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Original Research Article

Hematologic Evaluation of Beta Thalassemia Major Patients

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Abstract:

Background and Aim: Thalassemia is a common inherited illness, with Beta-thalassemia major being the most severe kind. The current study has the following goals:

To investigate several RBC parameters and their relationships in patients with multi-transfused beta thalassemia major. To evaluate changes in serum ferritin levels following blood transfusions, the efficacy of chelation therapy, and the prevalence of seropositivity among beta thalassemia major patients.

Material and Methods: The study included 100 individuals with significant beta thalassemia from a pediatric/medicine department of an Indian tertiary care centre. A thorough history was taken, as well as a physical examination and laboratory measures such as full blood counts. Over a year, all patients were evaluated for serum ferritin levels, chelation therapy, and screening for transfusion-transmitted illnesses. All of the data was collated, and statistical analysis was performed.

Results: The younger age group patients required less chelating agent at a lower transfusion frequency compared to the older age group patients who required a larger dose of chelating agents. Out of the 100 cases, 19 had the A+ blood group (19%), 12 had the AB+ blood group (12%), 29 had the B+ blood group (29%), and 38 had the O+ blood group (38%). There were 28 HCV+ patients (28%), 4 HIV+ cases (4%), and 66 nonreactive cases (66%). With p values of 0.005, serum ferritin levels were shown to be substantially linked with transfusion frequency. Serum ferritin levels were usually higher in patients who received two or more transfusions per month. Serum ferritin levels were shown to be substantially linked with medication and chelating agent dose, with p values 0.001.

Conclusion: Haematological metrics such as haematocrit, RBC mass, haemoglobin, and mean corpuscular volume have high covariances, whereas mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration have relatively narrow covariances.

Keywords: Beta Thalassemia, Blood Count, Chelating Agent, Haemoglobin.

Introduction

Thalassemia is a collection of inherited haemoglobin illnesses characterized by decreased synthesis of one or more globin chains, resulting in unbalanced globin synthesis, which is a crucial determinant in defining disease severity in the thalassemia syndromes. The thalassemias are the most frequent human monogenic disease in the world; they are a group of hereditary anaemias caused by mutations in the haemoglobin (Hb) gene clusters that slow the synthesis of one or more of the globin chain subunits of the Hbs tetramer.[1]

Thalassemias have been found in almost every racial group and geographical area on the planet. Beta-thalassemia is caused by a deficiency in the formation of beta globulin chains and can range from clinically silent heterogeneous thalassemia mild to severe transfusion-dependent thalassemia major.[2,3]

Beta-thalassemia major is a severe blood illness in which affected persons are unable to produce enough healthy red blood cells, leaving them completely dependent on blood transfusions throughout their lives.[3]This condition causes a variety of problems, including growth retardation, endocrine dysfunction, hypothyroidism, progressive liver failure, and aberrant renal function.[11] Trace metals, particularly iron, have been identified as causal factors in the excessive formation of free radicals, which can cause oxidative damage to erythrocytes.[4-12]

There are two main varieties of β thalassaemia, β0 thalassaemia, in which no β globin is produced, and

β+ thalassaemia. Less severe forms of thalassaemia are occasionally marked ++ to highlight that the - chain production deficiency is particularly mild. An high level of HbA2 in heterozygotes, which is observed in most forms of 0 and + thalassaemia, is the diagnostic hallmark of thalassaemia.[13] After a patient is diagnosed with thalassemia major, management includes regular 3 weekly filtered packed red cell transfusions, chelation therapy for iron overload, and management of iron overload and transfusion complications such as osteoporosis, cardiac dysfunction, endocrine problems, Hepatitis B & C, HIV infection, CMV, and so on.[14] Laboratory tests, such as Complete Blood Count (CBC), reticulocyte counts, and serum ferritin levels, are performed on persons who are frequently transfused to determine the success of the transfusion. RBC indices and their comparison pre and post transfusion are also useful in assessing patients' general health and predicting associated comorbidities.[15]

The current study has the following goals:

- To investigate various RBC parameters and their relationships in patients with multi-transfused beta thalassemia major.
- To analyse changes in serum ferritin levels following blood transfusions and the efficacy of chelation therapy.
- To determine the prevalence of seropositivity among beta thalassemia major patients.

Material and Methods

The study included 100 individuals with significant beta thalassemia from a pediatric/medicine department of an Indian tertiary care centre. Every case had informed parental consent obtained before to the study. The institute's ethics committee granted ethical approval. Patients with a known case of - Thalassemia major, aged 2 to 40 years, receiving frequent blood transfusions, receiving chelation therapy in the previous 6 months, and having clinical follow up for at least 12 months prior to the study were eligible for the study.

