Original Article

Detection of prevalence of β-thalassemia trait in children attending a tertiary pediatric university hospital for non-hematological disorders using red blood cell indices

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ABSTRACT

Background: Red cell indices have been reported to have a major role in detecting β-thalassemia carriers among children with hypochromic microcytic anemia, but still population studies show controversy on the choice of red cell indices and the cutoff values. **Objective:** The objective of this study was to detect the percentage of β-thalassemia carriers among children attending tertiary pediatric university hospital for non-hematological diseases and to test the sensitivity and specificity of different indices and formulas derived from automated hematology analyzer in detecting thalassemia carriers to determine the best index for screening in this age group. **Materials and Methods:** A cross-sectional study was conducted which included 200 children attending tertiary pediatric university hospital with non-hematological disorders for 6 months. Complete history was recorded for all children and complete blood picture was analyzed on Advia Sysmex analyzer, hemoglobin (hb) electrophoresis on Sebia Capillarys 2 instrument, and iron study in the form of serum iron and total iron-binding capacity. Gene mutation analysis for β-thalassemia was done by sequence-specific oligonucleotide hybridization for the common mutations for selected cases. **Results:** We detected a percentage of 7.5% β-thalassemia trait (TT) and 20% iron deficiency (ID) anemia among studied children. Hb distribution width is the parameter most useful in differentiating ID anemia and TT while Ehsani index was the best calculated index with the highest sensitivity and specificity. **Conclusion:** There is a lot of interpopulation difference in discriminative power of indices and cell parameters in differentiating ID and TT in children. We advocate larger studies in selected subpopulations to discriminate both diseases.

Key words: *Index, Iron deficiency, Sensitivity and specificity, β-thalassemia*

Thalassemia syndromes are the most common single-gene disorder worldwide. About 3% of the world population (150 million) carries the thalassemia genes. In Egypt, β-thalassemia is the most common genetically determined, chronic, hemolytic anemia, and carrier rate are estimated to be 8.5–10% [1,2]. Screening programs have been adapted to meet the needs of different communities and seem to reflect acceptable cultural practices. Screening is either mandatory for all couples before marriage or voluntary in high schools, before pregnancy and antenatally [3]. However, Egypt has a high level of consanguineous marriages which prevents proper premarital screening. Another obstacle is the high cost of genetic testing compared to the target population size and country resources. Apart from the socioeconomic burden, the quality of life of β-thalassemic patients is far behind their healthy peers [4].

Screening for β-thalassemia is technically difficult, mainly due to its genetic heterogeneity and the absence of a single pathognomonic finding to cover all variants. Despite these difficulties, many screening attempts have been made where differentiation from iron deficiency (ID) was most important. The most reliable methods for diagnosis of thalassemia trait (TT) include quantitative determination of hemoglobin A2 (HbA2), hemoglobin F, globin chain synthetic ratios, and DNA studies for specific mutations. These methods, though accurate, could not be a tool for initial mass screening [5,6]. Indices can be used in this context to detect subjects who would require appropriate follow-up and reduce unnecessary costs. The data published for younger age groups regarding discriminative power of indices are less, compared to adults. An ideal discrimination index has variable sensitivity and specificity in different ethnicities [2,7].

There is a paucity of data regarding screening the pediatric age group as most of the successful screening programs that led to the eradication of the disease were done in adulthood as mandatory premarital programs [8]. This makes it important to set the proper values on which the screening is made and to test the already validated indices on the current population, especially when attempting to screen the pediatric group. The study was conducted to test the validity of different blood indices and formulas derived from automated hematology analyzer in predicting β-TT (BTT) and differentiate it from ID.

