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Research Article

A Prospective Study on Correlation of Grade and Pattern of Liver Siderosis with Amount of Transfusion in β Thalassemia Major Patients Undergoing Splenectomy for Assessment of Hepatic Iron Overload

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Abstract: Chronic hyper transfusion therapy in β thalassemia major patients is designed to maintain a hematocrit of at least 27–30% so that ineffective erythropoiesis is suppressed. Transfused RBCs, being taken up and degraded by RES/macrophages, results in overloading of recycled iron and excess iron saturates the binding capacity of Transferrin, thereby getting stored as hemosiderin in liver. The purpose of this study was to determine amount of transfusion and its correlation with grades and the patterns of liver siderosis to assess extent of iron overload and whether splenectomy needs to be adopted for a patient on the basis of amount of transfusion to prevent advancing liver siderosis. A total of 30 patients of β thalassemia major have been evaluated. Mean amount of transfusion for patients getting Grade 2, 3 and 4 liver siderosis were 31, 37 and 46 units respectively with a lone patient of grade 1 liver siderosis having been transfused 21 units of blood. Mean amount of transfusion required for appearance of HH and Mixed (HH; non-HH) patterns of liver siderosis were 38 and 48 units respectively. Correlation between amount of transfusion and grade of siderosis was calculated using Spearman's Rho Correlation coefficient and it showed statistically significant correlation between Total number of units transfused and grade of liver siderosis (Spearman's Rho value of 0.692 and p-value < 0.01). So, splenectomy should be done before a patient gets to higher grade and Mixed (HH; non-HH) pattern of liver siderosis and amount of transfusion needs to be monitored accordingly for proper assessment.
Keywords: Blood transfusion,β Thalassemia major, Grade of Siderosis, Pattern of Siderosis, Hepatic Iron overload

INTRODUCTION

β thalassemia syndromes are a group of hereditary disorders characterized by a genetic deficiency in the synthesis of beta-globin chains. In the homozygous state, Beta thalassemia (i.e. thalassemia major) causes severe transfusion-dependent anaemia [1].

Many patients require chronic hyper transfusion therapy designed to maintain a hematocrit of at least 27–30% so that erythropoiesis is suppressed [2]. The commonly adopted criteria for splenectomy is a blood consumption greater than 50% above the mean annual transfusion requirement (volume of RBCs per kilogram of body weight per year) of the splenectomised population i.e. more than 200-250 ml/kg/year of pure red cell, to maintain a pre-transfusion Hemoglobin of 9 gm/dl [3].

Increased iron deposition resulting from multiple life-long transfusions and enhanced iron absorption results in secondary iron overload. Hepatic Iron overload resulting from multiple red cell transfusions over a long period of time is a complication of thalassemia major and other thalassemia like congenital anemias. Iron deposits are usually difficult to identify on usual stains unless they are abundant. Therefore, every liver biopsy should be routinely stained using not only hematoxylin-eosin (H&E) stain, but also iron stain (Pearl's Prussian blue). Pearls' staining of liver tissue is the most widely used, despite; it identifies Fe2+ (Ferrous Iron) only [4].

The pattern of hepatic iron deposits was first described by Scheuer et al in a classic article published in 1962 [5]. In their original publication, these authors studied iron deposits in liver biopsy sections stained by the Prussian blue method with grade 0 being negative and grades 1, 2, 3, and 4 representing increasing amounts of stainable iron. Shirin Nash *et al.* have, in their study, modified the original Scheuer method a little. They included "trace deposits" as heterogeneous iron deposits in periportal hepatocytes; grade 1, iron deposits homogeneous in periportal hepatocytes; grade 2, iron deposits filling hepatocytes up to the midlobule; and grade 3, iron deposits filling the entire lobule or

blue cirrhotic nodules. Intermediate grades (trace to 1+, or 2+ to 3+) were used if there was variability of iron deposits within the section [6]. Iron deposits in reticulo-endothelial (RE) cells were specified and similarly graded.

