Evaluation of a Modified Hematological Index to Differentiate between Iron Deficiency Anemia and Beta-Thalassemia Minor

Thesis

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Sally George Hanna Esehac

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List of Abbreviations

Abb.	Full term
ACD	*Anemia of chronic diseases*
AHSP	*Alpha hemoglobin stabilizing protein*
ANOVA	*One Way Analysis of Variance*
AUC	*Area under curve*
BTm	*Beta thalassemia minor*
CBC	*Complete Blood Count*
Chr	*Reticulocyte Hemoglobin Content*
FEB	*Free Erythrocyte Protoporphyrin*
Hb	*Haemoglobin*
Hct	*Hematocrit*
HPLC	*High performance liquid chromatography*
ID	*Iron deficiency*
IDA	*Iron deficiency anemia*
IQR	*Inter-quartile ranges*
K2-EDTA	*Potassium-ethylene diamine tetra acetic acid*
M/H ratio	*Ratio between percentage of microcytes & percentage of hypochromic cells*
MCH	*Mean corpuscular haemoglobin*
MCHC	*Mean corpuscular haemoglobin concentration*
MCV	*Mean corpuscular volume*
NPV	*Negative predictive value*
PPV	*Positive predictive value*
PQC	*Protein quality control*
RBC	*Red blood cell*
RBC-He	*Hemoglobin content of RBC*

List of Abbreviations (Cont...)

Abb.	Full term

RDW.....: RBC size distribution width
RETHe.....: Hemoglobin content of reticulocytes
ROC.....: Receiver operating characteristic curve
Tf.....: Transferrin
TfR.....: Transferrin receptors
TIBC.....: Total iron binding capacity
WHO.....: World Health Organization

Abstract

Background: Iron deficiency anemia and beta thalassemia minor are the most common causes of microcytic hypochromic anemia in childhood. It is essential to differentiate between them to avoid over treatment with iron and for genetic counseling to prevent thalassemia major. In this study we are evaluating the diagnostic reliablilty of different red blood cell indices in differentiation between both types of anemia. **Aim:** To determine the discriminating index with the highest efficiency in differentiating iron deficiency anemia from thalassemia minor to avoid missing the diagnosis of beta thassemia minor cases and save time and money. **Methods:** A cross sectional 6 months study; in patients aged 1-18 years with equal sex distribution with microcytic hypochromic anemia was done. A total of 110 patients were enrolled in the study. 50 patients had beta thalassemia minor (enrolled from referred cases to pediatric hematology clinic), 50 patients had iron deficiency anemia (enrolled from outpatient clinic) and 10 patients had both types of anemia. The three groups were compared as regard clinical presentation and investigations. Using an automated program, we calculated 9 discriminating indices and a new score; 11 T score based on the results of these 9 indices in addition to RBC count and RDW. The accuracy, sensitivity and specificity of different red blood cell parameters and discriminating indices were estimated. **Results:** No significant difference in clinical presentation was recognized except for positive family history of thalassemia. 11 T score had the highest accuracy 94.9% with sensitivity 88% and specificity 92%, followed by Green and king index then RDWI with accuracy 93.2% and 92.8% respectively. The single red blood cell parameter with the highest accuracy was red blood cell count with accuracy 82%. **Conclusion:** 11 T score is the best method to initially differentiate between iron deficiency anemia and thalassemia minor cases, followed by Green and King index and RDW index. RBC count has highest accuracy among different RBC parameters, yet its accuracy is lower than most calculated discriminating indices.

INTRODUCTION

Anemia affects about 800 million children and women worldwide ***(Matos et al., 2016)***. The most common microcytic hypochromic anemias are iron deficiency anemia (IDA) and beta thalassemia minor (BTm) ***(Sharma et al., 2015)***.

According to World Health Organization (WHO) estimates in 2004, IDA resulted in 273,000 deaths and the loss of 19.7 million disability adjusted life years, accounting for 1.3% of the global total, with 97% occurring in low and middle income countries ***(Matos et al., 2016)***. The developmental delay associated with iron deficiency motivate pediatricians to diagnose the iron deficient child as early and accurately as possible, ideally before the development of IDA ***(Hatoun et al., 2014)***.

Beta thalassemia minor (BTm) is commonly seen in Mediterranean region, Southwest Europe and Middle East ***(Chandra et al., 2016)***. Individuals with BTm are usually asymptomatic and may be unaware of their carrier status unless diagnosed by testing ***(Vehapoglu et al., 2014)***.

Differing management guidelines (e.g. iron supplementation) warrant correct differentiation between IDA and BTm ***(Sumera et al., 2012).*** A definitive differential diagnosis between these two types of anemia is based on the

results of hemoglobin electrophoresis and iron profile ***(Batebi et al., 2012)***.

While being accurate, these tests are too expensive and time-consuming for initial mass screening ***(Miri-Moghaddam and Sargolzaie, 2014)***. Several mathematical formulae have been used for identification of BTm, based on red blood cell indices ***(Sumera et al., 2012)***.

The purpose of using indices to discriminate anemia is to detect subjects who have a high probability of requiring appropriate follow-up and to reduce unnecessary investigative costs ***(Vehapoglu et al., 2014)***. Many authors calculated sensitivity and specificity of these discrimination indices in distinction between IDA and BTm. However, none of these indices has a sensitivity and specificity of 100% ***(Demir et al., 2002)***.

In this study we will reassess the accuracy of different red blood cells parameters provided by routine complete blood picture and calculated discriminating indices to differentiate between IDA and BTm.

