Efficacy of hydroxyurea on the rate of blood transfusion requirement and serum ferritin levels in β-thalassemia major

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INTRODUCTION

In 1925, Cooley and Lee first described a form of anemia that occurred early in life and was associated with splenomegaly and bone changes. They named it as Cooley’s anemia.1 They described it as a congenital hemolytic anemia syndrome showing microcytic hypochromic anemia of varying severity with well-defined features such as anemia, icterus, characteristic facial appearance, skeletal changes, splenomegaly, familial aspects and appearance of large number of normoblasts in peripheral blood film. Similar descriptions were published shortly afterward by Greppi and Michelli.2

There are more than 200 mutations in β-thalassemia most of which are rare. Hemoglobin is a conjugated protein of molecular wt. 64000 KD and consisting of an Iron
containing portion, the haem, and a protein portion called globin, which consists of two pairs of polypeptide chains. Haemoglobin exists in various forms differing in structure of globin chain only, whereas haem portion is same in all types of haemoglobin.\(^3\)

Haemoglobin A (HbA) consists of two globin α-chains and two globin β-chains.

Haemoglobin F (HbF) consists of two globin α-chains and two globin γ-chains.

Haemoglobin A\(_2\) (HbA\(_2\)) consists of two globin α-chains and two globin delta chains.

Thalassemias are most common single gene disorder in world and represent a major health burden. About 15 million have clinically apparent thalassemic disorder.\(^4\)-\(^6\) Individuals with thalassemia major have a quantitative defect in beta chain production and thus decreased levels of HbA. Such patients have severe anemia and hepatospleenomegaly and usually come to medical attention within the first two years of life. Without treatment, affected children suffer from severe failure to thrive and have shortened life expectancy.

Mainstay of treatment of severe β-thalassemia is regular blood transfusions. Blood transfusion is mandatory for children with thalassemia major and thalassemia intermedia who cannot maintain Hb above 7gm/dl, or those who show evidence of growth retardation and hyperspleenism. Complications secondary to transfusional iron overload can be prevented by adequate iron chelation with desferrioxamine, deferriprone and deferasirox over last 3 decades development of regular blood transfusion therapy and iron chelation has dramatically improved the quality of life. Hydroxyurea is an oral agent widely used in the treatment of myeloproliferative disorders with a good safety profile. Hydroxyurea was first used in a number of small scale non-randomized clinical studies that confirmed its HbF inducing activity in sickle cell disease. Present study was to evaluate the role of Hydroxyurea in β-thalassemia major patients in already resource constrained settings.

METHODS

This was a hospital based interventional study, and was conducted over a period of 10 months at thalassemia clinic in post graduate department of pediatrics at govt. medical college and hospital, Jammu. Patients of β-thalassemia major attending the clinic, which fulfilled the inclusion criteria were selected as cases as well as controls. An informed written consent was taken from the parents/guardian of the patients to ensure compliance and regular follow up.

Inclusion criteria

- Diagnosed cases of β-thalassemia major.
- Patient’s age ranging from 2 to 19 years.
- Patients regularly receiving blood transfusions with proper chelation.

Exclusion criteria

- Patients with poor compliance.
- Very sick patients.
- Patients with very severe anemia (<5 gm/dl) or bone marrow depression.
- Patient having any renal or liver disease.
- Patient hypersensitive to hydroxyurea.
- Patient on radiation therapy or chemotherapy.
- Patient who could not be followed up.

Therapy protocol

Cases: Seventeen patients meeting all the inclusion criteria were administered hydroxyurea in a dose of 15 mg/kg/day in a single dose. Folic acid 5 mg once daily was given during whole therapy. During the study patients were followed up after every thirty days

Controls: Cases selected for the study also acted as control, and transfusion requirement during last ten months was taken as control.

