Original Article

Role of Hydroxyurea in Reducing Transfusion Requirements in Patients with β-Thalassaemia Major

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ABSTRACT:

Background: *β thalassemia major (βTM) is one of the major genetic hematological diseases of Bangladesh. Blood transfusion is yet the mainstay of treatment. Hydroxyurea (HU) is known to reduce the transfusion requirements of patients with thalassemia intermedia and sickle cell anemia, but there are very limited evidences on its role in βTM.* ***Objective:*** *To evaluate the response of patients with βTM to Hydroxyurea.* ***Methods:*** *This prospective interventional study, included 28 diagnosed transfusions dependent βTM patients who took HU for one year (Group-I) and 28 diagnosed transfusions dependent βTM patients who did not take HU for one year (Group-II). After enrollment, baseline CBC was recorded. Patients were treated with HU at an incremental dose from 10 to 20 mg/kg over 10 weeks and then continued with 20 mg/kg for 42 weeks. Both clinical and laboratory response was observed at 3rd, 6th, 9th and at 12th month.* ***Results:*** *The mean Hb at 12 months was 7.42±1.31 g/dl in group I and 6.64±0.61 g/dl in group II. Mean MCV at 6 months was 68.34±7.09 fL (Gr-I) and 65.11±0.85 fL (Gr-II), at 9 month 71.19±6.29 fL (Gr-I) and 64.98±0.59 fL (Gr-II) and at 12 month 73.79±7.38 fL (Gr-I) and 64.76±0.52 fL (Gr-II). Mean serum bilirubin at 9 months was found 1.99±0.73mg/dl (Gr-I) and 2.42±0.44mg/dl (Gr-II) and at 12 month 1.66±0.71mg/dl (Gr-I) and 2.41±0.44mg/dl (gr-II) (p<0.05). Twelve (42.9%) patients required less amount of blood with or without prolong interval than baseline in group I and none in group II. Pre-HU therapy, mean RCC requirement were 1620.0±573.3 (ml) among 12 patients in Group-I, Post-HU therapy mean RCC requirements were 1111.7±458.8 ml and mean difference was 508.3±252.7 ml (p<0.05), and there was 31.2% reduction in their transfusion requirements. Two (7.1%) patients developed side effects in group I and none in group II.* ***Conclusion:*** *This study showed an improvement of Hb status and MCV, reduction of serum bilirubin as well as transfusion requirement were significantly more, among the children treated with HU.*

Key word: *Major beta-thalassaemia, hydroxyurea, haemaglobin F, blood transfusion.*

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INTRODUCTION:

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Beta-thalassaemia is most commonly inherited blood disorder in the world, results from a number of genetic defects in globin gene expression. Deficient globin production cause imbalance in the alpha / beta chain ratio and excess alpha chains precipitate within the red blood cells (RBC) resulting in hemolysis. The phenotypic presentation varies, but two forms of severe thalassaemia can be identified, major and intermedia, which generally correlate with the degree of alpha/beta imbalance.[1] Interaction of Hb-E and β-thalassaemia gene leads to heterozygous β-thalassaemia and Hb-E/β-thalassaemia disease that is major β thalassaemia syndrome in South- East Asian region. Hb-E/β-thalassaemia can be as severe as homozygous β thalassaemia.[2] The major modalities of treatment of these patients are regular blood transfusion with iron chelating agents. Blood transfusion itself has many complications like transmission of infectious agents e.g. HIV, HCV, HBV, and Treponema pallidum etc, and toxicity due to iron overload.

Iron chelating agents are costly and beyond reach of majority of the thalassaemic patients. Though bone marrow transplantation is currently the only curative therapy, it is very expensive and facilities are only available in some highly experienced centers. Drug therapy like hydroxyurea (HU), Na-phenylbutyrate and erythropoietin were tried in different studies.[2,3]

Hydroxyurea (HU) promotes fetal haemoglobin (HbF) production via a reactivation of γ-gene as a result of molecular mechanisms that are not yet elucidated.[4] This drug may act by promoting the transcription of γ-mRNA molecules. The clinical benefit induced by this compound in patient affected with sickle cell disease had been repeatedly demonstrated.[4]

A significant benefit could also be expected in patient with Beta-thalassaemia and HbE/β-thalassaemia disease, because the imbalance in alpha and beta globin chains could be ameliorated by the newly synthesized Ɣ-chains being able to neutralize the excess alpha chain which could partially correct ineffective erythropoiesis.

