RETICULOCYTE HAEMOGLOBIN CONTENT'S SIGNIFICANCE IN IRON-DEFICIENCY ANAEMIA DIAGNOSIS: AN OVERVIEW

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Abstract

Background: The utility of identifying CHr in peripheral blood samples for the diagnosis of iron deficiency has been shown in numerous research. It has been demonstrated to be a trustworthy iron marker for determining the effectiveness of iron therapy and providing an accurate evaluation of iron status.

Aim of work: To assess the importance of reticulocyte Hb (CHr) in the diagnosis of IDA and compare CHr with other iron indicators, particularly transferrin saturation and serum ferritin, in order to better manage children with iron deficiency anaemia.

Methods: Our study was a review article, interpreted in the out-patient hematology clinic, pediatrics department, Zagazig University Hospital during six months from September 2022 to February 2023. Approval was asked from the institutional review board (IRB) (9660-26-7-2022). Many studies related to our subject were collected, analyzed and a narrative review of our findings was performed.

Conclusion: The associations between CHr and common haematological and biochemical indicators of iron deficiency anaemia show that CHr can be a reliable indicator of IDA.

Key words: Reticulocyte; Iron; Anaemia; Haemoglobin.

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Introduction:

The most typical form of dietary anaemia is iron deficiency anaemia (IDA). Iron deficiency (ID) is one of the most widespread dietary deficits in the modern world. Since it is well recognized that ensuring an adequate intake of iron throughout growth and development has advantages, it is essential to identify ID early and correctly (1). Ferritin is one of the biochemical markers used to identify IDA. Serum iron, mean corpuscular volume (MCV), and transferrin saturation (TS) are additional measures. Despite the fact that these signs are available, there is ongoing disagreement about how well they work to diagnose IDA (2). Reticulocytes, the first erythrocytes that enter the bloodstream, circulate for one to two days before developing into mature erythrocytes. The amount of iron that is accessible in the bone marrow for the synthesis of haemoglobin is reflected in the reticulocytes' haemoglobin content. Reticulocyte haemoglobin (CHr) concentration has thus been proposed as a marker of iron status (3). The importance of CHr detection in peripheral blood samples for iron deficiency diagnosis has been shown in numerous investigations. It has been shown to be a reliable iron marker for determining the effectiveness of iron therapy and an accurate assessment of iron status (4). In the light of the previously mentioned data, we conducted this study aiming to assess the importance of reticulocyte Hb (CHr) in the diagnosis of IDA and compare CHr with other iron indicators, particularly transferrin saturation and serum ferritin, in order to better manage children with iron deficiency anaemia.

Methods:

Our study was a review article, interpreted in the out-patient hematology clinic, pediatrics department, Zagazig University Hospital during six months from September 2022 to February 2023. Approval was asked from the institutional review board (IRB) (9660-26-7-2022). Many studies related to our subject were collected, analyzed and a narrative review of our findings was performed

Iron deficiency anemia (IDA): a continuous threat

Iron deficiency anemia (IDA) is the most frequent hematological disorder of childhood and adolescence and the most common form of anemia, with an incidence in industrialized countries of 20.1% between 0 and 4 years of age and 5.9% between 5 and 14 years (39 and 48.1% in

developing countries). It is a hypochromic and microcytic anemia characterized by Hb values below the normal range for sex and age, reduced MCV, and MCH (5). Iron is an essential nutrient for the development of the fetus, infant, and child. The body's iron content is dependent on its intake and absorption with nutrition. The homeostasis of this nutrient is determined by the balance between its uptake and release from the cells where it is stored and recycled. Iron is released into the circulation, where it is carried by the plasma protein transferrin, into the duodenum by enterocytes that absorb dietary iron, and by macrophages which recycle senescent erythrocytes and liver reserves (6).

If iron levels in the body are inadequate, its intestinal absorption is enhanced; in case of excess, it is stored in the enterocytes as ferritin and the liver, spleen, and bone marrow as hemosiderin. The release of free iron ions in the plasma, essential for the maintenance of its homeostasis, is mediated by ferroportin, whose expression is subordinated to the activity of hepcidin (7). Children with any of the manifestations of IDA should obviously be investigated further. But considering that most children with IDA are asymptomatic and pallor is an unreliable sign, who should be screened for IDA? While the American Academy of Pediatrics recommends universal screening at approximately 1 y of age, there are no guidelines in many developed countries (8).

