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Original Article

Effect of Hydroxyurea Therapy on Renal and Liver Function Tests and Modulation of Red Cell Concentrate Requirement in Transfusion Dependent Beta-Thalassemia Patients

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ABSTRACT

Background: Raised fetal hemoglobin (HbF) levels in patients with Transfusion Dependent Beta Thalassemia (TDBT) are now known to contribute to increase in the total hemoglobin level, reduce ineffective erythropoiesis and reduce hemolysis due to prolonged survival of RBCs. Experience with hydroxyurea as an HbF inducer in Sickle Cell Disease (SCD) patients prompted researchers to conduct trials regarding this therapy in other hemoglobinopathies.

Material and Methods: It was a retrospective study. Non probability consecutive sampling technique was applied. A total of 265 splenectomized patients older than 2 years of age who had been treated with hydroxyurea for at least 12 months were selected. The patients were registered at the Hematology Day Care Center of the Hayatabad Medical Complex (HMC), Peshawar from January 2014 to January 2018.

Results: The number of transfused Red Cell Concentrate (RCC) units, reduced from 27.46 to 22.37 during the 12 months pre and 24 months post treatment period. The total hemoglobin (Hb) levels of 8.4 g/dL at 12 months pre-treatment increased to 8.9 g/dL at 12 months post-treatment, but without any statistical significance. Mean inter-transfusion time improved from 3.2 weeks to 7.4 weeks. No statistically significant difference was found in renal function tests in pre and post treatment groups. Although Alanine aminotrasferase (ALT) and Aspartate aminotransferase (AST) showed statistically significant differences in pre and post-treatment groups, yet these differences were not clinically significant.

Conclusion: Hydroxyurea therapy significantly decreased the RCC packs required to maintain adequate hemoglobin levels while increasing the time period between successive transfusions of RCC in patients with TDBT without any significant renal and hepatic adverse effects.

Keywords: Hydroxyurea, beta thalassemia major, renal function tests, liver function tests.

Introduction

Hemoglobin A (HbA), which constitutes more than 95 % of the adult human red cell hemoglobin content, is a tetramer of two alpha and two beta globin chains. Beta thalassemia patients suffer from a mutation in the beta globin gene leading to a decrease in the quantity of the beta globin and, hence the HbA produced. However, research over several years has shown a propensity of the gamma globin gene product to compensate for the decreased amount of the beta globin leading to higher levels of the alpha-gamma globin tetramer called fetal hemoglobin (HbF) [1, 2].

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The increase in HbF levels lead to increase in total hemoglobin,improvement in ineffective erythropoiesis and decrease in hemolysis due to prolonged survival of RBCs [3.]

Hydroxyurea (hydroxycarbamide) is an analogue of urea, with substitution of one hydroxyl group. It is a chemotherapeutic agent which reduces high blood cell counts in patients with myeloproliferative disorders. The drug acts as an inhibitor of ribonucleotide reductase, an enzyme essential for DNA synthesis by producing deoxy ribonucleotides from ribonucleotides [4, 5]. However, it was later found that it is also a potent HbF inducer and is associated with the proliferation of F cell clones in patients with sickle cell disease. [6] Although still widely disputed, consensus is now growing on the pathway involved in this process. Stress erythropoiesis has been the most common mechanism proposed [7]. More evidence from recent studies have suggested the production of nitric oxide

with subsequent soluble guanylyl cyclase activation leading to increased intracellular cyclic GMP causing raised gamma globin gene expression. [8,9]

Documented reduction in painful crises in sickle cell disease patients was the initial stimulus for approval of hydroxyurea (HU) as therapy for this disorder. [10,11,12] Subsequent trials on patients with beta thalassemia intermedia revealed benefit in raising HbF levels and improving the disease severity in these patients [13,14,15,16]. However, studies conducted on patients with TDBT or beta thalassemia major have not been very encouraging to facilitate the formulation of clear guideline towards approval of hydroxyurea for this subset of patients.

At an optimal dose hyroxyurea is not associated with hepatic and renal dysfunction and bone marrow suppression, but at high doses it can lead to these side effects[17]. ALT, AST, and bilirubin elevations can occur in a small number of patients on optimal doses of hydroxyurea. However, enzyme elevations are mostly not associated with symptoms and generally resolve rapidly, rarely requiring dose modification [18].