METHODS AND MATERIALS:

This prospective interventional analytical study was carried out in the Department of Paediatrics, Dhaka Medical College and Hospital and Bangladesh thalassaemia Hospital, Dhaka, from January 2016 to December 2017. Previously diagnosed cases of thalassaemia major attending at Paediatric Hematology-Oncology OPD at DMCH and Bangladesh Thalassaemia Hospital, Dhaka, were the study population, 28 diagnosed transfusion dependent β-thalassemia patients who took hydroxyurea (HU) for one year (Group-I) and 28 diagnosed transfusion dependent β-thalassemia patients who did not take hydroxyurea (HU) for one year (Group-II) were enrolled purposively in this study. Diagnosis was based on complete blood count with peripheral blood film and was confirmed by Hb-electrophoresis. Patient who has fulfilled inclusion criteria, physical examination was done thoroughly and 6 ml of venous blood was collected for hematological data with strict asepsis as per standard procedure. Blood sample data included, complete blood count like Hb%, MCV, MCH, MCHC, TC and DC of WBC, PBF, Platelet counts, serum creatinine, serum bilirubin, serum ferritin, SGPT. Blood was analyzed by auto-analyzer, Japan (Sysmex, Model- X-800i, Year-2008) during enrollment and then at follow-up. Serum creatinine and SGPT tests was assessed before study and then 3 monthly for any side effects of HU therapy. After taking written consent from well informed parents of each child, the said child was assigned to one of two groups purposively designated as group I and group II respectively, as per inclusion criteria. By single blinding, group I received hydroxyurea therapy and group II received placebo therapy. Evidence of hematological toxicity was defined as white blood cell count less than 3000/cmm (or neutrophil count less than 1000/cmm) or platelet count less than 100,000/cmm.[5] When toxicity developed as shown in data sheet, the hydroxyurea was discontinued for 2-3 weeks. After recovery from toxic conditions the hydroxyurea was reinstituted.

Patients were initially treated with a dose of hydroxyurea of 10 mg/kg/day in a single dose. This dose was maintained for approximately 10 weeks, at which point, in the absence of hematological

toxicity, the dose was increased by 3-5 mg/kg/day to 20mg/kg/day and continued for 42 weeks.

After initiation of HU therapy each patient was followed up 3 monthly for one year at outpatient department of Paediatric Hematology and Oncology of DMCH and Bangladesh Thalassaemia Hospital, Dhaka. During follow-up patients were assessed by careful history, physical examinations, drug compliance, and laboratory investigations. A follow-up sheet containing all these parameters for each patient was maintained. Red cell concentrate (RCC) transfusion was given after collecting 6 ml of blood for hematological study and Hb% was maintained at 8 gm/dl or more or as clinically indicated. All patients were given Folic acid 5 mg/day. Dietary advice regarding reducing of iron over load was given.

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Unpaired and paired t-test was used to analyze the continuous variables, shown with cross tabulation. P values <0.05 was considered as statistically significant.

RESULT:

Mean hemoglobin at 12 month was found 7.42±1.31 g/dl in group-I and 6.64±0.61 g/dl in group-II. Mean MCV at 6 month, 9 month and 12 month was found in group-I 68.34±7.09 fL, 71.19±6.29 fL and 73.79±7.38 fL and in group-II was 65.11±0.85 fL, 64.98±0.59 fL, 64.76±0.52 fL respectively, which were statistically significant (p<0.05) between two groups. Mean MCH and MCHC at baseline, 3 month, 6 month, 9 month and at 12 month were almost similar between two groups (Table I). Mean serum bilirubin at 9 month was found 1.99±0.73 mg/dl in group-I and 2.42±0.44 mg/dl in group-II. Mean serum bilirubin at 12 month was found 1.66±0.71 mg/dl in group-I and 2.41±0.44 mg/dl in group-II, which were statistically significant (p<0.05) (Table-II). Twelve (42.9%) patients required less amount of blood with or without prolong interval than baseline in group-I and not found in group-II. The difference was statistically significant (p<0.05) between the groups (Table-III). Among 12 patients in Group-I before HU therapy, mean value of RCC requirement were found 1620.0±573.3 ml, after treatment with HU, their mean value of RCC requirements were 1111.7±458.8 ml. Mean difference was 508.3±252.7 ml which was statistically significant. These patients exhibited 31.2% reduction in their transfusion requirements (Table-IV). Two (7.1%) patients developed side effects in group-I and not found in group-II. The difference was not statistically significant (p>0.05) between two groups (Table-V).

