INTRODUCTION

Beta-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 anemia. The disease is in aggregate among the most common inherited disorders of humans. The incidence may be as high as 10% in these areas.1 Hepatic iron overload resulting from multiple red cell transfusions over a long period of time is a complication of thalassemia major. Hepatocellular iron deposits with a decreasing gradient from periportal to centrilobular areas in the liver have been referred to as the HH pattern and those deposits primarily in reticuloendothelial cells and macrophages with occasional heterogeneous deposits in periportal hepatocytes are referred to as the non-HH pattern. Aims and Objectives: The purpose of this study was to evaluate patterns of liver siderosis in these patients and correlate with number of units of blood transfused. Materials and Methods: Thirty beta-thalassemia patients were selected, having splenomegaly and during splenectomy, liver biopsy was taken and sent for histopathology examination and pattern of siderosis was noted as HH (Hepatocytes), non-HH (Reticuloendothelial cells/Kupffer cells/macrophages) or HH; non-HH (Mixed). Results: Mean number of units transfused was 48.429 ± 9.53 (SD) for patients having HH pattern of liver siderosis whereas it was 58.667 ± 13.27 (SD) for patients having HH; non-HH pattern of liver siderosis. Independent-samples non-parametric Mann–Whitney U test was performed and P-value was found to be 0.028 which emphasized that total number of units of blood transfused vary significantly with two groups showing different patterns of siderosis. Conclusion: Hence, pattern of liver siderosis can be a good indicator for transfusional iron overload in beta-thalassemia major patients.

Key words: Blood transfusion; Beta-thalassemia; Siderosis; Hemosiderosis; Iron overload
siderosis in these patients and correlate with number of units of blood transfused.

**Aims and objectives**
1. The purpose of this study was to evaluate patterns of liver siderosis in these patients.
2. In addition to that, the aim was to correlate with number of units of blood transfused and see whether there are any statistically significant differences in amount of transfusion among patients with differing patterns of liver hemosiderosis.

**MATERIALS AND METHODS**

This was an institution-based and prospective study conducted in Department of Surgery, Medical College, Kolkata, India from January 2013 to June 2014 after obtaining proper informed consent and clearance from the Institutional Ethical Committee.

**Inclusion criteria**
The following criteria were included in the study:
1. β-Thalassemia major patients requiring repeated blood transfusions (at least 2 per month).
2. Patients who have not undergone chelation therapy.
3. Patients who are ≥12 years of age.

**Exclusion criteria**
The following criteria were excluded from the study:
1. Patients having any congenital or acquired liver disease.
2. Patients with chronic HepB or HepC infection or any other disease complicated by splenomegaly.
3. Patients whose liver biopsy has not been done.
4. Patients suffering from any malignancy.
5. Patients who refused to be part of study.

After careful considerations, 30 beta-thalassemia patients who were having splenomegaly and being planned for splenectomy were included in the study. All operable cases of beta-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. During the operative procedure, liver biopsy was taken and sent for histopathology examination and pattern of siderosis was noted as HH (Hepatocytes), non-HH (Reticuloendothelial cells/Kupffer cells/macrophages) or HH; non-HH (Mixed).

All statistical analyses were performed with SPSS® software version 26 for Windows 11 (SPSS, Chicago, IL, USA). Apart from descriptive statistics, non-parametric Mann–Whitney U-test was done was done to see whether there was statistical evidence that the means of those two groups are significantly different.

**RESULTS**

In this study, 30 beta-thalassemia major patients were studied. Out of 30 patients, seven were males (23%) and 23 females (77%). Overall mean age of patients was 15.23 years ± 1.431 (SD). Mean age of patients having HH (hepatocellular) pattern of liver siderosis was 15.29 years ± 1.27 (SD) and patients having HH; non-HH (mixed) pattern of liver siderosis was 15.11 years ± 1.83 (SD) (Table 1). There was not much difference between two groups in terms of age. Baseline relevant blood tests included MCV (Mean = 76.27 ± 4.3 fL), MCH (Mean = 23.31 ± 1.86 pg), and MCHC (Mean = 30.52 ± 1.16 g/dL). Iron studies included serum iron (Mean = 206.73 ± 27.89 μg/dL), serum ferritin (Mean = 1755.93 ± 595.45 ng/mL), and TIBC (Mean = 257.60 ± 27.31 μg/dL) (Table 1).