Objectives

To assess the effect of hyroxyurea therapy on renal and hepatic functions.

To evaluate the modification in transfusion dependence of TDBT patients with hydroxyurea treatment by calculating number of Red Cell Concentrate (RCC) packs transfused over 12 month before and 24 months after starting hydroxyurea therapy.

Materials and Methods

It was a retrospective study carried out at the Hematology Day Care Center,"the Thalassemia referral cente" affiliated with the department of Hematology, Hayatabad Medical Complex, Peshawar. A non-probability consecutive sampling technique was followed.

Records of the patients from January 2014 to January 2018 were evaluated. Patients were classified as having Beta Thalassemia Major based on Hb electrophoresis and a complete blood count along with peripheral smear. Those patients were included in the study who were older than 2 years, registered at least six months ago, and had been splenectomized. Those patients were excluded from the study; who had impaired renal function (serum creatinine > 2 times upper normal limit for age), who had elevated liver enzymes more than 2 times normal, thrombocytopenia (platelet count lower than 100,000/mm^3), neutropenia (WBC count under 1500/mm^3), active hepatitis B or C infection or who were on interferon therapy during the study period, who had not undergone a splenectomy before the start of HU therapy, who were splenectomized less than a month before the start of HU therapy, and who took HU less than three months. The clinical diagnosis of TDBT was based on two consecutive low Hb values before the second year of birth, and 1 to 2 months apart. As a policy, patients were put on a 2-4 weekly transfusion regimen of Red Cell Concentrates (RCCs) with target pre-transfusion Hb of greater than or equal to 9.0 g/dL. Guardians and parents were contacted and written consent was obtained. Patients were evaluated for clinical and laboratory response every two weeks. Signs of new onset of extramedullary hematopoiesis, including hepatosplenomegaly or bone changes were noted. Complete blood counts (CBC) were checked by Cell Dyne Ruby™(Abbot, USA) at every visit. Renal and liver function tests were performed on Alinity c (Abbott diagnostics). Serum ferritin levels were measured by ELISA every three months (not reported in study).

Out of total 1045 patients enrolled in the Hematology Day Care Center, 605 patients were registered during the time period of study. According to the inclusion and exclusion criteria, 281 patients were eligible to be included in the study. Sixteen patients, who consumed the drug for less than three months were excluded from the study on non-compliance basis. So overall 265 patients were part of the study.

Hydroxyurea was started in beta-thalassemia patients to reduce signs of ineffective erythropoiesis, improve Hb levels and decrease the transfusion dependency. Patients were started on drug at a dose of 10 mg/kg/per day. The dosage was escalated by 2 mg/kg/day every 8 weeks if hematological parameters permitted to do so. In case of any sign of drug toxicity, HU was withheld temporarily and restarted when the tests normalized. The critical cutoff for hematological toxicity was set at <1200/mm^3 for granulocytopenia, and <100,000/mm^3 for thrombocytopenia. A two-fold increase in ALT or AST was considered as hepatotoxicity while renal toxicity was set at > 50% of baseline creatinine level. Folate and calcium were administered to all subjects 3 months prior to start of therapy and during treatment as well. CBC, urea, creatinine, clinical side effects, and compliance with dosing were evaluated at every visit. Liver function tests including ALT, AST, bilirubin and alkaline phosphatase were measured 3-monthly. Total

Hb level was measured at every visit before packed cell transfusion. The transfusion volume was adjusted to achieve a target level of 9.0 g/dL. Pre-transfusion Hb levels at 12 months before start of HU therapy were measured followed by levels at 12 months after treatment. This was followed by measurement of Hb levels at the 12 month after the second set of measurements. Response to hydroxyurea therapy was stratified according to the recommendation of Musallam et al as; **Excellent Responders**: Independence from RCC transfusion requirement whileachieving a pre-transfusion target Hb level > 9.0 g/dL. **Good Responders:** Equal to or more than 50% reduction of RCC transfusion requirement compared to pre-treatment levels, whileat the same timemaintaining a pre-transfusion total hemoglobin level >9.0 g/dL; **Poor Responders:** Less than 50% reduction in transfusion requirement to achieve a pre-transfusion hemoglobin level > 9.0 g/dL; and **Non-Responders***:* No changein RCC requirement to reach a pre-transfusion hemoglobin level of >9.0 g/dL.[19] On the basis of reduction in RCC requirement, rise in Hb concentration and duration between transfusions, three groups were made. Group 1 was 12 months pre-treatment, group 2 was 12 months post-treatment and group 3 was 24 months post-treatment.

