

Hematologic Evaluation in Transfused β – Thalassemia Major Patients

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Abstract: Background: In β thalassemia major, there is impaired synthesis of β globin chain of hemoglobin moiety leading to severe anemia. As a therapy, patients are given regular blood transfusions. Transfusion associated hemosiderosis is a major complication in these patients. The ineffective hematopoiesis, hemolysis and associated hemosiderosis causes multitude of morphological changes in the peripheral blood cells. We hereby intend to highlight some of the possible causes leading to these changes. Aims & Objectives: To study the complete blood counts, red blood cell indices and peripheral blood smear findings in transfused β thalassemia major patients. Materials & Methods: Venous blood was collected in ethylenediaminetetraacetic acid vacutainers from 50 β thalassemia major patients who had taken more than 12 transfusions. Complete blood count was performed on 5 part automated hematology analyser ADVIA 2120i. Peripheral smears were stained with Leishman's stain and studied under light microscope. Results: Out of the 50 β -thalassemia major patients, 27 were males (54%) and 23 were females (46%). The age group ranged from 2 years to 19 years. The mean hemoglobin level was 8.32g/dl. 44% patients showed microscopic hypochromic red blood cell morphology followed by 30% showing dimorphic blood picture. Schistocytes, target cells, tear drop cells and erythroblasts were also seen. Conclusion: In transfused patients, the morphological changes in peripheral blood can be attributed not only to ineffective erythropoiesis or hemolysis but also transfusion associated hemosiderosis, infections, chronic liver disease, splenomegaly and associated nutritional deficiencies. Thus the peripheral smear and complete blood counts can serve as indicators of underlying co-morbidities.

Keywords: β thalassemia major, peripheral smear, complete blood counts, transfusion

1. Introduction

Hemoglobin is composed of heme and globin moieties and accounts for approximately 96% of the dry weight of the erythrocyte.[1] The globin portion consists of 2 α and 2 β polypeptide chains in the adult haemoglobin (HbA). The thalassemias are a heterogeneous group of inherited, single gene disorders of hemoglobin and are characterized by reduced or absent production of one of the globin chains. β - thalassemia mutations result in either a complete absence of β -globin chain termed β^0 thalassemia or in a variable reduction of β - globin output called as β^+ -thalassemia.

β - thalassemia major, also known as Cooley's anemia is the most severe form requiring regular blood transfusion therapy. The incidence of β thalassemia in India ranges from 1 – 17% and is highest amongst communities like Sindhis, Punjabis, Lohanas.[2] It is a severe disease causing emotional and financial burden to the affected families and to the society as a whole. Regular transfusions lead to many complications, the major ones being hemosiderosis and transfusion transmitted diseases. Associated nutritional deficiencies further contribute to the morbidity caused by this disease. Iron overload affects the liver leading to liver cirrhosis and failure, in the long run. Therefore, besides the disease, other complications also have an effect on hemoglobin levels and morphology of erythrocytes.

In regularly transfused patients, Complete Blood Counts (CBC) is an important tool to assess the need for then exttrans fusion. Red blood cell indices and peripherals mear examination not only help in initial diagnosis of these cases, but further contribute in analyzing the overall health of these patients once they are started on therapy in order to assess associated morbidities.

2. Aims and Objectives

a) To study the Complete Blood Count (CBC) and red blood

cell indices in transfused β thalassemia major patients.

b) To examine their peripheral blood smears for morphological abnormalities, especially of the erythrocytes.

c) To assess the cause of the various findings.

3. Materials and Methods

The prospective study was conducted after approval from Institutional Ethics Review Committee. 50 diagnosed (by High Pressure Liquid Chromatography) cases of β thalassemia major coming for their regular transfusion therapy were included in the study. The inclusion criterion was a minimum of 12 transfusions. A detailed clinical history was taken as to the number of transfusions, time gap between consecutive transfusions and drugs being taken especially iron chelating agents and duration of the same. Patients were examined to look for pallor, icterus, lymphadenopathy, edema, splenomegaly or any other systemic disease.

