Original Article

Pulmonary Hypertension in Hemoglobinopathies : A Neglected Entity

Subhrangsu Mukherjee[1], Subhabrata Ray[2], Avijit Moulick[3], Pasang Lahmu Sherpa[4]

Background : Hemoglobinopathies constitute a heterogeneous group of Hereditary Hemoglobin Disorders. Cardiovascular complications are among the leading causes of morbidity and mortality in Hemoglobinopathies. In the wide spectrum of Cardiovascular manifestations, Pulmonary Arterial Hypertension (PAH) holds a prominent place. Screening for Pulmonary Hypertension should be an essential component in assessment of patients with Hemoglobinopathies and may be accomplished by Transthoracic Doppler Echocardiography which is most established screening tool for Pulmonary Hypertension and is widely available and cost-effective.

Objective : This study aims to determine the presence of Pulmonary Hypertension in Hemoglobinopathies.

Method : Institution/Hospital based, Non-interventional, Observational Descriptive, Cross-sectional study conducted amongst 76 patients of Hemoglobinopathies (>12 years) in North Bengal Medical College and Hospital, Darjeeling. Detailed history and physical examination along with non invasive tests like ECG, Chest X-ray and Echocardiography were performed in all study population to detect PAH. Data was analysed using standard statistical method.

Result : 30.26% of patients with Hemoglobinopathy had PAH in Echocardiographic findings, of which 25% had mild and 5.26% cases had Moderate PAH. It was most prevalent in E-β thalassemia, followed by Sickle β and β thalassemia major respectively. The clinical indicators associated with increased risk of PAH in Hemoglobinopathies were presence of Severe Anemia, transfusion >10 U/year, Iron overload state, Splenectomy and their combinations.

Conclusion : Echocardiography serves as one of the most useful non-invasive screening tool for the diagnosis of Pulmonary Hypertension. Early detection and management is necessary to decrease the morbidity and mortality.

[*J Indian Med Assoc* 2022; **120(3):** 24-8]

Key words **: Hemoglobinopathies, Echocardiography, Pulmonary hypertension.**

Hemoglobinopathies recognised in India are Hb E-β thalassemia, Sickle-cell Anemia, HbE & HbD[1,2,3]. There is a sharp increase in frequency of β-Thalassaemia trait in Eastern and South Eastern India (Bengal, Odisha and Andhra Pradesh)[4-6]. Common Hemoglobinopathies prevalent in North Bengal are Hb E diseases and E-β Thalassaemia.

Cardiovascular complications are among the leading causes of mortality and morbidity in Hemoglobinopathies[7]. In the wide spectrum of Cardiovascular Manifestations, Pulmonary Arterial Hypertension (PAH) holds a prominent place. The Pathophysiology behind Pulmonary Hypertension is Chronic Anemia and Hemolysis. Free Hemoglobin released during Hemolysis scavenges the intrinsic vasodilator Nitric Oxide (NO) and releases arginase[8,9], which is an enzyme responsible for depletion of L-arginine, a substrate for NO Synthesis. So, there is depletion of NO leading to impaired NO dependent vasodilatation of Pulmonary Vasculature[10]. There is an increased Risk of Thrombosis due to factors released during Red Cell destruction leading to Platelet Activation, Thrombin Generation and tissue factors activation and Obliterative Pulmonary Vasculopathy.

Screening for Pulmonary Hypertension should be an essential component while assessing a patient with Hemoglobinopathy which may be accomplished by Transthoracic Doppler Echocardiography, the most established screening tool for Pulmonary Hypertension in general, widely available, cost-effective[11]. In a landmark study, 32% of adults with HbSS had TRV ≥2.5 m/s who were associated with a tenfold increase in risk of death[12].

Common features suggesting presence of PAH are

Editor's Comment :

- Screening for Pulmonary Hypertension should be an essential component while assessing a patient with Hemoglobinopathy which may be accomplished by Transthoracic Doppler Echocardiography, the most established screening tool for Pulmonary Hypertension in general, widely available, cost-effective. Early detection and management is necessary to decrease the morbidity and mortality.

