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Could Reticulocyte Haemoglobin Content (CHr) Help in Determination of Iron Status? Review article

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ABSTRACT

Background: Hemoglobin (Hb) carries oxygen throughout the body and is essential to survival, so maintaining healthy levels of Hb production is crucial. Due to their short lifespan (about 120 days), mature erythrocyte Hb is not a reliable indicator of Hb production. **Objective:** Review of the literature if Reticulocyte haemoglobin content (CHr) help in determination of iron status. **Methods:** We looked for data on Reticulocyte Haemoglobin Content in scholarly journals and databases including PubMed, Google Scholar, and Science Direct. The most recent or extensive studies published between October 2012 and August 2022 was taken into account. The writers also analysed similar works cited in their work. Lack of resources to translate documents written in languages other than English has led to their neglection. It was generally recognized that scientific research did not include research that was not published in a peer-reviewed journal, presented orally, or presented as a conference abstract or dissertation. **Conclusion:** CHr is unaffected by influences outside of iron metabolism and reflects the current state of Hb synthesis. In cases when it is challenging to estimate the ideal iron requirement, such as when providing an erythropoiesis-stimulating drug. CHr testing can help with the early detection of iron deficiency (ESA). Several investigations have shown that CHr detection in peripheral blood samples is a reliable method for identifying individuals with an iron shortage. It has been demonstrated to be a reliable indicator of iron status and a practical method for gauging the success of iron therapy.

Keywords: Reticulocyte haemoglobin content, Iron deficiency anemia.

INTRODUCTION

Children displaying any of the iron deficiency anemia (IDA) symptoms warrant additional evaluation. However, who should be evaluated for IDA given that most kids with the disease don't show any symptoms and pale skin isn't a good indicator? Although the American Academy of Pediatrics suggests starting screenings at the age of one year old, many wealthy countries lack even basic guidelines **(1)**. In the meantime, reticulocytes are released from the bone marrow into the peripheral circulation, where they undergo maturation into erythrocytes over the course of the next two days. Therefore, the reticulocyte Hb content (Hb-ret) may be an indicator of the most up-to-date Hb production status**(2)**.

Furthermore, iron, a crucial component of Hb, is a necessary element for virtually all human cells, with roughly 70% of the body's iron located in the reticuloendothelial system. Hb-ret is regarded a helpful method for evaluating acute iron metabolism in the body, provided that hematopoiesis is normal. Hb synthesis is affected by iron intake **(3)**. An iron deficit can induce anaemia and metabolic issues, while an iron overload can reduce the body's ability to effectively use the iron it already has. Furthermore, iron overload is linked to arteriosclerosis, carcinogenesis, hepatopathy, and diabetes because it causes the production of hydroxyl radicals via the Fenton and Haber-Weiss reactions **(4)**.

Because iron metabolism lacks a functional excretion system, maintaining normal blood iron levels and avoiding excessive iron supplementation are of paramount importance **(5)**. Traditional iron indices can provide a rough estimate of the body's iron status, but they are not always reliable because they are affected by variables outside iron levels. CHr, which may be measured immediately as Hb ret level by H*3 or ADVIA blood analyzers, is therefore anticipated to aid in the evaluation of iron deficiency or excess, leading to the correct prescription of iron deficiency medication **(6)**.

Iron storage, transport, and metabolism:

In a normal human being, the amount of iron is around 4.5 g to 5 g. Roughly 70% of this is found in the reticuloendothelial system (Hb, bone marrow, and reticuloendothelial macrophages), 30% in the liver and spleen as ferritin (the storage iron), and 10% in the muscles as myoglobin **(7)**. Iron metabolism is distinguished by the fact that iron is excreted exclusively through the regular shedding of skin and gastrointestinal mucosal cells. It lacks an active excretion route and has a mostly closed metabolic architecture. Iron absorption occurs predominantly in the duodenum and jejunal epithelial cells, and daily loss of iron is just 1-2 mg **(8)**.

