. Thein SL, Menzel S, Peng X, Best S, Jiang J, Close J, *et al.* Intergenic variants of HBS1L-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. Proc Natl Acad Sci U S A 2007;104:11346-51.
22. Old JM. DNA based diagnosis of the haemoglobin disorders. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, editors. Disorders of Haemoglobin-Genetics, Pathophysiology and Clinical management. Cambridge: Cambridge University Press; 2001. p. 941-8.
23. Tan AS, Quah TC, Low PS, Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha-thalassemia. Blood 2001;98:250-1.
24. Mancuso A, Maggio A, Renda D, Di Marzo R, Rigano P. Treatment with hydroxycarbamide for intermedia thalassaemia: Decrease of efficacy in some patients during long-term follow up. Br J Haematol 2006;133:105-6.
25. Rigano P, Pecoraro A, Calzolari R, Troia A, Acuto S, Renda D, *et al.* Desensitization to hydroxycarbamide following long-term treatment of thalassaemia intermedia as observed *in vivo* and in primary erythroid cultures from treated patients. Br J Haematol 2010;151:509-15.
26. Mtvarelidze Z, Kvezereli-Kopadze A, Kvezereli-Kopadze M, Mestiashvili I. Hematologic response to hydroxyurea therapy in children with beta-thalassemia major. Georgian Med News 2008;156:91-4.
27. El-Beshlawy A, El-Ghamrawy M, EL-Ela MA, Said F, Adolf S, Abdel-Razek AR, *et al.* Response to hydroxycarbamide in pediatric β-thalassemia intermedia: 8 years' follow-up in Egypt. Ann Hematol 2014;93:2045-50.
28. Italia K, Colah R, Ghosh K. Hydroxyurea could be a good clinically relevant iron chelator. PLoS One 2013;8:e82928.
29. Italia K, Chandrakala S, Ghosh K, Colah R. Can hydroxyurea serve as a free radical scavenger and reduce iron overload in

β-thalassemia patients? Free Radic Res 2016;50:959-65.
30. Roy P, Bhattacharya G, Mandal A, Dasgupta UB, Banerjee D, Chandra S, *et al.* Influence of BCL11A, HBS1L-MYB, HBBP1 single nucleotide polymorphisms and the HBG2 xmnI polymorphism on hb F levels. Hemoglobin 2012;36:592-9.
31. Neishabury M, Zamani F, Keyhani E, Azarkeivan A, Abedini SS, Eslami MS, *et al.* The influence of the BCL11A polymorphism on the phenotype of patients with beta thalassemia could be affected by the beta globin locus control region and/or the xmn1-HBG2 genotypic background. Blood Cells Mol Dis 2013;51:80-4.
32. 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.
33. Lettre G, Sankaran VG, Bezerra MA, Araújo AS, Uda M, Sanna S, *et al.* DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proc Natl Acad Sci U S A 2008;105:11869-74.
34. Sheehan VA, Luo Z, Flanagan JM, Howard TA, Thompson BW, Wang WC, *et al.* Genetic modifiers of sickle cell anemia in the BABY HUG cohort: Influence on laboratory and clinical phenotypes. Am J Hematol 2013;88:571-6.
35. Green NS, Ender KL, Pashankar F, Driscoll C, Giardina PJ, Mullen CA, *et al.* Candidate sequence variants and fetal hemoglobin in children with sickle cell disease treated with hydroxyurea. PLoS One 2013;8:e55709.
36. Ikuta T, Kuroyanagi Y, Odo N, Liu S. A common signaling pathway is activated in erythroid cells expressing high levels of fetal hemoglobin: A potential role for cAMP-elevating agents in β-globin disorders. J Blood Med 2013;4:149-59.
37. Basak A, Hancarova M, Ulirsch JC, Balci TB, Trkova M, Pelisek M, *et al.* BCL11A deletions result in fetal hemoglobin persistence and neurodevelopmental alterations. J Clin Invest 2015;125:2363-8.
38. Karimi M, Darzi H, Yavarian M. Hematologic and clinical responses of thalassemia intermedia patients to hydroxyurea during 6 years of therapy in Iran. J Pediatr Hematol Oncol 2005;27:380-5.
39. Dixit A, Chatterjee TC, Mishra P, Choudhry DR, Mahapatra M, Tyagi S, *et al.* Hydroxyurea in thalassemia intermedia-a promising therapy. Ann Hematol 2005;84:441-6.

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