MATERIALS AND METHODS

A cross-sectional descriptive study was conducted in Elshat by pediatric hospital and the subjects were screened for the duration

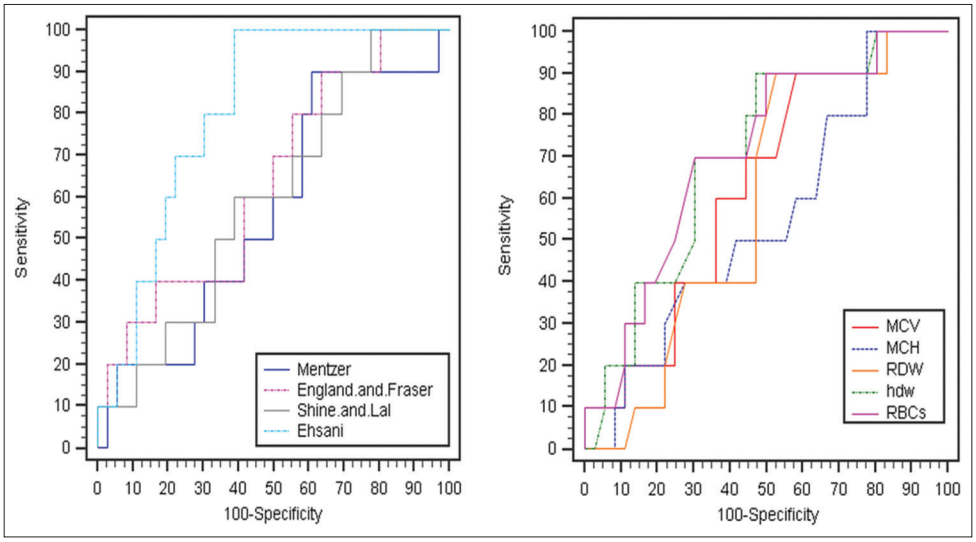

Figure 1: Receiver operating characteristic curve for different parameters to predict thalassemia trait versus iron deficiency

of 6 months starting from January 2017. A random selection of 200 children was done based on their non-hematological complaints such as infections, allergies, and rickets. Exclusion criteria were having a sibling with thalassemia major and chronic illnesses such as renal, cardiac, hepatic, and tuberculosis. Complete history was taken for all children. Complete blood picture was done on Advia Sysmex analyzer, Hb electrophoresis on Sebia Capillarys 2 instrument, iron study in the form of serum iron, and total iron-binding capacity (TIBC). Mutation analysis for β-thalassemia was done by sequence-specific oligonucleotide hybridization for the common mutations for selected cases.

Children were classified into microcytic and non-microcytic anemic patients, if Hb was <11 gm/dl and mean corpuscular volume (MCV) value <70 fl. For Hb electrophoresis, a cutoff was taken of 3.2% above which patients were classified as TT. Cases with HbA2 3.2–3.5% were subjected to gene mutation analysis. Children with serum iron of <55 mg/dl with a normal electrophoresis were diagnosed as ID anemia after documenting response to iron therapy. For the ID cases, electrophoresis was repeated after completion of iron therapy. The following indices were used to classify patients: Mentzer (MCV/RBC), England and Fraser (MCV–RBC – 5 × Hb – 3.4), Shine and Lal (MCV2 × MCH/100), Ehsani (MCV-10 × RBC), Srivastava (MCH/RBC), Green and King formula MCV2 × Red blood cell distribution width (RDW)/Hb × 100, and RDWI RDW × MCV/RBC [9]. The study protocol was approved by the ethics committee at Alexandria Faculty of Medicine, and an informed consent was obtained from all caregivers of the included children as participation was voluntary.

Data were entered and analyzed using IBM SPSS version 20. Data were described using number, percentage, mean median, and range according to variable type. Comparison between groups was done using Chi-square and Fisher's exact test for qualitative data and Kruskal–Wallis and Mann–Whitney tests for quantitative data as data were not normally distributed. Analysis was done at 5% level of significance. Accuracy of different studied indices for diagnosis of TT, namely, sensitivity, specificity, and area under the curve was tested at different coordinate points to verify the most accurate cutoff values using a receiver operating characteristic curve. Significance was judged at 0.5.

