



Diagnostic Utility of Red Blood Cell Indices-Derived Formulas in Discriminating Beta-Thalassemia Trait from Iron Deficiency Anemia

Thesis

*Submitted for Partial Fulfillment of
Master Degree in Clinical Pathology*

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2020

Acknowledgments

*First and foremost, I feel always indebted to **God** the Most Beneficent and Merciful.*

*I wish to express my deepest thanks, gratitude and appreciation to **Professor/ Amany Ahmed Osman**, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her meticulous supervision, kind guidance, valuable instructions and generous help.*

*Special thanks are due to **Professor/ Deena Samir Mohamed Eissa**, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her sincere efforts, fruitful encouragement.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Special dedication to the soul of my late father without whom nothing of this was possible. First of all my mother whose effort and love were the cornerstone all the way ever since my childhood. My husband who bore with me all the difficulties and whose encouragement that made me continue. My sister who helped me with every thing and bore tough times with me. Lastly, my little son Gaby the gift sent to me by God to give a meaning for all of this.

Maggie Ibrahim Shoukry

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List of Abbreviations

Abb.	Full term
APOEϵ4	<i>Apolipoprotein E ϵ4</i>
AUC	<i>Area under the curve</i>
CA	<i>Cellulose acetate</i>
CHr/Ret-He	<i>Reticulocyte hemoglobin content</i>
CKD	<i>Chronic kidney disease</i>
Col1A1	<i>Alpha-1 type I collagen</i>
CRP	<i>C- reactive protein</i>
DMT	<i>Divalent metal transporter</i>
EKLF	<i>Erythroid Krüppel like factor</i>
ERSD	<i>End stage renal disease</i>
ESA	<i>Erythropoietin stimulating agents</i>
Fe	<i>Iron</i>
Fe2+	<i>Ferrous iron</i>
Fe3+	<i>Ferric iron</i>
Hb	<i>Hemoglobin</i>
HBB	<i>Hemoglobin subunit beta</i>
HCP	<i>Heme carrier protein</i>
HCT	<i>Haematocrit</i>
HFE gene	<i>High Iron Fe (hemochromatosis gene)</i>
HPFH	<i>Hereditary persistence of fetal hemoglobin</i>
HPLC	<i>High performance liquid chromatography</i>
IDA	<i>Iron deficiency anemia</i>
IRIDA	<i>Iron-refractory iron-deficiency anemia</i>
IV	<i>Intravenous</i>
LCR	<i>Locus control region</i>
LED	<i>Light-emitting diode</i>
MCH	<i>Mean red cell hemoglobin</i>
MCHC	<i>Mean corpuscular hemoglobin concentration</i>
MCHD	<i>Mean cell Hb density</i>
MCV	<i>Mean red cell volume</i>
MDHL	<i>Mean density of Hb / litre of blood</i>

List of Abbreviations *cont...*

Abb.	Full term
Nitroso-PSAP	<i>2-Nitroso-5-(N-propyl-N-sulfopropylamino) phenol</i>
NRBC	<i>Nucleated red blood cells</i>
Oesr1	<i>Oxidative Stress Responsive Serine Rich 1.</i>
PPIs	<i>Proton-pump inhibitors</i>
RBC	<i>Red blood cell</i>
RDW	<i>Red cell width distribution</i>
RDWI	<i>Red cell distribution width index</i>
ROC	<i>Recover operating characteristics</i>
SLS	<i>Sodium lauryl sulphate</i>
TEB	<i>Tris / EDTA / borate buffer</i>
Tfn	<i>Transferrin</i>
TFR	<i>Transferrin receptor</i>
TI	<i>Thalassemia intermedia</i>
TIBC	<i>Total iron binding capacity</i>
TM	<i>Thalassemia major</i>
TPTZ	<i>2,4,6-Tri(2-pyridyl)-5-triazine</i>
TSAT	<i>Transferrin saturation</i>
TT	<i>Thalassemia trait (minor)</i>
UGT1A1	<i>UDP Glucuronosyltransferase Family 1 Member A1</i>
VDR	<i>Vitamin D receptor</i>
YI	<i>Youden's index</i>
β-TT	<i>Beta thalassemia trait</i>

INTRODUCTION

The two most frequently encountered microcytic hypochromic anemias are iron deficiency anemia (IDA) and β -thalassemia trait (β -TT) which need relatively expensive laboratory tests to be differentiated (*Ismail et al., 2016*).

It is well known IDA is the most prevalent nutritional disorder in the world (*Stoltzfus and Dreyfuss, 1998*). It is estimated that around 2.15 billion individuals suffer from iron deficiency anemia (*Abalkhail and Shawky, 2002*).

Beta Thalassemia is the most common chronic hemolytic anemia in Egypt (85.1% of all hemolytic anemias). A carrier rate of 9-10.2% has been estimated in 1000 normal random subjects from different geographical areas of Egypt (*El-Beshlawy et al., 1999*).

Considering the great similarity between IDA and β -TT, complementary lab methods are needed besides the routine blood examination. Currently, diagnosis of IDA is obtained by evaluating the iron metabolism, including serum iron, total serum iron binding capacity and serum ferritin measurements. Diagnosis of the β -TT is usually made by hemoglobin electrophoresis and HbA₂ levels being more than 3.5% (*Matos et al., 2016*).

Blood samples from β -TT and IDA subjects are usually associated with microcytosis and/or hypochromia. As early as

1970s, various red blood cell indices and formulas have been used as simple and inexpensive screening approach to differentiate between β -TT and IDA blood samples, to select which requires further investigations for these disorders (*Sirdah et al., 2007*).

