**Original Article** 

# Hunt for the Hidden Trait

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# ABSTRACT

**Objective:** To assess the efficacy of a peripheral smear examination as a screening tool for  $\beta$ -thalassemia trait. **Materials and Methods:** 17 623 Leishman-stained peripheral smears were evaluated during the period from July 2006 to September 2007. The following parameters were studied: hemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width. All the cases that showed microcytosis, hypochromia, erythrocytosis and absence of anisopoikilocytosis were suspected of having the thalassemia trait (TT), and all these cases were further evaluated with Alkaline Hemoglobin Electrophoresis for confirmation.

**Results:** Of the 17 623 smears examined, 60 cases were considered suspicious of having TT. Alkaline hemoglobin electrophoresis carried out on all these cases revealed an elevated  $HbA_2$  (Mean = 7.5%). Five cases evaluated were found to have other hemoglobinopathies (1 Sickle cell trait, 3 Hb-E, 1 thalassemia intermedia).

**Conclusion:** Careful screening of peripheral smear is an invaluable screening tool for thalassemia trait (PPV - 95%). There must be awareness among the peripheral centers about the importance of peripheral smear screening and the affected persons should be counseled.

Keywords: Iron deficiency anemia, peripheral smear, Thalassemia trait

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## INTRODUCTION

halassemia is considered the most common genetic disorder worldwide<sup>[1]</sup> supported by the fact that over 30 million people are carrying the defective gene.<sup>[2]</sup>  $\beta$ -thalassemia, results from mutations causing diminished production of mRNA and decreased synthesis of structurally normal globin, is the most common single gene disorder in India.<sup>[1]</sup>

The three  $\beta$ -thalassemia syndromes described are:

- 1. Thalassemia major (homozygous gene defect, present with severe anemia classically described by Cooley).
- 2. Thalassemia intermedia (homozygous gene defect associated with a moderate degree of anemia).
- 3. Thalassemia minor or thalassemia trait (TT) (heterozygous state with involved individuals being asymptomatic).<sup>[3]</sup>

Thalassemia constitutes a major cause of public health morbidity and early mortality in patients. Effective population screening for TT can dramatically decrease the incidence of thalassemia major births. Though quantitative assessment of abnormal hemoglobins by hemoglobin electrophoresis and DNA studies are the gold standard for diagnosis, they are too expensive and hence cannot be used for mass screening purposes. As thalassemia syndromes are invariably associated with microcytosis and hypochromia in the peripheral smear, peripheral smear examination and red cell indices can be used as preliminary indicators of diagnosis. Hence with this background, it was decided to assess the efficacy of peripheral smear examination as a screening tool for  $\beta$  TT.

## MATERIALS AND METHODS

During a one-year-period from July 2006 to September 2007, all peripheral smears stained with Leishman stain, which were done as part of investigations, were evaluated. The smears from patients with malignancy were excluded. The demographic details were retrieved from the charts.

The other parameters studied using automated Sysmex XT1800i were:

## RESULTS

1. Hemoglobin, 2. RBC count, 3. Mean corpuscular volume (MCV), 4. Mean corpuscular hemoglobin (MCH), 5. Mean corpuscular hemoglobin concentration (MCHC) and, 6. Red cell distribution width (RDW)

Perl's stain was used to stain the iron stores in bone marrow and was graded from 0 to 6. Grade 0 was considered as iron deficient, 1-3 as normal and 4-6 as increased iron stores.

All the cases showing a microcytosis and hypochromia on peripheral smear examination were categorized into two groups:

1. Suspected thalassemia trait (TT), 2. Suspected iron deficiency (IDA), which was considered as control group.

On correlation of peripheral smear findings and red cell indices, TT was suspected in cases that showed microcytosis, hypochromia, presence of target cells, erythrocytosis and absence of anisopoikilocytosis [Figure 1]. These cases were further evaluated with alkaline hemoglobin electrophoresis using cellulose acetate agar (Helene electrophoresis) for confirmation.

Iron deficiency was suspected in patients with microcytic hypochromic blood picture associated with anisopoikilocytosis and low RBC counts. Iron deficiency status was confirmed by serum iron studies including serum iron, serum ferritin (decreased) or bone marrow grade 0 iron stores [Figure 2]. Simple descriptive statistics of mean and percentage were used for comparison, and screening test of positive predictive value was used for assessing the efficacy of peripheral smear as a screening tool. Of the 17 623 smears examined, 60 smears were considered suspicious for TT.

A similar number of (n = 60) Iron deficiency anemia patients with microcytic hypochromic blood picture, were considered as control group and, were evaluated.

The mean value of parameters (1, 2, 3 and 6) is given as comparative statement in Table 1. The alkaline hemoglobin electrophoresis done on the 60 suspected TT cases showed an elevated Hb A2 (mean = 7.5%) indicating hemoglobinopathy. Of the 60 cases, 57 (95%) cases showed electrophoresis results consistent with TT. Three cases showed a marked increase of the Hb A2/C/E band (51.34, 52.19 and 35.82%) indicating hemoglobinopathy other than TT. These peripheral smears were re-evaluated and a diagnosis of Hb-E was suggested, which required further confirmation by acid electrophoresis method. All cases of suspected iron deficiency were confirmed with either serum iron studies or bone marrow iron stores that was depleted. The morphological differences are summarized in Table 2. [Figures 1 and 2]. In the bone marrow, erythroid hyperplasia with normoblastic maturation was seen in both conditions, but the degree varied. Micronormoblastic maturation was also seen in 5% of the IDA cases.

