Original Article

Co-relation of Hemolysis and Pulmonary Hypertension in Patients of Sickle Cell Disease

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ABSTRACT

Introduction : In sickle cell disease (SCD) Pulmonary Hypertension (PH) is a result of chronic hemolysis. It results in release of hemoglobin (Hb) in plasma. Plasma Hb can scavenge Nitric Oxide (NO) andcatalyze formation of Reactive Oxygen Species that leads to acute and chronic pulmonary vasoconstriction ultimately resulting in PH.

Aims &Objectives :

1.Evaluation of hemolysis and hemolytic factors in SCD population with PH.

2.Establishing relation of these factors with pulmonary hypertension (PH) in SCD.

Methods and materials : This observational cross sectional study was undertaken after taking approval from institute's Ethics Committee. Total 110 patients of Sickle Cell Disease (SCD), fulfilling inclusion and exclusion criteria were enrolled and evaluated. 88 patients had Sickle Cell Anemia (SS), 17 Sickle Cell Trait (AS) and 05 cases of Sickle Cell Thalassemia (ST). Depending on Tricuspid regurgitant velocity (TRV) these cases were divided into 2 groups. 1) With PH having TRV >2.5 m/s and 2) Without PH having TRV < 2.5 m/s.

Results : PH was found in 36.36% of SS patients and 23.52 % of AS patients. Vaso-occlusive crisis (VOC) and acute chest syndrome were commonest modes of presentations in SS group while VOC was common presenting feature in AS group. Mean duration of hospital stay number of hospital admissions in past and number of blood transfusions received had statistically significant association in SS cases with PH when compared with cases of SS without PH. Similarly, high serum Lactate Dehydrogenase (LDH), low Hemoglobin (Hb), reticulocytosis & elevated Bilirubin was found in SS with PH group when compared with SS without PH group and it was statistically significant difference.

Conclusion : PH affects mostly the SS cases. It is associated with chronic hemolysis suggested by decreased Hb, increased reticulocyte count, increased serum LDH and increased serum bilirubin which had significant relation with PH in present study.

Keywords : Sickle Cell Disease (SCD), SCD with Pulmonary Hypertension, LDH in SCD with PH

Introduction :

Prior to 1970s, mortality due to SCD was high with an estimated median survival of 14 years. Survival rate has improved over the last 3 decades due to advances in the management of SCD such as universal newborn screening, administration of prophylactic antibiotics, immunisation for life-threatening bacterial infections, Hydroxyurea treatment and stroke prevention with chronic transfusions[1]. As more children with sickle cell disease are surviving to adulthood, physicians caring for these patients are faced with a new challenge of recognising and treating the long-term complications of the disease. Pulmonary Hypertension is one of the long-termcomplications of SCD[2]. Pulmonary Hypertension is a result of chronic hemolysis, which results in release of haemoglobin in plasma. Plasma HBcan scavenge Nitric Oxide (NO) and catalyse formation of Reactive Oxygen Species that leads to acute and chronic pulmonary vasoconstriction ultimately resulting in PH[3]. So we evaluated various factors related to hemolysis like multiple hospitalisations,

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multiple blood transfusions, haemoglobin, reticulocyte count, serum bilirubin and serum LDH in SCD population with PH to establish their relation.

Objectives :

1. Evaluation of hemolysis and haemolytic factors like multiple blood transfusions, multiple hospitalisations, low haemoglobin, reticulocytosis, elevated bilirubin and elevated serum LDH in SCD population with PH.
2. Establishing relation of these factors with PH related to SCD.

Methods and materials :

It was an observational cross-sectional study of 110 SCD cases fulfilling inclusion and exclusion criteria, admitted to medical intensive care unit and medicine wards of GMC, Nagpur during November 2008 to October 2010. Institutional ethical committee has approved the study. Patients who had given informed written consent were enrolled in study. There were 3 different clinical variants i.e. Sickle Cell Anemia (SS), Sickle Cell Trait (AS) and Sickle Thalassemia (ST). Estimation of sample size was done in reference with assumption of prevalence of PH in SCD studied by Gladwin MT et al[4] as 32 with absolute precision of 5% and desired level of confidence interval 95%. Minimum estimated sample size was 84 while we included 110 cases.

Inclusion criteria :

SCD cases including SS, AS and ST with age > 12 years

Exclusion criteria :

Cases with an etiology of PH other than SCD like Valvular heart disease, congenital heart disease and connective tissue disorders.

Thorough clinical examination and detailed history of each case was noted. Age and gender were recorded. Disease characteristics like clinical presentation of vaso-oclusive crisis (VOC), acute chest syndrome, priapism, leg ulceration, stroke etc. were recorded. Blood investigations such as hemoglobin, reticulocyte count and Hb electrophoresis (in those patients who did not have previous report of Hb electrophoresis), serum creatinine and serum bilirubin were done on admission. Serum LDH was done for all cases included in study at steady state of illness. 2D-Echo and Doppler examination was done in each patient. TRV was used for estimating pulmonary artery systolic pressure. Depending on TRV > 2.5 m/s or < 2.5 m/s, all variants of SCD were divided into two groups as with PH and without PH respectively.

