

CORRELATION OF HAEMOLYTIC FACTORS AND HYDROXYUREA TREATMENT IN SICKLE CELL ANAEMIA WITH PULMONARY HYPERTENSION

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ABSTRACT

BACKGROUND

Retrospective and prospective studies¹⁻⁴ have shown that 20% - 40% of adult sickle cell patients have evidence of pulmonary hypertension on 2D Echocardiography evaluation. It is also an independent risk factor for death in sickle cell anaemia. Various studies⁴⁻⁶ reported 40% - 50% two-year mortality rate in sickle cell anaemia with pulmonary hypertension, which is quite high. Haemolysis is a proposed mechanism in development of PH in SS patients, while hydroxyurea has interesting relation with nitric oxide biology.

The objectives of this study are- 1) Evaluation of haemoglobin level, WBC count, platelet count, reticulocyte count and their relationship with SS patients; 2) Evaluation of serum bilirubin, serum LDH in relation with PH in SS patients; and 3) To establish relation of HU treatment with PH in SS patients.

MATERIALS AND METHODS

It is a hospital-based cross-sectional study; 88 cases of Sickle Cell Anaemia (SS) diagnosed as SS patterned on Hb electrophoresis were evaluated for various haemolytic factors, rheological factors and treatment with hydroxyurea to establish their relationship with pulmonary hypertension in sickle cell anaemia patient (SS pattern on Hb electrophoresis) and grouped in 2 groups as SS with Pulmonary Hypertension (PH) and SS without PH by 2D Echocardiography evaluation with Tricuspid Regurgitant Jet Velocity (TRV) of > 2.5 m/s and < 2.5 m/s respectively.

RESULTS

There were 66 male and 22 female cases; 32 (36.36%) cases had PH and 56 (63.64%) cases were without PH. There was no significant age and gender relation with PH in SS. Mean number of hospital admissions in the past were 3.63 ± 1.29 in SS with PH and 2.76 ± 1.08 in SS without PH having highly significant difference (p value = 0.001). Mean duration of illness from diagnosis of SS was not significant. Mean number of blood transfusions received in the past in SS with PH were 2.21 ± 1.82 and 1.03 ± 1.11 in SS without PH with significant difference. There was no relation in number of vaso-occlusive crises in past with PH. Mean Haemoglobin was 7.63 ± 0.69 in SS with PH and 8.25 ± 1.15 gm/dL in SS without PH group. This difference was statistically significant. Reticulocytosis also had positive relation with PH. Biochemical parameters such as high serum Lactate Dehydrogenase (LDH) had statistically significant relation with PH. Patients of SS without PH receiving Hydroxyurea were 26.78%, while only 6.25% of SS with PH patients were receiving Hydroxyurea. This difference was statistically significant. Zinc and Folic acid did not find significant difference in relation with PH.

CONCLUSION

Serum LDH is a good laboratory marker of haemolysis as well as PH in SS patients along with low haemoglobin and reticulocytosis. It may be used as a predictor of PH in sickle cell anaemia patients. There is high risk of development of PH in those sickle cell anaemia patients who were not receiving hydroxyurea suggesting its use in SS patients for primary prevention of pulmonary hypertension in sickle cell anaemia.

KEYWORDS

Hydroxyurea in Sickle Cell Anaemia with PH, LDH in Sickle Cell Anaemia, Sickle Cell Disease Related Pulmonary Hypertension.

HOW TO CITE THIS ARTICLE: Rathod BD, Bhaisare SD, Dave KK. Correlation of haemolytic factors and hydroxyurea treatment in sickle cell anaemia with pulmonary hypertension. J. Evolution Med. Dent. Sci. 2017;6(60):4398-4402, DOI: 10.14260/Jemds/2017/951

BACKGROUND

Retrospective and prospective studies¹⁻⁴ have shown that 20% - 40% of adult sickle cell patients have evidence of pulmonary hypertension on 2D Echocardiography evaluation.

Financial or Other, Competing Interest: None.

Submission 19-06-2017, Peer Review 14-07-2017,

Acceptance 20-07-2017, Published 27-07-2017.

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DOI: 10.14260/jemds/2017/951



It is also an independent risk factor for death in sickle cell anaemia. Various studies⁴⁻⁶ reported 40% - 50% two-year mortality rate in sickle cell anaemia with pulmonary hypertension, which is quite high.

