Imperial Journal of Interdisciplinary Research (IJIR)
Peer Reviewed – International Journal
Vol-4, Issue-1, 2018 (IJIR)
ISSN: 2454-1362, http://www.onlinejournal.in

Screening of β-Thalassemia Trait from Iron Deficiency Anemia on the bases of Absolute Indices

[1]Muneeba Musharaf Ali, Aemal Khan, Hina Khan Khilji, Mehwish Khan, Palwasha Zeeshan, Sabina Khan Khilji, [2] Fadieleh A.Sohail, [3]Rubina Ghani

Department of Biochemistry, [1,2 &3]Jinnah Medical and Dental College,
[3]Pathological & Molecular Laboratories, Karachi, Pakistan

ABSTRACT

Background A brief survey was carried out for the detection of hemolytic anemia present in our population of different ethnic origins. β-Thalassemia trait (BTT) often shows microcytosis, a normal or an increased red blood cell (RBC) count, and an elevated level of HbA_2, which provide the basis for laboratory screening. BTT is an important differential diagnosis of iron deficiency anemia (IDA). Donors with BTT have hemoglobin values comparable with normal; hence, they are accepted for donation and they usually escape diagnosis.

Aim The aim of this work was to differentiate beta thalassemia trait from IDA through blood indices performed in routine complete blood count.

Patients and methods Approximately 2, 02,600 blood samples of various age group were collected from different parts of Karachi. Hematological assessments were conducted with a range of laboratory techniques coming to Pathological and Molecular Laboratories for the complete blood count to rule out the reason of fever randomly. Complete blood count and mean corpuscular volume (MCV) were performed to all individuals. Hemoglobin electrophoresis was performed to samples with MCV less than 78 fl and to confirm the beta thalassemia minor

Results Prevalence of BTT in this study was 13%, whereas IDA represented 60% of total samples investigated. The MCV was68.16 fl incase BTT was low from IDA. Red blood cell distribution width level was28.03% when differentiate BTT from IDA. RBCs count at value above 5.89 million/mm^3 can differentiate BTT from IDA.

Conclusion The cutoff values of MCV 68.16 fl or less, RBC count above 5.89 /mm^3, and red blood cell distribution width 28.03% or less were suggested to be associated with a high probability of BTT.

Keywords: β-thalassemia trait, blood donors, iron deficiency anemia

Introduction

The hemoglobinopathies are a diverse group of inherited recessive disorders consisting of structural hemoglobin variants and thalassemias. A common cause of microcytic anemia and a significant public health problem worldwide is iron deficiency. The thalassemia syndromes are another cause of microcytosis, and although severe thalassemias generally are easily recognized, milder forms of both alpha and beta thalassemia may be misdiagnosed and treated as iron deficiency. (1, 2 & 3) The diagnosis of beta thalassemia minor is made readily by demonstration of elevated hemoglobin A2 levels in the absence of concomitant iron deficiency anemia that may result in normal hemoglobin A2 levels.

β- thalassemia trait or Iron deficiency frequently cause microcytic anemia. On the basis of certain hematological parameters only, β-thalassemia trait is inappropriately differentiated with microcytic hypochromic anemia. Similarly, in the case of iron deficiency, the mean cell volume (MCV), mean cell hemoglobin (MCH), hemoglobin and RBC count, tend to decrease. Therefore results in both microcytic hypochromic anemias can overlap.[4] Many formulas and indices that have been proposed, pose the ability to differentiate iron deficiency from β-thalassemia trait by using simple formulas that include a minimum of two CBC (Complete Blood Count) parameters (Hb, MCH, MCV, RBC count, red cell distribution width [RDW]) in various combinations. A confirmatory differntial diagnosis between β- Thalassemia trait and iron deficiency is based HbA2 electrophoresis. The main aim of this study is to differentiate between β-thalassemia trait and iron deficiency anemia. To evaluate the hematological and clinical diversity in these groups by using CBC indices to discriminate the microcytic hypochromic anemias is to detect a high probability of requiring appropriate follow-up and to reduce unnecessary diagnostic costs.

Material and Method

The sample was collected from Pathological & Molecular laboratories from January 2010 till June 2017. All the blood samples were collected in EDTA from 2,02,600 patients forCBC and Hb electrophoresis were evaluated. Automated cell counter was used for CBC test and Cellulose acetate electrophoresis for Hb electrophoresis. Individual reports of the patients were then analyzed on the basis of parameters given in the table below:

Parameters	Normal range	Iron deficiency anemia	Beta thalassemia trait
Hb gm/dl	15	8.39	10.16
RBC cu/mm	3.88	3.28	5.89
MCV fl	85.84	72.86	66.87
MCH pg	29.17	22.12	20.15
MCHC gm/dl	34.12	31.02	32.02
RDW %	14.27	17.81	28.19
Hb F %	-	-	5.21
Hb A2 %	2.1	1.98	6.21

Table1. Comparison of Clinical Data in Those with Iron Deficiency and Beta Thalassemia Minor From the Screening Clinic and in the Normal cases.