There is not much difference in means for MCV, MCH, MCHC, serum iron, and TIBC between patients having differing patterns of liver siderosis. However, serum ferritin has a mean of 1578.76 ng/mL and SD of 401.902 in patients having HH pattern of liver siderosis whereas it has a mean of 2169.33 ng/mL and S.D. of 778.985 in patients having HH; non-HH pattern of liver siderosis which requires further study to establish significance (Table 2).

Out of 30 patients, 21 patients had HH pattern of liver siderosis and nine patients had HH; non-HH pattern of liver siderosis (Involvement of Hepatocytes along with reticuloendothelial cells/Kupffer cells). None had liver siderosis.

| Blood Parameters | Range | Minimum | Maximum | Mean | SD |
|------------------|-------|---------|---------|------|----|
| Age (years)      | 6     | 12      | 18      | 15.23| 1.431|
| MCV (μL)         | 15.9  | 68.5    | 84.4    | 76.273| 4.3038|
| MCH (pg)         | 8.5   | 19.6    | 28.1    | 23.313| 1.8665|
| MCHC (g/dL)      | 5.5   | 28.6    | 34.1    | 30.527| 1.1596|
| Serum iron (μg/dL) | 165 | 89     | 254     | 206.73| 27.899|
| Serum ferritin (ng/mL) | 3067 | 1008   | 4075    | 1755.93| 595.452|
| TIBC (μg/dL)     | 106   | 206     | 312     | 257.60| 27.311|
Basak: A prospective study evaluating patterns of liver siderosis in beta-thalassemia major patients undergoing splenectomy

Siderosis involving only reticuloendothelial cells/Kupffer cells (Table 3 and Chart 1).

Transfusion history was taken and amount of transfusion is emphasized on. Out of 30 patients, mean amount of transfusion was 51.5 units±11.58 (SD). The range was from 31 units to 88 units. Mean amount of transfusion was 48.429±9.53 (SD) units for patients having HH pattern of liver siderosis whereas it was 58.667±13.27 (SD) units for patients having HH; non-HH pattern of liver siderosis (Table 4).

Chart 1 shows categorical field information of patterns of liver siderosis as written above. Charts 2 and 3 clearly show presence of outliers. Hence, one-way ANOVA or independent-samples t-test could not be done. Instead, independent-samples non-parametric Mann–Whitney U-test was performed. P value was found to be 0.028 (Table 5 and Chart 4).

Null hypothesis was defined as “The distribution of Total no. of units of blood transfused is same across categories of pattern of liver siderosis.” Mann–Whitney U test shows P value 0.028 (P<0.05). This results in rejection of null hypothesis. Hence, it shows that there are statistically significant differences in amount of blood transfusion among patients having different patterns of liver siderosis, namely, HH (hepatocellular) and HH; non-HH (mixed) (Table 6).

### Table 2: Comparison of means of baseline blood parameters, iron studies, and age among patients with different patterns of liver siderosis

| Pattern of siderosis | MCV (fL) | MCH (pg) | MCHC (g/dL) | Serum Iron (μg/dL) | Serum Ferritin (ng/mL) | TIBC (μg/dL) |
|----------------------|----------|----------|-------------|-------------------|-----------------------|--------------|
| HH                   | 75.438   | 23.024   | 30.481      | 202.24            | 1578.76               | 260.60       |
| SD                   | 3.6793   | 1.6428   | 1.0953      | 29.745            | 401.902               | 29.430       |
| HH; nonHH            | 78.222   | 23.989   | 30.633      | 217.22            | 2169.33               | 250.60       |
| SD                   | 5.2151   | 2.2691   | 1.3629      | 20.801            | 778.985               | 21.444       |

