RESEARCH PAPER

Efficacy of Fixed Low Dose Hydroxyurea in Indian Children with Sickle Cell Anemia: *A Single Centre Experience*

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Introduction: Data on the efficacy of hydroxyurea (HU) in Indian children with sickle cell anaemia (SCA) is limited. Hence, we have evaluated the efficacy of fixed low dose HU in Indian children.

Methods: The study cohort consisted of 144 children (<18 years of age) with SCA having severe manifestations (≥3 episodes of vasocclusive crisis or blood transfusions, or having ≥1 episode of acute chest syndrome or cerebrovascular stroke or sequestration crisis) who were started on fixed low dose HU (10 mg/kg/day). They were followed up for two years and monitored for the hematological and clinical efficacy and safety.

Results: There was significant increase in the fetal hemoglobin level (HbF%), total hemoglobin and mean corpuscular volume. Vasoocclusive crises, blood transfusions, acute chest syndrome, sequestration crises and hospitalizations decreased significantly. Baseline HbF% had significant positive correlation with HbF% at 24 months. There was significant negative correlation between baseline HbF% and change in HbF% from baseline to 24 months. No significant correlation was found between HbF% at baseline and clinical event rates per year after HU. No major adverse events occurred during the study period.

Conclusion: Fixed low dose HU is effective and safe in Indian children with SCA.

Keywords: *Child, Hydroxyurea, India, Low dose, Sickle Cell disease.*

Sickle cell disease is the most common hemoglobinopathy in the world and is linked to five major haplotypes [1]. The most common haplotype found in Indian sickle cell anemia (SCA) patients is the Arab-Indian haplotype which is associated with high baseline hemoglobin F (Hb F) levels [2-4]. Considerable clinical diversity has been seen in Indian patients with SCA [3-5].

Hydroxyurea (HU) therapy has been shown to ameliorate the severity of the disease in SCA, mainly by inducing HbF production [6,7]. In SCA patients with African haplotypes, escalation of the dose of HU to the maximal tolerated dose (MTD) significantly increases HbF levels yielding a good clinical response [6,7]. Few studies have demonstrated the clinical efficacy of HU in Indian patients with relatively higher HbF levels [8,9]. We also recently demonstrated a good clinical response to low fixed dose HU (10 mg/kg/day) in Indian SCA children [10]; these studies were limited by small number of subjects.

It is believed that the severity of SCA is less in those with high HbF [11]. Surprisingly, a significant proportion of Indian SCA patients have severe manifestations despite high HbF levels [4]. There is only one small study (25 children) which reports the correlation between baseline HbF and hematological response to fixed low dose HU in Indian children [8]. There are no studies that describe the correlation between baseline HbF and clinical response to HU. Hence, we have evaluated the clinical and hematological responses to HU in a large cohort of Indian SCA children and their correlation with baseline HbF.

METHODS

This prospective longitudinal study was conducted in a tertiary health care center, Government Medical College, Nagpur. Subjects were recruited between January 2005 and December 2009. The 30 subjects who received HU in our previous randomized controlled study [10] were not included in this study. A written informed consent was obtained from all subjects/parents/guardians. Approval from the institutional ethics committee was procured prior to the start of the study. Sickle cell anemia patients with ≥3 episodes of vasoocclusive crisis or blood transfusions, ≥1 episode of acute chest syndrome or cerebrovascular stroke or sequestration crisis were enrolled for the study. Subjects with pregnancy, human immunodeficiency virus infection, or medications that could potentially enhance HU toxicity were excluded from the study. Other exclusion

criteria were serum creatinine above the upper limit of normal for age and serum alanine aminotransferase (ALT) more than twice the upper limit of normal for age at the time of entry.

Subjects were started on 10 mg/kg/day of HU (ONDERA VHB, Mumbai). The liquid preparation of the HU is not available in India and is available only as 500 mg capsules. The HU capsules were opened and the required amount of the drug contents were weighted by a pharmacist and provided in new packages. No dose escalation was done irrespective of the clinical or hematological response. A clinical proforma was used for follow up which was done every month. A detailed clinical examination was done at each follow up. Laboratory investigations including complete blood count, HbF percentage, reticulocyte count, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum bilirubin (total and direct) were performed at the initiation of HU therapy and during each subsequent visit. The number of vasoocclusive crises, blood transfusions, cerebrovascular events (CVA), acute chest syndrome, sequestration crises, acute febrile illnesses and hospitalisations during the preceding one year and two years after initiation of HU were documented. Patient compliance was measured by checking the number of remaining capsules of HU during follow up visits. All the patients were advised to take folic acid (5 mg/day) and ensure adequate fluid intake.

