IMPACT OF HYDROXYUREA ON BLOOD TRANSFUSION RATE IN PATIENTS OF BETA THALASSEMIA MAJOR

ABSTRACT

Objective: To determine the effectiveness of Hydroxyurea on blood transfusion rate in patients of Beta thalassemia major.

Material and methods: This cross-sectional study was held in the department of Genetics and Molecular Biology/Pathology department at LUMHS Jamshoro, and Diagnostic and Research Laboratory, Hyderabad, Sindh from February 2015 to August 2015. All Patients of Beta thalassemia major diagnosed on the basis of clinical history Hb Electrophoresis, and their parents Hb Electrophoresis. All the patients with other Haemoglobinopathies, and other genetic diseases.

Results: Mean age of the patients was 11.02+3.93 years and males were in majority 68.0%. Positive family history was in 56.2% cases. Mean serum Ferritin level was 12824.39±300.60ng/ml and mean hemoglobin level was 7.52±1.67 gm/dl. Few patients did not report follow up, because some families had migrated to others areas of Sindh, and some cases went to other welfare hospitals/centers, for treatment, therefore, out of 40 patients, 30 were observed hydroxyurea and overall, this treatment showed a significant decrease in blood transfusion requirements (P-0.01).

Conclusion: As per study conclusion the hydroxyurea was observed to be the effective treatment to decrease the blood transfusion rate but patients should be treated under responsible and proper observation.

Key words: β Thalassemia, Hydroxyurea, transfusions

ACRONYMS:

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Results: The mean age of the patients was 11.02+3.93 years and males were the majority (68.0 %). Positive family history was found in 56.2 % of cases. The mean serum ferritin level was 12824.39+300.60 ng/ml and the mean haemoglobin level was 7.52+1.67 g/dl. Few patients did not report follow-up, because some families had migrated to other areas of Sindh, and some cases ...

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Comment [3]: Abbreviations should not be used in the abstract unless they are the standard ones and if they are used they should be stated here for the first time.

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INTRODUCTION

β--Thalassemia, the most common genetic blood disorder, is caused by a deficiency in the formation of -globin chain, that resulting in inadequate erythropoiesis caused by an imbalance in the creation of alpha and non-alpha globin chains.[1] Though -thalassemia is frequent amongst people from the Mediterranean, central Asia, southern China, Middle East and India, it is no longer exclusive to these regions due to migration to other parts of the globe.[2,3] In general, around 5 % of the worldwide population suffers from hemoglobin-related disorders, with thalassemia carriers accounting for around 1.7%.[4] Thalassemia affects about 5-8 percent of the Pakistani population, and approximately 5000 thalassemia major infants are born in Pakistan every year.[5] Regular blood blood transfusions are the standard treatment for B-TM, but in addition to the risk of transmitting blood-borne illnesses, blood transfusions gradually create iron overload in key organs like heart and liver, which could also lead to early mortality.[4,6] A hallmark of -thalassemia is an imbalance in the α/β-chain ratio, which can be lowered by trying to compensate for defaulted chain of β-globin molecule with improved productivity of the -globin, which eventually forms HbF, and this HbF initiation limits necessity for blood transfusions, as well as iron chelation, to avoid complications linked to overload of iron through transfusion therapy.[7,8] As a result, various medications have been studied in try to lessen the need for transfusions. Hydroxyurea (HU), a foetal Hb inducer that reduces /-globin chain imbalance and is expected to treat chronic anaemia and reduce the requirement for blood transfusions, has shown effective.[2,9] Hydroxyurea, which seems to be a ribonucleotide reductase inhibitor, can improve hemolytic symptoms by increasing HbF synthesis and partially rectifying the imbalance between globin chains and non-globin chains.[1] However, because therapy response differs from patient to patient, it is recommended that numerous factors that influence treatment response be identified.[5] On the other hand, various factors, including genetic alterations, globin chain formation, XmnI polymorphism, and other biochemical parameters, are thought to have a role in the therapeutic response to HU.[1] This study has been conducted to determine the effectiveness of Hydroxyurea on blood transfusion rate in patients of Beta thalassemia major.