RESULTS

The age of the studied children ranged from 1 to 15 years with a median of 2.5 years. The majority of them (86%) aged from 1 to 5 years. Based on the results of hemoglobin (Hb) level, Hb electrophoresis and serum iron studies were done. Of studied children (n=200), the number of confirmed BTT children was 15/200 (7.5%) while the number of ID was 40/200 (20%). Four cases (2%) were common between these two groups.

Eleven cases were confirmed to be TT while 36 cases showed typical ID profile with response to treatment. After iron treatment and reassessment, another four cases, which were categorized as ID proved to be of BTT. Only one case of 16 cases with Hb A2 >3.2 showed normal genetic pattern. Therefore, the number of confirmed BTT children was 15/200 (7.5%) while the number of ID was 40/200 (20%). Four cases were common between these two groups. Our cases were, therefore, divided into four groups; Group A which was normal regarding iron profile and Hb electrophoresis, Group B with ID anemia, Group C with BTT, and Group D with both.

With respect to Hb level, statistically significant difference was detected between Group A (11.7±1.3 g/dl) and Groups B and D (9.9±1.5 g/dl). No significant difference was found between Groups A and C. With respect to RBCs count, a highly significant difference was detected between Group A ($4.6\pm0.5 \times 10^6/mm^3$) and Group C ($5.2\pm0.5 \times 10^6/mm^3$). In addition, a highly significant difference was noticed between Group B ($3.9\pm0.5 \times 10^6/mm^3$) and Group C ($5.2 \times 10^6\pm 0.5$). RDW was highest in Group B followed by Group C and then Group A 14.9 (12.3–20.4), 14.5 (12.7–17.2), and 12.6 (10.6–137), respectively. Comparing MCH in different groups, all Groups B, C, and D were significantly lower compared to Group A. Similarly,

MCV was lower in the three groups compared to normal children. Hb distribution width (HDW) showed statistically significant difference between Group B and Groups A, C, and D (mean of 3.5 vs. 2.8, 2.8, and 2.9). We detected cutoffs for different cell indices to discriminate ID and TT. We found that HDW at a cutoff of 2.94 shows 90% sensitivity, 52% specificity, and area under the curve (AUC) of 0.682, while RBC count showed 90% sensitivity and 50% specificity at a cutoff of 4.82 × 10^6/mm^3, AUC of 0.691. RDW showed 91% sensitivity and 47% specificity, 0.588 AUC.

We further calculated some of the different indices previously studied including Mentzer, Shine and Lal, Ehsani, Srivastava, England and Fraser, and Green and King. The highest sensitivity and specificity were for Ehsani which showed a sensitivity of 100% and specificity of 61% at a cutoff of 17.5. This was followed by Mentzer index at a cutoff of 14 showing sensitivity of 90% and specificity of 39%. The other indices could separate groups with relatively low specificities (Fig. 1).

DISCUSSION

β-thalassemia has been the most common type of hereditary anemia, with a carrier rate of approximately 10%. Of 1.5 million live births, approximately 1000 babies are born with β-thalassemia major annually. In Egypt, the best age for screening for thalassemia remains debatable, in addition to many difficulties including social stigma and religious considerations. Another obstacle to the screening is the costly genetic testing and the lack of a single test or index to spot TT with efficient sensitivity and specificity.

We aimed at detecting the percentage of thalassemia carriers among children attending Elshat by pediatric hospital excluding children with hematological disorders and siblings of children with β-thalassemia major. We also aimed at detecting the best index obtained from complete blood count (CBC) for screening in this age group.

All 200 children tested had undergone a CBC on a Sysmex machine and were classified as anemic or non-anemic according to Hb level of 11 g/dl and were classified as a microcytosis by a cutoff of 70 fl. The microcytosis group had 58/200 (29%) children. All children were subjected to Hb electrophoresis and iron study. Cutoff for HbA2 was taken as 3.2 and further genetic testing was done for borderline cases. HbA2 reference range was taken as up to 3.2% which was previously studied and validated as such on Sebia Capillarys 2 [10]; therefore, we took this value as current reference but still did genetic testing for cases whose HbA2 was between 3.2% and 3.5%. Iron studies done were serum iron and TIBC which showed efficiency in screening for ID in pediatric population as reported by Vicinanza *et al*. [11].

