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Role of Hydroxyurea (HU) in reduction of Packed red blood cell (PRC)transfusion requirement amongst children having transfusion dependent beta-thalassemia major

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Abstract

Objective: To study effectiveness, safety profile, side effect of hydroxyurea (HU) to decrease need of PRC transfusions in children with β-thalassemia major.

Study design: Single-Centre open-label randomized control trial

Setting: Thalassemia Clinic of GMCH, Aurangabad

Participants: 58 patients are enrolled in this study out of which 27 were included in HU group and 28 were in control group, after exclusion and inclusion criteria.

Intervention: Randomization done by stratified block method with concealed allocation. Before starting hydroxyurea, all patient went through physical examination and routine biochemical laboratory tests. The intervention group receives Hydroxyurea10 to 20 mg/kg/day as a single dose daily for12 months while the control group receives a placebo. All were monitored for 12 months. All other standard treatment including iron chelation were continued. The statistical analysis was based on intention to treat.

Outcome: Reduction of PRC transfusion requirement during the treatment period was the primary outcome and to study the safety profile and side effect of hydroxyurea were considered as secondary outcome.

Result: Mean Hb ± 2SD was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in study group (P=0.0001), It was 7.41 ± 1.71 at enrolment and 8.53 ± 1.84 after 12 months in Control group (P=0.02). Percentage drop in Mean Annual blood requirement (ABR) was (ml/kg/year) in 21% in study group as compare to control group (8%). Mild (Hb rise 0-5%) response was observed in 2 (7.4%) Cases, Moderate (Hb rise 5-10%) in 23 (85.18%) and marked (Hb rise >10%) in 2 (7.4%) cases was observed. Hydroxyurea was well tolerated in and we got no remarkable adverse effect.

Conclusion: Hydroxyurea can be safely prescribed to transfusion-dependent β-thalassemia major patients for reduction their transfusion requirements.

Keywords: HU, ABR, PRC

Introduction

β-Thalassemia is one of the most common genetic diseases in the world [1]. It is estimated that 70000 children are born annually with β-thalassaemia worldwide [2]. The most severely affected patients develop profound anaemia during infancy that is life-threatening without blood transfusions [3]. The only existing cure is allogeneic haematopoietic stem cell transplantation, which is available only to a small subset of patients with human leucocyte antigen matched sibling donors [4]. All other patients, an overwhelming majority, are managed conservatively with supportive treatment with regular blood transfusions and iron chelation for life [5, 6]. Due to complications of the disease, these patients experience a poor quality of life and die prematurely in their fourth or fifth decade [7].

Adult haemoglobin (HbA) is composed of four protein globin chains two alpha and two beta which are arranged in a heterotetramer form. Thalassemia is a hemoglobinopathy in which patient have defect in one of the globin chain either alpha or beta which causes production of abnormal red blood cells. Thalassemia is classified according to defective chain of the haemoglobin molecule. In alpha thalassemia production of

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alpha globin chain is defective while in beta thalassemia production of beta globin chain is defective. The beta globin chain is encoded by a single gene on chromosome 11 and alpha globin chain is encoded by two closely linked genes on chromosome 16. Hence with two copies of chromosome in normal person two loci encode for beta chain and four loci encode for alpha chain. Deletion of any one loci has a high prevalence to develop thalassemia [8].

Main etiological factor associated is family history and ancestry. There is a 75% chance of the inheritance of mutated gene by an offspring. Even if a child does not develop beta thalassemia major or intermedia, they can still be a carrier with possibility of their offspring having beta thalassemia in future. Worldwide prevalence of gene causing beta thalassemia varies between 1 to 18% (Mean 9.5%) while in India its between 1 to 7% (Mean 3.3%) [9]. Nearly 8000 to 10,000 new thalassemia patients are born every year in India [10].

Treatment of affected children include regular blood transfusion. Confirmed laboratory diagnosis of thalassemia major and Hb levels less than 7gm per dL is prerequisite for transfusion. Packed red blood cells are leuco-reduced with a minimum of 40gm of haemoglobin content to minimize adverse reactions by contaminated white cells and platelet alloimmunization [11]. Limited supply of blood and risk of transfusion transmitted viral infections have encouraged to look for alternative approaches to manage beta-thalassemia especially in resource depleted country like India. Use of Hydroxyurea successfully in the treatment of sickle cell anaemia and thalassemia minor is well documented in previous literature. Hydroxyurea decreases the production of deoxyribonucleotides [12] via inhibition of the enzyme ribonucleotide reductase by scavenging tyrosyl free radicals which are involved in the reduction of nucleoside diphosphates [13]. It is a well-tolerated Food and Drug Administration (FDA) approved oral medication that is widely used in the treatment of cancers. However, it has been reported as a potent inducer of γ-globin in human erythroid cells in several preclinical studies [14, 15]. Although there is limited experience of efficacy of this agent in beta thalassemia major patients it needs to be evaluated for whether it can reduce transfusion needs in patients with beta-thalassemia major

With this perspective present study was undertaken with aim to study effectiveness, safety profile, side effect and ability of Hydroxyurea (HU) to decrease need of packed red cells transfusions in beta thalassemia patients.