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β-thalassaemia, the most common genetic blood disorder, is caused by a deficiency in the formation of globin chains, resulting in inadequate erythropoiesis caused by an imbalance in the creation of alpha and non-alpha globin chains.[1] Although thalassaemia is common among people in the Mediterranean, Central Asia, southern China, the Middle East and India, it is no longer exclusive to these regions due to migration to other parts of the world.[2,3]
Overall, about 5.0 % of the world's population suffers from haemoglobin-related disorders, and thalassaemia carriers account for about 1.7 %.[4] Thalassaemia affects between 5.0 % and 8.0 % of the Pakistani population, and approximately 5,000 children are born with thalassaemia major each year in Pakistan.[5] Regular blood transfusions are the standard treatment for B-TM, but in addition to the risk of transmitting blood-borne diseases, blood transfusions gradually create iron overload in key organs such as the heart and liver, which may also lead to early mortality.[4,6] A hallmark feature of thalassaemia is the imbalance in the α/β-chain ratio, which can be reduced by attempting to compensate for the defective β-chain globin molecule with increased globin-productivity, which ultimately forms HbF, and this onset of HbF limits the need for blood transfusions, as well as iron chelation, to avoid complications related to iron overload through transfusion therapy.7,8 As a result, several drugs have been studied to try to decrease the need for transfusions. Hydroxyurea (HU), a fetal Hb inducer that reduces the imbalance of the /globin chain and is expected to treat chronic anaemia and reduce the need for blood transfusions, is effective.[2,9]

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MATERIAL AND METHODS

This cross-sectional study was carried out in the department of Pathology/Molecular biology and Genetics department at LUMHS Jamshoro, and Diagnostic and Research Laboratory, Hyderabad, Sindh. Study duration was six months from February 2015 to August 2015. All the patient of Beta Thalassemia Major diagnosed on the Hb Electrophoresis, having Hb less than 7 g/dL and were on regular transfusion every 2-4 weeks were included. All the patients had other Hemoglobinopathies and other Genetic diseases were excluded. Patients were divided in two groups in equal numbers. Half of the patients underwent blood transfusion with treatment of hydroxyurea and half without hydroxyurea. After taking informed consent Hydroxyurea was started as range 8-14 mg/kg/day. A fixed attending hematologist visited the participants every two weeks to assess their clinical and analytical responses. They were clinically evaluated for any signs of new onset extramedullary hematopoiesis, such as hepatosplenomegaly or abnormalities in the facial bones. Complete blood count (CBC) was analyzed for basic hematological parameters by using automated cell analyzer (Sysmex XN 1000i Tokyo, Japan). The medicine was stopped if patients developed intolerance or if their laboratory tests revealed leucopenia, a low platelet count, or abnormal RFTs or LFTs. The LFT, CBC, RFT, and ferritin levels were evaluated on monthly during this time, as well as their height, weight, hepatic and spleen sizes. Patients' treatment responses and HU side effects were also tracked. In comparison to those who did not receive hydroxyurea treatment, treatment response was defined as the capacity to maintain hemoglobin above 9g/dl or a reduction of at least 50% of baseline transfusion requirements. All the data was collected via study proforma. Data was analyzed on (SPSS) version 26.

RESULTS

Mean age of the patients was found 11.02+3.93 years, ranging from a minimum of 8 years to maximum 20 years. males were higher than females, 68.0% compared to 32.0%. Positive family history was found in 56.2% of the cases, but 43.8% patients had no family history of thalassemia being present. This is unusually higher negative

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1- The methodological design does not define what type of research was conducted. It is not enough to state that it is a cross-sectional study, more details are needed. From what the authors refer to, it seems to be a quasi-experimental study.

2-The sample size is not defined at any point and later in the results, an n=80 is expressed. This element should be made clear in the methodology.

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Comment [27]: The number must be separated from the unit of measurement symbol.

history of patients under study. The possible explanation can be the fact that many patients were not aware of such cases in their families. Mean serum Ferritin level was 12824.39±300.60ng/ml. Mean hemoglobin level was 7.52±1.67 gm/dl. Table.1

In this study 10 patients did not report follow up, because some families had migrated to others areas of Sindh, and some cases went to other welfare hospitals/ centers, for treatment. Therefore, out of 70 patients, 30 were on treatment with hydroxyurea. Overall, this treatment has caused a significant decrease in blood transfusion requirements(P-0.01). Table.2

(G-C) mutated patients had particularly shown good response with use of hydroxyurea as seen by significantly reduced blood transfusion rate with significant difference (p-0.001). Table.3

Table.1 Descriptive statists of the demographic characteristics n=80

Variables		**Statistics (n=80)**
Age (years)		11.02+3.93 years
Gender	**Males**	68.0%
	Females	32.0%.
Family history	**Positive**	45/56.2%
	Negative	35/43.8%
Serum Ferritin level		2824.39±300.60 ng/ml
Haemoglobin level		7.52±1.67 gm/dl

Table. 2 Average blood transfusion with and without hydroxyurea n= 70

Variable	Without hydroxyurea n= 40	With hydroxyurea n= 30	P-value
Blood transfusions (average)	2.2±2.3	1.1±1.4	0.01

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Comment [30]: This table describes the demographic characteristics of the study population. I consider that the variables serum ferritin level and haemoglobin level are not demographic variables but clinical-diagnostic variables, therefore I recommend renaming this table or separating these variables into a different table or graph.