Of the studied cases, 11 were confirmed TT (5.5%), 36 children (18%) showed ID anemia while four children showed both diseases (2%). Seven cases of the microcytic group showed both normal iron profile and HbA2. The total prevalence of BTT in our population was, therefore, 7.5% and ID 20%. The rate we detected for ID was much lower than reported by an Egyptian investigator who reported an incidence of 64% among children from 6 months to 12 years of age [12], while the prevalence of β-thalassemia was similar to published data [2]. A study done by Chung King Ling on a larger population all with thalassemia minor stated that 31% of cases had coexisting ID. We hereby determine similarly that 26.6% of current TT patients had concomitant ID [13].

We further tested the indices derived from CBC parameters to discriminate anemia of ID and TT. Similar to Vehapoglu *et al.*, we showed that RBC count which was previously considered a perfect index for differentiation is not a reliable tool for differentiation of ID and BTT as we showed that RBC count had only 50% specificity. This may be explained by Aslan and Altay observations, who reported that high erythrocyte count is common in ID anemia in infants and young children [14,15].

We showed that HDW at the level of 2.94 or less can differentiate BTT from iron deficiency anemia (IDA) with sensitivity 81.8 and specificity 55.5. The HDW was superior to RDW in differentiation of cases of ID and BTT (AUC of 0.682 vs. 0.588). This is explained by Liu *et al*. who stated that the erythrocyte CH content is constant through different sizes of RBCs and concentrations of Hb for a particular individual, as demonstrated by direct measurement [16]. d'Onofrio *et al.* also showed that the CH is largely unchanged through reticulocyte maturation while the MCV decreases by approximately 20%. CH is a better reflection of the state of hemoglobinization during erythropoiesis; therefore, the cell hemoglobin distribution width is a better indicator of variability than RDW [17].

The current study showed that Ehsani index was superior to other indices in differentiating IDA and BTT (100% sensitivity and 61% specificity), superior to Mentzer index (90% sensitivity and 37% specificity). Contrary to this study, another done by Nalbantoğlu *et al.* showed that the highest sensitivity was obtained with Shine and Lal (87.1%), while the highest specificity was obtained with E&F formula (100%) [18]. A study on Chinese children showed that Green and King index had the highest reliability, whereas Srivastava index and Shine and Lal index had lowest reliability [19]. Another study in pediatric population showed that none of the indices showed sensitivity and specificity of 100% in the patients older than 10 years, and in the patients younger than 10 years, only Shine and Lal index showed sensitivity close to 90% and specificity of 100%. The most accurate discriminative index for patients younger than 10 years was Shine and Lal and for those older than 10 years, it was RDW index. This would probably explain the contradictory results we got as current population involved both groups of children above and below 10 years of age [20]. Another recent study showed that sex differences should be respected when studying indices for discriminating ID and TT [21].

Ferrara *et al.* studied the formulae in differentiating BTT and ID and concluded that none of RBC indices or formula appears reliable to discriminate between BTT and ID subjects [22]. Studies in pediatric age groups are scarce and their results are conflicting. Differences in the effectiveness of various RBC indices for discriminating β-TT from IDA could be attributed to differences in the mutation spectrum of the thalassemia disease

in different populations [23]. Age and sex differences also affect these indices.

The current population was selected randomly; therefore, we recommend further selection of larger populations detected as ID and TT and retrospectively examining their CBCs to determine cutoffs and test the efficiency of all known indices on the current pediatric population. It is also recommended to test the efficacy of using multiple indices and incorporating them into CBC machines to give a definitive diagnosis for microcytic anemia.

CONCLUSION

Hemoglobin distribution width is the best parameter obtained from complete blood picture to differentiate between children with TT and those with ID, while Ehsani index proved to be the best index in our studied population.

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