Hepatocellular iron deposits with a decreasing gradient from periportal to centrilobular areas in the liver recently have been referred to as the HH pattern [7]. Iron deposits primarily in RE cells and macrophages with occasional heterogeneous deposits in periportal hepatocytes are referred to as the Non-HH pattern [7]. If iron deposits were present in hepatocytes with a portal to central lobular gradient and iron also was noted in macrophages and sinusoidal lining cells, we considered the pattern to be a combined HH with superimposed Non-HH pattern [7].

Liver parenchymal iron overload is usually the result of excessive iron absorption by the enteral route, such as in HHC (Hereditary Hemochromatosis) and anemias with ineffective erythropoiesis (iron loading anemias), but may also reflect enhanced internal redistribution of transfused erythrocyte iron recycled from the RE cells, as observed in the more advanced stage of transfusional iron overload [8-11].

The purpose of this study was to determine amount of transfusion and its correlation with grade and the pattern of liver siderosis to assesss extent of iron overload and whether splenectomy needs to be adopted for a patient on the basis of amount of transfusion to prevent advancing liver siderosis.

MATERIAL AND METHODS

This was an institution-based, prospective study conducted in Department of Surgery, Medical College, Kolkata, India from January 2013 to June 2014 (16 months). Informed consent was taken from all the patients. The study got clearance from Institutional Ethical Committee.

30 β Thalassemia patients were selected, having splenomegaly and is being planned for splenectomy.

Inclusion Criteria

The following patients were included in the study population:

- Patients who are β-Thalassemia major requiring repeated blood transfusions (at least 2 per month).
- Patients who have not undergone chelation therapy.
- Patients who are > 12 years of age.

Exclusion Criteria

The following patients were excluded from the study population:

- Patients having any congenital or acquired liver disease.
- Patients with chronic Hepatitis B or Hepatitis C infection or any other disease complicated by splenomegaly.
- Patients suffering from any malignancy.
- Patients who refuses to be part of study.
- Patients whose liver biopsy has not been done.

All operable cases of β-thalassemia patients requiring repeated blood transfusions and who have not taken iron chelation therapy were subjected to a detailed history using a structured questionnaire and examined clinically.

Baseline investigations of the patients were done. Serum iron studies were done along with ultrasound of abdomen to assess splenomegaly and rule out other pathologies.Patient was adequately prepared before surgery. After the patients were found fit for surgery from anaesthetic point of view, splenectomy with or without cholecystectomy was done.

During the operative procedure liver biopsy is taken and sent for histopathology examination (HPE) along with the specimens of spleen and gallbladder. Histological examination of specimen of liver tissue using formalin-fixed, paraffin-embedded, hematoxylin and eosin (H&E) along with Pearl's Prussian blue stained slides representing degree of siderosis and pattern of distribution of iron.

Grade of siderosis and pattern of siderosis in liver is noted. Grade is scored as 0 - 4 (Mild / Intermediate / Severe).
Pattern of Siderosis noted as HH (Hepatocytes only), non-HH (RE cells/Kupffer cells / macrophages) or HH; non-HH (Mixed).

All statistical analyses were performed with SPSS® software version 21.0 for Windows 8.1 (SPSS, Chicago, IL, USA).Spearman's Rho Correlation coefficient was calculated as one of the variables is parametric and the other one is non-parametric. All bivariate analysis was appropriately done and p value < 0.01 was considered significant.

RESULTS

In this study, 30 β Thalassemia major patients were studied. Out of 30 patients, 7 were males (23%) and 23 females (77%).

Descriptive Statistics of Grade of Liver Siderosis

Only one patient presented with Grade 1 liver siderosis. Grade 2 siderosis was found in 3 patients. Grade 3 and Grade 4 siderosis were found in 9 and 17 patients respectively.

Table-1: Descriptive Statistics of Grade of Liver Siderosis.