Under all aseptic precautions, venous blood was collected in vacutainers containing ethylenediamine tetraacetic acid (EDTA). Complete Blood Counts were performed on 5-part differential automated cell counter – ADVIA2120i. The principle of estimation of hemoglobin was by cyanide-free method. White Blood Cell (WBC) count was analysed using a 2 angle laser light scatter signals. WBC Differential count was estimated in this analyser by the peroxidase method while the Red Blood Cell (RBC) and platelet count were by impedancemethod.

In order to study the red blood cell morphology, smears were prepared with a drop of EDTA anticoagulated blood on clean and dry glass slides, followed by Leishman staining. Morphology was studied under X400 (high-power) and X1000 (Oil immersion)

4. Results

Out of the 50 β -thalassemia major patients, 27 were males (54%) and 23 were females (46%). The age group ranged from 2 to 19 years. 43 patients had been started on iron chelation therapy (86%). The transfusion details of these patients are summarised in Table 1.

Table 1: Categorizing patients depending on the number of blood transfusions taken.

No. of Transfusions Taken	No. of Patients
012 – 050	20
051 – 100	14
101 – 150	4
151 - 200	3
201 – 250	2
251 – 300	3
301 – 350	2
351 - 400	2
TOTAL	50

3 out of 50 (6%) patients were infected with Hepatitis C virus (HCV).

The lowest hemoglobin (Hb) recorded was 3.1g/dl and the highest was 11.3g/dl with a mean \pm SD of 8.32 ± 1.625 g/dl. The mean \pm SD of the total leucocyte count (TLC) and platelets were 9536 ± 3838.004 cells/cu.mm and 3.068 ± 1.199 lakhs/cu.mm respectively. The red blood cell count (RBC) showed a mean \pm SD of 3.168 ± 0.655 million/cu.mm. Thus, only the hemoglobin of these patients was on the anaemic side, although they were taking regular saline washed packed red blood cell transfusions while rest of the parameters fell in the normal range.

In order to get into the details of anemia, we evaluated the red blood cell indices of these patients. The mean \pm SD of the mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haematocrit (Hct) and red cell distribution width (RDW) were as summarised in Table 2.

Table 2: Red blood cell indices

Sr. No.	Parameter	Mean	Standard Deviation
1	MCV (fl)	80.646	5.012
2	MCH (pg)	26.43	2.284
3	MCHC (g/dl)	32.772	1.695
4	Hct (%)	25.436	5.095
5	RDW (%)	16.926	3.494

The peripheral smear examination showed that 22 out of 50 patients (44%) had microcytic hypochromic blood cell

morphology characteristic of thalassemia major, followed by 15 patients having dimorphic blood picture (30%), out of which maximum (26%) showed microcytic and normocytic hypochromic red blood cells. These findings are summarised in Table 3 and Figure 1.

Also seen were nucleated red blood cells, codocytes (target cells), schistocytes, tear drop cells, occasional pencil cells

and basophilic stippling in varying proportions in all the smears studied. Moderate to marked anisopoikilocytosis and polychromasia were the other features (Figure 2-5). The WBC and platelets were normal in morphology and distribution in most of the cases except in one case, that had pancytopenia attributable to marked splenomegaly. No hemoparasites were noted in any of the smears examined.

5. Discussion

The basic defect in β - thalassemia of reduced or absent production of β - globin chains leads to relative excess of α - chains. These excess α - chains combine with residual β chains in cases of β thalassemia and undergo oxidation or proteolysis. All these mechanisms cause death of the RBC precursors in the bone marrow contributing to ineffective erythropoiesis, the hallmark of β - thalassemia major. The RBCs which are released into the peripheral blood are also prematurely destroyed by the reticuloendothelial system, further contributing to anemia by hemolysis.