Department of Medicine, North Bengal Medical College, Darjeeling 734101
[1]MD, Senior Resident
[2]MD, Assistant Professor
[3]DM, Associate Professor
[4]MD, Associate Professor and Corresponding Author

Received on : 31/01/2022
Accepted on : 01/02/2022

as following[13]

1. Right atrial enlargement
2. Right ventricular enlargement and dysfunction
3. Small under filled left-sided heart chambers
4. Interventricular septal flattening
5. Tricuspid regurgitation with elevated velocity
6. Reduced Tricuspid Annular Plane Systolic Excursion (TAPSE).
7. PASP: Pulmonary Arterial Systolic Pressure, calculated by assessing TR velocity and Right Atrial Pressure (RAP). RAP is calculated by IVC size and collapsibility. It is easy to measure. If > 2.1 cm size with <50% collapsibility, the estimated RAP is 15 mm of Hg.

PASP = 4 TRV^2 + RAP.

Cut off value of PASP is 35 mm Hg[14].

8. mPAP: Mean Pulmonary Arterial Pressure calculated by assessing PR velocity and Right Atrial Pressure.

mPAP = 4 PRV^2 + RAP. It is relatively easy to measure. Cut off value is 25 mm Hg.

9. AT (RVOT): Right Ventricular Outflow Tract acceleration time is easy to measure, reliable and reproducible. Cut off value is 100 ms. <100 ms is critical.

mPAP is calculated by the formula from AT of RVOT[14]:

$mPAP = 79 - (0.45 \times AT^{RVOT})$

The estimation of Pulmonary Hypertension in Hemoglobinopathies is necessary for early detection and management along with the risk stratification and prevention.

Materials and Methods

This study was Hospital based, Non-interventional, Observational Descriptive, Cross-sectional study, conducted among the indoor patients and those attending Medicine OPD of North Bengal Medical College.

Patients with co existing Pulmonary and Heart Diseases, Connective Tissue Disease, HIV with history of drug and Toxin causing Pulmonary.

Hypertension were excluded from the study.

76 patients of Hemoglobinopathies (>12 years) were evaluated for determining the presence of PAH from 1st April, 2019 to 31st March, 2020.

Detailed history, general and systemic examination were conducted. Blood investigations like complete Hemogram with Peripheral Blood Smear, Liver Function test, Urea, Creatinine, Fasting and Postprandial Blood Sugar, Serum Iron Profile Study, Hepatitis B and C were done. ECG, Chest X-ray and Echocardiography were performed to see the presence of features suggestive of PAH in all the study subjects.

Doppler Echocardiography were performed with measurement of Right Atrial Pressure (RAP) by IVC collapsibility, presence of Tricuspid (TR) and Pulmonary Regurgitation (PR). The patients with TR were further evaluated for TR gradient and TR velocity. Pulmonary Arterial Systolic Pressure (PASP) was deducted from TR velocity and RAP. The patients with PR were further evaluated for PR gradient and PR velocity. Mean Pulmonary Arterial Pressure (mPAP) was deducted from PR velocity and RAP. Indirectly, another value of mean Pulmonary Arterial Pressure (mPAP) were deducted from RVOT acceleration time for all patients irrespective of presence of PR.

The entire data was collected with consistency and completeness and entered in Microsoft excel software to prepare a master Table and analysed using various statistical methods like Chi square test, Fisher's Exact, unpaired T-chart and Mann- whitney U test where ever applicable.

Analysis and Results

In this study, majority of the subject were male (56.58%) and the mean age of overall study population was 20.68 ± 9.30 years. (Male 21.14 ± 8.96 years > Female 20.09 ± 9.98 yrs) and most of study population were below 20 years of age. Majority of them belonged to the Rural areas (73.68%) of North Bengal and adjoining areas attending hospital for the treatment of Hemoglobinopathies. 27.63% were Rajbangshi, most of them (23.68%) had Hb E trait. While remaining of the study subject had Hb E β thalassemia (22.37%), followed by, β thalassemia major (17.11%) and Hemoglobin E homozygous state (14.47%) which shows that HbE is predominant in this area.