Grade of Liver Siderosis	Frequency/Number of cases	Percentage
1	1	3.33
2	3	10
3	9	30
4	17	56.67

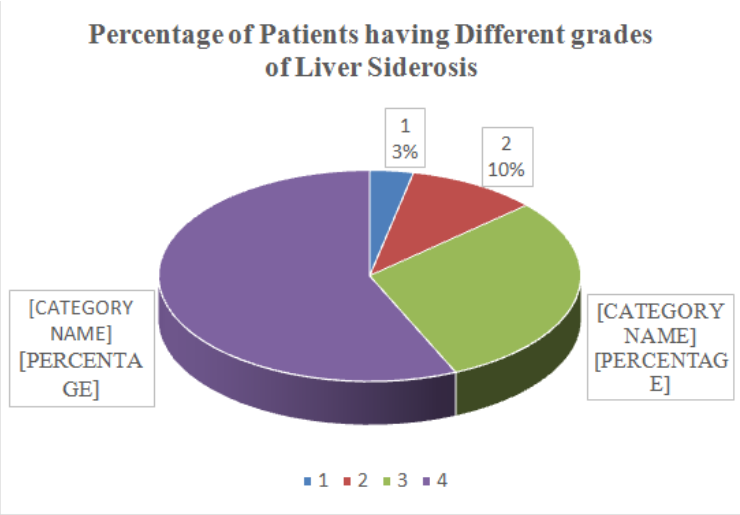

Chart-1: Pie Chart of Distribution depicting descriptive statistics of Grade of Liver Siderosis

Descriptive Statistics of Pattern of Liver Siderosis

Out of 30 patients, 21 patients had HH pattern of liver siderosis (involvement of hepatocytes only) and 9 patients had HH; non-HH pattern of liver siderosis (involvement of hepatocytes along with RE cells/kupffer cells). No one had only non-HH pattern of liver siderosis (involving only RE cells/kupffer cells).

Table-2: Descriptive Statistics of Pattern of Liver Siderosis.

Pattern of Siderosis	Frequency/Number of cases	Percentage
HH	21	70
Non-HH	0	0
HH; non-HH	9	30

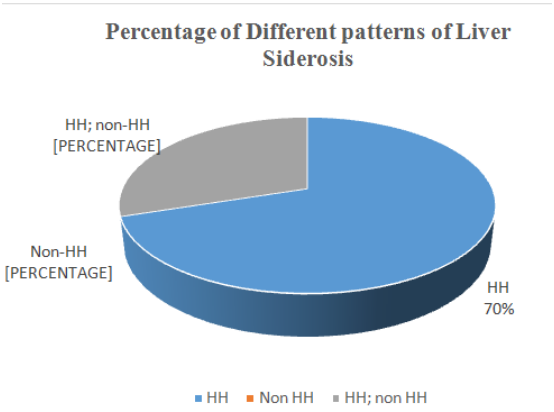

Chart-2: Pie Chart of Distribution depicting descriptive statistics of Pattern of Liver Siderosis.

Descriptive Statistics of Age

Out of 30 patients, minimum age of presentation was 12 years and maximum age of presentation was 18 years with a standard deviation of 1.431(Table-3).

Out of 30 patients, there was not much difference in the age distribution according to grades of liver siderosis.(Table-4).

Table-3: Descriptive Statistics of Age.

	N	Range	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Age	30	6	12	18	15.23	.261	1.431
Valid N (listwise)	30						

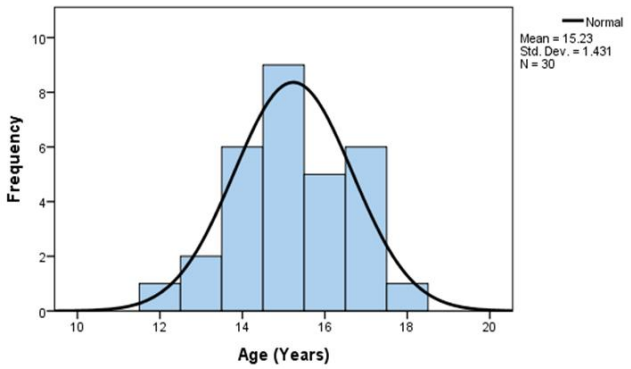

Chart-3: Histogram and Distribution curve depicting descriptive statistics of Age.