Only events for which the patient presented to the hospital for medical care were recorded for analysis. A painful event/crisis was defined as pain in the extremities, back, abdomen, chest, or head for which no other explanation could be found. Severe anemia was defined as a hemoglobin level less than 5 g/dL. Sequestration crisis was defined as a decrease of the hemoglobin or hematocrit level of at least 20% from baseline accompanied by an increase in palpated spleen size of at least 2 cm from baseline. A febrile episode was defined as a hospitalization with fever (≥101º Fahrenheit). Confirmatory malarial diagnosis was defined as an identification of malaria parasite in a peripheral blood smear. Bacteremia was defined as an acute event with growth of bacteria in blood culture samples. Meningitis was defined as abnormal cerebrospinal fluid (CSF) findings and yield of pathogenic bacteria on CSF culture. Acute chest syndrome was defined by the following three symptoms: fever, tachypnea, and observation of new pulmonary infiltrates on *X*-ray. Stroke or CVA was defined as an acute neurologic syndrome secondary to the occlusion of an artery or to hemorrhage with resultant neurologic symptoms and signs.

During the follow up, the drug was stopped temporarily if ALT was elevated above two times the upper limit of normal for age and serum creatinine was elevated above the upper limit of the normal, reticulocyte count was <80,000/µl when the Hb was <9 g/dL, absolute neutrophil count was < 1500/µL or platelet count was <80,000/µL. The abnormal parameters were closely followed for recovery. If recovery occurred, the drug was restarted at the same dose (10 mg/kg/day) with close monitoring for reappearance of side effects.

Complete blood count was done on the Sysmex K 1000 haematology counter. Reticulocyte count was done using brilliant cresyl blue staining. Haemoglobin analysis was done on the Variant Haemoglobin Testing System (BioRad Laboratories, Inc, Hercules, CA, USA). Renal and liver function tests were performed using an autoanalyser.

Statistical analysis: Statistical analysis was done with SPSS software version 16. Paired *'t'* test was used to compare the baseline findings with those after HU therapy at 24 months. Pearson's correlation coefficient (r) was used to calculate correlation between two variables. A $P<0.05$ was considered statistically significant.

RESULTS

One hundred and forty four children with SCA (92 males and 52 females; age range: 3.5 to 17.9 years) were started on hydroxyurea. There were no drop outs out of 144 children, followed for two years. The mean age of the study population at the time of registration in our sickle cell clinic was 6.7±3.3 years and the mean age at which HU started was 13.8±4.7 years. The numbers of subjects with various indications for initiation of HU are listed in ***Table* I**.

Hematological response: Hematological parameters at the start of HU and at 24 months and clinical event rates per year during the preceding year and 2 years after initiation of HU therapy are shown in ***Table* II**. There was a significant increase in the HbF levels from baseline to 24 months. Total hemoglobin and mean corpuscular volume also increased significantly. There was significant decrease in the leucocyte count, platelet count and reticuloyte count.

Clinical response: When compared to the event rate before initiation of HU, there was a significant decrease in the frequency of vasoocclusive crises, blood transfusions, sequestration crises, stroke, acute chest syndromes and hospitalizations at 24 months. Frequency of vasoocclusive crises was decreased by 96.4% and that of blood transfusion by 79.3%. None of the subjects had stroke, acute chest syndrome or sequestration crisis after

TABLE I INDICATIONS FOR INITIATION OF HYDROXYUREA

Indications	*Number of subjects*
Vasoocclusive crises ≥3	113
Vasoocclusive crises ≥3 and blood transfusion ≥3	4
Vasoocclusive crises ≥3 and stroke	6
Vasoocclusive crises ≥3 and acute chest syndrome	3
Vasoocclusive crises ≥3 and sequestration crises	5
Blood transfusion ≥3	9
Stroke	1
Acute chest syndrome	1
Sequestration crisis	1
Acute chest syndrome and sequestration crisis	1

initiation of HU. Frequency of hospitalizations was also decreased by 93.5%.