Data were subjected to statistical analysis using SPSS version 23. Means of continuous variables were derived. Means of the variables like number of RCC units consumed, Hb levels and duration between transfusions were compared between 12 months pre and 24 months post treatment groups by using independent t-test. The three groups of patients i.e. group 1 (12 months pre-treatment), group 2 (12 months post-treatment) and group 3 (24 months post-treatment) for the assessment of biochemical profile were compared by using one way anova. Similarly duration between transfusions, number of RCC transfused and Hb levels among different categories of drug responders were compared by the help of one way anova. A value of <0.05 at 95% confidence interval was considered as significant.

Results

Out of total265 patients, 145 were males and 120 were females. Mean age of the study population was 20.37 ± 4.56 years with a range of 2–40 years. Mean RCC requirement decreased significantly after treatment over two years. The mean Hb levels before and after 24 months of HU therapy did not increase significantly while duration between transfusions showed significant increase.

Table.1: Change in mean Hb levels and RCC requirements 1 year before treatment and over years after Hydroxyurea treatment.

Characteristic	12 months pre HU treatment	24 months after starting HU treatment	P value
Red Cell Concentrate units transfused (Mean ± SD)	27.46 ± 5.24	22.37 ± 3.36	<0.05#
Hemoglobin (g/dL) (Mean ± SD)	8.4 ± 2.56	8.9 ± 3.15	0.543
Duration (Weeks) between transfusions (Mean ± SD)	3.2 ± 0.76	7.4 ± 1.56	<0.05#

#: Significant p value on independent t test, HU: Hydroxyurea, SD: Standard deviation

On analysis renal function tests did not show significant differences among all the three groups. ALT and ALP showed statistically significant differences but without any clinical significance.

Table 2: Mean values of renal and liver function tests in before and after hyroxyurea treatment groups

Biochemical parameters	12 months pre HU treatment (Mean ± SD)	12 months post HU treatment (Mean ± SD)	24 months post HU treatment (Mean ± SD)	P value on anova	Group 1 VS 2 on anova	Group 2 VS 3 on anova	Group 1 VS 3 On anova
Urea (mg/dl)	32.56 ± 5.57	30.15 ± 3.84	28.54 ± 3.04	0.07	0.05	0.06	0.02#
Creatinine (mg/dl)	0.82 ± 0.18	0.79 ± 0.16	0.75 ± 0.10	0.5	0.42	0.1	0.07
ALT (U/L)	25.28 ± 4.58	32.56 ± 5.28	46. 30 ± 6.27	0.01#	0.06	0.02#	<0.001#
AST (U/L)	28.52 ± 3.38	33.86 ± 3.90	44.59 ± 5.55	0.03#	0.09	0.04#	0.01#
ALP (U/L)	80.12 ± 15.60	90.30 ± 17.90	102.20 ± 18.80	0.1	0.20	0.14	0.04#
Bilirubin (mg/dl)	1.9 ± 0.31	2.1 ± 0.55	1.9 ± 0.42	0.09	0.06	0.07	0.52

SD: Standard deviation, HU: Hydroxyurea, VS: Versus, #: Significant p value on one wayanova

The improvement in duration (weeks) between successive transfusion episodes to maintain a stable Hb of 9.0 g/dL or near 9.0 g/dL was substantial. The average duration between successive transfusions, before patients started hydroxyurea, was three to four weeks but 12 months after therapy, the duration increased in 97 patients i.e. once every 6-7 weeks instead of once every 4-5 weeks (good responders) and improved only slightly in 102 patients (Poor responders). On the other hand, 66 patients showed no response in duration of their transfusion requirements (non-responders). A total of 75.09 % of our subjects responded to treatment but, 36.6% of our subjects reduced their transfusion requirement to more than 50 % of their pre-treatment transfusion needs. So 36.6% of patients were good responders while the rest of patients (38.49%) were poor responders. None of them could be weaned off transfusion to fit into excellent responder category.