Table I: Mean hemoglobin, MCV, MCH and MCHC in different follow up

	Group-I (n=28)	Group-II (n=28)	P value
	Mean±SD	Mean±SD	
Hemoglobin (g/dl)			
Baseline	6.86±1.07	6.81±0.66	0.834^{ns}
At 3 month	6.87±1.06	6.76±0.66	0.643^{ns}
At 6 month	6.98±1.16	6.75±0.61	0.357^{ns}
At 9 month	7.24±1.27	6.75±0.60	0.070^{ns}
At 12 month	7.42±1.31	6.64±0.61	0.006^{s}
MCV (fL)			
Baseline	66.60±4.72	65.17±0.74	0.119^{ns}
At 3 month	65.77±6.00	65.14±0.69	0.583^{ns}
At 6 month	68.34±7.09	65.11±0.85	0.020^{s}
At 9 month	71.19±6.29	64.98±0.59	0.001^{s}
At 12 month	73.79±7.38	64.76±0.52	0.001^{s}
MCH (pg)			
Baseline	20.51±2.41	21.41±2.40	0.167^{ns}
At 3 month	20.63±2.61	21.37±2.35	0.270^{ns}

At 6 month	21.48±2.47	21.40±2.38	0.902[ns]
At 9 month	22.14±2.73	21.46±2.38	0.325[ns]
At 12 month	22.64±3.16	21.49±2.37	0.129[ns]
MCHC (g/dl)			
Baseline	30.58±2.00	30.91±1.68	0.507[ns]
At 3 month	30.60±1.64	30.90±1.69	0.503[ns]
At 6 month	30.91±1.61	30.94±1.71	0.946[ns]
At 9 month	31.32±1.84	31.01±1.72	0.517[ns]
At 12 month	31.75±1.80	31.07±1.71	0.153[ns]

s= significant, ns=not significant

P value reached from unpaired t-test

Table II: Mean serum bilirubin in different follow up

Serum bilirubin (mg/dl)	Group-I (n=28)	Group-II (n=28)	P value
	Mean±SD	Mean±SD	
Baseline	2.52±0.79	2.46±0.47	0.731[ns]
At 3 month	2.29±0.68	2.45±0.47	0.310[ns]
At 6 month	2.14±0.68	2.44±0.47	0.060[ns]
At 9 month	1.99±0.73	2.42±0.44	0.010[s]
At 12 month	1.66±0.71	2.41±0.44	0.001[s]

s=significant; ns=not significant

P value reached from unpaired t-test

Table III: Clinical effects of hydroxyurea scored by the test subjects on the scale of amount and interval of blood transfusion

	Group I (n=28)		Group II (n=28)		P value
	n	%	n	%	
Patients required less amount of blood with or without prolong interval than baseline	12	42.9	0	0.0	0.001[s]
Patients required same (as per HU) schedule of blood transfusion	16	57.1	28	100.0	

s= significant

P value reached from chi square test

Table IV: Patients required less amount of blood with or without prolong interval than baseline in group-I (n=12)

	Mean±SD	Range
Last 12 months before HU therapy (ml)	1620.0±573.3	900-2750
12 months after HU therapy (ml)	1111.7±458.8	660-2300
Difference (ml)	508.3±252.7	150-1000
p value	**0.001[s]**	
Change of RCC requirement (%)	31.2%	16.4-42.3

s= significant

P value reached from paired sample t test

Table V: Side effects of hydroxyurea therapy by the test subjects and placebo therapy by control cases

Side effects	Group I (n=28)		Group II (n=28)		P value
	n	%	n	%	
Patients developed side effects	2	7.1	0	0.0	
Patients developed without any side effects	26	92.9	28	100.0	0.245[ns]

ns= not significant

P value reached from chi square test

DISCUSSION:

Response was observed at 3rd month, 6th month, 9th month and finally at 12th month with clinical as well as hematological parameters. Red cell concentrate transfusion was given to maintain Hb% gm/dl or more or as clinically indicated.

This study shows that mean hemoglobin at 12 month was 7.42±1.31 g/dl in group-I and 6.64±0.61 g/dl in group-II, which was statistically significant (p<0.05). Singer et al.[6] conducted a multicenter trial of hydroxyurea among patients with HbE-β-thalassaemia in which 50% patients showed a significant increase in haemoglobin concentration after 2 years of therapy. This lack of a considerable and steady effect could underlie the relatively modest change in Hb in thalassemia as a result of hydroxycarbamide treatment as previously noted.[7] Hydroxyurea treatment also resulted in an overall increase in Hb from 6.8±0.8 to 7.4±1 g/dl (P <0.001). Hossain et al.[8] study revealed a statistically significant increase of total Hb concentration in 12 of 32 patients from 7.0±0.9 to 8.8±1.0 gm/dl (mean 1.8±0.3, P<0.001) which are more or less similar to the values reported in many studies done in different countries [9,10,11] but slight lower extent than the value reported by others [18] may be due to relatively less dose of HU and small number of study case.