AIM OF THE WORK

To determine the discriminating index with highest efficiency in differentiating between iron deficiency anemia and thalassemia minor in order to:

- Avoid missing the diagnosis of beta thassemia minor cases.
- Save time and money.

Chapter 1

BETA THALASSEMIA

Definition of beta thalassemia syndromes:

The β-thalassemias are genetic disorders of impaired hemoglobin synthesis characterized by deficient (β+) or absent (β0) synthesis of the β-globin subunit of hemoglobin molecule ***(Galanello and Origa, 2010)***. This results in imbalanced globin chain production, ineffective erythropoiesis, and hemolytic anemia ***(Pognatti and Galanello, 2009)***. Variable phenotypes are observed, ranging from severe anemia to clinically asymptomatic carriers ***(Galanello and Origa, 2010)***.

Structure of hemoglobin:

It is a conjugated protein which consists of globin that is tightly bound to four heme molecules. Globin is a protein with four polypeptide chains joined together by non covalent bonds. Several different types of hemoglobin are normally found in humans. They differ in the primary structure of the peptide chains of globin ***(Ahmed et al., 2002)***.

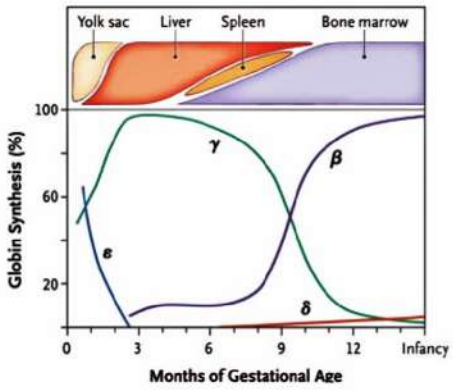

Figure (1): Molecular regulation of the fetal-to-adult hemoglobin switch ***(Sankaran, 2010)***.

Transient embryonic hemoglobins include Hb Portland (ζ2γ2), Hb Gower 1 (ζ2ε2) and Hb Gower 2 (α2ε2) ***(Weatherall and Clegg, 2001)***. Hemoglobin F (α2γ2) is the main hemoglobin of fetal life and comprises the major proportion of hemoglobin found at birth. It accounts for only 1% of adult human hemoglobin ***(Manca and Masala, 2008).***

Hemoglobin A is the major hemoglobin in adult (97%). Its globin contains two α-chains and two βchains (α2β2). Each polypeptide chain has a helical structure and folded into eight stretches labeled from A to H creating a pocket inside it for heme binding ***(Manca and Masala, 2008).*** Hemoglobin A2 (α2δ2) accounts normally for about 2% of adult hemoglobin ***(Nagel and Steinberg, 2001)***.

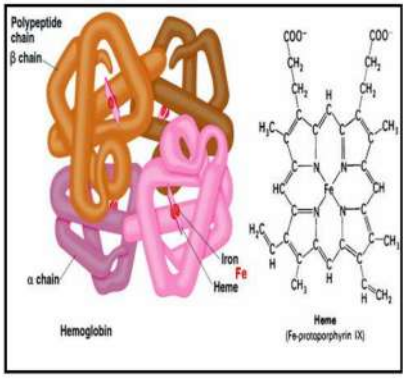

Figure (2): Structure of hemoglobin ***(Yamada, 2004)***.

Pathogensis:

The molecular defects in β-thalassemia result in absent or reduced β-chain production, while alpha chain synthesis is unaffected. The inbalance in globin chain production leads to an excess of alpha chain. Free alpha-globin chains are highly unstable, precipitate in red cell precursors and form intracellular inclusions which interfere with red cell maturation ***(Giardina and Rivella, 2013)***.

Thalassemia syndromes are heterogeneous due to the different possible mutations affecting the human globin chain loci. These mutations include gene deletions, as well as globin chain initiation, translation and termination ***(Clegg et al., 1968; Nathan et al., 1971).***

The clinical syndromes associated with thalassemia arise from combined effects of inadequate hemoglobin production and unbalanced accumulation of globin subunits. The former

causes microcytosis and hypochromia; the latter leads to ineffective erythropoiesis ***(Griffin, 2000)***.

Following synthesis, α-globin forms a complex by binding to its carrier protien, α-hemoglobin stabilizing protein (AHSP) ***(Weiss and dos Santos, 2009)***. AHSP facilitates folding of α-globin and prevents formation of misfolded aggregates. α-Globin mutations that impair interaction with AHSP are associated with microcytosis and anemia in humans ***(Yu et al., 2009)***. Loss of AHSP has also been shown to impair erythropoiesis ***(Kong et al., 2004)***. Evidence suggests that AHSP levels may affect the phenotype of β-thalassemia ***(Lai et al., 2006)***.

Once the capacity of AHSP is exceeded, α-globin forms aggregates and inclusions that damage cell membrane and membranes of intracellular organelles. Aggregated alpha chains also trigger the formation of reactive oxygen species, which also damage the protein and lipid contents of cell membranes ***(Rund and Rachmilewitz, 2005)***.

The formation of alpha chain inclusions occurs early during erythropoiesis and reaches the peak in the polychromatophilic erythroblasts, leading to cellular apoptosis ***(Mathias et al., 2000)***. Almost all cells have some capacity to detoxify and remove damaging proteins through multiple biochemical pathways called protein quality control (PQC) which participate in the degradation of alphan globin, but their