Divalent metal transporter 1 (DMT1) transports nonheme iron to the intestinal epithelial cells, while heme iron is carried in its native form. Ferroportin, which is expressed in the vascular lumen, is responsible for the absorption of both forms of iron into the blood. Ferrous iron, a very toxic type of iron, enters cells through the bloodstream through binding to the membrane protein transferrin receptor (TfR) **(4)**.

Hematopoiesis necessitates an iron content of 0.8-1.0 mg/h, but serum iron level is only 3-4 mg, thus the organism can rapidly enter a state of iron insufficiency if iron is not given constantly into the blood **(6)**. It is the reticuloendothelial system that primarily facilitates the recirculation of iron into the blood, while the intestinal absorption rate is just 1-2 mg/day. In addition, the liver secretes a peptide called hepcidin, which controls iron metabolism.

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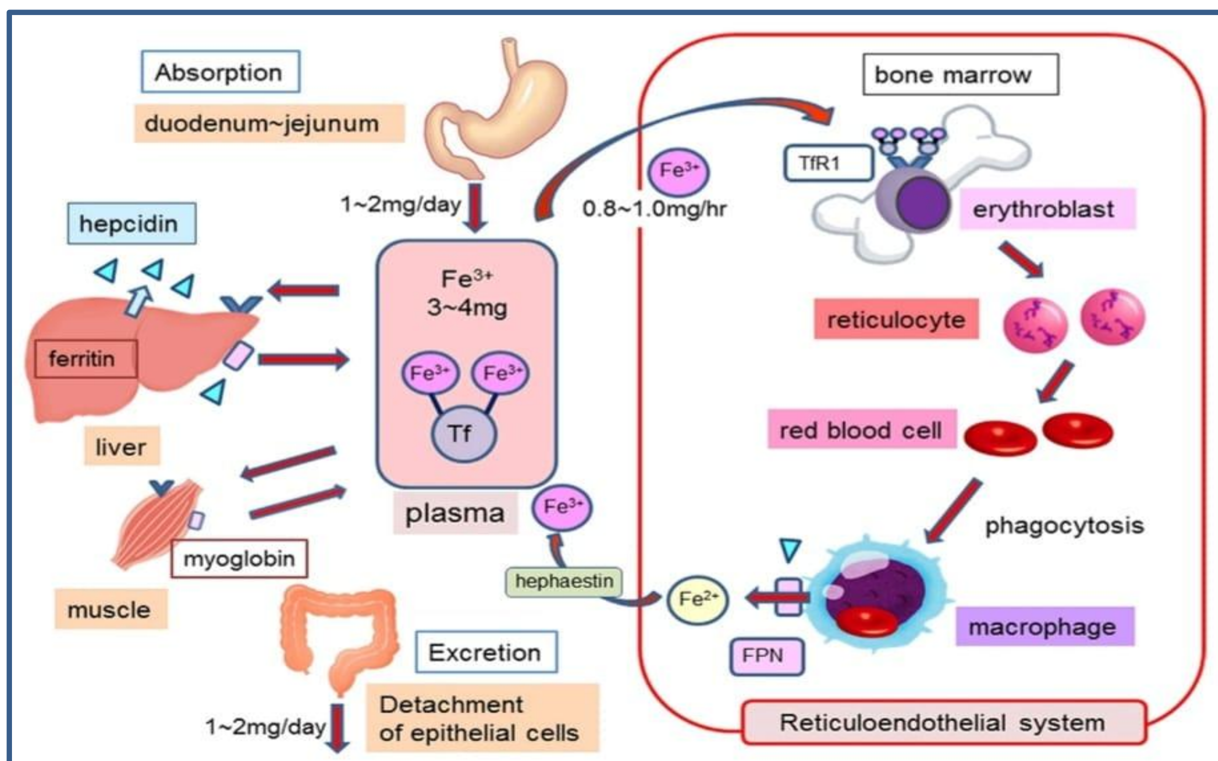

Figure (1): Metabolism of iron [7].