Table-4: Descriptive Statistics showing distribution of age according to Grade of liver siderosis.

	Grade of Liver Siderosis							
	1		2		3		4	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
Age in yrs	16	.	15	2	15	1	15	1

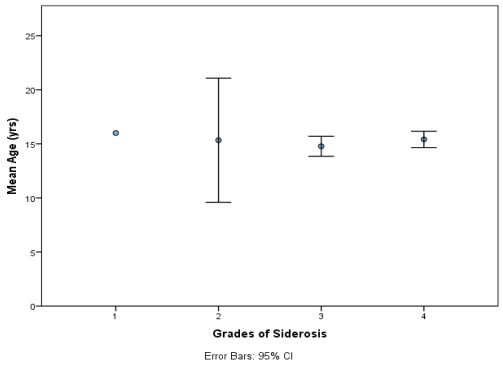

Chart-4: Diagram showing Age Distribution (Mean +/- S.D in Years) of presentation of different grades of Liver Siderosis.

Descriptive Statistics of Amount of Blood Transfusion according to Grade and Pattern of Liver Siderosis

Total duration of transfusion varied widely among the 30 patients. So amount of blood transfused is taken into consideration. Mean amount of transfusion was 41 units ± 10.22 (S.D). The range was from 21 units to 68 units.

Table-5: Descriptive Statistics of Amount of Blood Transfusion.

	N	Range	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic

Total Number of units Transfused	30	47	21	68	41.00	1.866	10.222
Valid N (listwise)	30						

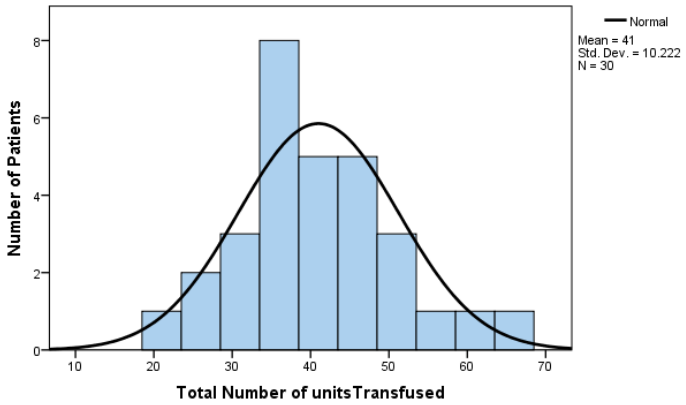

Chart-5: Histogram and Distribution curve depicting descriptive statistics of Amount of Blood Transfusion

The only patient in whom grade 1 liver siderosis was found, was transfused a total of 21 units of blood. Mean total number of units transfused in patients presenting with Grade 2, 3 and 4 liver siderosis were 31, 37 and 46 respectively.

Table-6: Distribution Total units of blood transfused according to Grade of liver Siderosis.

		Grade of Liver Siderosis			
		1	2	3	4
Total number of Units Transfused	Mean	N=21	N=31	N=37	N=46
	Maximum	21	34	44	68
	Minimum	21	28	28	34
	Standard Deviation	.	3	6	9

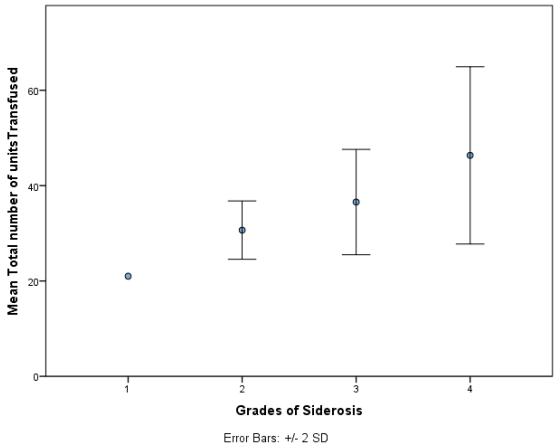

Chart-6: Distribution of Mean Total number of Units Transfused according to grades of liver siderosis(Mean +/- 2 S.D)