A novel mechanism of the clinical sequelae of haemolysis and its effect on Nitric Oxide (NO) biology has been proposed as mechanism for PH in SS.⁷ Other mechanisms are endothelin pathway,⁸⁻⁹ asplenia, procoagulant state, nocturnal hypoxaemia and iron overload.¹⁰⁻¹²

The therapeutic efficacy of Hydroxyurea (HU) is attributed to induction of foetal haemoglobin and potentially to reduction of white blood cells as well as platelet counts, improved rheology and decreased endothelial red blood cell adhesion. Investigators have demonstrated an interesting relationship between HU and NO.¹³⁻¹⁵

Considering this background, we evaluated SS patients for various parameters like age, gender, haematological parameters such as haemoglobin, reticulocyte count, WBC count and platelet count, biochemical parameters like serum LDH and treatment with HU, zinc and folic acid in relation to PH.

Objectives

- 1) Evaluation of haemoglobin level, WBC count, platelet count, reticulocyte count and their relationship with PH in SS patients.
- 2) Evaluation of serum bilirubin, serum LDH in relation with PH in SS patients.
- 3) To establish relation of HU treatment with PH in SS patients.

MATERIALS AND METHODS

It was a hospital-based cross-sectional study of 88 Sickle cell anaemia (SS) patients fulfilling inclusion and exclusion criteria, admitted to Medical Intensive Care Unit and Medicine Wards at our tertiary care hospital, Central India, during the period of November 2008 to October 2010 randomly. Estimation of sample size was done in reference with assumption of PH 32% quoted by Gladwin et al⁴ with absolute precision of 5% and desired level of confidence interval of 95%, estimated sample size was 384 while we evaluated 88 cases of SS. We included 88 cases of SCD patients having Hb electrophoresis report of SS pattern (i.e. identified cases of SS pattern).

Inclusion Criteria

SS patients aged > 12 years.

Exclusion Criteria

Patients with an aetiology of PH other than SCD like valvular heart disease, congenital heart disease and connective tissue disorders.

Thorough clinical examination and history was noted. Patient’s demographic characteristics like age and gender, duration of illness from time of diagnosis, number of blood transfusions received in the past and number of hospitalisations in the past were recorded. Investigations such as haemoglobin, WBC count, platelet count, reticulocyte count and serum bilirubin were done on admission. Serum LDH was done in all patients after steady state of illness (on discharge mostly at least after one week). 2D Echo with colour Doppler was done on each patient. TRV was used to estimate pulmonary arterial systolic pressure. Depending on TRV > 2.5 m/s or < 2.5 m/s, they were grouped in 2 groups as SS with PH and SS without PH respectively. All patients were evaluated retrospectively regarding what treatment they were receiving like zinc, folic acid and HU.

Statistical Analysis

Data reported as Mean ± SD when normally distributed. Discrete variables were expressed in actual number and percentage. For categorical data, Chi square test was used and Fisher exact test was used for small numbers.

For continuously distributed variables, student ‘T’ test was used. P value of < 0.05 was considered statistically significant, while P value of < 0.001 was considered highly significant. Statistical software SPSS Version 17 and Primer was used for analysis.

RESULTS

88 cases of SS were evaluated and grouped in two groups as SS with PH and SS without PH (Table 1). There were 66 males and 22 females; 32 (36.36%) cases had PH and 56 (63.64%) cases were without PH. There was no significant relation of gender with PH. Maximum number of patients from both SS with PH and SS without PH were 20 - 29 years and 10 - 19 years with no significant relation of age with PH (Table 2).

Difference of mean number of hospital admissions in SS with PH and SS without PH group was highly significant (t=3.384 with 95 degrees of freedom, p= 0.001). Difference of mean number of blood transfusions received in SS with PH and SS without PH group was also significant (t=3.782 with 95 degrees of freedom, p < 0.001). Mean duration of illness from diagnosis of SCD and number of VOCs in past had no significant relation with PH (Table No. 3).

Among rheological factors difference of mean haemoglobin (t= -2.774 with 95 degrees of freedom, p= 0.007 i.e. < 0.01) and mean reticulocyte count in SS with PH group and SS without PH group was highly significant (t= 6.031 with 95 degrees of freedom, p < 0.001) (Table No. 4).