Detailed history including age at presentation, age at diagnosis, H/O Consanguinity of parents, frequency of blood transfusion required, HIV/HBsAg/HCV status, as well as detailed physical examination and following investigations were done at the time of enrollment at start of therapy and repeated at 3 monthly intervals:

- Complete haemogram.
- Liver function tests.
- Renal functional tests.
- HIV and HBsAg and HCV.
- Serum ferritin level by ELISA.
- Serum calcium and phosphorus.

Estimation of blood transfusion levels: Blood transfusion requirements were given to keep Hb above 10 gm/ dl. The need for blood transfusions was assessed at 3 monthly intervals and compared to the blood transfusion requirements in the 10 months prior to intervention.
Estimation of serum ferritin

Serum ferritin is estimated by Chemiluminiscent method done by ADVIA centaur CP ferritin assay. It is a two way sandwich immune assay using direct Chemiluminometric technology which uses constant amounts of two antiferritin antibodies. Serum ferritin levels were measured at 3, 6 and 10 months after starting treatment and compared to baseline serum ferritin levels which acted as control.

Mean, standard deviation and standard error of mean (SEM) were calculated from the data collected.

Results obtained were analyzed statistically by using student’s paired T-test and Chi-square test.

RESULTS

A total of 17 patients meeting all the inclusion criteria were selected to receive oral hydroxyurea. The mean age ± SD of the subjects at the time of enrollment was 10.05 ± 2.89 years, while the age at the time of diagnosis was 12.11±11.46 months. The mean weight and height at the time of enrollment were 24.82 ± 8.02 kg and 105.47 ± 11.06 cm respectively. Among 17 cases of thalassemia major there were 8 (47.06%) male cases and 9 (52.94%) female cases. Out of total 17 patients, 1 (5.8%) patient was splenectomised. None of the patient was HIV, HBsAg or HCV positive at enrollment as well as the end of the study.

Table 1: Baseline epidemiological profile of the study subjects at enrollment.

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<thead>
<tr>
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<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age at enrollment for study (years)</td>
<td>10.05 ± 2.89</td>
<td>2-19 years</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>24.82 ± 8.02</td>
<td>12-47 kg</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>105.47 ± 11.06</td>
<td>80-132 cm</td>
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<tr>
<td>Mean age at diagnosis (months)</td>
<td>12.11 ± 11.46</td>
<td>4-60 months</td>
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<tr>
<td>Duration of chelation therapy (years)</td>
<td>6.8 ± 2.97</td>
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</tr>
<tr>
<td>No. of patients with splenectomy</td>
<td>1 patient (5.8%)</td>
<td></td>
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<tr>
<td>Spleen size at time of enrollment (cm)</td>
<td>8.58 ± 2.36</td>
<td>8-11 cm</td>
</tr>
<tr>
<td>n=22</td>
<td>15 mg/kg/day</td>
<td></td>
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<tr>
<td>Male/Female</td>
<td>8/9</td>
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All of the patients were on chelation treatment for a mean duration of 6.8 ± 2.97 years. 15 (88.23%) of the patients were on mono chelation therapy and 2 (11.76%) were on combined chelation therapy. Out of 17 thalassemia cases studied, weight and height of 9 and 12 patients respectively were below <3rd percentile. Weight of 5 and height of 5 patients were between 3rd to 50th percentiles. Only 3 patient’s weight was more than 50th percentile. None of the patient has weight or height more than 90th percentile. These findings indicate a high incidence of stunting and wasting among thalassemics.

Figure 1: Showing weight and height distribution of the study population.

The mean haemoglobin level at the time of enrollment in the study was 7.02 gm/dl, ranged between 6.5 to 9.0 gm/dl. The mean haemoglobin level calculated from the values of pre transfusion haemoglobin in the preceding 10 months to the study was 7.03 gm/dl, ranged between 6.2 to 8.0 gm/dl. At 15 days, 1 month and 2 months no significant increases was observed in the mean haemoglobin levels (P>). At 3 months, the mean haemoglobin level increased to 7.50 gm/dl but this increase was also not statistically significant. The mean Hb level at 6 months was 7.62 gm/dl, haemoglobin increased and it was statistically significant (P<) as compared with the value of 6 months prior to the study.