The whole blood was taken in the sterilized test tubes and the cells were washed thrice with normal saline (0.85 %). The hemolysate was prepared by mixing one part of washed packed cells with six parts of hemolysate reagent (Helena cat. no.5125) and stored at -20^{o}C till further analysis..

Result:

The present study focused on the diagnosis of beta thalassemia trait in the population of Karachi, an area of rapidly changing social and economical conditions with a high prevalence of hemolytic anemia. The results were evaluated in the screening clinic proved to have microcytosis caused by iron deficiency as shown in table 1, were 60% and beta thalassemia minor was diagnosed in 13 % patients in whom hemoglobin levels were normal but hemoglobin A2 levels were elevated as shown in figure 1. During our study we also found other abnormal hemoglobin variants is also shown in figure.

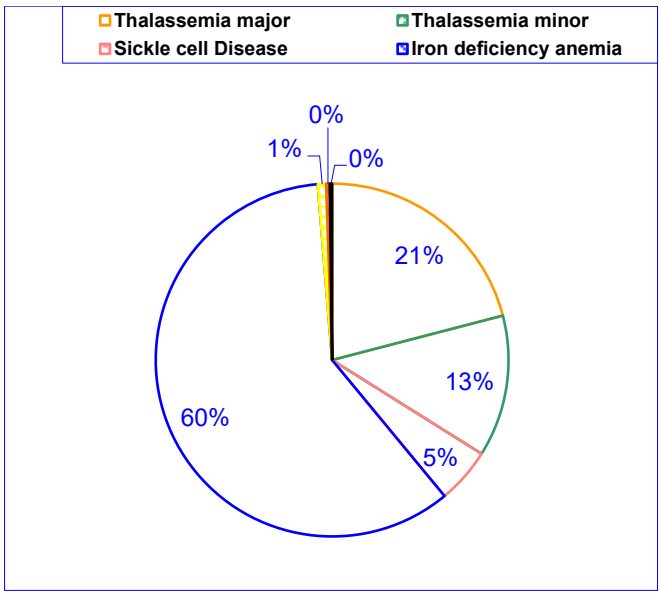

Figure 1. The pie distribution showing prevalence of common hematological disorders found in the population of (2,02,600) Karachi (different ethnic groups). 6 % had one or more combination of these disorders.

The cellulose acetate electrophoresis of hemoglobin at pH 8.6 was performed and the beta thalassemia minor was identified by measuring Hb A2 and the result was compared with control cases shown as in figure 2. The clinical characteristics of these cases and the hematologically normal group are given in Table 1.Those with beta thalassemia were less anemic when compared with those with iron deficiency and had higher RBC counts and a greater degree of microcytosis; however, the MCHC was lower in iron deficiency.

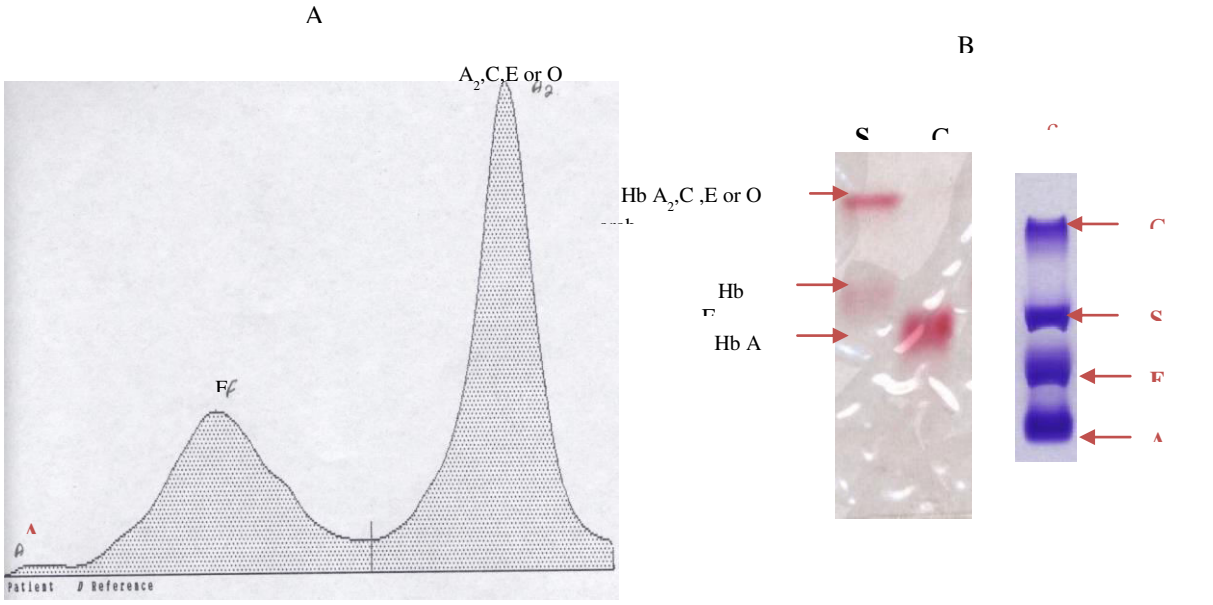

Figure 2: Densitometry quantitation of electrophoretical strip showing the increased Hb A_2 level with Hb F and low Hb A. In (b) the cellulose acetate strip is shown along with the control strip