### Table 3: Descriptive statistics of patterns of liver siderosis

| Pattern of siderosis | N  | Percentage | Mean | SD  |
|----------------------|----|------------|------|-----|
| HH                   | 21 | 70         | 48.429 | 9.5319 |
| Non-HH               | 0  | 0          | 0    | 0   |
| HH; non-HH           | 9  | 30         | 58.667 | 13.2759 |

### Table 4: Group statistics of patterns of liver siderosis and total number of units transfused

| Pattern of liver siderosis | N | Mean | SD |
|----------------------------|---|------|----|
| Total no. of units transfused |  | HH   | 21  |
|                             |   | HH; non-HH | 9  |
|                             | Total | 30  |  |
DISCUSSION

In this study, 30 beta-thalassemia major patients, fulfilling the selection criteria of this study, were examined. Out of 30 patients, seven were males (23%) and 23 females (77%). Since beta-thalassemia is a form of inherited autosomal recessive blood disorder, not much inference should be drawn from this observation. Overall mean age of patients was 15.23 years±1.431 (SD). Mean age of patients having HH pattern of liver siderosis was 15.29 years±1.27 (SD) and patients having HH; non-HH pattern of liver siderosis was 15.11 years±1.83 (SD). There was not much difference between two groups in terms of age. Baseline relevant blood tests included MCV (Mean=76.273±4.3 fL), MCH (Mean=23.313±1.86 pg), MCHC (Mean=30.527±1.16 g/dL). MCV, MCH, and MCHC decreases in microcytic hypochromic anemia like thalassemia. Hence, this is an expected finding in these patients.

Other blood tests included iron studies such as serum iron (Mean=206.73±27.89 μg/dL), serum ferritin (Mean=1755.93±595.45 ng/mL), and TIBC (Mean=257.60±27.31 μg/dL). There is not much difference in means for MCV, MCH, MCHC, serum iron, and TIBC between patients having differing patterns of liver siderosis. However, serum ferritin has a mean of 1578.76 ng/mL and SD. of 401.902 in patients having HH pattern of liver siderosis whereas it has a mean of 2169.33 ng/mL and SD. of 778.985 in patients having HH; non-HH pattern of liver siderosis which requires further correlation studies to establish significance. Moreover, since serum ferritin ranges from 1008 ng/mL to 4075 ng/mL, it can be said that early measurement of serum ferritin is necessary for proper evaluation. This is consistent with the findings of Takatoku et al.7

Following splenectomy, specimen of liver tissue is sent for HPE examination using H and E staining and Pearl’s Prussian blue staining. Pattern of liver siderosis and grade of siderosis is determined. Out of 30 patients, 21 patients had HH pattern of liver siderosis (Involvement of Hepatocytes only) and nine patients had HH; non-HH

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Table 6: Hypothesis test summary showing rejection of null hypothesis

| Null hypothesis                                                                 | Test                                | Sig.    | Decision                |
|---------------------------------------------------------------------------------|-------------------------------------|---------|-------------------------|
| 1. The distribution of Total number of units of blood transfused is the same across categories of Pattern of Liver Siderosis. | Independent-Samples Mann-Whitney U Test | 0.028a  | Reject the null hypothesis |

Asymptotic significances are displayed. The significance level is 0.050. *Exact significance is displayed for this test

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Chart 3: Categorical field information of Total number of units transfused

Chart 4: Independent-samples Mann–Whitney U test graph
pattern of liver siderosis (Involvement of Hepatocytes along with reticuloendothelial cells/Kupffer cells). As per studies by Pootrakul et al., and Halliday et al., in Thalassemia major abnormalities in hemoglobin can decrease erythrocyte life span, the pool of erythrocyte precursors is markedly expanded, leading to increased enteral absorption of dietary iron.\textsuperscript{4,6} According to Olivieri et al., aggressive transfusion therapy suppresses endogenous erythropoiesis and corrects the severe anemia, but leads to its own complications, the worst of which is iron overload.\textsuperscript{8} In iron overload secondary to ineffective erythropoiesis, iron is deposited in the liver hepatocytes in a lobular distribution with a decreasing perportal-to-pericentral gradient\textsuperscript{6} whereas in transfusional iron overload, iron deposition is panlobular and typically involves Kupffer cells and portal macrophages (reticuloendothelial cells).\textsuperscript{9,10} In my study, it is seen that mostly hepatocellular and mixed patterns of liver siderosis are found whereas isolated deposits in RE cells/Kupffer cells were not seen. It can be explained by their late presentation which is evident since the patients already had serum ferritin above 1008 ng/mL and are already undergoing repeated monthly blood transfusions (>2). Increasing the awareness of both patients and their first points of contact like primary health workers must be done to minimize irreversible organ damage and subsequent complications. Non-invasive methods of assessment of hemosiderosis should be considered to detect early deposition of iron in liver to prevent lasting organ damage.

