

Efficacy of Hydroxyurea (HU) in Reduction of Packed Red Cell (PRC) Transfusion Requirement Among Children Having Beta-thalassemia Major: Karachi HU Trial (KHUT)

Saqib H. Ansari, MBBS, DCH, DPGN, MPhil, Tahir S. Shamsi, MBBS, FRCPath, Fahad J. Siddiqui, MBBS, MSc, Muhammad Irfan, MBBS, FCPS, Kausar Perveen, BSc, Tasneem Farzana, MBBS, MCPS, Vinodh K. Panjwani, MBBS, Ayesha Yousuf, MBBS, and Tabassum Mehboob, PhD

Background: Packed red blood cell (PRC) transfusion with iron chelation is the mainstay of treatment for patients with beta-thalassemia major. Hemoglobin F augmentation is another approach to treat this hemoglobinopathy. This study evaluates the efficacy and safety of hydroxyurea (HU) in minimizing PRC transfusions in patients with beta-thalassemia major.

Method: Twenty-three patients with beta-thalassemia major received HU at a mean dose of 16 mg/kg/d. The results were analyzed at the end of 24 months. Transfusion requirement during the 6 months preceding the study was considered as the control.

Result: Twenty patients were evaluable after 24 months. The mean volume of PRC transfused was reduced from 2126.45 mL to 1489.59 mL ($P < 0.001$). The interval between transfusions was increased by 68.7%. Grade I myelosuppression was observed in 4 patients and diarrhea in 2 patients.

Conclusions: HU was found to be safe in patients with beta-thalassemia major, and resulted in reduction in the

transfusion requirements and in increase of the intervals between transfusions.

Key Words: hydroxyurea, beta-thalassemia major, transfusion volume

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Beta-thalassemia is the most prevalent genetically transmitted blood disorder with a carrier rate of 5% to 8%; around 5000 children are diagnosed each year in Pakistan.^{1,2} Efforts are being made to prevent this disorder.³ Limited supply of blood and risk of transfusion-transmitted viral infections prompted researchers to look for alternative approaches to manage beta-thalassemia.⁴ Affected infants maintain normal hemoglobin levels till the first couple of months after birth. Methylation of gamma-globin gene at this time switches off its synthesis. Mutated beta-globin gene is unable to provide functional beta-globin chains to combine with alpha-globin chains, resulting in severe anemia. Hemoglobin F (HbF) augmentation is an exciting concept; if gamma-globin gene can be reactivated in postnatal life, then gamma-globin chain synthesis will restart. This will reduce the imbalance of alpha-beta/nonalpha globin chain ratio in red cells and ameliorate the biochemical defects in hemoglobin molecule and partially correct the ineffective erythropoiesis.⁵

There are a number of pharmacologic agents at different stages of development that can induce HbF production.^{6,7} 5-Azacytidine was the first HbF-inducing agent tested in patients with sickle cell disease (SCD).^{6,7} Further studies were abandoned because of the concerns regarding its carcinogenic potential. rHuEPO treatment has shown an increase in HbF levels in transfusion dependent beta-thalassemia patients in various studies.⁸ Variable degree of response has also been seen in small groups of patients during therapy with arginine butyrate in homozygous beta-thalassemia.^{9,10} In contrast to 5-azaC, hydroxyurea (HU) is an oral agent widely used in the treatment of myeloproliferative disorders with a good safety profile. HU was first used in a number of small-scale nonrandomized clinical studies that confirmed its

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From the Bismillah Taqee Institute of Health Sciences and Blood Disease Center.

Contribution of authors: Dr Saqib H. Ansari designed the protocol and was involved directly in patient care throughout the study. He did the literature search and wrote the manuscript. Dr Tahir S. Shamsi is the mentor of the group. He gave intellectual input, defined strategy, and defined questions that needed to be addressed. He was involved in patient care and contributed in writing and editing the manuscript. Dr Fahad J. Siddiqui is a Biostatistician. He contributed in the statistical analysis of data. Dr Muhammad Irfan was involved in patients care and contributed in data collection and literature search. Ms Kausar Perveen was actively involved in data collection and biostatistical analysis. Dr Tasneem Farzana participated in patient care and manuscript writing. Dr Vinodh K. Panjwani was actively involved in patient care, data collection, and literature search. Dr Ayesha Yousuf participated in writing the protocol and designing patient data form. She was also involved in patient care. Dr Tabassum Mehboob participated in manuscript writing.