Discussion

In Southeast Asian countries, including India and Pakistan the other major health problems were infection and malnutrition (*5*). The discrimination between iron deficiency and thalassemia has an important clinical implication. Microcytosis is a classical laboratory feature of thedisorders of hemoglobin synthesis such as iron deficiency or the thalassemias and may be seen in the anemias of chronic disorders, plumbism, and other rare types of anemia. (1 and 6).

Therefore, a reliable diagnosis is needed in order to reduce unnecessary laboratory testing and avoid inappropriate treatment. A wide range of parameters are available to facilitate this differentiation between iron deficiency and thalassemia. However, no single marker or any combination of tests will be optimal for this discrimination.(7) Iron deficiency often occurs in combination with other diseases that complicate the differential diagnosis. It regulates the Hb A2 synthesis, resulting in reduced HbA2 levels in patients with iron deficiency. An increment in the HbA2 level is, so far, the most significant parameter for identifying β-thalassemia carriers.(8) On the other hand, patients with thalassemia and concomitant iron deficiency may show normal or low HbA2 levels. Hence, diagnosing patients with concomitant thalassemia and iron deficiency is even more challenging. Thalassemias are common recessive autosomal disorders. β-thalassemias posses heterogeneity at the molecular level, with > 150 molecular defects identified till date.(9) Despite of this, every "at-risk" population has its own spectrum of some common mutations, usually from 5 to 10.(10). A homozygosity for β thalassemia leads to THALASSEMIA MAJOR, which is often "Transfusion-Dependent" and, rarely "Non-Transfusion Dependent" in mild conditions (molecular diagnosis is used to define genotypes with mild forms). Application of the CBC indices is recommended for screening iron deficiency and β-thalassemia. The main idea of using different indices in microcytic hypochromic anemia discrimination is to screen the patients having a high probability of requiring appropriate

follow-up to reduce unnecessary investigations and costs.

Conclusion

The present study was performed to promote screening programs to detect thalassemic heterozygotes (β-thalassemia trait). We used common hematological indices (included in a routine CBC). These indices were found to be highly specific, sensitive and most accurate method for detecting β-thalassemia trait. The main idea of our study was to create awareness and to focus on pre-marital counseling so that any two individuals before getting married should undergo thalassemia screening in order to have a healthy progeny. It is reported that annually, over 9 million β-thalassemia carriers give birth to a new one.

Acknowledgements

This work was not supported in part by a grant. All the data was collected from Pathological & Molecular lab and the patients sample was collected for the routine or referred by consultant and these patients came to lab for the diagnosis.

Reference

1. Cunningham LO, Rising JA: Erythrocytic microcytosis: Clinical implications in 100 patients. Am J Med Sci 1977; 273:149-155
2. Pearson HA, O'Brien RT, Mcintosh S: Screening for thalassemia trait by electronic measurement of mean corpuscular volume. N Engl J Med 1973; 288:351-353
3. Walford DM, Deacon R; Alpha-thalassemia trait in various racial groups in the United Kingdom: Characterization of a variant of alpha-thalassemia in Indians. Br J Haematol 1976; 34:193-206
4. Ou Z, Li Q, Liu W, Sun X. Elevated hemoglobin A2 as a marker for β-thalassemia trait in pregnant women. Tohoku J Exp Med. 2011; 223(3):223-6.
5. Fucharoen, S. and Winichagoon, P.,(1992). "Thalassemia in Southeast Asia: Problems and strategy for prevention and control." Southeast Asian J. Trop. Med. Public Health, 23(4): 647-655.
6. Okuno T, Chou A: The significance of small erythrocytes. Am J Clin Pathol 1975; 64:48-52.
7. Aulakh R, Sohi I, Singh T, Kakkar N. Red cell distribution width (RDW) in the diagnosis of iron deficiency 5. with microcytic hypochromic anemia. Indian J Pediatr. 2009 Mar;76(3):265-8.
8. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int. 2002 Dec;44(6):612-6
9. Schoorl M, Schoorl M, Linssen J, Villanueva MM, NoGuera JA, Martinez PH,Bartels PC. Efficacy of advanced discriminating algorithms for screening on iron-deficiency anemia and β-thalassemia trait: a multicenter evaluation. Am J Clin Pathol. 2012 Aug;138(2):300-4
10. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008 Aug; 30(4):324-330.