Pulmonary Hypertension was more prevalent in male patients (37.21%) than female patients (21.21%) but the difference was statistically insignificant. The mean age of patients (18.96 ± 6.35) was lower with PAH. Most of our study subjects with PAH had classically two Cardiovascular Symptoms ie, Shortness of breath (58.33% with p value = 0.037) & syncope (71.43% with p value = 0.024) and both of the symptoms were statistically significantly associated with PAH. Presence of Jaundice (41.30% *versus* 13.33% with p value = 0.009), weight loss (52.38% *versus* 21.82% with p value = 0.010) and Anorexia (39.53% *versus* 18.18% with p value = 0.045) were statistically significantly associated with PAH. Most of the patients with Pulmonary Hypertension had Chelation Therapy (50% *versus* 26.56%) but it was not statistically significant (p = 0.168). All the patients who had undergone Splenectomy had PAH (100% with

splenectomy *versus* 24.29% without splenectomy) and it was statistically significant. History of blood transfusion were more in mild (31.82%) and moderate (9.09%) PAH and it was statistically significant (p value = 0.038). Mean Pulmonary Arterial Pressure was directly proportional to mean total yearly transfusion (Table 1).

PAH was strongly related with Degree of Anemia and was more prevalent in patients with Severe Anemia (57.14%), 36.84% of Moderate Anemia, 23.81% of Mild Anemic patients. Patients of PAH had lower mean Hemoglobin level than others (7.64 ± 2.46 g/dL in PAH *versus* 9.20 ± 3.02) which was statistically significant (p value = 0.032) and mean Pulmonary Arterial Pressure was inversely related with of Hemoglobin level. Mean rank of Platelets was lower (34.00 with PAH *versus* 40.45 without PAH) and Mean rank of Albumin was lower (35.61) in patients with PAH. Mean rank of Ferritin (52.83 with PAH *versus* 32.28 without PAH) were higher in the patients of PAH. It was observed that mean Pulmonary Arterial Pressure, Pulmonary Arterial Systolic Pressure proportionately increase and RVOT Acceleration time proportionately decrease with mean Ferritin level. Mean iron (197.17 ± 63.32 μg/dL with PAH *versus* 137.62 ± 52.16 without PAH) and mean Transferrin saturation (69.49 ± 20.77% with PAH *versus* 50.16 ± 20.60%) were higher in patients with PAH and they were statistically significant. Mean TIBC (282.17 ± 33.09 μg/dL) was also higher among the patients with PAH.

Table 1 — Prevalence of PAH in various clinical settings

Parameters		% of PAH
H/O Chelation	Present	50%
	Absent	26.56%
H/O Splenectomy	Present	100%
	Absent	24.29%
Severe Anemia (Hb <8)	Present	42.86%
	Absent	22.92%
Mean total transfusion (>10 U / yr)	Present	44.12%
	Absent	30%
Iron Overload (Ferritin Male >300, Female >200)	Present	36.96%
	Absent	20%
H/O Chelation + Splenectomy	Present	100%
	Absent	25.40%
H/O Splenectomy + Iron Overload	Present	100%
	Absent	20%
H/O Splenectomy + Iron Overload + Severe Anemia (Hb <8)	Present	100%
	Absent	20.69%
Iron Overload + Mean total transfusion (>10 U / yr)	Present	43.33%
	Absent	33.33%
H/O Chelation + Severe Anemia (Hb <8)	Present	50%
	Absent	22.92%
H/O Splenectomy + Iron Overload + Severe Anemia (Hb <8) + Mean transfusion (>10 U/yr)	Present	100%
	Absent	33.33%

Majority of the patients with Pulmonary Hypertension had TR (52.5%) and PR (58.6%) and it was statistically significant. The mean rank of TR Gradient (60.98) and PR Gradient (55.46) were higher in patients with PAH and both of them were statistically significant.The mean AT (110.69 ± 12.93) was lower and mean Right Atrial Pressure (6.21 ± 2.69 mm of Hg) was higher among the patients with PAH and were statistically significant (p value = 0.000 for both) (Table 2).