Figure 2: Demonstrates the mean haemoglobin level of the patients at different time periods of the study.
The mean haemoglobin levels at 10 months of study was 7.72 gm/dl, a statistically significant difference was found when this value was compared with the value of mean haemoglobin level at 6 months prior to study (P).

The average number of transfusions before enrollment in the study was $14.93 \pm 5.209$ while during the study at 6 and 10 months were $7.86 \pm 3.46$ and $4.20 \pm 2.17$ respectively. This figure shows a statistically significant decrease in the number of transfusion during the hydroxyurea therapy at 10 months (P <0.05). There was also a statistically significant increase in the interval between the transfusions during the study from a mean of $22.93 \pm 7.10$ days to $27.93 \pm 8.81$ days at 6 months and $29.33 \pm 11.15$ days at 10 months.

**Figure 3: Demonstrates the number of transfusions requires and average interval between the transfusions at different time periods of the study.**

The mean value of serum ferritin in our patients at the time of enrollment in the study was found $4502.04 \pm 1662.65$ ng/dl. The ferritin level observed in 10 months prior to study from the records was $2409.33\pm1854.09$. Over 10 months of study period serum ferritin levels decreased significantly in all cases 3rd months onwards. The observed mean were $3782.73 \pm 1901.04$ ng/dl at 3 months of the therapy. The observed mean were $3782.73 \pm 1901.04$, $2902.06 \pm 1784.40$, $2416.4 \pm 1258.93$ at 3, 6 and 10 months of therapy respectively. Close scrutiny of above data shows that serum ferritin levels significantly decreased in all patients after 3 months of therapy.

**Figure 4: Demonstrates the mean serum ferritin levels at different time periods of the study.**

**DISCUSSION**

**Epidemiological profile**

A total of 17 patients were selected after applying inclusion and exclusion criteria and taking consent from among the patients attending the thalassemia day care center of govt. medical college, Jammu. The selected patients were comparable with respect to sex (8 (47.06%) male cases and 9 (52.94%) female cases. All the patients were non-reactive for HbsAg, HCV and HIV at the beginning of the study and none converted serologically positive for the above mentioned diseases at the end of the study. Among the 17 thalassemia cases studied, weight of 9 and height of 12 patients were respectively below 3rd centile whereas Weight and height of 5 patients were between 3rd to 50th centile for CDC normogram for their age and sex indicating a high incidence of growth retardation among thalassemics. This finding in our study is supported by a longitudinal study done by Saxena et al. on 90 thalassemic patients. The dose of hydroxyurea used in the study period was 15 mg/kg/day as a single oral dose: this was similar to dose of hydroxyurea used in previous studies viz Zamani F et al. with mean dose of hydroxyurea was 10 mg/kg/day (8-15 mg/kg). The mean follow up time was 60 months, Kosaryan M et al. with dose of hydroxyurea was 15.5 ± 6.4 mg/kg/day and duration was 5.2 ± 2 years (ranging 0.5-9 years) and Fucharosen S et al. who administered HU (10-20 mg/kg/day) for 5 months in 13 patients with beta thalassemia major/HbE.