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TABLE: 3. Blood transfusion rate according to gene mutation with hydroxyurea n= 30

Genes	Decrease transfusion rate	No change Transfusion rate	P- value
IVS 1 - 5 (G-C)	14	03	
IVS 1 - 1 (G-T)	04	01	
Fr 8 - 9	01	00	
CD 30 (G-A)	01	01	0.001
Fr - 16 (-C)	01	01	
Fr 41 - 42	01	00	
Del 619	00	01	
CD 5 (-CT)	01	00	

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DISCUSSION

In our study mean serum Ferritin level was found to be 2824.39±300.60 ng/ml. Azhar U et al[12] reported levels of Ferritin 4236.5 ng/ml, which is significantly higher than normally accepted levels. Ferritin is the body's principal iron-storage protein. the synthesis of it is regulated via iron levels via interactions between cytoplasmic proteins attached to messenger ribonucleic acid (mRNA), now known as iron regulatory proteins, and certain mRNA structures, known as iron-responsive elements.[13] Because it binds to and sequesters intracellular iron, it plays a crucial function in iron homeostasis. Serum ferritin testing is becoming a frequent clinical finding, with elevated concentrations being a common finding. Increased serum ferritin levels are related with or without iron overload in a wide range of genetic and acquired disorders. In beta-thalassemia trait comparative investigations, high concentrations of serum ferritin were found, and even individuals who've never been transfused had clinical and biochemical symptoms of hemochromatosis.[14-16]

In this series the best efficacy of Hydroxyurea (HU) treatment in patients with thalassemia, was evidenced in terms of significant transfusion reduction rate (p-0.001). Consistently Kosaryan M et al[17] found an excellent response in 44.7% of thalassemia major patients with the mean Hb of 10g/dl. The remaining patients needed transfusions less frequently after treatment with HU. The changes in Hb and HCT before and after HU were also statistically significant in their study (p <.0001). Another study conducted by Bradai M et al[18] noted that good improvement in hematology with HU

and regression of extramedullary hematopoietic masses in β Thalassaemic cases. They also reported that a reduction in extramedullary haematopoiesis has resulted in decrease in size of spleen and decreased number of circulating erythroblasts. It has also been reported in some studies that the higher age at first transfusion and higher baseline Hb correlated with a better response.[19] In our study, the thalassaemic patients showed better response to HU as compared to the late first transfusion starters which is comparable with the findings of Ansari et al.[20] Furthermore, we identified IVS 1 -5 (G-C) mutant individuals that responded well to Hydroxyurea treatment in terms of decreases in the blood transfusion (p-0.001), while IVS 1-1, on the other hand, had an equally positive response (G-T). However, a limited sample size does not allow for firm conclusions to be formed.

CONCLUSION

As per study conclusion the hydroxyurea was observed to be the effective treatment to decrease the blood transfusion rate but patients should be treated under responsible and proper observation. This was a small sample size and single center study; hence further large-scale studies are recommended to assess the role of Hydroxyurea in reducing the frequency of transfusion among patients of β -thalassemia.

REFERENCES

1. Bordbar MR, Silavizadeh S, Haghpanah S, Kamfiroozi R, Bardestani M, Karimi M. Hydroxyurea treatment in transfusion-dependent β-thalassemia patients. Iranian Red Crescent Medical Journal. 2014 Jun;16(6).

2. Algiraigri AH, Wright NA, Paolucci EO, Kassam A. Hydroxyurea for nontransfusion-dependent β-thalassemia: a systematic review and meta-analysis. Hematology/oncology and stem cell therapy. 2017 Sep 1;10(3):116-25.

3. E.P. Vichinsky, Changing patterns of thalassemia worldwide. Ann N Y Acad Sci, 1054 (2005), pp. 18-24

4. Ravangard R, Mirzaei Z, Keshavarz K, Haghpanah S, Karimi M. Blood transfusion versus hydroxyurea in beta-thalassemia in Iran: a cost-effectiveness study. Hematology. 2018 Aug 9;23(7):417-22.