The HH pattern of liver siderosis was found in patients receiving a mean amount of transfusion of 38 units whereas, HH; non-HH(Mixed) pattern of liver siderosis was found in patients receiving a mean amount of transfusion of 48 units. Minimum amount of transfusion required for appearance of HH and Mixed (HH; non-HH) patterns of liver siderosis were 21 and 34 units respectively. None was found with only Non-HH pattern of liver siderosis.

Table-7: Distribution Total units of blood transfused according to Pattern of liver Siderosis.

		Patternof Liver Siderosis	
		HH	HH; non-HH
Total number	Mean	N=38	N=48

ofunitsTransfused	Maximum	61	68
	Minimum	21	34
	Standard Deviation	9	11

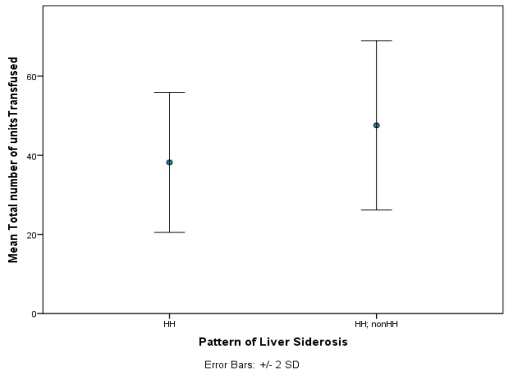

Chart-7: Distribution of Mean Total number of Units Transfused according to Pattern of liver siderosis (Mean +/- 2 S.D)

Correlation of total number of units of blood transfused with Grade of Liver Siderosis

Correlation between amount of transfusion and grades of liver siderosiswas calculated using Spearman's Rho Correlation coefficient as one of the variables is parametric and the other one is non-parametric. P-value was calculated. Since Spearman's Rho value is 0.692 and p-value is < 0.01, it can be concluded that there is a statistically significant correlation between Total number of units transfused and grade of liver siderosis.It is also seen that combined HH; non-HH pattern of liver siderosis is seen only in grade 4 liver siderosis.

Table-8: Correlation of Total number of units of Blood Transfused with Grade of Liver Siderosis

Spearman's Rho	p-value
0.692	< 0.01

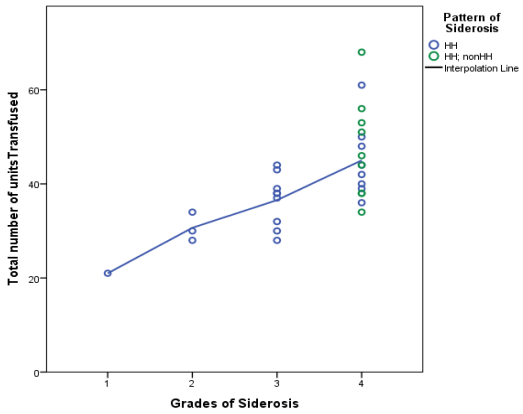

Chart-8: Scatter Dot Graph showing Correlation of Total units of Blood Transfused with Grade of Liver Siderosis with Interpolation line.

DISCUSSION

In this study, 30 β-thalassemia major patients, fulfilling the selection criteria of this study, were examined.The mean age of the study population was 15.23 years ± 1.431 (S.D). The range was from 12 years to 18 years.Most of the patients have started transfusion at the age of 5-7 years and so there is not much difference in the duration of transfusion among the groups.

Out of 30 patients, 7 were males (23%) and 23 females (77%). Since β-thalassemia is a form of inherited autosomal recessive blood disorder, not much inference should be drawn from this observation. Transfusion history was taken and amount of transfusion is considered. Out of 30 patients, Mean amount of transfusion was 41 units ± 10.22 (S.D). The range was from 21 units to 68 units.