Intestinal cells, reticuloendothelial macrophages, and hepatocytes all release iron, however only ferroportin is the only cellular iron exporter [5].

Hepcidin inhibits ferroportin's ability to transport iron out of cells and into the circulation for about two to three days after attaching to the protein. Functional iron shortage occurs when there is an inadequate supply of iron to the blood despite an acceptable amount of iron in the body due to iron excess or inflammation, when iron and inflammatory signals work together to upregulate hepcidin synthesis [3].

Hb synthesis is bolstered by signals from the hematopoietic system and from hypoxia, which boost iron absorption from the gut and iron delivery to cells. Hepcidin is the principal regulator of iron metabolism at the organismal level, whereas the iron responsive element and iron regulatory protein system are involved at the cellular level [9].

When intracellular iron content is low, intestinal cells generate more of the iron-importing transport proteins TfR and DMT1, and when intracellular iron concentration is high, intestinal cells create more of the iron-storing proteins ferritin and ferroportin. Therefore, ferrokinetics in the body are tightly regulated at both the organismal and cellular levels [6].

Synthesis of erythrocytes:

Beginning as hematopoietic stem cells, erythroid progenitors progress through the stages of proerythroblasts, erythroblasts, reticulocytes, and adult erythrocytes. Renal anemias are caused by a lack of the cytokine erythropoietin, which is mostly produced in the kidney [2]. Further, proerythroblast development relies on transcription factors such hypoxia-inducible factor-1 (HIF-1), which promotes erythropoietin production in low-oxygen conditions, and GATA-binding protein 1 (GATA1), which is involved in heme (Hb) synthesis [4].

Hemoglobin (Hb) synthesis initiates in proerythroblasts and is primarily an erythroblast process throughout hematopoiesis. TfR is membrane-expressed, thus iron can enter cells and be used in Hb production after binding to TfR. Rapid Hb production occurs during the early stages of erythroblast differentiation into basophilic and polychromatic erythroblasts, followed by 4-5 rounds of cell division and subsequent transformation into orthochromatic erythroblasts [10]. They then develop into reticulocytes after undergoing enucleation. A suitable Hb level in reticulocytes cannot be ensured if iron availability is inadequate throughout this phase. It takes 1-2 days for reticulocytes to mature into erythrocytes after being discharged from the bone marrow into the peripheral circulation [5].

Spleen reticuloendothelial macrophages phagocytose adult erythrocytes after about 120 days, recycling the iron they contain. Because of this haematological mechanism, Hb-ret is useful for assessing iron concentration required for Hb synthesis and analysing the current status of Hb synthesis, both of which are difficult to assess using mature erythrocyte Hb alone [11].