Most of the patient of Hb E β thalassemia (23.5%) had Moderate Pulmonary Hypertension, while those with Sickle β Thalassemia (33.3%) had Mild Pulmonary Hypertension had but it was not statistically significant (Table 3)There was significant increase in prevalence of PAH due to Splenectomy, mean total transfusion >10 u/yr, severe Anemia, Iron overload and their combinations (Table 4).

Discussion

Seven subtypes of Hemoglobinopathies were identified in this region mainly Hb E trait (23.68%), Hb

Table 2 — Mean Echocardiographic parameters assessing Pulmonary artery pressure in different subgroups

	BTM (n=13) 17.11%	BTT (n=10) 13.16%	EBT (n=17) 22.37%	HEH (n=11) 14.47%	HET (n=18) 23.68%	SBT (n=3) 3.95%	SC (n=4) 5.26%	Total (n=76) 100%
Study population (n =76)								
RAP (mm of Hg)	4.15± 2.19	3.50± 1.58	5.88± 2.87	3.45± 1.51	4.39± 2.30	3.00± 0.00	3.00± 0.00	4.30± 2.28
AT (mili second)	126.77± 23.28	135.30± 21.88	123.41± 19.17	142.64± 13.82	142.72± 15.42	135.00± 13.23	139.25± 22.56	134.20± 19.68
m PAP(by AT) (mm of Hg)	21.96± 10.37	18.05± 9.65	23.31± 8.58	14.85± 6.13	14.85± 6.91	18.33± 5.86	16.38± 10.06	18.60± 8.75
TR (n = 40)								
	BTM (n=5) 12.5%	BTT (n=5) 12.5%	EBT (n=11) 27.5%	HEH (n=5) 12.5%	HET (n=8) 20%	SBT (n=3) 7.5%	SC (n=3) 7.5%	Total (n=40) 100%
RAP (mm of Hg	6.00± 2.74	4.00± 2.24	6.09± 3.05	4.00± 2.24	4.88± 2.59	3.00± 0.00	3.00± 0.00	4.85± 2.59
TRV (m/s)	2.62± 0.22	2.34± 0.50	2.59± 0.70	2.66± 0.35	2.11± 0.51	2.40± 0.26	2.43± 0.42	2.45± 0.52
TRG (mm of Hg)	27.60± 4.67	23.00± 8.75	27.81± 13.14	29.00± 7.00	19.00± 8.96	23.67± 4.93	25.00± 7.94	25.05± 9.56
PASP (mm of Hg	33.52± 6.38	26.80± 6.76	33.62± 15.00	32.70± 7.83	23.73± 11.40	26.17± 4.54	23.63± 8.50	29.12± 10.86
PR (n = 29)								
	BTM (n=3) 10.34%	BTT (n=3) 10.34%	EBT (n=11) 37.94%	HEH (n=4) 13.79%	HET (n=6) 20.69%	SBT (n=1) 3.45%	SC (n=1) 3.45%	Total (n=29) 100%
RAP (mm of Hg	6.33± 2.89	3.00± 0.00	6.55± 2.91	4.25± 2.50	4.67± 2.58	3.00± 0.00	3.00± 0.00	5.21± 2.72
PRV (m/s)	2.23± 0.64	2.37± 0.21	2.06± 0.54	1.53± 0.31	1.73± 0.37	2.40± 0.00	1.70± 0.00	1.97± 0.50
PRG (mm of Hg)	20.67± 10.21	25.00± 5.29	18.06± 8.33	9.88± 3.47	12.90± 5.00	23.00± 0.00	12.00± 0.00	16.81± 7.86
m PAP(by PR) (mm of Hg	27.33± 13.43	28.00± 5.29	24.76± 10.20	13.88± 5.45	17.28± 7.33	26.00± 0.00	14.50± 0.00	22.00± 9.56

Table 3 — Degree of Pulmonary hypertension in subtypes of Hemoglobinopathies (n = 76).