To avoid much more expensive, time-consuming, and complicated procedures for discrimination between these disorders, researchers attempt to use either RBC indices such as MCV, MCH, and RDW, or formulas derived from these indices. This process helps to select appropriate individuals for more detailed examination (*Soliman et al., 2014*).

AIM OF THE WORK

This study aims at evaluating the diagnostic utility of different discrimination formulas derived from red blood cell indices from complete blood count in the differentiation of β -TT from IDA.

*Chapter 1***IRON DEFICIENCY ANEMIA****Definitions**

The World Health Organization (WHO) has defined anemia in adults as a hemoglobin of <13 g/dL in males (a hematocrit [Hct] of about 39) and <12 g/dL in females (Hct about 36) (*Barth and Hirschmann, 2007*). Iron deficiency anemia starts with a state called iron deficiency in which reduction of iron stores precedes development of overt iron-deficiency anemia. It may persist without progression or it can progress to a more severe condition i.e. Iron Deficiency Anemia in which low levels of iron are associated with anemia and the presence of microcytic hypochromic red cells in the circulation, the relative number of which reflects the severity of the iron deficiency (*Goodnough et al., 2010*).

Prevalence

Anemia is a widespread problem affecting people worldwide. Iron deficiency and iron-deficiency anemia (IDA) are common medical conditions seen worldwide (*Kassebaum et al., 2014*). The estimated prevalence of iron deficiency worldwide is twice as high as that of IDA. IDA severely affects the lives of young children and premenopausal women (particularly those of low-income or in developed countries) (*McLean et al., 2009*).

In developing countries, iron deficiency and iron-deficiency anemia typically result from inadequate dietary intake and/or blood loss due to intestinal worm colonization, or both. In higher-income countries, certain eating habits such as vegetarian diet and chronic blood loss or malabsorption are the most common causes. Iron deficiency in developed countries is especially high in the elderly (*Kassebaum et al., 2014*).

One study in Egypt found that iron deficiency anemia (IDA, low Hb and low ferritin) was recognized among 18.5% of whole sample population, with high prevalence for mothers (25.1%). Prevalence of iron deficiency without anemia (low ferritin with normal Hb) reach 26.0% in whole population, and adolescents showed highest prevalence (29.4%) (*Tawfik et al., 2015*)

Iron Homeostasis and mechanism of erythropoiesis

Iron (Fe) is crucial to biologic functions, including respiration, energy production, DNA synthesis, and cell proliferation (*Hentze et al., 2010*). All cells need a small amount of iron; however, erythroid precursors require substantial amounts to synthesize hemoglobin. Accordingly, anemia is a prominent manifestation of iron deficiency. Three cell types are important in iron homeostasis: the duodenal enterocytes which absorbs iron, the hepatocyte that serves a depot function (removing excess iron from circulating plasma and safely storing it until it is needed), and the tissue

macrophages that recognize and phagocytose old and/or damaged erythrocytes, recovering their iron for reuse and storage (*Donovan et al., 2006*).

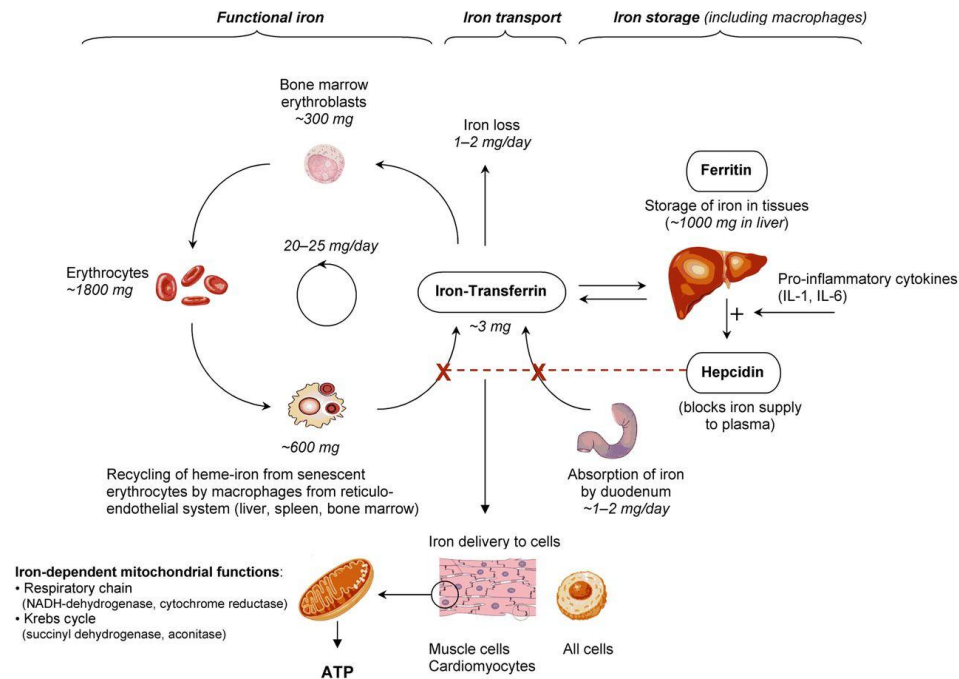


Figure (1): Iron Homeostasis and mechanism of erythropoiesis.

Molecular signals coordinate the operations of each of these cell types. No efficient, regulated excretion mechanism for iron exists, emphasizing the importance of meticulous regulation of iron acquisition and distribution. Since excretory mechanisms for eliminating iron from the body are lacking and excess levels of iron in tissues may be toxic, iron absorption is limited to 1–2 mg daily. About 95% of the iron needed daily (about 25 mg per day in normal state) is provided through the recycling by macrophages that phagocytose senescent erythrocytes (Fig. 1). Fe absorption from water-soluble forms