Statistical analysis :

Discrete variables were expressed in actual number or percentage. Data reported as mean $\pm$ SD when normally distributed. For categorical data, 'Chi square test' was used and for continuously distributed variables, 'Student-T test' was used. For small numbers, 'Fisher exact test' was used. Multivariate analysis was done by multiple logistic regressions. P value of < 0.05 was considered statistically significant while P value of < 0.01 was considered highly significant. Statistical software SPSS Version 17 and Primer was used for analysis.

Results :

Out of total 110 cases of SCD, sickle cell anemia (SS) was outnumbering the AS and ST patients. Being small numbers, in univariate analysis of variables we did not include ST patients. The overall prevalence of PH was 36.36% (40 of 110) ***(Table 1)***

Table 1 : **Variants of SCD with PH**

Sr. no.	Variant of SCD	With PH (TRV>2.5 m/s)	Without PH (TRV <2.5 m/s)
1	SS (n=88)	32 (36.36%)	56 (63.64%)
2	AS (n=17)	04 (23.52%)	13 (76.48%)
3	ST (n=05)	04 (80%)	01 (20%)
	Total n=110	40 (36.36%)	70 (63.64%)

There was no statistically significant difference in clinical presentation of subjects in SS Group with or without pulmonary hypertension ***(Table 2)***

Table 2 : **Clinical presentation of SCD**

Sr. no.	Clinical Presentation	SS with PH (n=32)	SS without PH (n=56)	P value	AS with PH (n=04)	AS without PH (n=13)	P value
1.	VOC	25 (78.12%)	51 (91.07%)	0.168	04 (100%)	13 (100%)	1.00
2.	ACS	06 (18.75%)	02 (3.75%)	0.046	0	0	-
3.	LU	03 (9.37%)	01 (1.78%)	0.266	0	0	-
4.	Priapism	02 (6.25%)	01 (1.78%)	0.617	0	0	-
5.	Stroke	04 (12.5%)	01 (1.78%)	0.107	0	0	-

Abbreviations- VOC- Vaso-occlusive crisis, ACS- Acute chest syndrome, LU- Leg ulceration

In univariate analysis of various biochemical parameters, haemoglobin and mean reticulocyte count showed significant association with SS related PH group than others, while mean serum LDH value, number of hospitalizations and number of blood transfusions received showed highly significant association to PH in SS with PH group than SS without PH group. ***(Table 3)*** However in AS variant this was not significant.

Table 3 : **Clinical and biochemical parameters and their relation with PH**

Sr. no.	Parameters	SS with PH Mean ± SD	SS without PH Mean ± SD	P value	AS with PH Mean ± SD	AS without PH Mean ± SD	P value
1	No. of hospital admissions in past	3.63 ± 1.29	2.76 ± 1.08	**0.001**	2.75 ± 0.43	1.76 ± 0.57	0.027
2	No. of blood Transfusions received in past	2.21 ± 1.82	1.03 ± 1.11	**0.001**	0.75 ± 0.43	0.46 ± 0.63	0.513
3	Hemoglobin (gm/dl)	7.63 ± 0.69	8.25 ± 1.15	**0.007**	9.02 ± 0.67	9.03 ± 1.41	0.989
4	Reticulocyte count (mg/dl)	1.66 ± 0.74	0.98 ± 0.31	**0.001**	0.65 ± 0.16	0.64 ± 0.34	0.956
5	Sr. Bilirubin	1.639 ± 0.69	1.39 ± 0.66	0.098	1.05 ± 0.295	0.89 ± 0.42	0.493
6	Sr. LDH	475.90 ± 212.83	303.50 ± 85.51	**<0.001**	269.5 ± 36.91	240.92 ± 49.31	0.305

In multivariate analysis reticulocyte count, serum bilirubin and serum LDH were found to be significantly associated with PH in SS group than non PH SS group while number of blood transfusion and haemoglobin did not show any statistically significant association. ***(Table 4)***

Table 4 : **Multiple logistic regression analysis of SS patients (n=88)**

Sr. no.	Parameters	Odds Ratio	95% confidence Interval	P value
1	No. of blood transfusions received in past	1.113	0.514-2.408	0.785
2	Hemoglobin	0.856	0.400-1.831	0.689
3	Reticulocyte count	24.385	1.62-365.25	**0.021**
4	Sr. Bilirubin	0.114	0.022-0.573	**0.008**
5	Sr. LDH	1.008	1.005-1.017	**0.036**

On univariate analysis, systolic blood pressure had highly significant difference with mean of 123.69 ± 9.09 mm hg in SS with PH and 120.14+5.26 mm hg in SS without PH group (t = 7.157 with 86 degree of freedom, p < 0.001). But on multiple logistic regression analysis, it was not significant, with p value of 0.297.