Mean serum LDH value in SS with PH group was higher as compared to SS without PH group with highly significant difference (t= 5.368 with 86 degrees of freedom, p < 0.001). Although difference of mean serum bilirubin in SS with PH and in SS without PH group was not significant (t= 1.675 with 95 degrees of freedom, p= 0.098) (Table No. 5).

Patients were already receiving medications like Hydroxyurea (HU), Zinc and Folic Acid; 26.78% of SS without PH cases and only 6.25% of SS with PH were receiving HU. There was statistically significant difference in relation with PH in SS cases by Chi square test (4.271 with 1 degree of freedom, p= 0.039 i.e. < 0.05). Hence, patients of SS not on HU have a higher risk of PH. Zinc and Folic Acid did not find significant difference in relation with PH (Table 6).

Group	Male	Female	P value
SS with PH (n = 32)	25 (78.12%)	7 (21.88%)	0.798
SS without PH (n = 56)	41 (73.21%)	15 (26.79%)	

Table 1. Gender Distribution and its Relation with PH

Group	Mean Age ± SD in Years	Confidence Interval	P value
SS with PH (n=32)	25.09 ± 11.58	-3.027 to 4.067	0.771
SS without PH (n=56)	24.57 ± 5.08		

Table 2. Age Distribution and its Relation with PH

Sl. No.	Variables	SS with PH Mean ± SD	SS without PH Mean ± SD	P value
1	Number of hospital admissions in past	6.12 ± 2.184	4.45 ± 0.89	0.001
2	Duration of illness from diagnosis of SCD (in Years)	4.24 ± 4.16	3.56 ± 2.5	0.340
3	Number of blood transfusions received in past	2.21 ± 1.82	1.03 ± 1.11	0.001
4	Number of VOCs in past	3.03 ± 1.31	2.67 ± 0.91	0.132

Table 3. Clinical Variables and Relation with PH

Sl. No.	Variable	SS with PH (n=32) Mean ± SD	SS without PH (n=56) Mean ± SD	P value
1	Haemoglobin (gm/dL)	7.63 ± 0.69	8.25 ± 1.15	0.007
2	WBC count (/cu. mm)	8251 ± 1900.51	7683 ± 2605.36	0.279
3	Platelets count (lakh/cu. mm)	2.97 ± 1.18	2.94 ± 0.93	0.895
4	Reticulocyte count (%)	1.66 ± 0.74	0.98 ± 0.31	0.001

Table 4. Haematological Variables and Relation with PH

Sl. No.	Variable	SS with PH (n=32) Mean ± SD	SS without PH (n=56) Mean ± SD	P value
1	Serum LDH (IU/L)	475.90 ± 212.83	303.50 ± 85.51	0.001
2	Serum Bilirubin (mg/dL)	1.639 ± 0.69	1.39 ± 0.66	0.098

Table 5. Biochemical Variables and their Relation with PH

Sl. No.	Medicines	SS with PH (n=32)	SS without PH (n=56)	P value
1	Hydroxyurea	2 (6.25%)	15 (26.78%)	0.039
2	Folic Acid	28 (87.5%)	48 (85.71%)	0.930
3	Zinc	26 (81.25%)	44 (78.57%)	0.980

Table 6. Treatment of SCD and its Relation with PH

DISCUSSION

There were 88 cases of sickle cell anaemia; 32 (36.36%) had PH and 56 (63.64%) cases had no PH. So prevalence of PH was 36.36% in SS cases in our study. Ataga et al³ and De Castro et al¹⁶ reported PH ranging from 30% - 36% in SCD. Age and gender have no significant relation with PH in SS patients in our study. The mean age was 25.09 ± 11.58 years and 24.57 ± 5.08 years in patients of SS with PH and SS without PH respectively. Mustafa San et al¹⁷ reported similar findings, while some studies^{4,18} found higher mean age

ranging from 37 ± 13 years to 40 ± 14 years in SS patients with no significant difference in relation to PH.