Reprints: Dr Saqib H. Ansari, MBBS, DCH, DPGN, MPhil, Bismillah Taqee Institute of Health Sciences and Blood Disease Center, ST-19, Block-5, Rashid Minhas Road, Gulshan-e-Iqbal, Karachi (e-mail: ansarisaqib@hotmail.com).

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HbF inducing activity in SCD.^{11,12} Nitric oxide has been implicated in the mechanism of HbF synthesis induced by HU.¹³ Zileuton, a structural analog of HU, has been found to have a dose-dependent effect in increasing gamma-globin expression in K562 cell line.¹³

Clinical and hematologic benefits have been reported in patients with thalassemia intermedia treated with HU.^{5,14} The response of HU in thalassemia major has been studied in small series of patients.¹⁵⁻¹⁸ These studies have shown promising results in respect to the increment of Hb level. In one study, 61% of beta-thalassemia patients became transfusion-independent on HU therapy.¹⁹

In Pakistan, blood is in short supply and blood-banking facilities are primitive even though they are developing gradually. Voluntary blood donation is not a common practice. Generally, the available amount of blood is transfused to thalassemic children throughout the country with the exception of 4 big cities, as whole blood. In the absence of national guidelines, institutions use different cut off Hb limit for transfusing patient, which is based on their particular circumstances. These limits range between 6 and 7 g/dL. Owing to social and economic reasons, patients could not maintain the ideal pretransfusion hemoglobin of 9 to 10.5 g/dL. On the basis of these observations, we designed a clinical trial to evaluate the safety and efficacy of HU in beta-thalassemia major.

METHODS

Bismillah Taquee institute of health sciences and blood diseases center is a tertiary care center for hematologic disorders, which also provides bone marrow transplantation facilities. We conducted a clinical trial from July 2003 to July 2005. Institutional ethical committee approved this trial, which followed the good clinical practice guidelines of Helsinki Declaration. Written informed consent in local language was obtained from the parents of the patients.

Twenty-three children, residents of Karachi, were treated with HU (Cap. Hydrea, Bristol-Myers Squibb, Latina, Italy) at a mean dose of 16 mg/kg/d (10 to 20 mg/kg/d mg) for 24 months.

Inclusion criteria included diagnosis of beta-thalassemia major, having been registered at our center for at least last 6 months, being of age between 2 and 18 years and requiring more than 8 transfusions a year. Exclusion criteria included evidence of any other chronic illness unrelated to thalassemia and known hypersensitivity to HU. Criteria for noncompliance were to not attend the clinic before one-third of the study was completed or failure to keep his/her appointments 50% of the times at the first 12 months of the study.

Outcome variable was packed red blood (PRC) transfusion required during the 24 months on HU. The same patients being observed for 6 months preceding the intervention served as control.

TABLE 1. Characteristics of Children Having Beta-thalassaemia Major Enrolled in KHU Trial, Karachi, Pakistan (2003-2004) (n = 23)

Characteristics	n (%)
Age (y)*	8.7 (2-18)
Female	8 (38)
Age at first transfusion (mo)*	8 (4-18)
Viral profile	
Hepatitis C	4 (19.04)
Hepatitis B	1 (4.7)
HIV	0 (0)
Genetic mutations	
Homozygous IVS1-5 (G-C)	9 (42.8)
IVS1-5 (G-C) and Fr 41-42	2 (9.5)
IVS1-5 (G-C) and Del 619	2 (9.5)
Others	8 (38)
Splenectomy	3

*Mean (range).

