Original Research Article

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20172697

Efficacy and safety of high dose hydroxyurea in transfusion dependent thalassemic children: a quasi experimental study

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Received: 10 May 2017 Accepted: 03 June 2017

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ABSTRACT

Background: This study was conducted to find out whether high dose hydroxyurea is an effective and safe modality, in inducing haemoglobin synthesis to decrease blood transfusion requirement in transfusion dependent thalassemics. **Methods:** This quasi experimental un-control before and after comparison study was conducted in Thalassemia Day Care Centre, Department of Pediatrics over a period of six months after obtaining an approval from the Institute's ethical committee. Fifty transfusions dependent thalassemic children belonging from 2 to 18 yrs were given hydroxyurea in dose of 20mg/kg after getting consent. Pre and post intervention haemoglobin and HbF levels were obtained using Hb electrophoresis by HPLC. Paired t test was applied to find out statistical significance and p value <0.05 was taken as significant.

Results: Significant rise in haemoglobin pre and post intervention (p<0.001) but the rise in HbF was not significant (p=0.110). One patient had bone marrow depression which was reversible with drug withdrawal and one patient had rise in s. creatinine.

Conclusions: High dose hydroxyurea is an effective and safe drug in inducing Hemoglobin synthesis in transfusion dependent thalassemics.

Keywords: Haemoglobin F, High dose hydroxyurea, Transfusion dependent thalassemia

INTRODUCTION

β-Thalassaemia major is a hereditary anaemia characterized by ineffective erythropoiesis haemolysis.1 The underlying mechanism is defective production of haemoglobin β- chains, resulting in excess of α-chains, which are unstable and precipitate to form bodies.^{2,3} inclusion This intracellular intracellular deposition of α-chain material is responsible for accelerated apoptosis of the erythroid precursors and for peripheral haemolysis of the erythrocytes.³ By the age of 3-6 months, severe anaemia develops leading to

increased intestinal iron absorption. To maintain haemoglobin at a level of 10–12 g/dl, patients suffering from β -thalassaemia major require repeated blood transfusions. Frequent blood transfusions not only aggravate the iron overload but are associated with risk of transfusion transmitted infections like HIV, heapatitis C and hepatitis B.

Hydroxyurea (or hydroxycarbamide) primarily a cytotoxic, antimetabolic, and antineoplastic agent also induces Hb F synthesis by stimulating γ -globin production. Besides stress erythropoeisis which is

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considered to be the primary mechanism, production of nitric oxide and the soluble guanylyl cyclase and cyclic guanosine monophosphate—dependent protein kinase pathway gene have been proposed as being responsible for inducing γ -globin synthesis. In addition to its known effects in stimulating γ -globin production, hydroxyurea may have a more general role in augmenting globin synthesis, including β -globin in some patients who maintain the capacity to express normal β -globin chains. Thus hydroxyurea not only induces Hb F but overall production of Hb also. After being identified as a potent Hb inducer, hydroxyurea became one of the key therapeutic agents for the management of patients with Sickle cell anemia, and has been widely evaluated in thalassemia intermedia, with varying results.

After early case reports of documented hematological improvements in β -thalassemia patients treated with hydroxyurea, several studies have evaluated the efficacy and safety of the drug in this patient population with a dose ranging from 10-20 mg/kg/day.^{5,7} Most of the studies done with fixed low dose (10mg/kg/day) of hydroxyurea showed variable rise in Hb ranging from 5 to 25 g/l. Dose as high as 35 mg/kg day has been used in sickle cell anemia.⁸ In β thalassemia a dose up to 20 mg/kg/day has been well tolerated. Bone marrow depression was the major toxicity observed at dose exceeding 20 mg/kg/day of hydroxyurea. This toxicity was dose dependent and was completely reversible on reduction of dose.

As hydroxyurea at a dose up to 20 mg/kg/day has been tolerated well in previous studies, we wish to utilize this dose in β thalassemia children to see its effects. We want to see whether this high dose (20 mg/kg per day) can induce more Hb synthesis in comparison to previous studies utilizing low dose, without producing any adverse effects. If high dose results in more induction of Hb synthesis, it would decrease the dependency on blood transfusion and will improve the ultimate outcome in β thalassemic children.

METHODS

A Thalassemia Day Care Centre is being run by Department of Pediatrics, Umaid hospital for blood transfusion facility of thalassemic children. Before every transfusion Hb was estimated. A thalassemia register was maintained for the same, in which clinical, demographic and laboratory details of all thalassemia patients were entered. From the thalassemia register 50 patients fulfilling the following inclusion and exclusion criteria were randomly selected. Details of the study including anticipated effects and side effects of the drug and duration of treatment were explained to the parents and patients, and those who were willing to participate and who gave written consent were only be included in the trial. As most of children visit every fortnightly for blood transfusion, so follow up was done every 15 days. Blood transfusion was given at 10ml/kg. Study was conducted

for six months after obtaining ethical approval from Institute's ethical committee.