Type of Hemoglobinopathies	Pulmonary hypertension			Total	Statistical test
	Absent No. (%)	Mild No. (%)	Moderate No. (%)		
Beta thalassemia major	9 (69.23)	4 (30.77)	0 (0)	13 (100)	Chi square = 15.976, df = 12, p value = 0.192
Beta thalassemia trait	7 (70)	3 (30)	0 (0)	10 (100)	
Hb E Beta thalassemia	9 (52.94)	4 (23.53)	4 (23.53)	17 (100)	
Hb E hemoglobinopathy	8 (72.73)	3 (27.27)	0 (0)	11 (100)	
Hb E trait	15 (83.33)	3 (16.67)	0 (0)	18 (100)	
Sickle beta Thalassemia	2 (66.67)	1(33.33)	0 (0)	3 (100)	
Sickle Cell anemia	3 (75)	1 (25)	0 (0)	4 (100)	
Total	53 (69.74)	19 (25)	4 (5.26)	76 (100)	

Table 4 — Association of degree of PAH with History of Blood Transfusion

Blood transfusion	Pulmonary hypertension			Total	Statistical test
	Absent No. (%)	Mild No. (%)	Moderate No. (%)		
Yes	26 (59.09%)	14 (31.82%)	4 (9.09%)	44 (100%)	Chi square = 6.551, df = 2, p value = 0.038
No	27 (84.38%)	5 (15.62%)	0 (0.0%)	32 (100%)	
Total	53 (69.74%)	19 (25%)	4 (5.26%)	76 (100%)	

E β thalassemia (22.37%) and β Thalassemia major (17.11%). 30.26% of them were having PAH (Mild 25% & Moderate 5.26%). PAH was predominantly seen in Hb E β Thalassemia (47.06%) followed by Sickle beta thalassemia (33.33%) & β Thalassemia major (30.77%)(Fig 1). This finding corroborates with the study done in Thailand by Chueamuangphan N *et al* where that 29.02% of study population of Hemoglobinopathies were having PAH (Mild 17.86%, Mod 7.59%, Severe 3.57%) and among them majority were having E β thalassemia[15]. In our study, 25% of Sickle Cell Disease patients were suffering from PAH. Similar study on PAH in Sickle Cell Disease shows the prevalence ranges from 20 to 40% in Echocardiographic findings[10]. PAH was least prevalent in Hb E Trait (16.67%). Symptoms of Abdominal pain, Fatigability, Bone pain and Anorexia were common in overall all the groups of Hemoglobinopathies. Classical Cardiovascular symptoms like shortness of breath and syncope were seen in those with PAH. 15.79% of total population (comprising only BTM 30.77% & EBT 47.06%) are under Chelation Therapy with deferasirox from the hospital. PAH was more prevalent in the patients receiving Chelation Therapy (50% with chelation therapy vs 26.56% without therapy). Azami M *et al* concluded that 47.22% of total population had PAH and Chelation history was more commonly found in patients of PAH (88.2% with PAH *versus* 73.7%)[16]. 7.89% of study population had undergone Splenectomy surgery and mostly in E β thalassemia (23.53%). Splenectomy was one of the major reason for PAH in Hemoglobinopathies (100% in splenectomised *versus* 24.29%). Similarly, another study by Meera V, Jijina F, Ghosh K, that among 21 splenectomised patients of Hemoglobinopathies 28.57% of subjects had PAH with mPAP of 46.28 ± 28.17 mmHg. The Platelet Count was significantly high in splenectomized patients due higher Pulmonary flow, Thromboembolic occlusion of the vessels and Plexiform Arteriopathy[17].

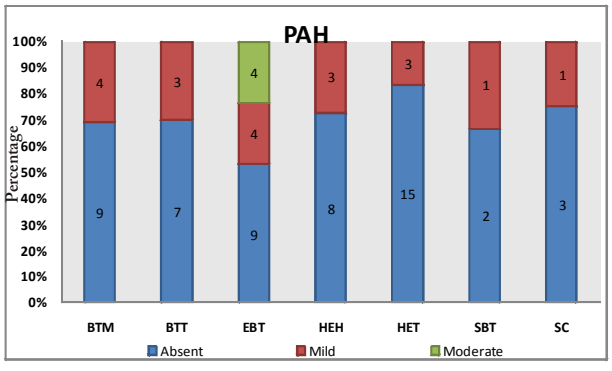

Fig 1 — Degree of Pulmonary hypertension in different subgroups of Hemoglobinopathies (n=76)