Patients with Thalassemia Intermedia who were not receiving frequent blood transfusions and who had not received chelation therapy in the previous 6 months were excluded from the trial.

The following procedures were performed on all subjects:

Detailed history taking: A questionnaire was intended to be completed.

1. Demographic information
2. Medical background
3. Management regime

A thorough physical and clinical assessment

A thorough clinical examination is performed, with special attention paid to the presence of pallor, jaundice, and symptoms of thalassemic characteristics. Hepatosplenomegaly is diagnosed through an abdominal examination.

Investigations in the laboratory

Venipuncture was used to collect blood in both simple and EDTA vacuettes. The serum was isolated from the ordinary vacuette, and RBC hemolysis was avoided. The serum samples were carefully examined to ensure that they were not tainted. The collection tubes were devoid of iron. Serum was kept in Stopper tubes at 2-8 °C until time for laboratory tests, which included:

1. Iron status as measured by serum ferritin levels, which were checked three times a month during the trial.
2. Detection of hepatitis virus infection using Hepatitis B surface antigen (HBsAg) and RNA-PCR testing for HCV positive and HIV patients at the start of the trial.
3. A complete blood count (from the EDTA Vacuette) was performed at the beginning and end of the longitudinal study to assess changes in haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular hemoblobin concentration (MCHC). The Horiba 5 part Penta XLR analyser was used to perform complete blood counts.

1. Level 1 serum ferritin, Diametra, CA06034, Italy Ferritin determination by direct immunological enzymatic method in human serum or plasma.
2. Prechek Bio, Inc. CA92806, USA, Diagnostic kit for hepatitis B viral surface antigen (ELISA), one-step incubation, double-antibody Sandwich concept.
3. HCV-PCR: A nested RT-PCR technique was used to detect HCV-RNA in serum in a qualitative manner.
4. HIV-PCR: A nested RT-PCR technique was used to detect HIV in serum in a qualitative manner.

Statistical Analysis

The collected data was assembled and input into a spreadsheet program (Microsoft Excel 2007) before being exported to the data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). The confidence level and level of significance for all tests were set at 95% and 5%, respectively.

Results

In the current study, 60 patients were males and 40 patients were girls. The average age is 16.10 6.25 years, with a range of 2-37 years.

In the current study, 69% (N = 69) of cases required twice-monthly transfusions, whereas 31.0% (N = 31) required once-monthly transfusions. It was discovered that elderly patients had a higher frequency of transfusion while younger patients had a lower frequency.

It was also discovered that younger age group patients needed less chelating agent at a lower transfusion frequency than older age group patients who needed more chelating agents.

Out of the 100 cases, 19 had the A+ blood group (19%), 12 had the AB+ blood group (12%), 29 had the B+ blood group (29%), and 38 had the O+ blood group (38%). There were 28 HCV+ patients (28%), 4 HIV+ cases (4%), and 66 nonreactive cases (66%). (Table 1)

A splenectomy was performed in 16 cases (16%). Splenomegaly was present in 84 (84%) of the patients.

In the current investigation, random successive 2 times pre transfusion CBCs were taken in multi transfused diagnosed patients with beta thalassemia major to compare and determine transfusion adequacy.

The Initial Pre-Transfusion CBC

RBC distribution is typical, with a mean of 3.50. The Hb (gm%) distribution is normal, with a mean of 9.15. The HCT% distribution is normal, with a mean of 28.12.

A second pre-transfusion CBC was performed.

RBC distribution is typical, with a mean of 3.58. The Hb (gm%) distribution is normal, with a mean of 9.10. The HCT% distribution is normal, with a mean of 27.80. It was discovered that parameters such as Hb, HCT%, and RBC Count are affected by factors such as sample time and point of sampling, and hence have no effect on the number of transfusions, average serum ferritin level throughout the year, age or sex, and chelating agent dose. MCV and MCH were found to be low normal values and moderately reduced, with no dependence on transfusion frequency, chelation therapy, or ferritin levels. MCHC results were found to be generally stable, with little fluctuation in values regardless of sampling site, transfusion frequency, serum ferritin levels, or chelating agent utilised.

Using the cut off of 10 gm% Hb as a marker to qualify patients for sufficient chelation therapy, we discovered that 33 instances were adequately transfused with levels greater than 10 gm%. Using a limit of 10 gm% Hb as a marker to qualify patients for effective chelation therapy, we discovered that 37 instances were adequately transfused with levels greater than 10 gm%.

