

Effect of Hydroxyurea on Clinical and Haematological Profile of Children with Sickle Cell Anaemia

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ABSTRACT

Introduction: Sickle Cell Anaemia (SCA) is a common haemolytic disorder prevalent in Eastern Ghats range of India. Children with SCA from this part of country suffer from multiple episodes of vaso-occlusive crisis (VOC) and require multiple blood transfusions (BT) which significantly affect their quality of life. Till now no study has been undertaken to evaluate the beneficial effect of Hydroxyurea (HU) in SCA patients in this geographic region. This study was undertaken to evaluate the clinical and haematological response to standard recommended dose (20mg/kg/day) of HU in SCA patients of more than 5 years of age.

Method: 114 children in the age group 5-14 years were enrolled in the study which was conducted in a tertiary care Paediatric Institute from November 2017 to October 2019. SCA patients having severe manifestations (>3 episodes of VOC or >3 BT or having >1 episodes of acute chest syndrome or cerebrovascular stroke or sequestration crisis) who were started on standard therapeutic dose of 20mg/kg/day.

Results: It was observed that HU treated children had reduced episode of VOC by 50%, BT by 56% and painful crisis by 50% with improved quality of life. HU improved school attendance and scholastic performance in these children. The haematological parameters improved significantly with rise in mean Hb concentration by 52%, HbF by 51% and MCV, MCH and MCHC by 7%, 3%, and 2.5% respectively. Some patients did not show any

improvement in HbF concentration but all of them had a good clinical recovery in terms of less number of VOC and less no of transfusions. No major adverse events occurred during the study period.

Conclusions: Standard therapeutic dose of Hydroxyurea is safe and effective in Indian children with SCA.

Key Words: Hemoglobinopathy, Sickle cell crisis, Blood transfusion, Antimetabolite, Vaso-Occlusive Phenomenon, fetal haemoglobin.

INTRODUCTION

Sickle Cell Disease (SCD), a disease of inheritance affecting African, Arabian and Indian population.¹ Out of five major haplotypes Arab-Indian haplotype is the most common haplotype found in Indian sickle cell anaemia patients which is associated with high baseline HbF levels.²⁻³ SCD is common among all ethnic groups in India, high prevalence being reported among tribal population.⁴ The highest frequency of sickle cell gene in India is reported from Odisha followed by Assam, MP, UP, TN, Gujarat. The average frequency of SCD in India is 4.3% and that of Odisha is 9.1%. The prevalence of SCD was found to be 16.55% in under 15yr children with considerable clinical diversity⁴

HU has opened a new horizon in the treatment of Sickle cell Disease and is trying to return a happy smile in SCD

children⁵ HU is the only effective drug proven to reduce the frequency of painful episodes as per drug research and trial. It raises the level of HbF and Hb level. HU decreases the painful episodes by 50% and it decreases the rate of (acute chest syndrome). ACS episodes and blood transfusion nearly by 50%.⁶ HU is safe and well tolerated in children older than 5 year of age with no clinical and laboratory toxicity. The primary toxicity is limited to myelo-suppression which is reversible on cessation of drug.⁶ HU therapy began in infancy preserve splenic function, improve growth and reduce the incidence of ACS, painful crisis and dactylitis⁶ There was significant decline in mean level of leucocytes, PMN, reticulocyte and dense sickle cells and significant increase in the levels of haemoglobin, PCV, MCV, HbF, F cells and F reticulocytes.⁷ HU has been found to be an alternative to blood transfusion in sickle cell stroke. HU improves quality of life and is cost effective.

Many studies have shown beneficial effect of HU in SCA. Though prevalence of SCA is quite high in our region, no searched article in English literature on internet was found, to evaluate the beneficial effect of HU in this region. Hence we undertook this study to evaluate the clinical and haematological response to HU in SCA patients of more than 5 year.

MATERIALS & METHODS

The prospective cohort study was conducted at a tertiary paediatric centre from November 2017 to October 2019.

2.1 Inclusion Criteria:

All children with SCD of age group 5 years-14 years

2.2 Exclusion Criteria:

Those who refused to take the drug.
Children who were already in HU therapy.
Children with active liver and kidney disease.

2.3 Study Design-

Two groups were taken in the study Cohort H+ group were treated with HU and Cohort H- group who were not. Children who had relatively more number of VOC and BT were included in Cohort H+ group.

2.4 Documentation:

Signed written consent was obtained from parent/guardian of patients. It included discussion about the treatment, method of monitoring, potential toxicity, potential teratogenicity/carcinogenicity

2.5 Baseline Investigation:

Documented complete physical exam (all vital signs including weight, height, and SpO₂)

Hb electrophoresis with quantitative HbF %
CBC with Differential and reticulocyte count

Liver function tests.(AST, ALT)

Renal Function.(BUN, serum Creatinine)

2.6 Dosing and Administration

Initial dose of 20 mg/kg/day were given as a single daily dose at bed time preferably. Capsules of strength 500 mg with brand names Hydrea, Durea were given according to body weight on daily or alternate day basis.