Inclusion criteria

- Age group: 2-18 years
- Diagnosed cases of thalassemia major diagnosis based on quantification of HbF and HbA2 by HPLC
- Transfusion dependent: requiring blood transfusion two to three times per month

Exclusion criteria

- Pre-existing hepatic disease defined as rise of serum ALT or AST, more than two times of upper limit of normal (ULN - 45 units per litre for both)
- Pre-existing renal disease defined as serum creatinine more than 1 mg/dl
- Thrombocytopenia platelet count <100,000/mm³
- Neutropenia (PMN) <1,500/mm³

Before starting hrdroxyurea, HbF, Baseline average pretransfusion Hb of last six months, Average blood transfusion requirement of last six months, Baseline neutrophil and platelet count, Serum AST and ALT level and Serum creatinine level were obtained. During the intervention period, neutrophil, platelet count, serum AST, ALT and creatinine level were monitored every two months and hemogram every 15 days. Clinical side effects of the drugs were also noted. Mean of pretransfusion Hb during 6 months after start of hydoxyurea was calculated. At the end of study (six months from start of hyroxyurea), HbF was again measured. Hydroxyurea was temporarily stopped if Absolute Neutophil count <1,500/mm³ or platelet <100,000/mm³) or more than two -fold rise in ALT or AST or >50% increase in serum creatinine. Equipments used were electronic counter (Sysmex K-3000) for complete blood count and HPLC (Bio-Rad Variant) for HbF and HbA2. Our primary outcome is to find the rise in mean Hb (Mean pretransfusion Hb post intervention - Mean pre transfusion Hb pre intervention). Response was graded as- rise >2 g/dl – good, 1–2 g/dl– partial and < 1g/dl - no response.⁹ Secondary outcome is to calculate the rise in Hb F and assessment of side effects/toxicity of drug.

Statistical analysis

All statistical analyses were performed using SPSS software version 21. For all statistical purposes p value less than 0.05 was considered significant. Paired t test was applied to find out statistical significance

RESULTS

Out of 50 patients enrolled, 2 patients did not turn up, 11 patients were not compliant (by using Morisky Medication Adherance Score) to hydroxyurea and 2 patients developed side effects. Out of 35 patients, 24 (68.5%) were males and 11 (31%) were females.

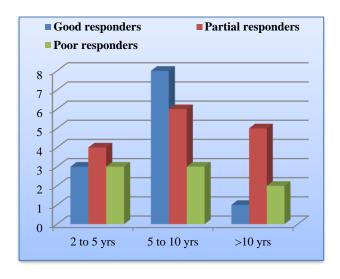


Figure 1: Age wise distribution of responders to hydroxyurea.

There were 12 (34.28%) good responders, 15 (42.85%) partial and 8 (22.8%) poor responders. Descriptive statistics of all observed parameters have been summarised in Figure 1.

Rise in mean pre-transfusion haemoglobin in good and partial responders (p<0.001) was significant but not that of poor responders (p-0.092). Mean pre-transfusion Hemoglobin rise in all patients was found to be significant (p<0.001) (Table 1).

The rise in HbF was found to be significant only in good responders (p=0.0138). Mean Spleen size was reduced in patients but the reduction was not significant (Table 2). Regarding the side effects of drug, 5 patients were reported to have epigastic discomfort, 1 had petechia owing to thrombocytopenia (on complete blood count found to have ANC=1200/mm³) and 1 patient had increase in S. Creatinine (S. Creatinine=1.9). All side effects resolved after discontinuation of drug.

Table 1: Comparison of Pre-transfusion haemoglobin parameters.

Responder	Parameters (Mean±SD)	Pre HU	Post HU	P value
Good	Hemoglobin (g/dl)	8.29±0.55	10.46±0.56	< 0.0001
Partial	Hemoglobin (g/dl)	8.3±0.38	9.4 ± 0.47	< 0.0001
No	Hemoglobin (g/dl)	8.5 ± 0.42	8.86±0.32	0.092
Total	Hemoglobin (g/dl)	8.27±0.46	9.63±0.78	< 0.0001

Table 2. Comparison of HbF and spleen size.

Responder	Parameters (Mean±SD)	Pre HU	Post HU	P value
Good	HbF	1.83±1.43	3.46±1.57	0.0138
	Spleen size	3.2 ± 2.01	2.52 ± 1.43	0.349
Partial	HbF	4.84 ± 4.25	5.89 ± 4.98	0.538
	Spleen size	3.4 ± 2.38	3.07±1.24	0.538
No	HbF	5.0±6.73	5.11±6.65	0.92
	Spleen size	3.5 ± 2.32	3.3±2.29	0.957
Total	HbF	6.05 ± 3.216	7.24 ± 2.94	0.110
	Spleen size	3.357±2.188	2.88±2.01	0.345