Both Mild (31.8%) and Moderate (9.1%) PAH was more common with the history of blood transfusion. Mean Total Transfusion (14.70 ± 9.41 U/yrs) was directly associated with the PAH in Hemoglobinopathies. Similarly, Chuncharunee S *et al* concluded in their study that 37.3% of E β Thalassemia patients had PAH and early age of transfusion and total yearly transfusion are directly associated with PAH like our study. PAH was more common with high WBC count (5997.82 with PAH *versus* 5786 without PAH) and Low Platelet Count (Platelet rank 34 with PAH *versus* 40.45). PAH was more predominant in the individuals with Iron overload state (higher value of Ferritin with mean rank 52.83 *versus* 32.28, mean iron 197.17 ± 63.32 μg/dL and mean Transferrin saturation 69.49 ± 20.77% with p value 0.000). Hamdy AM, Zein El Abdin MY, Abdel Hafez MA concluded in their study that, Cardiac dysfunction is a major cause of death in patients with β Thalassemia. Patients with higher serum ferritin level significantly associated with Pulmonary Hypertension[18]. Similar studies also show mean Ferritin level was much higher (3,759.54 + 3267.67 *versus* 2,307.66 + 1886.46) in the patient

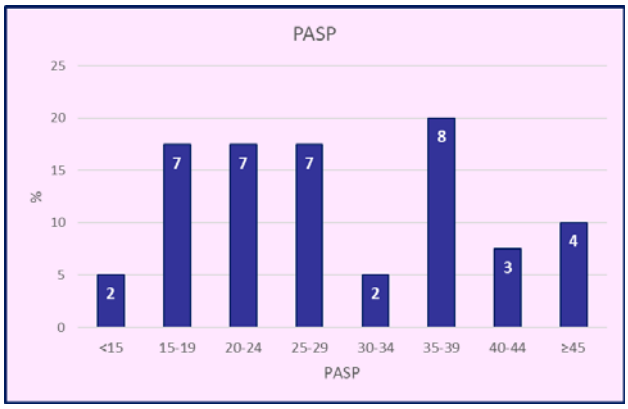

Fig 2 — Distribution of PASP in study population (n=76)

group having PAH[15].

The mean PASP was 29.12 ± 10.86 mm of Hg with maximum in E β Thalassemia (33.62 ± 15.00) (Fig 2). 38.16% subjects have Pulmonary Regurgitation with PR jet velocity of 1.97 ± 0.50 m/s and PR gradient of 16.81 ± 7.86 mm of Hg with maximum value in β Thalassemia trait and Sickle β Thalassemia. According to estimated mPAP from acceleration time, 26.32% of the population having mPAP ≥25 mm of Hg with 1.32% of > 40 mm of Hg.

In literature also clinical indicators which increase the PAH in the cases of hemoglobinopathies were E β Thalassemia, Splenectomy (67.69% of patients of PAH), transfusion >6 U/yr, Ferritin > 1000 ng/dL[15]. Similarly, in our study, we also establish the clinical indicators which increase chance of PAH, like- E β Thalassemia, Splenectomy, Severe Anemia, Transfusion >10 U/year, Iron Overload state.

Conclusions

We conclude from this study that many patients with Hemoglobinopathies have PAH which could be detected by the most widely and easily available Doppler Echocardiography. Most classically, PAH in Hemoglobinopathy is associated with Cardiovascular symptoms like Shortness of Breath and Syncope. Splenectomy increases the prevalence of PAH in the patients of Hemoglobinopathies. Transfusion is a major risk for developing PAH. Mean total transfusion is directly related to the prevalence of PAH. Degree of Anemia Jaundice and Iron Overload State is also associated with degree of PAH.

However, a large scale, multicentric study needs to be conducted to assess the overall prevalence of Pulmonary Hypertension in Hemoglobinopathies and a definitive diagnosis always requires Right Heart Catheterization.