The distribution of the thalassemia gene is not uniform in India and the prevalence is very high among certain communities such as Sindhis and Punjabis from Northern India, Bhanushalis, Kutchis, Lohanas from Gujarat, Neobuddhists, Kolis and Agris from Maharashtra & Gowdas, with lower incidence in the southern tribes. Overall frequency of β - thalassaemia carriers in Maharashtra was 2.7% while in Gujarat it was 3.5%. Among the 35 districts in Maharashtra, Ahmednagar district had the highest prevalence of β - thalassaemia in the state (6.0%). Mumbai and a few other districts (Bid, Satara, Raigad, Thane, Nashik and Dhule) also had higher frequencies (3.9–5.2%) while the frequencies were much lower in the southern and eastern districts of the state (1.0–2.7%). [3] With this, the thalassemia burden is high and the treatment options till date are packed cell transfusions and hematopoietic stem cell transplantation. Within the Indian scenario, very few patients can avail from the latter. Also the immunosuppressive therapy following the transplant has its own disadvantages. Hence, the widely used treatment modality is in the form of blood transfusions.

Repeated blood transfusions subject these patients to a variety of transfusion related complications, viz allergies, haemolytic transfusion reactions, febrile non haemolytic transfusion reaction, alloimmunization, lung injury, graft versus host disease, transmission of infections like HIV, HCV and HBsAg. Iron overload is a major adverse event in cases of repeated blood transfusion as each unit of blood has about 250mg of iron. [4]

Table 3: RBC morphology on peripheral smear examination.

RBC Morphology		No. of Patients	Percentage (%)
Microcytic Hypochromic		22	44
Normocytic Normochromic		10	20
Normocytic Hypochromic		3	6
Dimorphic Picture (30%)	Microcytic + Normocytic Hypochromic	13	26
	Normocytic Normochromic + Microcytic Hypochromic	2	4
TOTAL		50	100