DISCUSSION

Hemoglobin F induction has been a longstanding therapeutic goal for the treatment of β-thalassemia. Three classes of HbF inducing agents have been introduced. They are hypomethylating agents (such as HU, decitabine and 5-azacytidine), histone deacetylase inhibitors (like sodium phenylbutyrate and isobutyrate) and finally recombinant erythropoietin. These agents have been shown to increase total Hb levels by 1-5 g/dL above baseline if administered for at least 3-6 months. Hydroxyurea is the most widely accepted HbF inducer, and its efficacy was investigated in several studies. In present study, patients were belonging to the age group of

2-18 yrs similar to the study conducted by Mancuso et al in which the patients were of age 4-16yrs. ¹³ They were transfused regularly like that of our study. Dose used in their study was variable 15-30 mg/kg and rise in pretranfusion haemoglobin was 5g/dl which is much greater than our study i.e. 1.36 g/dl. In study conducted by De Paula et al, the rise in pretransfusion haemoglobin was 4g/dl. ¹⁴ In present study, the rise in HbF was found to be significant only in good responders (p<0.001). Similar to that our study, post-HU HbF level was significantly higher in good responders compared with partial and non-responders (p<0.001), also in partial responders, a significant higher level of post-HU HbF was observed compared with non-responders (p=0.036) in study done by Mohammad Reza Bordbar et al. ¹⁵ In

present study, 12 out of 35 were good responders whereas studies conducted by Mancuso et al had 11 responders out of 18 pts; De Paula et al had 1 responder out of 4 pts; Koren et al had 9 responders out of 11; Karimi et al had 106 responders out of 120 and Bradai et al had 20 responders out of 45 patients. 13,14,16-18

This difference is supposed to be due to the interaction of many factors, including different genetic mutations, α and γ globin chains production, XmnI polymorphism and other biochemical factors contribute to the therapeutic response to HU.

Total Hb Dose Transfusion Responders Reference Follow up Increment Age vs total Mg/kg Regt (g/dl) Mancuso et al¹³ 4-16 yrs 12 months 5-30 Regularly 11/18 +5 13-21 To keep pt alive Pre Rx-4.5±0.9 Bradai et al¹⁹ 7/7 9-48 months 18.3 ± 3.5 months Pre tx-4.2g/dl Post Rx-7.9±0.8 De Paula et al¹⁴ 01/04 16-22 months 6-96 months 10-20 Regularly +4.124-60 Yavarian et al²⁰ 81/133 10-15 8-31 months To keep Hb>6 At end of study 10.3 months Karimi et al¹⁷ 106/120 4-35 yrs 8-12 To keep Hb>7 Upto 6 yrs At end of study 9.5 ± 1.5 To keep pt alive Bradai et al¹⁸ 20/45 12 yrs 12 months 17.4 ± 2.4 Tx need decreased Pre tx-4.2g/dl by 70%

6-60 months

Table 3: Comparison of various studies of hydroxyurea on thalassemic patients.

We administered HU with dose of 20mg/ kg/day, similar to Bradai et al but much lower than some other investigators Karimi's and Yavarian's. ¹⁷⁻²⁰ Comparison of various studies is listed below in Table 3.

9-34 yrs

9/11

Koren et al16

Limitations of present study included the fixed interval of transfusion fortnightly rather than giving transfusion at a pre-fixed haemoglobin which can prove effectiveness of hyroxyurea by alteration in duration of transfusion. This is because most of the patients visit the clinic fortnightly only.

Similar to present study, Mancuso et al and De Paula et al conducted the study in which transfusion was given at regular interval. ^{13,14} On the contrary to present study, Yavarian et al kept pre-transfusion Hemoglobin >6; Karimi et al kept it >7 and Bradai et al kept it >4.2g/dl to keep patient alive and demonstrated the decreased frequency of transfusion as well as increase in pre transfusion hemoglobin. ^{17,18,20}

The other limitation of present study was the relatively short study period to assess the long-term safety and efficacy of the drug. An education program is needed to convince transfusion-dependent thalassemia patients to consume HU regularly and for a long time, since most of the thalassemia major patients are adapted to transfusion, and they hardly accept to share in other treatment regimens. In conclusion, HU as the most widely studied HbF inducer can be safely prescribed to some of transfusion-dependent β -thalassemia patients in order to

diminish their transfusion requirements and bring about a feeling of well-being.

48 months after Tx-

 8.2 ± 0.7

Ml of blood/yr

137±49

HU is a safe medication in thalassemic patients. Saving in blood transfusion costs and disease complications is remarkable. Relatively mild and transient side effects are tolerable, yet patients are to be supervised periodically.

Funding: No funding sources Conflict of interest: None declared

Ethical approval: The study was approved by the

Institutional Ethics Committee

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 10.9 ± 3.0

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Cite this article as: Suthar K, Sharma P, Verma M, Goyal VK. Efficacy and safety of high dose hydroxyurea in transfusion dependent thalassemic children: a quasi experimental study. Int J Contemp Pediatr 2017;4:1514-8.