In the current study, it was observed that mean MCV at 6 month was 68.34±7.09 fL in group-I and 65.11±0.85 fL in group-II and at 9 month was 71.19±6.29 fL in group-I and 64.98±0.59 fL in group-II. Mean MCV at 12 month was found 73.79±7.38 fL in group-I and 64.76±0.52 fL in group-II, which were statistically significant (p<0.05) between the groups. This increase in MCV with HU therapy has got correlation with the increased MCV found in other studies,[11,12] Fucharoen et al.[5] study observed that MCV was found 64.8±7.1 fL at baseline and 74.2±6.2 fL after 5 month HU therapy. The difference was statistically significant (p<0.05) between the group.

In the present study, it was observed that mean MCH at baseline, 3 month, 6 month, 9 month and at 12 month were almost similar between two groups. Other studies observed that in the responders, a significant increase was seen in MCH (p<0.01).[8,12,13,14,15,16]

This study showed that mean serum bilirubin at 9 month and at 12 month were statistically significant (p<0.05) between the groups. Italia et al.[13] observed a significant decrease in serum total bilirubin concentration (p<0.01) in all the patients. Hossain et al.[8] study showed a significant (p<0.001) reduction of serum bilirubin concentration from 2.9±1.2 mg/dl to 1.8±0.6 mg/dl (mean 1.06±0.6 mg/ dl).

In the present study, it was observed that 12(42.9%) patients required less amount of blood with or without prolong interval than baseline in group-I and not found in group-II. The difference was statistically significant (p<0.05) between two groups. Italia et al.[13] observed that four of the 11 patients who had clinically severe disease (36.3%) became transfusion independent (responders) after 2-6 months of treatment, and four (36.3%) showed a reduction in their transfusion requirements of 30-50% (partial responders). However, no change in transfusion requirements was seen in three patients (27.2%) (non-responders). Hossain et al.[8] showed, regarding the need of blood transfusion, 8 of 32 patients need less amount of blood at prolonged interval than pre-HU therapy and 4 of 32 patients

need no blood transfusion at all after HU therapy which are statistically significant (P<0.001) compared to the placebo-assigned patients. Need of less amount of blood or no need of blood transfusion by the patients after HU therapy have also been found by many other investigators.[10,11,17] So, our results support improvement of ineffective erythropoiesis of these patients. But we cannot definitely say whether this improvement was achieved due to change of HbF and HbE level of our cases as we could not measure HbF and HbE because of technical limitations.

The present study observed that pre-hydroxyurea therapy, mean value of RCC requirement were 1620.0±573.3 ml among 12 patients in Group-I, Post- hydroxyurea therapy their mean value of RCC requirements were 1111.7±458.8 ml, mean difference was 508.3±252.7 ml which was statistically significant. These patients exhibited 31.2% reduction in their transfusion requirements. Italia et al.[13] showed a very good responsiveness (74%) in thalassemia intermedia patients. However, only a third of his patients with thalassemia major had more than 50% reduction in their transfusion requirements. Similarly Bradai et al.[18] tried HU in 45 patients with thalassemia major with a mean dose of 17 mg/kg. About half of his patients exhibited more than 70% reduction in their transfusion requirements which by our definition meant good response. However, increasing the dose of HU had no impact on the transfusion needs.

The current study observed that 2(7.1%) patients developed side effects in group-I and not found in group-II, and was not statistically significant (p>0.05) between the groups. One developed abdominal pain, fatigue, anorexia, and vomiting and one patient had increased liver enzyme. However, adverse reactions were resolved after a short term of discontinuing HU. Hossain et al.[8] reported that only 4 patients out of 33 with HU therapy, developed few side effects like pain in the extremities, anorexia and increased general weaknesses. Only one patients developed severe anaemia and bicytopenia.

So from the above discussion, the present study supports the hypothesis that hydroxyurea (HU) reduces red cell concentrate transfusion requirements in patients with β-thalassemia major.

CONCLUSION:

It is concluded from this study that improvement of haemoglobin status and MCV, reduction of serum bilirubin as well as transfusion requirement were significantly more among the children with β-thalassemia major treated with hydroxyurea. But for generalization of this finding, a large multi-centric study is required.

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