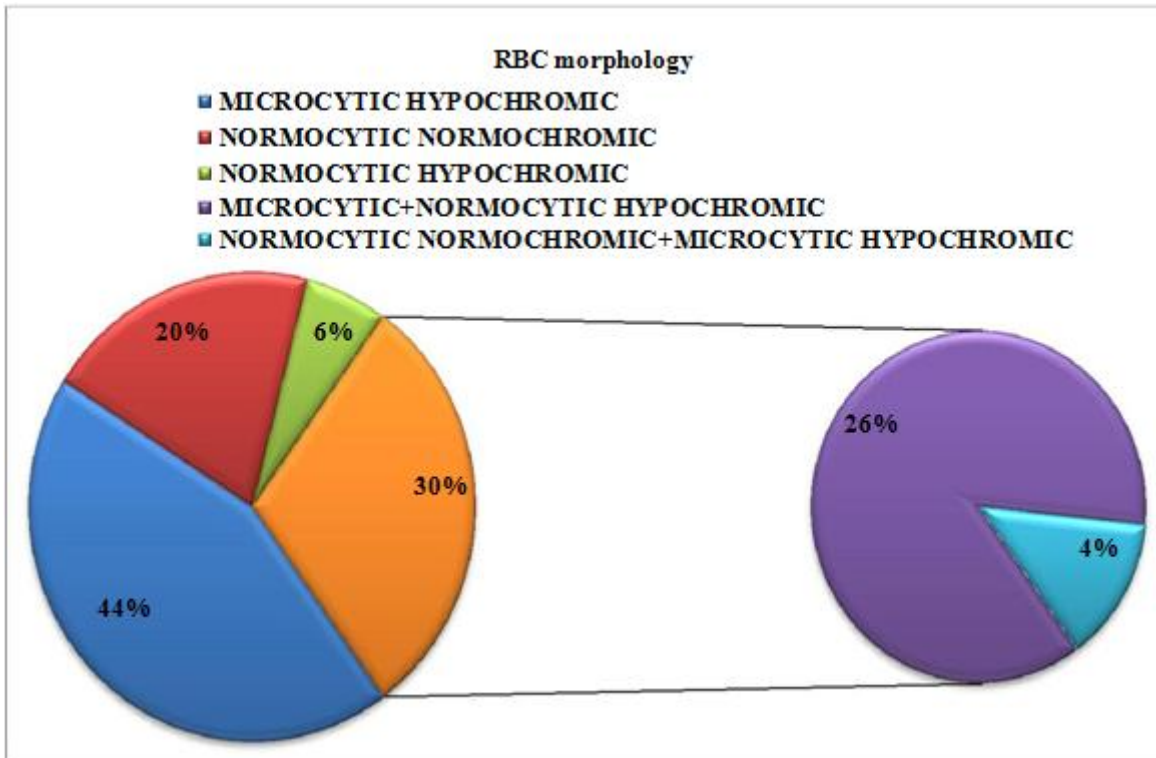


Figure 1: Pie-chart showing distribution of patients according to red cell morphology

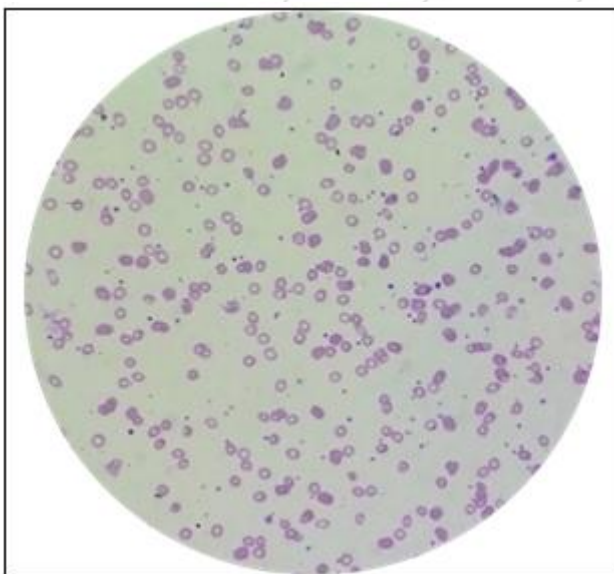


Figure 2: Leishman stained peripheral smear showing microcytic hypochromic red blood cells (X400).

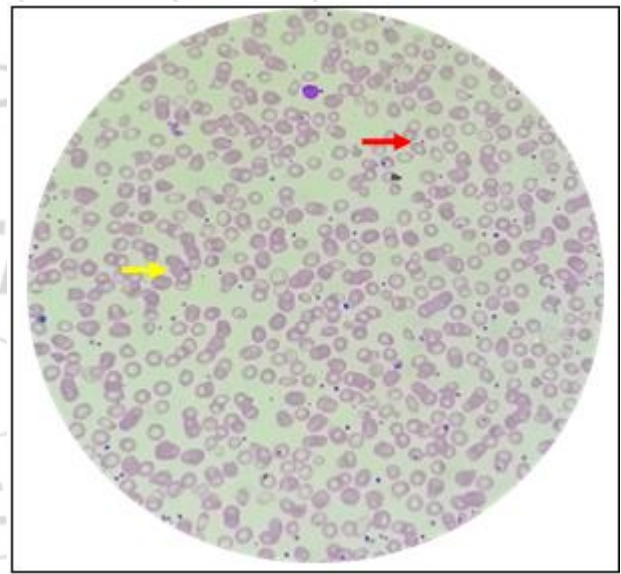


Figure 3: Leishman stained peripheral smear showing dimorphic red cell morphology – Microcytic hypochromic (Red arrow) and Normocytic normochromic (Yellow arrow) red cell morphology. (X400).