2.7 Monitoring Visits

All children were followed up once in 3 weeks to record weight, BP, vital sign as pulse & SpO₂ to look for toxicity and haematological status. In some cases earlier follow up dates were assigned for reinforcement of parents those lacking education or compliance. Laboratory evaluation done every 3 months interval included CBC, Differential and Reticulocyte count and at 6 month interval serum bilirubin, ALT, BUN, creatinine quantitative HbF.

Side-effects like GI symptom/nausea, rash/pigment changes, alopecia, unusual bleeding tendency and drug allergy were documented. Treatment

continued until patient had any evidence of toxicity.

2.8 Toxicity

HU therapy was stopped if any toxicity detected like bone marrow suppression, AKI, hepatotoxicity (elevated enzymes) as per organ toxicity criteria. Unexplained hair loss or skin rash were also considered as toxicity mandated termination of therapy.

Bone Marrow toxicity criteria- Absolute neutrophil counts < 1000-1500/cmm, Absolute reticulocyte count < 80, 000/mm associated with Hb <9 gm/dL, Platelet count < 80, 000/mm and fall of > 20% Hb concentration

Renal toxicity criteria- >50% or more increase in serum creatinine or an increase of > 0.5 mg/dL of serum creatinine.

Hepatic/Gastrointestinal toxicity criteria- 100% increase in ALT, >100% increase in GGT

2.9 Toxicity Management

In the event of bone marrow, hepatic or renal toxicity, HU was held for 4-7 days and lab values repeated. If values remain abnormal, treatment interrupted until values return to baseline. After monitoring at 2 weeks if values had returned to baseline, the drug was restarted at the same dose. Other potential toxicity were carefully evaluated.

Cessation of therapy was considered if the patient failed to turn up for follow up, not taking drug consistently, developed toxicity or faced any problem as exclusion criteria. The risk and benefits of HU therapy were discussed with parents along with written consent and submitted to complete clinical data in a special format.

2.10 Case Definition

Painful Crisis - A painful crisis is a sudden attack of pain, which may be mild or extremely severe, often occurring in bones and joints. For this study, which was limited to patients with moderately severe disease. (at least 3 attacks/year), a crisis was a visit to a medical facility for pain not due to another disease, lasting at least 4 hours, and

treated with injections of a narcotic pain-killer or NSAID.

Repeated Blood Transfusion - Blood transfusion 3 or more per year in a patient

Adverse Event -An adverse event was defined as death or life threatening clinical event likely to interfere patient's ability to continue or tolerate HU therapy.

2.11 Subject Analysis

Data regarding Age, Sex, chief complaints, history, previous treatment history, general examination and systemic examination were documented. Confirmation of case was done by Sickling test and HPLC. Blood CBC, HbF level, Total and direct bilirubin, ALT, AST, blood urea, serum creatinine were ascertained. All patients were advised for USG abdomen and pelvis, chest X-ray PA view, X-ray pelvis including both hip joint to look for progression or toxicity.

Haematological investigations- CBC, Haemoglobin Estimation (Sahli's method), Total Leukocyte Count, Differential Count and Comment on Peripheral Smear, Reticulocyte Count, Sickling Test, LFT, RFT and Automated High Performance Liquid Chromatography (HPLC) Data were expressed as mean Standard deviation. Clinical and laboratory variables were compared before and after HU therapy. We used student t both paired and unpaired test for analysing sample. Sample analysis done by *SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc*

RESULTS

Total hundred patients fulfilling standard guideline criteria were selected. HU were given to 52 children and were enrolled under cohort H+ group. Rest 48 subjects were not given any drug and enrolled under cohort H- group. At the time of statistical analysis it was seen that roughly 14 subjects were lost to follow up from both cohort H+ and cohort H- group. Base line CBC, HPLC, RFT, LFT, BT, hospitalisation stay and clinical profile documented. HU at a dose of 20mg/kg/day

administered taking consent to cohort H+ Group and avoiding same in cohort H- group initiated. Follow up was done every 4 weeks for three months then every three monthly for two years with CBC, HPLC, RFT, LFT, h/o BT, VOC and organomegaly. Clinical events and toxicity were monitored at each visit with counselling. Fourteen patients lost to follow up due to poor compliance after 3months. Data with cohort H+ group and clinical haematological profile with cohort H- group analyzed. Mean Age of the study subjects: 8.52 ± 2.134 years. Prevalence of disease was more in 5-10 year of age. It was observed that the patient treated with HU required less number of BT with a mean of 5.42 compared to 5.21 in cohort H- group. (Table-1) Also we observed a decrease in VOC episodes in patient under HU therapy compare to other group.(Table-2) Paired t-test was applied to know the difference in blood transfusion before and after HU therapy in cohort H+ group. It showed significant decrease in frequency of BT after