Toxicity: Clinical adverse events in the study subjects are listed in ***Web Table* I**. There were 21 episodes of laboratory toxicities leading to hydroxyurea withdrawal (***Web Table* II**). The indications for HU withdrawal were abnormal liver function (8 episodes in 6 patients), abnormal kidney functions (3 episodes in 2 patients), neutropenia (5 episodes in 4 patients), thrombocytopenia (n= 4 episodes in 4 patients), acquired immunodeficiency syndrome (1 patient). In 16 episodes of HU withdrawal, the abnormalities were transient and HU could be restarted.

Correlation of Baseline HbF with clinical and hematological response: Fetal hemoglobin response to HU was variable. However, there was significant negative correlation between baseline HbF levels with change in HbF levels from baseline to 24 months (r = -0.61, $P < 0.001$) suggesting a higher increase in HbF levels in patients with lower baseline HbF levels. Baseline HbF levels had a significant positive correlation with HbF levels at 24 months (r = 0.78, P <0.001) and baseline MCV (r = 0.31, P <0.001). There was no significant correlation between baseline HbF levels with any other baseline parameters or parameters at 24 months. Fetal hemoglobin levels at 24 months had significant positive correlation with MCV at 24 months (r = 0.22, P = 0.008). There were no significant correlations between HbF levels at 24 months with any other parameters at baseline or at 24 months.

DISCUSSION

This study reports the hematological and clinical efficacy of low fixed dose HU in a large number of Indian SCA children. Many long term studies have clearly demonstrated the efficacy of HU in African children with SCA [12,13]. There are very few studies which have reported the efficacy of HU in Indian children with SCA who have higher HbF compared to other population groups. We have recently reported the efficacy of fixed low dose HU in a small number of SCA children in a randomized controlled trial. In that study subjects had very severe phenotypes with mean VOC rate of 12.13±8.56. The study clearly showed the clinical benefits of this regimen along with lesser side effects [10]. In the present study we have used the same regimen in a large number of relatively less severe SCA children. This study has also demonstrated significant clinical and hematological improvement with HU. Similar to our

TABLE II COMPARISON OF CLINICAL EVENT RATES PER YEAR AND HEMATOLOGICAL PARAMETERS BEFORE AND 24 MONTHS AFTER HYDROXYUREA THERAPY OF THE WHOLE STUDY COHORT

Character	*Baseline*	*After 24 months HU*	*P value*
Vasoocclusive crisis*	4.27 ± 1.99	0.15 ± 0.47	<0.001
Blood transfusion*	0.77 ± 1.33	0.15 ± 0.58	<0.001
Hospitalization*	4.57 ± 1.77	0.29 ± 0.73	<0.001
Cerebrovascular event*	0.04 ± 0.21	0.0	<0.001
Acute chest syndrome*	0.03 ± 0.18	0.0	0.025
Sequestration crisis*	0.04 ± 0.21	0.0	<0.001
Hemoglobin (g/dL)	8.53 ± 1.74	9.66 ± 1.58	<0.001
Mean corpuscular volume (fl)	79.21 ± 23.74	88.3 ± 11.10	<0.001
Leucocyte count ×1000/mm^3	12.36 ± 8.92	8.79 ± 3.74	<0.001
Platelet count × 1000/mm^3	244.23 ± 133.58	199.61 ± 124.72	0.004
Reticluocyte × 1000/mm^3	258.2 ± 144.6	168.43 ± 123.5	<0.001
Hb F (%)	16.45 ± 7.66	21.98 ± 5.22	<0.001

**event rates are defined per year.*

previous study we did not escalate the dose of HU since most of the possible benefit was observed with a low dose and this regimen was proved to be associated with greater safety. A recent study from eastern India has also demonstrated good efficacy of low fixed dose HU (10 mg/kg/day) in SCA subjects. The frequency of painful crises was reduced by 71.5% in pediatric cases. The efficacy was more in adults with reduction of 92.2% of painful crises. Hydroxyurea therapy resulted in transfusion independency in 95% of patients [14]. Few studies from outside India have also documented the efficacy of low dose HU in SCA patients [12,15,16]. There has been no direct comparison of fixed dose to MTD in children or adults with SCA. Indirect comparison of multiple studies that escalated to MTD compared to fixed dose or escalation to clinical effect, supports greater improvement in beneficial laboratory measures (increased total Hb concentration, Hb F, decreased WBC) in children treated at the MTD [17]. Further studies with direct comparison of fixed dose to MTD may provide better information on this issue.