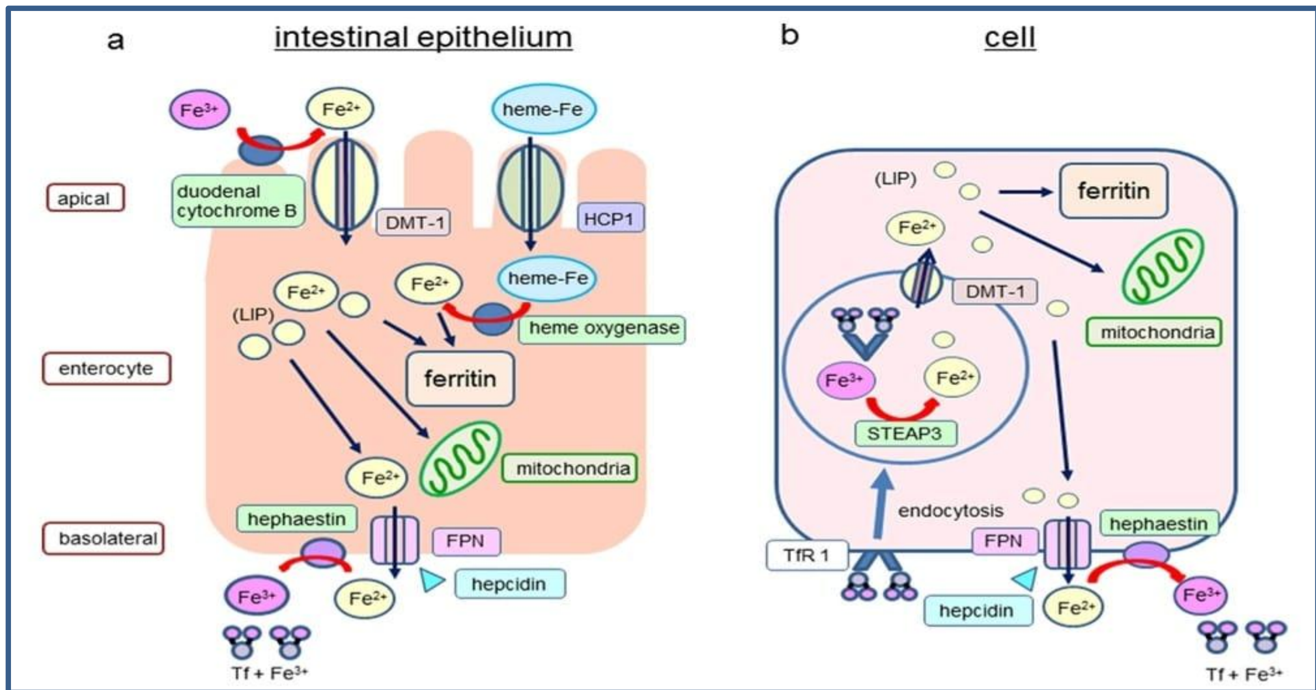

Figure (2): The Role of the Intestinal Epithelium and Cells in Iron Metabolism [7].

Principles of CHr measurement and its unique properties:

The H*3 Blood Analyzer makes it simple and precise to measure CHr, also known as Hb-ret (Bayer Diagnostic, Tarrytown, NY, USA). A reticulocyte measuring reagent (Oxazine 750, buffer, surfactant) is added to the sample mixture and incubated, spheroidizing and immobilising the erythrocytes without altering their volume [12].

Flow cytometry is then used to measure the volume and Hb concentration of each cell in these pretreatment samples, yielding a cytogram of the erythrocytes. Cytograms can tell mature erythrocytes apart from reticulocytes because RNA in the latter appears blue [3].

Reticulocytes' cell volume and Hb concentration are used to derive an average CHr value. As a result, CHr is interpreted as a direct reflection of both the bone marrow's ability to synthesise Hb and the amount of iron available for hematopoiesis [7].

Comparison with other iron indices:

CHr reveals equal to or superior diagnostic power even in persons with stable general health, when standard iron indices for absolute iron deficiency anaemia are somewhat more powerful [2].

New markers representing iron metabolism, such as soluble transferrin receptor (sTfR) and percent hypochromic red cells (percent HYPO), have been described in several investigations. Functional iron deficiency can be diagnosed by sTfR and percent HYPO, however several studies have found that CHr has a higher diagnostic power [4].

Serum ferritin:

Because inflammatory cytokines drive hepcidin production and cause iron trapping in cells, serum ferritin levels rise during inflammation even if total body iron concentration remains same [3].

Serum iron:

Although serum iron levels are inversely proportional to the amount of useable iron, the iron in serum is rapidly depleted. Because serum iron levels are affected by both diet and the diurnal oscillation, it is difficult to determine iron deficiency or excess in the body with a single examination [6].