Materials and Methods

Study design and setting

This study is an ongoing single-centre, randomised, placebo-controlled clinical trial to. To study the effect of hydroxyurea in packed red cell (PRC) transfusion reduction in βThalassemia Major patients the study is conducted at the pediatric thalassemia clinic
government medical college, Aurangabad.

Study population and eligibility criteria

All patients of admitted in ward under Department of Pediatrics at tertiary care hospital fulfilling inclusion and exclusion criteria were enrolled for the study Selection of participants.

Inclusion criteria

58 patients of beta thalassemia major (27 Study group & 28 Control Group) diagnosed on
High Performance Liquid Chromatography (HPLC) and Haemoglobin Electrophoresis.

Exclusion criteria

1. All patients with inconclusive HPLC reports.
2. Sickle β-thalassaemia.
3. Coexisting chronic liver disease.
4. Coexisting chronic kidney disease.
5. Coexisting viral hepatitis.
6. Patients with contraindications for hydroxyurea (e.g., hypersensitivity, bone marrow depression, pregnancy).
7. Patients who have undergone bone marrow transplantation.
8. Patients on immunosuppressant therapy.

Procedure

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All eligible patients who fulfil inclusion criteria will be given a patient information sheet to read and time to clarify doubts with investigators before consenting. Informed written consent from all participants will be obtained before recruiting into the study (figure 1). All Patients are below age of 18 years, so consent will be obtained from one of the parents. At the time of enrolment, a detailed history and physical examination including age of presentation, history of consanguinity of parents and other relevant details were recorded. Patients were examined for clinical findings like pallor, icterus, haemolytic facies and hepatosplenomegaly. Complete hemogram and serum ferritin levels were evaluated at the time of enrolment and then repeated after 12 months. Information on socio-demographic background, family history, medical history. Height and weight will be recorded and abdominal examination will be done to assess hepatic and splenic sizes. Then patients will be randomised into intervention or placebo group using a stratified block randomisation method with concealed allocation. The intervention group will receive hydroxyurea, while the control group will receive a placebo. Patients continued to get regular blood transfusion along with chelation therapy during follow-up period. If during the visit pre-transfusion haemoglobin was recorded to be more than 10.0gm/dL, blood transfusion was withheld for next one week and patient called for follow-up. On follow-up visit again transfusion was decided on the basis of pre-transfusion haemoglobin.

Ethics and Dissemination

Patient management decisions (other than hydroxyurea treatment) which will be done by the clinical team caring for subjects. All decisions regarding blood transfusions will be taken by the clinical management team according to unit protocols. Patients who develop severe adverse events (haematological or clinical) related or unrelated to the treatment during the study period and those who are unable to tolerate hydroxyurea will be discontinued from the study. Suspected adverse events will be reported according to the national guidelines. Participants will have the right to withdraw from the trail at any point without providing explanations.

Ethical approval of institutional ethics committee was taken prior to commitment of the study the study was undertaken in the department of Pediatrics at the tertiary care hospital GMCH Aurangabad.

CTRI Registration Number for this Trial is CTRI/2020/07/026938 Intervention

27 patients of study group were given hydroxyurea 10-20 mg/kg/day as a single dose (To nearest 250 mg capsule). Hydroxyurea is available in market in 500 mg capsules only, so the drug was re-dispensed in 250 mg capsules under all aseptic precautions in the pharmacy department of the institute. All patients also received concurrent iron chelators, folic acid and calcium supplementation.