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5. Asif N, Anwar T, Chaudary H, Mehmood K, Yaqoob N, Tahir M, Hassan K. Treatment response to hydroxyurea in beta thalassemia. JIMDC. 2014;392:48-52.

6. Choobineh H, Dehghani S, Alizadeh S., et al. Evaluation of leptin levels in major beta-thalassemic patients. Int J Hematol Oncol Stem Cell Res. 2009;3(4):1–4

7. Iqbal A, Ansari SH, Parveen S, Khan IA, Siddiqui AJ, Musharraf SG. Hydroxyurea treated β-thalassemia children demonstrate a shift in metabolism towards healthy pattern. Scientific reports. 2018 Oct 11;8(1):1-9.

8. Ansari SH, Shamsi TS, Ashraf M, Perveen K, Farzana T, Bohray M, Erum S, Mehboob T. Efficacy of hydroxyurea in providing transfusion independence in β-thalassemia. Journal of pediatric hematology/oncology. 2011 Jul 1;33(5):339-43.

9. Musallam KM, Taher AT, Cappellini MD, Sankaran VG. Clinical experience with fetal hemoglobin induction therapy in patients with β-thalassemia. Blood, The Journal of the American Society of Hematology. 2013 Mar 21;121(12):2199-212.

10. Kosaryan M, Vahidshahi K, Karami H, Ehteshami S. Effect of Hydroxyurea on Thalassemia Major and Thalassemia Intermedia in Iranian Patients. Pak J Med Sci 2009;25(1):74-78.

11. Raza S, Farooqi S, Mubeen H, Shoaib MW, Jabeen S. Beta thalassemia: prevalence, risk and challenges. International Journal of Medicine and Health Research. 2016;2(1):5-7.

12. Azhar U. Audit Of Beta-Thalassemia Cases At Sheikh Zayed Medical College/Hospital, Rahim Yar Khan. JSZMC 2015;6(2):811-815

13. Kannengiesser C, Jouanolle AM, Hetet G, Mosser A. A new missense mutation in the L ferritin coding sequence associated with elevated levels of glycosylated ferritin in serum and absence of iron overload. Haematologica 2009;94: 335-339

14.Fargion S, Taddei MT, Cappellini MD, Piperno A. The iron status of Italian subjects with beta-thalassemia trait. Acta Haematol.982; 68: 109-114.

15. Fargion S, Piperno A, Panaiotopoulos N, Taddei MT. Iron overload in subjects with beta-thalassaemia trait: role of idiopathic haemochromatosis gene. Br. J. Haematol.1985; 61: 487-490.

16. Piperno A, Mariani R, Arosio C, Vergani A. Haemochromatosis in patients with beta-thalassaemia trait. Br. J. Haematol. 2000;111: 908-914

Comment [42]: Asif N, Anwar T, Chaudary H, Mehmood K, Yaqoob N, Tahir M, Hassan K. Treatment response to hydroxyurea in beta thalassemia. JIMDC [Internet]. 2014 [cited -/-/-];392:48-52. Available from: https://jimdc.org.pk/jimdc/Volumes/3-2/Treatment%20Response%20to%20Hydroxyurea%20in%20Beta%20Thalassemia.pdf

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Choobineh H, Dehghani S, Alizadeh S, Ghobadi Dana V, Saiepour N, Meshkani R, et al. Evaluation of Leptin Levels in Major beta-Thalassemic Patients. Int J Hematol Oncol Stem Cell Res [Internet]. 2009 Oct [cited -/-/-] 1;3(4):1-4. Available from: https://ijhoscr.tums.ac.ir/index.php/ijhoscr/article/view/223

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17. Kosaryan M, Vahidshahi K, Karami H, Ehteshami S. Effect of Hydroxyurea on Thalassemia Major and Thalassemia Intermedia in Iranian Patients. Pak J Med Sci 2009;25(1):74-78.

18. Bradai M, Abad MT, Pissard S, Lamraoui F, Skopinski L, de Montalembert M, et al. Hydroxyurea can eliminate transfusion requirements in children with severe β thalassemia. Blood. 2003;102(4):1529– 30

19 Bradai M, Pissard S, Abad MT, Dechartres A, Ribeil JA, Landais P, et al. Decreased transfusion needs associated with hydroxyurea therapy in Algerian patients with thalassemia major or intermedia. Transfusion 2007; 47(10):1830–6.

20. Ansari S, Shamsi T, Siddiqui F, Irfan M, Perveen K, Farzana T, et al. Efficacy of hydroxyurea in reduction of pack red cell transfusion requirement among children having beta-thalassemia major. J Pediatr Hematol Oncol, 2007; 29: 743-46