To summarize, factors like number of blood transfusions received, haemoglobin levels, serum bilirubin, reticulocyte count, serum LDH had significant difference in SS patients in relation to PH on univariate analysis but reticulocyte count, serum LDH levels and serum bilirubin had significant difference in relation to PH on multivariate analysis also, stating they are independent predictors of PH in SS cases.

Discussion :

Prevalence of SCD is high in central India. It ranges from 1.9 % to 33.5 % in various communities of Maharashtra. Elevated reticulocyte count, serum bilirubin and serum LDH have been postulated as markers of hemolysis in various studies. Some studies has evaluated the various risk factors for mortality in SCD but our study is unique in that we have tried to establish relation of haemolytic factors with PH which might be helpful in early screening of SCD patients for evidence of PH by 2D ECHO.

The prevalence of PH detected by 2D-Echo was found to be 36.36% (40 of 110 patients). It was also 36.36% (32 of 88) in SS variant while it was 23.52% (4 of 17) in AS variant. Ataga et al[5] and DeCastro et al[6] reported PH as 30% to 36% in SS patients while Haque and coworkers[7] reported it as 25% in AS patients.

Age and gender have no significant relation with PH in SCD cases in our study. The mean age was 25.09 ± 11.58 years and 24.57 ± 5.08 years in SS with and without PH respectively. Similar findings were seen by Mustafa San et al[8] while some studies[4,5] reported higher mean age ranging from 37 ± 13 to 40 ± 14 years in SCD patients with no statistically significant difference in relation to PH.

Patricia Houston-Yu and coworkers[9] in their study reported frequent hospitalisation and prolonged hospital stay in both SS and AS variants with PH. In our study, mean duration of hospital stay and number of hospitalisations were more in both SS and AS variant with PH when compared to the cases without PH. The difference was statistically significant ***(table 3).*** Gladwin and colleagues[4] showed significant co-relation stating patients with more frequent blood transfusions (>10) had PH while patients without PH had lesser blood transfusions (<10) in SS patients. We found more number of blood transfusions (mean 2.21 ± 1.82 with minimum of 1 and maximum of 6 blood transfusion) in SS with PH cases although such findings were not seen in AS patients. Usually patients of SCD requiring more hospital stay or more frequent hospitalisations or more blood transfusions have either more hemolysis, acute chest syndrome, VOCs or other complications of the disease like autosplenectomy, pro-coagulant state and iron overload which are the pathophysiological triggers for PH in SCD[10-13].

Kato GJ et al[14], Gladwin et al 4 and Liem et al[15] found significant higher level of LDH in patients of SCD 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 in relation to PH in SS variants. Similarly, studies[5,16] demonstrated low Hb, high reticulocyte count and increased bilirubin had significant relation with PH in SCD. In our study, mean Hb was 7.63 ± 6.9 gm//dl, mean reticulocyte count was 1.66 ± 0.74% and mean bilirubin was 1.639 ± 0.69 mg/ dl in SS with PH group having significant relation on comparing with SS without PH group. In AS variant, it was not significant.

Elevated serum LDH, increased reticulocyte count, increased bilirubin and low Hb are due to chronic hemolysis of RBCs in SCD. A novel mechanism of the clinical sequelae of hemolysis and its effect on nitric oxide (NO) biology has been proposed for the development of PH in SCD[10]. Cell-free plasma haemoglobin released during intravascular hemolysis leads to scavenging of NO. In addition hemolysis releases Arginase, which degrades Arginine- the substrate for endothelial NO synthase,

resulting in decreased NO production[17]. NO is a potent vasodilator and plays an important role in vascular endothelial hemostasis. Its depletion leads to vasoconstriction, endothelial dysfunction, platelet activation, oxidative stress and proliferative vasculopathy that ultimately lead to PH in SCD and other haemolytic anemias[10,18]. Another mechanism is the 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[19].

Neely and coworkers[20] demonstrated serum LDH as a marker of intravascular hemolysis. Gladwin et al[4] co-related serum LDH with PH and death in SCD. Liem et al[14] established serum LDH as predictor of PH in children and young adults with SCD. No other marker of hemolysis like low Hb, elevated bilirubin and increased reticulocyte count co-related as well as serum LDH. Thus, LDH is emerging as the most useful marker of hemolysis-related complications of SCD like PH over the years.

In conclusion, serum LDH is a good laboratory marker of hemolysis as well as PH in SCD alongwith low Hb, reticulocytosis and increased serum bilirubin. Serum LDH can be used as a predictor of PH in patients of SCD.