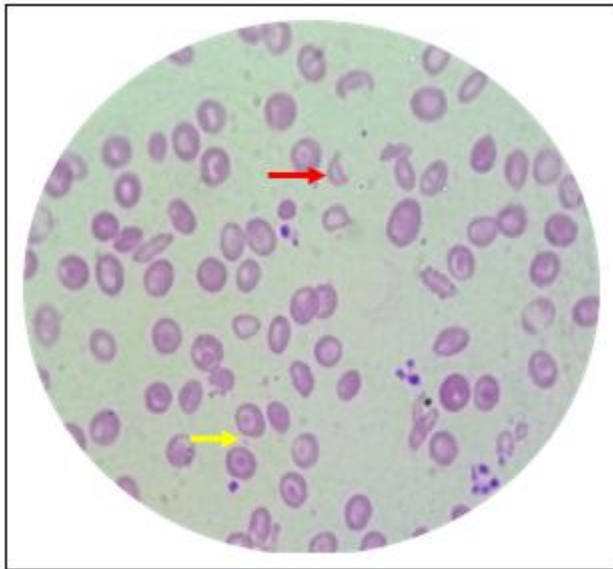


Figure 4: Leishman stained peripheral smear showing target cells (Yellow arrow) and schistocytes (Red arrow) (X1000).

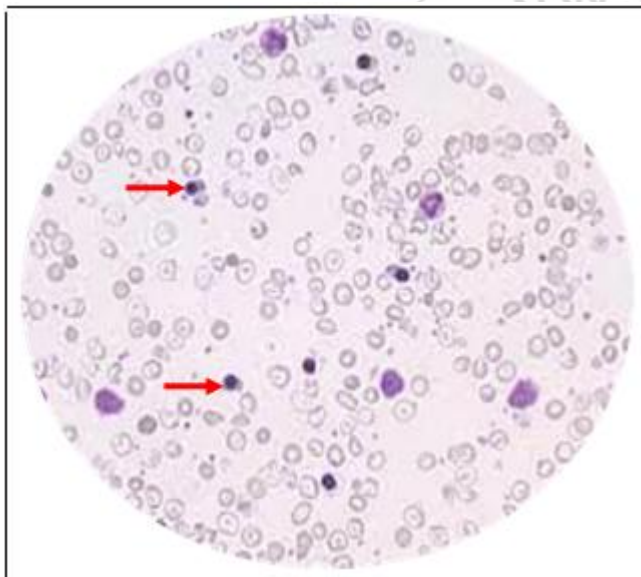


Figure 5: Leishman stained peripheral smear showing nucleated RBCs (Red arrow) and target cells (X400).

Hemosiderosis predominantly affects the heart, liver, endocrine glands, joints and brain. Liver cirrhosis and failure complicate the picture and are one of the causes for morphological changes in erythrocytes.

In β - thalassemia major, the characteristic morphology of the RBCs is microcytic hypochromic and the MCV and MCH are typically in the range of 60-70fl and 12-18pg/cell.[5]

We studied cases who had taken multiple, regular (at 3-4 weeks interval) transfusions and still the average hemoglobin was 8.32g/dl, a little lower than the target hemoglobin of 9-10g/dl. This can be explained as with increasing age and weight the amount of blood to be transfused per visit has to be increased and also at times, the interval between two consecutive transfusions needs to be shortened, especially if there has been an episode of intercurrent illness. These patients are susceptible to infections because of their compromised immune responses. One major reason for this is

iron overload. Lactoferrin is a prominent component of granules of polymorphonuclear leucocytes and it is bacteriostatic. High transferrin saturations with iron overload compromise bacteriostatic properties of this protein leaving these patients susceptible to infections like *Yersinia enterocolitica*, *Rhizopus oryzae* (mucormycosis) and *Listeria monocytogenes*.[5]

The mean MCV and MCH in our study were 80.646fl and 26.43pg respectively. The peripheral blood smear post-transfusion is expected to show dimorphic blood picture with normocytic normochromic transfused RBCs and microcytic hypochromic RBCs of the patient. However, in our study, maximum cases (44%) showed microcytic hypochromic RBCs and dimorphic morphology was seen in 30% cases. 20% cases showed a predominant normocytic normochromic RBCs. Microcytic hypochromic picture predominant even after transfusion might indicate the need to transfuse at shorter intervals since apparently the transfused RBCs have been destroyed, one of the cause being increasing splenomegaly as the age increases.