Main clinical and biologic characteristics recorded are mentioned in Table 1. Diagnosis was based on DNA mutation analysis for thalassemia gene. Beta-thalassemia mutations were characterized by polymerase chain reaction on the basis of the allele-specific priming known as Amplification Refractory Mutation System (ARMS). Initially patients were reviewed every week for 1 month and then every 15 days for 24 months. On every visit, thorough clinical examination and blood counts were performed. Baseline investigations included complete blood count, reticulocyte count, liver function, renal functions, uric acid, serum calcium, phosphate, and serum ferritin. Anti-HIV antibodies, anti-HCV antibodies, HBsAg, DNA analysis for beta-thalassemia mutations, Hb electrophoresis, abdominal ultrasound, and electrocardiography. At 3rd, 6th, 12th, and 24th months, serum ferritin, echocardiography, and abdominal ultrasound were repeated. Biochemistry was performed using Merck Germany kits and CBC was done on Sysmex SF 3000 analyzer, (Sysmex Corp, KOBE, Japan). Viral markers were performed on Ortho-Clinical Diagnosis, Johnson & Johnson, Vitros Eci Immunodiagnosis System, Rochester. Hb electrophoresis were carried out on Helena Biosciences electrophoresis system, Helena Laboratories, TX. Echocardiography and abdominal ultrasound were done on logic book X.P, G.E Medical system). Criteria for transfusing a patient after initiating the intervention were Hb of 6 g/dL, symptoms or signs of anemia including lethargy, dyspnea, tachycardia, edema, loss of appetite, or infection including high grade fever (= 38°C), diarrhea, etc. (even if the Hb > 6 g/dL). HU was withheld if there were side effects like marrow suppression, gastrointestinal symptoms like diarrhea, vomiting, or elevation of creatinine levels above the 2-times the normal range.

Statistical Analysis

Means and SD were reported where data was distributed normally otherwise median and ranges were reported. Paired *t* test was used for estimating significant

difference between the preintervention and postintervention values. Treatment observation period was 4 times that of the preintervention period, and thus, the total volume of PRC transfused during treatment was divided by 4. We also analyzed any statistically significant reduction in frequency of PRC transfusion as measured by average interval between 2 transfusions before and after the treatment and serum ferritin levels by using paired *t* test.

RESULTS

Twenty out of 23 patients were studied at the end of 24 months on HU. Two patients were dropped because of noncompliance. In all patients, mean volume of PRC transfused was reduced. The hematocrit of PRC unit was between 55% and 60%. Difference of mean PRC volume required before and after intervention was 637.3 mL [95% confidence interval (CI): 402.8, 871.8; *P* value: < 0.001]. This translates into 42.8% reduction in PRC volume transfused. Interval between 2 transfusions also increased by 68.7%, corresponding to a mean increase of 12.1 days (CI: -18.0, -6.3; *P* value: < 0.001). Mean base line Hb was 8.6 g/dL and mean pretransfusion Hb level was 7.2 g/dL. One patient maintained her mean Hb of 8.2 g/dL, did not receive any transfusion during the 2 years. Hence time to next transfusion was at least 730 days. This made an extreme outlying value. Mean (SD) interval between transfusions with and without this value was mentioned in Table 2. At the end of the study, statistically insignificant increase was noted in serum ferritin levels with mean difference of 658.0 ng/L (95% CI: -1428.6, 112.6; *P* value: 0.09). Results are mentioned in Table 2. Linear negative correlation has been found between late first transfusion and response to HU (R2: 15.4%; *P* value: < 0.001).

One death was observed in an 18-year-old boy after 15 months on HU. He had severe iron overload and developed cardiac arrhythmia and did not respond to intensive therapy including continuous intravenous infusion of desferrioxamine. No other severe adverse event was observed. Four children (20.0%) developed mild myelosuppression, which was reversed within a week of withdrawal of the HU. Two patients (10.0%) developed diarrhea. HU was withdrawn for 1 week, which relieved their symptoms.

Subsequently, HU was restarted in all these 6 patients and was tolerated well. No other previously reported side effects with HU including confusion, headache, hallucinations, rashes, pruritis, alopecia, dys-urea, oligurea, or polyurea were observed. All laboratory parameters including liver function, renal function, blood counts remained comparable to the baseline levels throughout the study. One patient, who had splenomegaly at the onset of the trial, developed hypersplenism after 14 months on therapy and was splenectomized. She did not receive HU after the surgery.