The last Ferritin (4th) evaluation yielded an average result of 4720.05±336.15 ng/mL. During the study period, the mean of average yearly ferritin readings was 4725±286.10 ng/mL. With p values of 0.005, serum ferritin levels were shown to be substantially linked with transfusion frequency. Serum ferritin levels were usually higher in patients who received two or more transfusions per month. Serum ferritin levels were shown to be substantially linked with medication and chelating agent dose, with p values 0.001. For almost a year, the trend of Serial Serum Ferritin monitoring was followed in these 10 patients, and it was discovered that although 45 patients had an increasing trend of Serum Ferritin levels, 55 patients had a declining trend due to their appropriate chelated status.

The serum ferritin was measured three times a month for a year using the ELISA method. Serum ferritin levels were discovered to be linearly proportional to chelation therapy and cheating agent dose. The individual required more chelating agent as ferritin levels increased. Serum ferritin had no relationship with the number of transfusions given to a patient with well-balanced/titrated dosages. Serum Ferritin was utilised to grade the patients into three groups.

Table 1: Blood group with sero status

Variable		Blood Group						Total
		A-	A+	AB+	B-	B+	O+	
Sero status	NR	1	11	10	1	20	25	66
	HCV + ve	0	8	2	0	9	9	28
	HIV + ve	0	0	0	0	0	4	4
Total		1	19	12	1	29	38	100

Discussion

Beta-thalassemia, one of the most common genetic illnesses in Asia and much of the world, has already piqued the interest of scientists. Thalassemia syndromes are a collection of genetic and severe illnesses caused by being homozygous for one of the thalassemia or haemoglobin Lepore genes in childhood or infancy. There is metabolic dysregulation, iron excess, persistent hypoxia, and cell damage. All of these physiological alterations lead to inefficient erythropoiesis, hemolysis, and anaemia.

Most patients rely on blood transfusions and bone marrow transplants to survive.[16-21]

Anaemia is severe, often hypochromic and microcytic, with low mean cell haemoglobin (MCH) and mean cell volume (MCV). Red-cell indices produced from electronic cell counts may not always accurately indicate the degree of hemoglobinization of red cells. Many factors may be at work in this discrepancy, including cell population heterogeneity, with large numbers of extremely small cells that cannot be seen by the electronic cell counter, as well as artefacts produced by the large numbers of nucleated red cells, white cells, and platelets that are almost always present after splenectomy.[22]

Iron overload is a serious problem in the treatment of thalassemia major, which should be regularly monitored and kept under limits via phlebotomy or chelation therapy. Because it can take a long time to considerably reduce iron depending on the organ, the ideal method is to act early and, in fact, attempt to prevent major iron loading from the start.23 Iron overload treatment demands frequent assessment of the body's iron reserves.[24] Serum Ferritin is one of the few quantitative, non-invasive procedures for assessing body iron that is safe, accurate, and widely available. In a study conducted by Bandyopadhyay et al., patients of all ages had elevated serum ferritin levels. They discovered that the average blood ferritin level in children aged 1 to 5 years was 1750 ng/ml, and this climbed to 3650 ng/ml in patients aged 11 to 15.[24] The blood ferritin level was not successfully managed because only a few individuals followed the prescribed routine at home. Our findings are comparable to those of similar regional and worldwide research. Our final ferritin (4th) evaluation resulted in an average value of 4720.05±336.15 ng/mL. During the study period, the mean of average yearly ferritin readings was 4725±286.10 ng/mL.

In addition, all of the patients in this study were receiving some type of chelation therapy from the government-run thalassemia ward. Approximately 3% (3 cases) of patients were inadequately chelated, 13% were under controlled chelation and required serum ferritin levels to be monitored, and 83% had extremely high ferritin levels > 2500 ng/mL and required intensive chelation therapy. When compared to Shah N, et al [25]'s research at IRCS, Ahmedabad. Chelation treatment was used by 96 (67%) of the participants in our research.

In our investigation, no patients had transfusion-related reactions or received mismatched transfusions. Only 3% of occurrences were on triple saline cleaned blood components, and its occurrence was noted in patients over the age of 24, maybe due to full immunisation. The prevalence of HCV was 27% (27 cases) while HIV was 3% (3 cases). This high frequency of thalassaemic individuals with HCV in the post-testing era is concerning since it has an additional effect in iron-depleted people.

Conclusion

Haematological parameters such as Haematocrit, Red Blood Cell mass, Haemoglobin, and Mean Corpuscular volume had large covariances, whereas Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration had very narrow covariances. Serum Ferritin levels were positively correlated with chelation therapy and transfusion frequency among thalassaemic patients, and Serial Serum Ferritin Levels is to be used.

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