Implication of Study :

Reticulocytosis, increased bilirubin as well as elevated serum LDH are markers of hemolysis. But our study had shown positive correlation of these factors with PH in SS group, which can be used to assess for evidence of PH in SCD patients.

Limitations of Study :

This is a cross sectional observational study with small sample size. A prospective cohort study with large sample size is required to assess role of these factors in PH in SCD patients.

References :

1. National Heart, Lung and Blood Institute. Sickle Cell Research for treatment and Cure. NIH publication. Bethesda, MD : 2002: 02 : 521-524.
2. Quinn CT, Rogers ZR, Buchanan GR. Survival of children with Sickle cell disease. Blood 2004 : 103: 4023-4027.
3. Reiters C D, Wang X, Tanus-Santos J E. Cell free haemoglobin limit NO bioavailability in sickle cell disease. Nat. Med. 2002: 8: 1383-1389.
4. Gladwin M T, Sachdev V, Jison M L, Schizicuda Y, Plehn J, Minter K. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N. Eng. J. Med. 2004 : 350: 886-895.
5. Shahla G. Naoman, Mehdi Nouraie, Oswaldo L Castro. Echocardiographic findings in patients with sickle cell disease. Ann. Of Hematol.2010 : 89: 61-66.
6. Laura M. De Castro, Jude C. Jonassaint, Felicia L G, Koch A A, Telen M J. Pulmonary hypertension associated with sickle cell disease: Clinical and laboratory endpoints and disease outcomes : Am. J. Hematol. August 2007 : 19-26.
7. Haque A K, Gokhale and Rampy B A. Pulmonary hypertension in sickle cell hemoglobinopathy : A clinic-pathologic study of 20 cases. Hum. Pathol. Oct 2002: 33(10): 1037-1043.
8. Mustafa San, Mustafa Demirtas, Refik Burgul. Left ventricular systolic and diastolic functions in patients with sickle cell anemia. Int. J. Angiology May 1998: 17: 185-187
9. Patricia Houston-Yu, Sohail R Rana, Betsy Beyer, Oswaldo Castro. Frequent and prolonged hospitalisation : A risk factor for early mortality in sickle cell disease patients. Am. J. Hematol. 2003 : 72: 201-203.
10. Rother R P, Bell L, Hillmen P, Gladwin M T. The clinical sequelae of intravascular hemolysis and extravascular plasma hemogobin: A novel mechanism of human disease. JAMA : 2005 : 293 : 1653-1662.
11. Hsu LL, Champion H C, Campbell-Lee S A. Hemolysis in sickle cell mice causes pulmonary hypertension due to global impairment in nitric oxide bioavailability: Blood 2007: 109: 3088-3098.
12. Ataga K I, Orringer E P. Hypercoagulability in sickle cell disease: A curious paradox. Am. J. Med. 2003: 115: 721-728.
13. Neumayr L, Morris C, Harmatz P. Pulmonary hypertension in chronically transfused patients with sickle cell and thalassemia: A report from multicentre study of iron overload (Abstract). Presented at the 29th Annual Meeting of the National sickle cell disease programme, Memphis, TN: April 8-12, 2006.
14. Kato G J, McGowan V, Machado R F, Little J, Taylor J, Morris C R, James S et al. Lactate dehydrogenase as a biomarker of hemolysis associated nitric oxide resistance in priapism, leg ulceration, pulmonary hypertension and deathin patients with sickle cell disease. Blood: 2006: 107: 2279-2285.
15. Robert I Liem, Luciana T. Young, Alexis A, Thompson. Tricuspid regurgitant jet velocity is associated with hemolysis in children and young adults with sickle cell disease evaluated for pulmonary hypertension. Haematological: 2007: 92 (11):
16. Castro O, Hoque M, Brown B D. Pulmonary hypertension in sickle cell disease: Cardiac catheterisation, results and survival. Blood 2003: 101: 1257-1261.
17. Morris C R, Morris S M Jr, Hagar W. Arginine therapy a new treatment for pulmonary hypertension in sickle cell disease. Am. J. Respir. Crit. Care Med. 2003: 168: 63-69.
18. Gladwin M T, Kato G J. Cardiopulmonary complications of sickle cell disease: role of nitric oxide and haemolytic anemia. Hematol(Am. Soc. Hematol. Edu. Programme) 2005: 51-57.
19. Ergul S, Brunson CY, Hutchinson J. Vasoactive factors in sickle cell disease : in vitro evidence for endothelin 1 mediated vasoconstriction. Am. J. Hematol. 2004: 76: 245-251.
20. Neely C L, Wajima T, Kraus A P, Diggs L W, Barrera S. Lactic acid dehydrogenase activity and plasma hemoglobin elevation in sickle cell disease. Am. J. Clin. Pathol. 1969: 52: 167-169.