Operational definitions
Response to hydroxyurea

1. Marked (Hb rise >10%).
2. Moderate (Hb rise 5-10%).
3. Mild (Hb rise 0-5%.

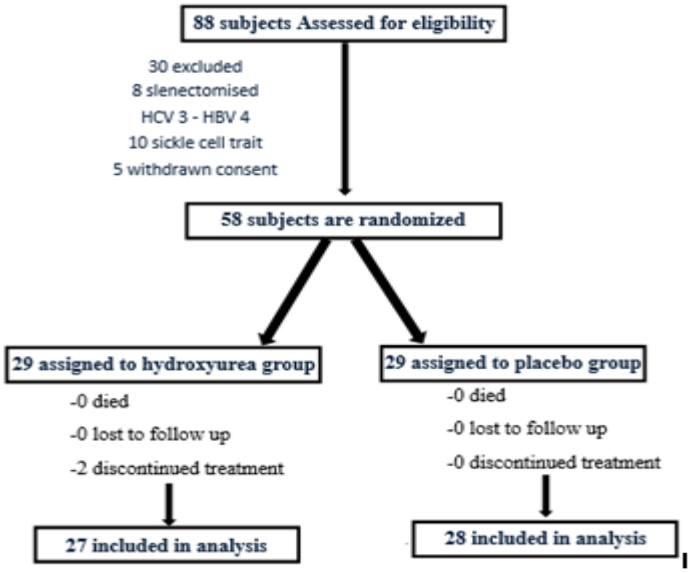

Fig 1: Study design

Observation & Result

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Table no. 1: Shows distribution of cases with Demographic Variables. Mean ± 2SD for age in study group was 7.91 ± 3.38. In control group it was 8.3 ± 3.36. Male to female ratio was 1:2 in study group whereas it was 3:4 in control Group. Family history was present in 23 (41.81%) study cases and 25 (45.45%) control cases. Splenomegaly was present in 27 (49.09%) study cases and 28 (50.9%) control cases.

Table no. 2: Shows distribution of different blood parameters amongst study and control group. Hb Mean ± 2SD was 7.41 ± 1.71 at enrolment and 9.25 ± 1.81 after 12 months in study group (P=0.0001). It was 7.29 ± 1.67 at enrolment and 8.53 ± 1.83 after 12 months in Control group (P=0.02). Enrolment and 9.25 ± 1.81 after 12months in study group (P=0.003). It was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in Control group (P=0.0002). MCH Mean ± SD was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 After 12 months in study group (P<0.0001). It was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in Control group (P=0.454). Serum ferritin Mean ± SD was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in study group (P=0.05). It was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in Control group (P=0.251). Haematocrit Mean ± SD was 38.70 ± 6.44 at enrolment and 42.03 ± 6.15 after 12 months in study group (P=0.0001). RBC Mean ± SD was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in study group (P<0.0001). It was 7.29 ± 1.67 at enrolment and 9.25 ± 1.81 after 12 months in Control group (P=0.005). MCV Mean ± SD was 7.29 ± 1.67 at It was 31.85 ± 6.70 at enrolment and 34.07 ± 7.61 After 12 months in Control group (P=0.478).

Table 1: Demographic variables

Variable	Study Group	Control Group	Odds Ratio (95% CI)	P Value
Age (Mean ± 2SD)	7.91 ± 3.38	8.3 ± 3.36	t=0.429	0.66
Gender (Ratio M:F)	1:2	3:4	-	-
Family History Present N (%)	23 (41.81%)	25 (45.45%)	0.69	0.649
Splenomegaly Present N (%)	27 (49.09%)	28 (50.9%)	0.964	0.985

Table 2: Haematological Variables

Outcome Variable	Study Group		Control Group		P Value	
	At Enrolments	After 12 months	At Enrolments	After 12 months	Study Group	Control Group
Hb (gm/dl) Mean ±2 SD	7.29 ± 1.67	9.25 ± 1.81	7.41 ± 1.71	8.53 ± 1.84	0.0001	0.02
RBC (million/dl) Mean ± SD	2.94 ± 0.55	4.26 ± 0.85	3.22 ± 0.87	3.95 ± 1.01	<0.0001	0.005
MCV(fl) Mean ± 2SD	78.14 ± 10.84	86.6 ± 9.71	79.42 ± 9.21	87.25 ± 4.91	0.003	0.0002
MCH (pg) Mean ± 2SD	28.94 ± 0.09	32.58 ± 3.71	28.57 ± 2.76	29.11 ± 2.60	<0.0001	0.454
Haematocrit (%) Mean ± 2SD	38.70 ± 6.44	42.03 ± 6.15	31.85 ± 6.70	34.07 ± 7.61	0.05	0.251
SF (ng/ml) Mean ± 2SD	235.70 ± 56.77	151.88 ± 88.34	235.46 ± 68.49	247.96 ± 62.45	0.0001	0.478

* SF: Serum Ferritin,

Table no. 3: Shows Hydroxyurea treatment Variables. Haematological Side effects was present in 1 (3.7%) case, Non-Haematological in 15 (55.55%) and both in 11 (40.74%) cases.
Mild response was observed in 2 (7.4%) Cases, Moderate in 23 (85.18%) and Marked in 2 (7.4%) cases.