Funding : None

Conflict of Interest : None

References

1 Chatterjee JB — Some Aspects of Haemoglobin E and its Genetic Interaction with Thalassaemia. Indian Journal of Medical Research. 1965;53(5):377-383.
2 Gupta SC, Mehrotra TN, Mehrotra VG — Haemoglobin-E-thalassaemia in Uttar Pradesh. *Indian Journal of Medical Research* 1970; **58:** 857-62.
3 Shukla RM, Solanki BR — Sickle-cell trait in Central India. *Lancet* 1985; **1:** 297-8.
4 Balgir RS — Scenario of haemoglobin variants in Central-East coast of India. Current science. 2006: 1651-7.
5 Munshi A, Anandraj MP, Joseph J, Shafi G, Anila AN, Jyothy A — Inherited hemoglobin disorders in Andhra Pradesh, India: a population study. Clinica Chimica Acta. 2009;400(1-2):117-9.
6 Dolai TK, Dutta S, Bhattacharya M, Ghosh MK — Prevalence of hemoglobinopathies in rural Bengal, India. *Hemoglobin* 2012; **36:** 57-63.
7 Balgir RS — Genetic epidemiology of the three predominant abnormal hemoglobins in India. *The Journal of the Association of Physicians of India* 1996; **44(1):** 25-8.
8 Kim PS, Iyer RK, Lu KV, Yu H, Karimi A, Kern RM, *et al* — Expression of the liver form of arginase in erythrocytes. *Molecular genetics and metabolism* 2002; **76(2):** 100-10.
9 Azizi E, Dror Y, Wallis K — Arginase activity in erythrocytes of healthy and ill children. *Clin Chim Acta* 1970; **28:** 391-6.
10 Morris CR, Kuypers FA, Kato GJ, Lavrisha L, Larkin S, Singer T, *et al* — Hemolysis-associated pulmonary hypertension in thalassemia. *Annals of the New York Academy of Sciences* 2005; **1054:** 481.
11 The management of the cardiac complications of thalassaemia major. In: Cappellini M-D, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A (eds). Guidelines for the Clinical Management of Thalassaemia.2nd revised ed. Nicosia, Cyprus: Thalassaemia International Federation. 2008: 83-91.
12 Borgna-Pignatti C, Galanello R — Thalassemias and related disorders: Quantitative disorders of hemoglobin synthesis. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, Rodgers GM (eds). Wintrobes Clinical Hematology. 13th ed. Philadelphia: Lippincott Willams and Wilkins; 2014: 862-913.
13 Mclaughlin VV, Humbert M — Pulmonary Hypertension. In: Zipes DP, Libby P, Bonow RO, Mann DL, Tomaselli, GF (eds). Braunwald's Heart Diseases: A Textbook of Cardiovascular Medicine. 11th edition. Philadelphia: Elsevier;2019: 1699-1719.
14 Ferrara F, Kasprzak JD, Voilliot D, Stanziola AA, Bossone E — Role of echocardiography in Pulmonary hypertension. In: Navin C Nanda (eds). Comprehensive textbook of echocardiography. 2nd edition. Jaypee. 2020: 1094-1105.
15 Chueamuangphan N, Wongtheptien W, Nawarawong W, Sukornthasarn A, Chuncharunee S, Tawichasri C, *et al* — Clinical indicators for pulmonary arterial hypertension in thalassemia. *Journal of the Medical Association of Thailand* 2012; **95(1):** 16.
16 Azami M, Sufi Nia A, YektaKooshali MH, Nikpay S, Madmoli Y, Malekshahi M, *et al* — Prevalence and risk factors of pulmonary arterial hypertension in thalassemia major patients of Ilam, 2014. *Evidence Based Care* 2017; **6(4):** 74-8.
17 Meera V, Jijina F, Ghosh K — Pulmonary hypertension in patients with hematological disorders following splenectomy. *Indian Journal of Hematology and Blood Transfusion* 2010; **26(1):** 2-5.
18 Hamdy AM, Zein El Abdin MY, Abdel Hafez MA — Right ventricular function in patients with beta thalassemia: relation to serum ferritin level. *Echocardiography* 2007; **24(8):** 795-801.