Bester et al. have shown that RBC ultrastructure is changed significantly in the presence of iron overload. They analysed this with the help of four microscopic techniques, light microscopy and axial ratios, scanning electron microscopy, atomic force microscopy and confocal microscopy. They concluded that poorly liganded iron is the main culprit and plays a fundamental role in the development of this pathology. The main kind of mechanism by which iron overload causes this damage is considered to be free radical toxicity caused by the excessive levels of this poorly liganded metal. Hydroxyl radicals ($\text{OH}\cdot$) are especially well-known culprits in causing damage to biomolecules, and iron catalyzes the redox-based production of hydroxyl radicals via the Fenton reaction. RBCs are very sensitive to unliganded iron changes.[6] This view was further supported by the study conducted by Naithani et al. They analysed malondialdehyde (MDA), nitric oxide (NOx), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) along with serum iron and ferritin, liver functions and uric acid in 50 transfusion dependent thalassemics. Plasma MDA was analyzed to indicate the oxidative parameters, whereas the erythrocyte SOD, GPx, and plasma NOx were measured to show the antioxidant status of the children. Markers of free radical injury such as MDA and antioxidant enzyme SOD and NOx levels were significantly elevated in thalassemic children while mean GPx levels were decreased in patients compared to controls.[7] All this could explain the varied morphologies we saw in our study, since all our patients were transfused multiple times (maximum number of patients had taken at least 50 transfusions and iron overload begins with 10-12 transfusions) and had transfusion induced hemosiderosis.[8] Although iron chelation therapy had been initiated, compliance was poor due to lower socioeconomic conditions. Moreover, they have disproportionately low levels of hepcidin, allowing more and more iron to be absorbed, even in the presence of overload.[5] Therefore, morphological changes in erythrocytes in multi-transfused patients should be evaluated taking into consideration the iron overload status.

Liver is another major organ affected in hemosiderosis leading to cirrhosis and liver failure. Besides, 3 of our patients were infected with HCV further contributing to liver damage. Thus, in these transfused patients, anemia of liver disease can also complicate the picture. This, apparently results from a combination of intravascular dilution due to volume overload, shortened red cell survival, and impaired ability of the marrow to respond optimally to the anemia. Reduced red cell survival in liver disease can be attributed to abnormal erythrocyte metabolism. The activity of the pentose phosphate shunt is decreased leading to glutathione instability. This metabolic abnormality renders the cell sensitive to oxidant hemolysis.[5] Another metabolic abnormality encountered occasionally in liver disease is hypophosphatemia, with reduced erythrocyte adenosine triphosphate levels and consequent hemolysis.[9] Characteristic alterations in red cell membrane lipids, occurring in patients with liver disease further contribute to the problem. Increase in both cholesterol and lecithin is noted in the membrane. These changes result in an increased cell-surface area associated with the target cells or thin macrocytes. The loss of sialic acid from the red cell surface may contribute to impaired viability of the cell.[10] This explains the presence of normocytic cells and also the pokilocytosis.

Besides these, folic acid deficiency is common in thalassemics. It is an effect of increased folate utilization caused by the enhanced total, both effective and ineffective erythropoiesis, further contributing to the change in RBC morphology.[11]

Thus, the varied morphologies seen in multi-transfused patients are not only attributable to thalassemia, but a wide variety of causes. As explained above, target cells can indicate underlying liver disease and normocytic hypochromic erythrocytes may be because of underlying folate deficiency and/or liver disease. Fragmented RBCs may be because of iron overload causing free radical injury causing membrane damage.

6. Conclusions

With this study, we tried to explain the multitude of comorbidities in thalassemics and their effect on erythrocytes based on the underlying molecular events. Thus, Complete Blood Counts and peripheral smear in transfused thalassemic patients should always be evaluated keeping the complete clinical history and physical examination in mind. A smear can serve as a mirror to some underlying disease process and should, therefore be a must to examine in all cases. In thalassemics, besides anemia, hemosiderosis, liver damage and nutritional deficiencies exist and these need to be diagnosed as well as catered to immediately in an effort to improve their quality of life.

7. Clinical Significance

In thalassemics, CBC should not only be done to monitor transfusion therapy, but it should be kept in mind that this simple test combined with peripheral smear examination can serve as a window to underlying disease processes that have yet not become symptomatic.

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