Table 3: Treatment Variables

Hydroxyurea Treatment	Number of Cases N (%)	Total
Side effects		
Haematological	1 (3.7%)	27 (100%)
Non-Haematological	15 (55.55%)	

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Both	11 (40.74 %)	
Response		27 (100%)
Mild (<5%)	2(7.4%)	
Moderate (5 to 10%)	23(85.18%)	
Marked (10%)	2(7.4%)	

Table no. 4: Annual blood requirement

Annual blood requirement was 190 ml/kg/year in 27(49.09%) cases at enrolment and After 12 months it became150 ml/kg/year in 27(49.09%) in study group (P). Annual blood requirement was 190 ml/kg/year in 28(50.9%) cases at enrolment and After 12 months it

became 175ml/kg/year in 28(50.9%) in Control group i.e. Percentage drop in Mean Annual blood requirement (ABR) was (ml/kg/year) 21% in study group.

Table 4: Annual blood requirement

ABR/group	Mean ABR at enrolment (ml/kg/yr)	Mean ABR after 1 year (ml/kg/yr)	Percentage reduction in mean ABR
Study group	190	150	21
Control group	190	175	8

Discussion

Despite being one of the first genetic diseases to be characterised precisely at the molecular level, β-thalassaemia remains a life-limiting disorder without an effective cure. Nonetheless, several attempts exploring novel pathways are currently underway to develop a cure for β- thalassaemia. Most of these emerging therapies use advanced experimental technologies like gene therapy or genome editing [15-18], hence may not be available to a majority of patients living in low-income and middle income countries. 29 In this clinical trial, we aim to determine the efficacy and safety of oral hydroxyurea in minimising transfusion requirements and improving clinical outcomes of patients with transfusion-dependent β-thalassaemia. Hydroxyurea is an already FDA approved drug which is currently being used for other indications including sickle cell disease and non-transfusion-dependent β-thalassaemia.

HbF induction is nowadays a therapeutic goal for the treatment of β-thalassemia. Different HbF inducing agents are hypomethylating agents like Hydroxyurea (HU), decitabine and 5- azacytidine, histone deacetylase inhibitors like sodium phenylbutyrate and isobutyrate and recombinant erythropoietin[6]. Previous studies have found that they increase total Hb levels by 1 to 5 g/dL above the baseline if administered for at least 3 to 6 months [19. Hydroxyurea (HU) is most widely accepted inducing agent as its efficacy was investigated in various studies. In a study by Ansari *et al.* [20] they found 80% response rate in transfusion-dependent thalassemia patients on taking Hydroxyurea (HU). In our study 85.18% showed moderate and 7.4% showed marked response. In a study by Alebouyeh *et al.* [21] they found rise in Hb post- Hydroxyurea (HU) treatment as well as a decrease in serum ferritin. In our study statistically, significant rise was found in Hb from 7.29 ±1.67 at enrolment to 9.25 ± 1.81 after 6 month (P=0.0001). In a study by Italia *et al.* [22] one third of patients with thalassemia major had more than 50% reduction in their transfusion requirements. In our study transfusion requirements was dropped from 220 ml/kg/year to 180 ml/kg/year after 6 months in all study group. Bradai *et al.* [23] found half patients exhibited more than 70% reduction in their transfusion requirements. They also commented that increasing the dose of HU have no impact on transfusion needs.

Limitations

One important limitation of this trial is that these patients are on regular transfusion regimens. It was deemed unethical to stop transfusions in these patients for us to evaluate the full efficacy of hydroxyurea to see whether these patients could maintain a steady-state, safe although lower haemoglobin level of approximately 7-8g/dL without transfusions. All patients in the trial will continue to receive standard treatment with transfusions when their haemoglobin drops below 10g/dL. Therefore, we will only be able to evaluate the reduction in transfusion requirement as an outcome measure. Similarly, we may not be able to evaluate the effects on HbF fully. However, this is acceptable as most previous clinical trials on transfusion dependent β-thalassaemia have demonstrated reduction in transfusion burden rather than complete cessation of transfusions.

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Conclusion

In conclusion hydroxyurea 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. Hydroxyurea is a safe medication in thalassemia patients. It will help to save costs of blood transfusion and also complications associated. Relative mild and transient side effects are tolerable but patients are needed to be supervised periodically. Hence it is suggested to use hydroxyurea in thalassemia major patients to minimize or even obviate the need for regular transfusion and concomitant iron overload during therapy.

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