Total Iron-Binding capacity:

When an individual is genetically predisposed to an iron deficit, the liver's ability to synthesise iron improves, however this ability declines in the face of starvation, inflammation, and diminished liver function. Therefore, the TSAT calculated from the TIBC and serum iron levels is also not a reliable diagnostic tool [2].

sTfR:

The majority of isolated TfR was found in erythroblasts, the cells at the heart of hematopoiesis. Therefore, not only does iron deprivation cause a rise in sTfR levels, but so does increased hematopoiesis [12].

Percentage of hypochromic cells:

Hypochromic mature red cells develop from hypochromic reticulocytes when iron is lacking during hematopoiesis. Their proportion, shown as a HYPO value, represents Hb synthesis directly but does not give a real-time figure because it includes only fully mature erythrocytes in its calculation [14].

Clinical applications of CHr:

Iron deficiency in children:

Iron requirements for growth vary substantially from early infancy to puberty, making it simple for children to become iron deficient if their iron intake does not keep up with these variations. Therefore, from infancy through early adulthood, iron deficiency anaemia is

widely recognised as the most prevalent blood condition worldwide [15].

Even in the absence of anaemia, long-term iron deficiency in young children has been shown to have negative effects on motor skills, intelligence, and mental activity; furthermore, delaying treatment may not improve the condition even with iron supplementation [3].

Adolescents have higher iron needs because of secondary growth, higher iron losses because of menstruation, and a higher prevalence of iron deficiency anaemia because of increased physical activity. Iron deficiency has been linked to worse cognitive performance in children and adolescents, according to a number of studies conducted on the topic [16].

Because of this, research on iron insufficiency in children and adolescents have been undertaken employing CHr to aid in early detection. Based on these studies' findings, it's not required to take into account sexual differences in CHr measurement the way that's done with Hb in healthy children; rather, the mean CHr measured by ADVIA is somewhat greater in adolescence, but there are no sexual differences [2].

Further research is needed, however based on the current findings, CHr has the potential to become a helpful criterion for diagnosing iron insufficiency. These variations may be influenced by age- and iron-deficiency-specific reference values [3].

Signs of IDA include hypochromia and microcytosis in erythrocytes, low levels of serum ferritin and iron, a high total serum transferrin saturation, and an elevated total iron binding capacity. In IDA, a low serum ferritin level is crucial but should not be automatically linked to the disease. The fact that it is a normal level in healthy people does not rule out IDA, however, and the underlying cause must be determined and controlled [6].

In contrast to these circumstances, iron excess decreases iron usage efficiency and increases oxidative stress production. Other dependable laboratory test criteria used to characterise IDA include free erythrocyte zinc protoporphyrin (ER-ZPP), soluble transferrin receptor (sTfR), and reticulocyte haemoglobin content (CHr or Ret-He). Elevated levels of the soluble transferrin receptor in erythrocytes are associated with a rapid decline in iron status and the development of IDA [5].

Important factors in determining sTfR level include erythropoietic activity in the bone marrow and the need for iron intracellularly. Aplastic anaemia is reduced and other forms of iron deficient anaemia (sickle cell anaemia, megaloblastic anaemia, thalassemia, polycythemia, etc.) are associated with elevated sTfR levels [2].

Serum sTfR levels should be between 3.5 and 8.5 mg/L. As an early and sensitive diagnostic for the diagnosis of IDA, a high sTfR level (>8.5 mg/L) is well-established. Diagnosing IDA can also be determined by comparing the sTfR concentration to the logarithmic ferritin level. Anemia due to chronic disease is indicated by a ratio lower than 1, whereas an IDA ratio greater than 2 is considered favorable [4].

A high concentration of ER-ZPP (80 g/dL) in erythrocytes is linked to iron deficiency because low iron levels promote zinc transport in the intestines. However, due to automation challenges, using ER-ZPP measurements on a regular basis is arduous and time-consuming [3].