Table.3: Stratification of response to hydroxyurea therapy based on target Hb level, reduction in transfusion requirement and duration between transfusions.

Response Strata	Excellent	Good	Poor	None	P value
N (%)	0 (0.00%)	97 (36.6%)	102 (38.49%)	66 (24.90%)	
Mean duration (weeks) between successive transfusion of RCCs in 12 months before treatment	0	4.35 ± 0.93	3.89 ± 0.72	3.90 ± 0.67	0.01#
Mean duration (weeks) between successive transfusion of RCCs in 12 months after treatment	0	6.37 ± 1.23	4.21 ± 0.84	3.27 ± 0.65	0.03#
Mean duration (weeks) between successive transfusion of RCCs in 24 months after treatment	0	6.85 ± 1.46	5.4 ± 1.50	3.95. ± 1.56	0.01
Mean RCCs transfused in 12 months pre- treatment	0	23.34 ± 5.56	27.87 ± 7.20	20.03 ± 4.85	0.03#
Mean RCCs transfused in 12 months after treatment	0	18.26 ± 2.28	22.77 ± 3.12	24.78 ± 6.12	0.58
Mean RCCs transfused in 24 months after treatment	0	10.26 ± 3.21	13.97 ± 4.12	24.78 ± 6.12	0.47
Mean pre-transfusion Hb levels before treatment	0	6.4 ± 1.24	7.2 ± 2.45	7.2 ± 2.03	0.01#
Mean pre-transfusion Hb levels after 12 months of treatment	0	8.2 ± 2.98	7.2 ± 2.10	7.3 ± 2.19	0.04#
Mean pre-transfusion Hb levels after 24 months of treatment	0	8.9 ± 3.10	7.5 ± 2.32	7.3 ± 2.35	0.02#

RCCs: Red cell concentrates, Hb: Hemoglobin, #: Significant p value on one wayanova

The only significant adverse events encountered in this study were of cytopenias in 43 cases over 4 years but they were offset by adjusting dosage. Fifteen patients reported nausea at commencement of therapy which resolved spontaneously or with anti-emetics.

Discussion

Our study evaluated the effect of hydroxyurea treatment on transfusion requirement, duration between transfusions and effects of hydroxyurea on renal and hepatic functions in this cohort. Response rates for patients with TDBT have been reported to vary from 30% to 70%. Ansari et al. showed a therapeutic response rate of 80% in 152 transfusion-dependent thalassemia patients taking HU after a mean duration of 65 days of therapy [20]Alebouyeh et al. found similar response, and their subjects exhibited a rise in post-HU treatment Hb, as well as a decrease in serum ferritin [21]. Similarly, Bradai and colleagues conducted a study on 45 beta thalassemia major patients with a mean dose of 17 mg/kg of the drug. About half of their patients exhibited more than 70% reduction in their transfusion requirements. [22]. Our results were comparable to the results concluded by Ansari et al and more promising than Bradai and colleagues.

In our study RFTs did not reveal any decline in the renal functions. These findings were consistent with the study done by Chik and co-workers [23].

On the other hand, although there were significant differences in the mean values of ALT, AST and bilirubin among the pre and post-treatment groups, yet the changes were without any clinically remarkable side effect. These findings were also consistent with the findings of Chik et al [23]and Ghasemi et al [24]. Chik and co-workers reported no significant renal and hepatic adverse effect. Similarly, Ghasemi et al reported transient and moderately raised LFTs in only 7.4% of patients. Ghasemi and colleagues also recommended to start therapy with a dose of 10mg/kg/day and increase it gradually, which is exactly same as our dosage protocol.

The mean duration of HU treatment in our study was 64 weeks. Decrease of transfusion requirement began in the first four months of HU therapy in our study. Our results were comparable to the results drawn by Ansari et al. [20], Alibouyeh et al. [21], Bradai et al. [22], Zamani et al.[25] and Bordbar et al [26]. All of them reported a comparable period of 4-6 months during which first effects of the medication were observed.

Conclusion

This study concludes that hydroxyurea treatment can decrease the transfusion requirement of patients with Transfusion Dependent Beta Thalassemia and increase the time period between successive transfusions of Red Cell Concentrate. There are no significant adverse effects of the treatment on hepatic or renal functions when drug is given in an optimal dose.

Conflict of Interest: Authors declare no conflict of interest.

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