Transfusion history was taken and amount of transfusion is emphasized on. Out of 30 patients, mean amount of transfusion was 51.5 units±11.58 (SD). The range was from 31 units to 88 units. Mean number of units transfused was 48.429±9.53 (SD) for patients having HH pattern of liver siderosis whereas it was 58.667±13.27 (SD) for patients having HH; non-HH pattern of liver siderosis. Charts 2 and 3 clearly show presence of outliers. Hence, one-way ANOVA or Independent-samples t-test could not be done. Null hypothesis was defined as “The distribution of total number of units of blood transfused is the same across categories of Pattern of Liver Siderosis.” The significance level was predetermined to be 0.050 (i.e., P<0.05 was considered significant). Independent-samples non-parametric Mann–Whitney U Test was performed and P-value was found to be 0.028. Null hypothesis was, therefore, rejected. From this test, it is obvious that total number of units of blood transfused vary significantly between two groups showing different patterns of siderosis. Hence, it can be inferred that as transfusion increases iron deposition in liver starts occurring in Kupffer cells or portal macrophages apart from hepatocytes. Therefore, it is imperative that proper evaluation of a thalassemia patient including baseline parameters must be done before commencing of blood transfusion. Along with that careful follow-up and proper reassessment of progress regarding treatment must be adhered to. Blood chelation agents should be started at appropriate times so that the chances of patients ending up for surgery can be minimized.

According to Deugnier et al., in cases of hereditary hemochromatosis patients have an estimated 240-fold increased relative risk of developing hepatocellular carcinoma, with the degree of risk correlating with the amount and duration of iron overload and degree of fibrosis.\textsuperscript{11} Although mechanism of hemosiderosis is different in thalassemia and hereditary hemochromatosis, it cannot be ignored that iron deposition per se can have several liver related complications, even life-threatening ones and further research is necessary in this regard.

**Limitations of the study**

The study period was limited and correlation studies among blood parameters was not included.

**CONCLUSION**

There is not much difference in means for age, MCV, MCH, MCHC, serum iron, and TIBC between patients having differing patterns of liver siderosis. Serum ferritin has a mean of 1578.76 ng/mL in patients having HH pattern of liver siderosis whereas it has a mean of 2169.33 ng/mL in patients having HH; non-HH pattern of liver siderosis. Mean total units of blood transfused (amount of transfusion) are 48.429±9.53 (SD) units for patients having HH pattern of liver siderosis (hepatocellular deposition) whereas it was 58.667±13.27 (SD) units for patients having HH; non-HH pattern of liver siderosis (mixed deposition). In addition, on performance of Mann–Whitney U test P-value was found to be 0.028 (<0.05), that is, it is significantly higher in patients with HH; non-HH pattern of liver siderosis over patients with HH pattern of liver siderosis. This emphasizes that as transfusion increases iron deposition in liver starts occurring in Kupffer cells or portal macrophages apart from hepatocytes. The more the amount of transfusion, the more widespread is the deposition of iron in liver in the patients of beta-thalassemia major who has come for and undergone splenectomy in Medical College, Kolkata. Hence, pattern of liver siderosis can be a good indicator for transfusional iron overload in beta-thalassemia major patients. However, further research is necessary in regards to iron deposition in non-hepatic organs to properly assess progress of disease and determine timing for aggressive chelation therapy.
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SB- Concept and design of the study; prepared first draft of manuscript; Interpreted the results; reviewed the literature and manuscript preparation; and revision of the manuscript.

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