Hemoglobin in reticulocytes (or CHr) is an indication of cell hemoglobination that reflects the quality of recently formed reticulocytes. Continual reticulocyte production leads to the formation of microcytic, hypochromic RBCs when iron levels are low. Therefore, compared to haemoglobin and hematocrit, RET-He provides an earlier indicator of decreasing haemoglobin status [6].

After Hb synthesis, erythroblasts release reticulocytes into the circulation, where they travel to the periphery and eventually mature into erythrocytes. As a result, CHr should be taken into account as the primary parameter for Hb synthesis in real time. Except in cases of haematological diseases, iron is the single factor influencing the haemoglobin concentration of reticulocytes [17].

By measuring RET-He, iron status can be ascertained. The average forward light scattering intensity of adult red blood cells and reticulocytes stained with a polymethine dye is measured by automated fluorescence flow cytometry to calculate RET-He. The results are a measure of the amount of haemoglobin found in reticulocytes [18].

The haemoglobin concentration of reticulocytes is superior to other measures involved in iron metabolism in terms of its ability to diagnose iron deficiency, determine the presence of early iron deficiency anaemia, and distinguish beta-thalassemia features. CHr is a less variable measure that responds better to intravenous (IV) iron therapy than ferritin, hence it can be used as a more accurate iron diagnosis [16].

Iron deficiency in postpartum:

Increased iron requirement is the usual cause of ID during pregnancy. There is also no agreement on the best target Hb level during pregnancy because of the hemodilution that occurs in pregnancy (up to a 45% increase in the volume of circulating plasma). Furthermore, anaemia commonly develops due to haemorrhage during delivery; hence, it is challenging to separate IDA from other causes of anemias [9].

To address this, a study was done to see if the reaction of CHr and MCV to oral iron preparations could distinguish IDA from other causes of anaemia in postpartum women. Patients with a creatinine clearance (CCr) of 28 pg/mL or an MCV of 80 fL were shown to be at an increased risk for IDA and a favourable response to iron preparations. Patients who do not satisfy these conditions may not gain any health benefits from taking iron supplements and should thus only do so if absolutely necessary [10].

Iron depletion in blood donors:

There have also been attempts to use CHr to identify blood donors with low iron levels. Donating whole blood can result in an iron deficiency because of the loss of 200-250 milligrammes of iron. One patient who received a whole blood transfusion was reported to have experienced restless leg syndrome. However, it is time consuming and costly to measure serum ferritin (a marker of iron stores in the body) [19]. As a result, the value of less complex and less expensive CHr has been calculated. The results demonstrated that a higher detection rate of iron depletion was attained when percent HYPO was combined with CHr as opposed to Hb alone. It has also been found that this combination can help identify blood donors with early signs of functional iron insufficiency [2].

Differentiation of beta thalassemia trait:

Most cases of microcytic hypochromic anaemia can be traced back to either IDA or beta thalassemia trait. Defective generation of beta globulin chains, which are Hb-constitutive proteins, is the cause of beta thalassemia trait, an autosomal dominant condition. The Mediterranean coast, Africa, and Southeast Asia are hotspots for the disease. Because beta thalassemia trait mainly causes minor microcytic hypochromic anaemia, treating it is not necessary [20].

But since costly procedures like Hb quantitative analysis and genetic tests are needed for the diagnosis, it's crucial to find easy-to-use alternatives that can tell the difference between IDA and beta thalassemia phenotype. Studies have assessed CHr's value as a measure of hemopoietic capacity to help with this problem [20].

CONCLUSION

CHr is unaffected by influences outside of iron metabolism and reflects the current state of Hb synthesis. In cases when it is challenging to estimate the ideal iron requirement, such as when providing an erythropoiesis-stimulating drug, CHr testing can help with the early detection of iron deficiency (ESA). Several investigations have shown that CHr detection in peripheral blood samples is a reliable method for identifying individuals with an iron shortage. It has been demonstrated to be a reliable indicator of iron status and a practical method for gauging the success of iron therapy.

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- **Competing interests:** Nil.

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