**Change in hemoglobin level**

In our study the mean hemoglobin level at the time of enrollment was 7.02 gm/dl, (range 6.5-9.0 gm/dl). The mean hemoglobin level calculated from the values of pre-transfusion hemoglobin in the preceding 10 months to the study (control value) was 7.03 gm/dl (range 6.2-8.0 gm/dl). The mean hemoglobin level at 3, 6 months and 10 months were 7.50, 7.62 and 7.72 gm/dl respectively, hemoglobin increases and a statistically significant increase was found when these values were compared with the control value. Once increased, the rise in hemoglobin was consistent and progressive with increasing duration of HU therapy. Similar findings were reported by Bradaï M et al. reported a marked elevation of initial mean Hb levels from 65.0 to 105.0 gm/l (6.5-10.5 gm/dl) in two children with thalassemia intermedia, and from 45.0 ± 9.0 to 79.0 ± 8.0 gm/l (4.5 ± 0.9 to 7.9 ± 0.8 gm/dl) in 5 children with thalassemia major. Zamani F et al. also observed 8.2% increases in the mean hemoglobin over 60 months of follow up time. Fucharosen S et al. also observed slight (10%) but statistically significant increase in hemoglobin levels. In another study done by Ansari et al a rise in mean pre-transfusion Hb level 7.2g/dl to 8.6 g/dl was observed. Singer ST et al have also reported a mean 1.3g/dl steady-state increase in Hb in 40% of patients, and a milder response (≤1 g/dl) in the others.
Change in the transfusion requirement

A statistically significant decrease in the average no. of transfusion during the hydroxyurea therapy was observed. There was also a statistically significant increase in the interval between the transfusion during the period of study from a mean of 22.93 days at enrollment to 27.93 (18-46) and 29.33 (18-50) days at the end of 6 and 10 months of study respectively. None of our patients became transfusion independent, though duration between transfusions was significantly increased (over up to 42 days in one patient). Similar findings have been reported by Zamani F et al. who observed a reduction in mean packed red cell transfusions during one year of starting of hydroxyurea from 22.75 units to 6.02 units after treatment (P <0.01). Ansari et al. showed benefits of HU in 23 TM patients in form significant reduction in transfusion requirement (42.8%) and increased interval between two transfusions (68.7%). Similar outcomes were observed by Sachdeva A et al. They observed that 25/70 (36%) patients had a complete response as need for transfusion was obviated, 15/70 (21%) patients had a partial response as the interval between transfusions increased and 24/70 (34%) patients had no response to HU therapy.

Changes in serum ferritin levels

The mean value of serum ferritin of the patients in our study at the time of enrollment was 4502.04ng/dl. The ferritin level of patients in 10 months prior to the study was 2409.33ng/dl (taken as control). The observed mean at 3rd, 6th, and 10th month of therapy were 3782.73 ng/dl, 2902.06 ng/dl and 2416.4 ng/dl respectively. Close analysis of above data shows that serum ferritin levels significantly start decreasing in all patients after 3 months of hydroxyurea therapy. Iron overload is decreased and so simultaneously given chelation therapy becomes more effective. Our results are supported by study of Albebouryeh M et al. where serum ferritin decreased from pre-HU level of 1877 ng/ml to post-HU level of 525ng/dl, this translates into a 72.03% decrease in iron overload over a period of 6 months (mean: 9 months). Zamani F et al also reported a serum ferritin level decrease from the mean ferritin level 2751.44 ng/ml to 1594.20 ng/ml after one year of hydroxyurea therapy during the 60 months of follow up period (P <0.001).

Hydroxyurea is not only effective but also a safe drug. Only 2 patients suffered from minor side effects of hydroxyurea. Previous studies by Kosarayan M et al. etc. do not report any serious adverse effects.

There were a lot of limitations in the present study. The total No. of patients included were too small. The duration of follow up was also very short. This was not a randomized controlled trial as same patients in the previous ten months before initiating treatment with HU were considered as controls. Nevertheless this study provides some evidence about the efficacy of HU in thalassemia patients and larger studies with longer follow up are needed before arriving at a consensus.

CONCLUSION

The demonstrable significant decrease in the transfusion requirements as well as improvement in laboratory parameters including serum ferritin levels after initiating treatment with oral hydroxyurea in β-thalassemia major patients apparently suggests that oral hydroxyurea may find place in the management of β-thalassemia major children. However larger trials are warranted to derive optimum dosing schedule and confirm its efficacy so that it can be incorporated into future Management guidelines.

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REFERENCES


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