HU therapy. (Table 3) Statistically the difference in VOC before and after HU therapy in cohort H+ group showed there was significant decrease in incidence of VOC after HU therapy.(Table 4) In cohort H+ group there were significant increase in all haematological parameters including HbF where as decrease in all parameters in other group.(Table-5) Myelotoxicity encountered in 2 cases(3.8%) which were neither symptomatic nor required any therapeutic intervention. 96.2% cases did not develop any myelotoxicity throughout the course of treatment.

Table 1 : BLOOD TRANSFUSION REQUIRED PER YEAR IN EITHER OF THE GROUPS

NO.OF BT/YR	COHORT H+	COHORT H-
<5	27	34
>5	25	14
MEAN	5.42	5.21

Table 2 NUMBER OF VASO-OCCLUSIVE CRISIS EPISODES PER YEAR

NO.OF VOC/YR	COHORT H+	COHORT H-
<5	18	20
>5	34	28
MEAN	6.33	5.83

Table 3 CLINICAL CHANGES IN COHORT H+ GROUP AFTER HU TREATMENT WITH RESPECT TO BLOOD TRANSFUSION REQUIREMENT.

Blood Transfusion	No.Of PT(N)	Mean	Standard Deviation	Standard error of mean	T value and p value
Before Hydroxyurea	52	5.42	1.446	0.201	t=19.288
After Hydroxyurea	52	2.40	1.445	0.200	p<0.001, df=51

Table 4 CLINICAL CHANGES IN COHORT H+ GROUP AFTER HU TREATMENT WITH RESPECT TO VASO-OCCLUSIVE CRISIS EPISODES

VOC	N	Mean	Standard Deviation	Standard error of mean	T value and p value
Before Hydroxyurea	52	6.33	1.410	0.196	t= 16.138,
After Hydroxyurea	52	3.19	1.253	0.174	p<0.01, df=51

Table 5 COMPARISON OF HAEMATOLOGICAL PARAMETERS BETWEEN BOTH COHORT H+ & COHORT H- GROUP

Parameter	Mean ± SD.(Hydroxyurea)	Mean ± SD .(Non-Hydroxyurea)	t value	p value
Hb				
Baseline Hb	5.622 ± 0.6406	5.042 ± 0.4057	5.380	<0.001
Mean Hb at 6, 12 and 18 months	7.3647 ± 0.54103	4.8410 ± 0.32784	28.052	<0.001
MCH				
Baseline MCH	30.568 ± 1.2810	27.942 ± 0.4667	13.588	<0.001
Mean MCH at 6, 12 and 18 months	31.1080 ± 1.07039	27.7118 ± 0.43865	20.698	<0.001
MCHC				
Baseline MCHC	31.588 ± 1.0718	28.579 ± 0.4500	18.245	<0.001
Mean MCHC at 6, 12 and 18 months	31.9660 ± 0.97237	28.3729 ± 0.43033	23.812	<0.001
MCV				
Baseline MCV	82.942 ± 2.1781	79.735 ± 1.0195	9.393	<0.001
Mean MCV at 6, 12 and 18 months	86.2620 ± 1.60449	79.3715 ± 0.91476	26.247	<0.001
HbF				
Baseline HbF	8.844 ± 0.8825	6.796 ± 0.9027	11.357	<0.001
Mean HbF at 6, 12 and 18 months	11.6793 ± 1.11875	6.3956 ± 0.91322	25.387	<0.001

DISCUSSION

In our study 69(69%) were male and 31(31%) were female. This shows that SCD is more prevalent in males in comparison to females. Similar study done in 1985 had observed male preponderance in their population.⁸ Maximum numbers of subjects(68%) were between age group 5-10 year followed by 10-14 years(22%). Mean age of subjects in our study was 8.52 ± 2.134 years. A searched publication also suggests mean age of patient in their study was found to be 8 years.⁹ This study found a strong correlation between the incidence of disease and h/o consanguineous marriage. 52 numbers of subjects (52%) in our study had positive h/o consanguinity. Two other Asian studies suggested the same correlation most recent published article in 2018.¹⁰⁻¹¹ this consanguineous marriage is found mostly in Middle East and South India making expressive some autosomal recessive diseases, including very rare or new syndromes.¹² A significant reduction in frequency of blood transfusion was noted from 5.4 units per year to 2.4 units per year in cohort H + group and is statistically significant. HU had a definite beneficial effect on frequency of transfusion. In contrast, cohort H- group showed transfusion rate increase from 5.21 units per year to 5.94 per year.($P < 0.001$) This might reflect increased need of BT in SCA with advancing age. Similar study showed that mean number of transfusions decreased from 3.9 units per year to 0.43 unit in per year in those subjects who received HU therapy. A study from Belgium also showed similar result.⁽¹³⁻¹⁴⁾ The no. of VOC per year significantly decreased from 6.33 times per year to 3.19 times per year in cohort H+ subjects i.e. 50 % reduction in incidence of VOC.($P < 0.01$) Similar findings with 50% of reduction in VOC after HU therapy. The median time between two consecutive VOC in our study increased from 2 months to 4 months. In cohort H- group the incidence of VOC increased from 5.83 episodes per year to 6.06 episodes per year. HU has a definite role in reducing number of VOC episodes