DISCUSSION

HU increases the fetal hemoglobin production in SCD. It promotes fetal hemoglobin production via reactivation of gamma-globin gene. A series of clinical trials with HU for SCD proved that this medicine is effective and significantly reduces painful crises, occurrence of chest syndrome, and the frequency of transfusions.²⁰⁻²² Patients with thalassemia intermedia have also shown clinical and hematologic improvements.^{15,23} Such effects of HU in patients with thalassemia major have not been adequately evaluated. However, available data shows clinical and hematologic benefits.²⁴⁻²⁶

In our study, total PRC volume required for 24 months after starting medicine was reduced in all 20 patients. Our findings are consistent with other studies, which also have shown an overall decrease in PRC volume transfusion, with 35% of their patients needed less than half of the initial requirement.²⁵ Bradai et al²⁶ reported cessation of transfusion requirement in all 7 patients treated with HU. Their results may be due to the fact that these patients had initiated transfusion therapy in older age. In our cohort too, linear negative correlation has been found between late first transfusion and response to HU (R2: 15.4%; *P* value: < 0.001), where median age at first transfusion was 7 (range: 4 to 18) months. One of the patients who was receiving PRC twice a month, did not receive any transfusion during 24 months on HU. Increase in interval between 2 transfusions was observed in our group, which is consistent with decrease in their transfusion requirements.

Increase in fetal Hb which neutralizes the excess alpha-globin chain in red cells lead to longer red cell life span, decreased red cell lysis hence decreased free iron, which is toxic radical and causes further cell lysis. Therefore, breaking of the vicious cycle may be responsible for increased intervals and decreased volume of PRC

TABLE 2. Effects of HU on Children Having Beta-thalassaemia Major in KHU Trial, Karachi, Pakistan (2003-2005)

Characteristics	Base Line, Mean (SD)	End of Therapy, Mean (SD)	<i>P</i>
PRC transfusion	2126.45 (612.3)*	1489.13 (672.8)†	< 0.001
Interval between transfusions (d) (outlier included)	21.4 (4.8)	68.41 (156.3)	0.19
Interval between transfusions (d) (outlier excluded)	21.4 (5.0)	33.59 (14.6)	< 0.001
Serum ferritin (ng/mL)	3807 (1810)	4465 (2351)	0.09
Urea (mg/dL)	20.7 (5.8)	23.0 (8.4)	0.35
Creatinine (mg/dL)	0.51 (0.9)	0.46 (0.9)	0.14
SGPT (μ/L)‡	105.0 (64.7)	109.8 (67.7)	0.82
Alkaline phosphatase (μ/L)	670.0 (155.9)	643.3 (212.2)	0.01
Spleen size (cm ²)	10.3 (2.6)	11.8 (2.8)	< 0.001
Liver size (cm)	12.6 (2.0)	13.3 (2.2)	0.01

*Volume of PRC transfused in preceding 6 mo.

†Volume of PRC transfused during 24 mo on HU (adjusted).

‡Median (interquartile range).

required. It has been postulated that the effect of HU on gamma-globin gene expression is associated with the G-158 C > T polymorphism (Xmn I).¹⁸ We could not demonstrate any correlation between Xmn I and response to HU in 11 of our patients, whom we analyzed for this polymorphism.

The spacing between transfusions observed in our trial will somewhat relieve families from financial burden besides reducing pressure on demand of blood and its products. A healthy psychosocial effect is also expected on children. In addition to decreased PRC requirement, nearly every child experienced a sense of well being as indicated by improved exercise tolerance, increased appetite, weight gain, better school attendance, and mother's perception of improved socialization as seen in other study.¹⁸

According to previous studies that have shown that HU is a safe medicine in pediatric patients of SCD,^{11,21} we did not observe any severe adverse event and all our patient tolerated the medicine well. The patient who expired, was a splenectomized 18-year-old male, had organomegaly and high serum ferritin before enrollment in the study and through out the study. He was admitted for refractory cardiac arrhythmias due to secondary hemochromatosis, unresponsive to aggressive iron chelation therapy given for a month. Therefore, probably this death is not associated with HU therapy. The observed increase in serum ferritin levels was not statistically significant and it could be due to the fact that most of the children continued to get PRC transfused with suboptimal chelation therapy compliance.

CONCLUSIONS

We found a significant reduction in transfusion requirement, increased interval between transfusions, and associated improvement in the sense of well being in children with beta-thalassemia major treated with HU. HU can be used safely in children with beta-thalassemia major. Additional studies with longer follow-up and diverse population are required to further establish the role of HU in the treatment of thalassemia syndrome.

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