## DISCUSSION

Increasing social burden from thalassemic patients can be reduced only by awareness and use of effective tools to



Figure 1: Peripheral smear showing Thalessemia trait (showing microcytic hypochromic RBCs) and presence of target cells (Leishman stain  $\times 1000$ )



**Figure 2:** Peripheral smear showing iron deficiency anemia. Note the marked anisopoikilocytosis (Leishman stain ×1000)

Table 1: Comparison of hematologicalparameters in TT and IDA			
Parameter	Thalassemia trait	Iron deficiency	
Hemoglobin (g/dl)	11.39	5.44	
RBC Count (millions/mm <sup>3</sup> )	5.74	3.19	
MCV (femtoliters)	62.3	64.9	
RDW-CV%	18.78	22.58	

diagnose heterozygous carriers of the gene (TT).

Microcytosis is a common red cell change seen in anemias of varying etiology. These include IDA, thalassemia, chronic disease and sideroblastic anemias. The microcytosis of heterozygous β-thalassemia needs to be distinguished from non-thalassemic microcytosis for its role in thalassemia control.<sup>[4]</sup>. Comparison of both groups showed significant differences between the two groups [Table 2].

Onofrio *et al* used H\*1 hematology analyzer, in which the percentile of microcytes and hypochromic cells were obtained. They found that there was an inverse relationship between the percentages of the microcytes (MCV < 60 fl) and the hypochromic cells (MCHC 280 g/L) in IDA and TT. Percentile of microcytes (mean 33.1%) was higher than the hypochromic cells (mean 13.9%) in TT. On the contrary, in IDA, the percentage of hypochromic cells (mean 34.6%) was higher than the percentage of microcytes (mean 12.8%).<sup>[5]</sup>Bentley *et al* used Technicon H\*1 analyzer, which provides direct MCHC measurement (termed CHCM), in addition to the conventional computed value (Hb/PCV). The mean values for both MCHC and CHCM were significantly lower in patients with IDA parallel to the above study.<sup>[6]</sup>

Kotwal *et al*, in their study found that the prevalence of IDA as a cause for microcytosis in India is high.<sup>[4]</sup> Hence, there are controversies on the choice of red cell indices to be used and assign cut-off values for distinction from TT. They overcame this difficulty by plotting Receiver Operating Characteristic (ROC) curves, and re-calculating the cutoff values for red cell indices in an Indian setting. The cut-off values defined by them were: MCV < 76 fL, RBC count  $\geq 4.9 \times 10^{12}/L$  and RDW  $\geq 18\%$ . Of these they observed RBC count to be the most efficient single test for distinguishing TT cases.<sup>[4]</sup> In the present study, significant differences were found in the mean values of Hb percentage, RBC count and RDW with a positive predictive value of 95% [Table 1].

Various discriminative functions (DF) have been described using red cell indices and RBC count. Some of them are: 1. Mentzer ratio (MCV/RBC count); 2. England and Fraser

Table 2: Morpho and IDA	ological differences	between TT
Parameter	TT	IDA
Microcytosis	++	+
Hypochromia	+	+++
RBC count	Mild erythrocytosis	Anemia
Anisopoikilocytosis	-	+

index (MCV-  $(5 \times Hb) - 8.4$ ; 3. MCH/RBC count and; 4. (MCV)2 × MCH.

Afroz *et al* used Mentzer ratio to discriminate between IDA and TT with a positive predictive value of 91%. Mentzer ratio >14% was marked as IDA.<sup>[7]</sup> In the present study, the mean Mentzer ratio in TT was 10.8 and in IDA was 19.7.

Various opinions have been suggested as to the role of DFs in the differential diagnosis of microcytosis. Eldibany *et al* analysed four groups of patients (normal,  $\beta$ -thalassemia,  $\alpha$ -thalassemia and IDA). They statistically analyzed RBC count, Hb, MCV, MCH and RDW by stepwise multivariate discriminant analysis and calculated by Fischer linear DF. They identified RBC count, MCV and RDW as the best set of indices to differentiate the diagnoses. The Fischer linear DF resulted in mean weighted sensitivity of 80.4%.<sup>[8]</sup> On the other hand, Bentley *et al* noted in 67% of the cases they studied that use of a DF did not resolve the diagnosis to the extent that Hb concentration and quantitation studies were no longer indicated.<sup>[6]</sup> In the present study, mean Hb, RBC count and RDW were found to be significantly different in the two groups evaluated.

#### CONCLUSION

Careful examination of the peripheral smears along with red cell indices are invaluable screening tools for TT (positive predictive value = 95%). Creating awareness among the peripheral centers of thalassemia belts in using the above method will go a long way in reducing thalassemia major births.

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