Patricia Houston-Yu and colleagues¹⁹ found frequent hospitalisation in SS with PH cases with significant difference. In our study, mean number of hospitalisations were 3.63 ± 1.29 and 2.76 ± 1.08 in SS with PH and SS without PH respectively having highly significant relation with PH (p value= 0.001). A study by Gladwin and co-workers⁴ showed significant relation of blood transfusions in SS patients stating patients requiring more blood transfusions had PH, while patients without PH had required lesser blood transfusions. We found mean number of blood transfusions received were 2.21 ± 1.82 in SS with PH and 1.03 ± 1.11 in SS without PH with highly significant difference (p value= 0.001, i.e. < 0.01). Patients of sickle cell anaemia requiring frequent hospitalisation or more blood transfusions have either haemolysis, acute chest syndrome, VOCs or other complications of the disease such as auto-splenectomy procoagulant state and iron overload which are the pathophysiologic factors responsible for PH in SS anaemia patients.^{7,10,12,20}

Studies^{4,21,22} found significant higher level of serum LDH in SS cases with PH with values of 508 ± 51 IU/L, 491 ± 196 IU/L and 488 ± 191 IU/L respectively. This was 475.9 ± 212.13 IU/L in our study with significant difference when compared to SS without PH group. Naoman et al¹⁸ and Castro O et al⁶ demonstrated low haemoglobin and reticulocytosis having significant relation with PH in SCD. In our study, mean Hb was 7.63 ± 0.69 gm/dL and mean reticulocyte count was 1.66 ± 0.74% in SS with PH group having significant relation when compared to SS without PH group.

Higher serum LDH, reticulocytosis and low Hb are due to chronic haemolysis of RBCs in SCD, which has effect on NO biology resulting in PH.⁷ Chronic haemolysis leads to cell-free plasma haemoglobin which scavenges NO. In addition, haemolysis releases Arginase, which degrades Arginine the substrate for endothelial NO synthase resulting in decreased NO production.²³ NO is a potent vasodilator and plays an important role in vascular endothelial haemostasis. Its depletion leads to vasoconstriction, endothelial dysfunction, platelet activation, oxidative stress and proliferative vasculopathy that ultimately leads to PH in SCD and other haemolytic anaemias.^{7,24} Another mechanism is endothelial pathway. Endothelin-1 promotes pulmonary artery smooth muscle contraction, proliferation and hypertrophy. It is over-expressed in SCD, potentially causing the development of PH.⁸⁻⁹ Other factors are asplenia, procoagulant state, nocturnal hypoxaemia and iron overload.¹⁰⁻¹²

26.78% patients of SS without PH group were on HU as compared to 6.25% patients of SS with PH group. The difference was statistically significant. Hence, HU may have a role in preventing PH in SS patients. Ataga et al³ and De Castro et al¹⁶ had 33% and 29% of SCD with pH on HU respectively, while 62% and 70% of patients of SCD without PH were on HU. Both studies found significant difference. There was no significant difference in relation to pH for folic acid and zinc treatment.

Although the exact mechanism of action remains uncertain, the therapeutic efficacy of HU is attributed to induction of foetal haemoglobin and potentially to reduction of white blood cell and platelet counts, improved rheology and decreased endothelial red blood cell adhesion. This has

been shown to reduce painful events and acute chest syndromes as well as improve survival in sickle cell anaemia patients.²⁵⁻²⁷ Several investigators have also demonstrated an interesting relationship between HU and NO, that HU increases the level of NO which plays a role in HU-induction of foetal haemoglobin. Given the critical role of NO depletion in the pathogenesis of PH in SCD, these observations may support important role of HU in treatment of PH in SCD.¹³⁻¹⁵

Limitations of Study

Being small sample size, it has limitations and requires large randomised trials.

CONCLUSION

Serum LDH in steady state of disease is a good laboratory marker of haemolysis as well as pulmonary hypertension in sickle cell anaemia along with low haemoglobin and reticulocytosis. It may be used as predictor of PH in SCD patients. As the survival of sickle cell anaemia patients is increasing, the complication like pulmonary hypertension is more evident nowadays. Once the pulmonary hypertension develops, the morbidity and mortality in SCD increases as secondary prevention by various medications for PH still have no promising results. Hydroxyurea reduces the risk of PH in sickle cell disease. Hence, can each patient of sickle cell anaemia, particularly young adults be put on hydroxyurea to primarily prevent this complication? To answer this, it may require formulation of some solid guidelines to put these patients on hydroxyurea other than already existing guidelines after doing large randomised trials.

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