Following splenectomy, specimen of liver tissue was sent for histopathology examination using H&E staining and Pearl's Prussian blue staining. Pattern of liver siderosis and grade of siderosis was determined. Out of 30 patients, 21 patients (70 %) had

HH pattern of liver siderosis (Involvement of Hepatocytes only) and 9 patients (30%) had HH; non-HH pattern of liver siderosis (Involvement of Hepatocytes along with RE cells/kupffer cells). None had liver siderosis involving only RE cells/kupffer cells (Non-HH only). It was also noted that combined HH; non-HH pattern of liver siderosis was seen only in grade 4 liver siderosis.Apart from that, the HH pattern of liver siderosis was found in patients receiving a mean amount of transfusion of 38 units whereas, HH; non-HH (Mixed) pattern of liver siderosis was found in patients receiving a mean amount of transfusion of 48 units. Minimum amount of transfusion required for appearance of HH and Mixed (HH; non-HH) patterns of liver siderosis were 21 and 34 units respectively. This shows that with increasing amount of transfusion, hemosiderin also gets deposited in RE cells apart from hepatocytes.

Out of 30 patients, only one patient (3.33%) presented with Grade 1 Liver siderosis. Grade 2 siderosis was found in 3 patients (10%). Grade 3 and Grade 4 siderosis is found in 9 (30%) and 17 (56.67%) patients respectively. The only patient in whom grade 1 liver siderosis was found, was transfused a total of 21 units of blood. In addition, mean (along with S.D) amount of blood transfused in Grade 2, 3 and 4 siderosis were 31 ± 3, 37 ± 6 and 46 ± 9 units respectively. So the patients having higher grade of siderosis gave history of more amount of transfusion which occurred because of transfusional iron overload.

Spearman Rank correlation (Rho/ρ) test was used to correlate the amount of transfusion with grade of liver siderosis (non-parametric variable in ordinal scale).The Spearman's Rho for correlation of Total units of Blood Transfused with grade of liver siderosis was 0.692 with a p-value <0.01. Thus, there was statistically significant correlation between Total number of units Transfused and grade of liver siderosis. This finding corroborates with findings related to transfusional iron overload [8-11].

From these results, it is evident that amount of blood transfusion has statistically significant correlation with grade of liver siderosis. So, more is the transfusion, more is the iron overload, and more is the deposition of iron in liver in β-thalassemia major patients who has come for and undergone splenectomy. The pattern of deposition of iron in liver also changes with increasing amount of transfusion.

CONCLUSION

Meanamount **o**f transfusion required for the patient getting Grade 1, 2,3 and 4 liver siderosis were found to be in increasing order (21, 31, 37 and 47 units respectively). The HH pattern of liver siderosis was found in patients receiving a mean amount of transfusion of 38 units whereas, HH; non-HH (Mixed) pattern of liver siderosis was found in patients receiving a mean amount of transfusion of 48 units. Minimum amount of transfusion required for appearance of HH and Mixed (HH; non-HH) patterns of liver siderosis are 21 and 34 units respectively. None was found with only Non-HH pattern of liver siderosis.

Correlation between amount of transfusion and grade of siderosis was calculated using Spearman's Rho Correlation coefficient. Since Spearman's Rho value was 0.693 with p-value is < 0.01, it can be concluded that there is a statistically significant correlation between Total number of units transfused and grade of liver siderosis.

The more the amount of transfusion, the greater is the grade of siderosis in the patients of β-thalassemia major who has come for and undergone splenectomy in Medical College, Kolkata. Apart from that, it also shows that with increasing amount of transfusion, hemosiderin gets deposited in RE cells apart from hepatocytes So, any intervention, like splenectomy needs to be done before a patient gets to higher grade of siderosis and amount of transfusion should be monitored and counted accordingly for proper assessment and management.

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