per year and at the same time, subjects who did not receive HU may experience higher number of VOC.^{7, 14-15} Effect of HU on the frequency of painful crisis subjects who received HU had less number of VOC per year with $P < 0.03$.^{7,14-15} Red cell indices like MCV, MCH, MCHC improved significantly from 82.9fl to 8.6 fl; 30 pg to 31.18 pg; 31.58 to 32.27gram/dl respectively after 6, 12 and 18 months of HU therapy.

In searched published article, MCV was shown to be increased by 14% after 2 years of HU therapy.¹⁶ Similarly, different other studies also support improvement in red cell indices after 2 years.⁹ Statistical Studies showed MCHC value increased from 32 pg to 34.5 with (P -value < 0.02) after 2 year of HU therapy.^{9,16-19} Different studies support a beneficial role of HU in improving MCV, MCH and MCHC in multiple observational studies but in contrast, our study revealed no significant improvement of MCV, MCH and MCHC.^{9,16-19}

The base line Hb increased from a mean of 5.5 gram/dl to 8.4 gram/dl after 2 years of HU therapy in cohort H+ group. Maximum rise of Hb was observed after 12 months of HU therapy from 7.8 grams/dl to 9 grams/dl over 2 year after treatment.^{9, 16} In subjects who did not receive HU, Baseline Hb decreased from a mean of 5 grams/dl to a mean of 4.7 grams/dl after 2 years. Similar published article explaining role of HU in increasing baseline level of Hb was compared with our research.^{9,16,18} The mechanism of action of HU by which it improves general wellbeing of a SCA patient is its ability to increased foetal Hb concentration. We observed a rise of HbF by 51% after 2 years of HU therapy. In our study subjects, mean HbF before HU was 6-13% which increased to 9%--16.6% after HU . Maximum rise of HbF was seen with in 6 month of starting therapy. In cohort H – group mean HbF level was 6.7% which was further reduced to 6.2%. In other studies done, similar effect of HU in improving HbF concentration was e.g. a rise of 47% in

HbF level after 2 years of HU treatment.^{9,20}

There were no significant changes in serum creatinine before and after HU therapy i.e. HU was not nephrotoxic. But after 2 years of HU therapy serum Bilirubin decreased significantly from mean 3.1 mg/dl to 2.9 mg/dl. ALT also decreased significantly from mean 41.76 to 40 IU/L after 2 year of HU therapy. Similar observation a transition of total bilirubin from 3.6 to 2.5 mg/dl.¹⁶ Two patient developed neutropenia and thrombocytopenia after 3 months of HU therapy. Dose of HU was decreased for both the patients but pancytopenia persisted, so HU treatment was discontinued for both the patients. Almost similar myelosuppression (10 %) was seen in other studies.⁹ The haematological parameters improved significantly with rise in mean Hb concentration by 52%, HbF by 51% and MCV, MCH and MCHC by 7%, 3% & 2.5% respectively. Some patients did not show any improvement in HbF concentration but all of them had a good clinical recovery in terms of less number of VOC and less number of transfusions. The hypothetical mechanism by which these patients benefited might be due to sustained red cell hydration and vasodilatation through release of Nitric Oxide. This Nitric Oxide mechanism is supported by other studies but more evidence with more sample size is required to prove this hypothesis.

CONCLUSION

Children with sickle cell disease treated with HU had a significant impact on reduction of VOC by 50%, need of BT by 56%. HU therapy is cost effective as compared with economic burden of hospitalization. Haematological marker like Hb, HbF, MCV, MCH, MCHC improved significantly. Reduction in total serum Bilirubin and ALT were noted in children treated with HU whereas as no significant change was found in level of Serum creatinine in these subjects. HU therapy improved the school attendance and scholastic performance in children. Small

sample size, lack of long term follow up, technical design errors associated with the laboratory analysers were the limitations of the study. More number of multi-institutional studies with larger sample size is needed to validate more vividly the role of Hydroxyurea in Sickle Cell Anaemia patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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