In the present study, both the sickle cell related events like painful events and other side effects like nausea/vomiting, headache, diarrhea were less common than that in HUG-KIDS phase I/II trial in which MTD of HU was used (***Web Table* I**). Laboratory toxicities like neutropenia, reticulocytopenia and thrombocytopenia were also less common in our study than that in HUG-KIDS phase I/II trial (***Web Table* II**). Majority of the laboratory toxicities in the HUG-KIDS phase I/II trial[6] were due to neutropenia (< 2000/µl) and in most of these cases the neutrophil counts were between 1500-2000/µl. In our study, we have defined neutropenia as < 1500/µl. This may be partly responsible for disproportionately higher occurrence of neutropenia in the HUG-KIDS study. Despite these differences laboratory toxicities in our study were much less where we have used low fixed dose HU [6]. Even in BABY-HUG [18] study in which a low fixed dose (20 mg/kg/day) of HU was used, the frequency of neutropenia was much higher despite using a stricter definition of neutropenia (<1250). Episodes of thrombocytopenia were also higher in BABY-HUG study. Similar to our study, the frequency of reticulocytopenia was very negligible in BABY-HUG study. Frequencies of ALT elevation in our study were similar to those in HUG-KIDS and BABY-HUG studies. However, unlike HUG-KIDS and BABY-HUG studies, there were 3 episodes of creatinine elevation in our study. Two episodes in one patient were attributed to recurrent urinary tract infection while the third episode was associated with acute upper respiratory infection and decreased fluid intake. Low fixed dose HU may require less frequent or no laboratory monitoring and cause fewer episodes of cytopenia as seen with 20 mg/kg/day dose used in the BABY HUG Study and 10 mg/kg/day dose used in our study. This may be particularly valuable in regions with limited resources for health care [17].

There was a predominance of boys in our cohort. This may be due to gender bias, still prevalent in our country, where boys are brought more to health care than girls. This is reflected by a higher number of boys registered in our sickle cell clinic. All our patients were followed-up regularly which may be attributed to a well-established sickle cell clinic for the last 20 years at our center. All subjects were compliant to HU therapy. This may be attributed to the free provision of the drug, large clinical benefits and lower toxicity due to the fixed low dose regimen.

In the western population, HbF response was considered as the gold standard to assess the response to HU. There are many clinical and genetic predictors of HbF response to HU [19,20]. Baseline HbF levels is shown to predict HbF response to HU in few studies [21, 22]. In the current study, baseline HbF levels had significant positive correlation with HbF at 24 months suggesting attainment of higher HbF levels in those who had higher baseline HbF levels. However, there was a negative correlation between baseline HbF levels with change in HbF levels from baseline to 24 months suggesting a lesser increase in HbF levels in those children with higher baseline HbF levels.

There was no significant correlation between baseline HbF levels and frequency of vasoocclusive crises. This may be due to selection of subjects with severe manifestations irrespective of baseline HbF levels. There was no significant correlation between frequency of vasoocclusive crises at 24 months and HbF levels at 24 months or change in HbF from baseline to 24 months. This may be due to other mechanisms of action of HU which include improvement of the rheological properties by decreasing the adhesiveness and absolute number of neutrophils and reticulocytes, decrease in hemolysis by improving hydration, causing macrocytosis and thereby hampering the sickling mechanism and vasodilatation due to release of nitric oxide [23]. This may also be due to very small number of vasoocclusive crises occurring at 24 months.

In conclusion, low fixed dose HU (10 mg/kg/day) causes significant increase in HbF levels with significant clinical benefits in Indian children with SCA and is associated with lesser HU associated laboratory toxicities.

Contributors: DLJ: was involved in study planning, patient

recruitment and patient management; MA, SD, AG, AB and HLJ: were involved in patient recruitment, data collection and review of literature; VS: was involved in review of literature, data analysis, initial drafting of the manuscript; RC and KG: were involved in the hematological investigations and critical review of the manuscript.

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