Laboratory reveals microcytic-hypochromic anaemia (reduced Hb, MCV, MCH, elevated RDW) with reduced reticulocyte count. Low ferritin, sideremia, a saturation of transferrin, and high unsaturated serum transferrin are noted. Usually, ferritin is the reference test for assessing the state of iron deposit; however, the lower limits vary according to age and sex (5). False normal to high levels of ferritin are typical of the inflammatory or infectious state. Therefore, all these issues impair the usefulness of ferritin as an accurate marker of iron status. Free erythrocyte protoporphyrin increases in iron deficiency anaemia, but it is a non-specific parameter. Tests used for diagnosis differ depending on the stage in the progress of IDA (7).

Reticulocyte haemoglobin content (CHr): a light at the end of the dark tunnel

Hemoglobin (Hb) plays the role of transporting oxygen to the whole body and is the basis for life support; thus, a proper control of Hb synthesis is important for the body. Because the lifespan of mature erythrocytes is approximately 120 days, mature erythrocyte Hb cannot sensitively reflect Hb synthesis (9). Meanwhile, reticulocytes are released into peripheral blood from the bone marrow, and they subsequently differentiate into mature erythrocytes in the following 1–2 days. Thus, the Hb content of reticulocytes (Hb-ret) can possibly reflect the latest Hb synthesis status (10). In addition, iron, which is a key constituent of Hb is an essential element for almost all cells in the human body, with approximately 70% of iron in the body found in the reticuloendothelial system. Provided that there is no abnormality in hematopoiesis because Hb synthesis is influenced by iron intake, Hb-ret is considered a useful means for assessing acute iron metabolism in the body (11).

Although iron deficiency leads to anemia and metabolic disorders, iron overload causes a decrease in iron utilization efficiency. Moreover, iron overload produces hydroxyl radicals, the most toxic type of active oxygen species, via the Fenton and Haber–Weiss reactions and is implicated in arteriosclerosis, carcinogenesis, hepatopathy and diabetes (12). Therefore, it is important to maintain body iron at a proper level, and as an active excretion pathway is unavailable in iron metabolism, any inappropriate administration of iron supplements must be avoided (4). However, as conventional iron indices are influenced by not only iron content but also other factors, it is often difficult to accurately determine the iron status in the body. Therefore, the use of reticulocyte hemoglobin content (CHr), which directly measures as Hb ret level by H*3 or ADVIA blood analyzers, is expected to help in the assessment of iron deficiency or overload, leading to an appropriate iron deficiency treatment (13).

CHr and IDA: future perspectives

CHr reflects the latest Hb synthesis status and is not affected by factors other than those participating in iron metabolism. Therefore, diagnosis using CHr becomes useful in cases with a background that involves serum ferritin, serum iron, and TIBC; in cases where it is difficult to determine the optimum iron requirement, such as when administering an erythropoiesis-stimulating agent (ESA); in cases where an early diagnosis of iron deficiency is necessary; and in cases wherein determination of the therapeutic effects of iron supplementation at an early stage is desired (14). As humans' physical size changes dramatically and the iron content required for growth fluctuates greatly from premature infancy to adolescence, it is easy for children to fall into an iron-deficient state if the corresponding iron intake is insufficient. Therefore, iron deficiency anemia is considered the most common blood disorder from early childhood to childhood (9). Furthermore, it has been reported that even without the presence of anemia,

prolonged iron deficiency in early childhood may damage motor function, cognitive function, and mental activity, and delayed treatment may not improve the condition even with iron supplementation (15).

Moreover, in adolescence, in addition to increased iron requirements due to secondary growth, there is increased iron loss due to menstruation and increased prevalence of iron deficiency anemia due to excessive sports. Several studies reported the presence of reduced cognitive ability due to iron deficiency in the schooling period and adolescence (10). Therefore, early diagnosis of iron deficiency is important, and studies on iron deficiency from childhood to adolescence have been conducted using CHr. The results of these investigations revealed that in healthy children, the mean CHr measured using ADVIA was slightly higher in adolescence, but there were no sexual differences, indicating that there is no need to consider sexual differences as is necessary with Hb (4). In addition, these differences could be affected by the differences in the reference values of iron deficiency and age, and further studies are necessary; however, based on the available data, CHr could become a useful parameter for diagnosing iron deficiency in the future (11).

IDA is characterized by hypochromia and microcytosis in erythrocytes, decreased serum ferritin and serum iron levels, TSAT, and increased total iron binding capacity. Low serum ferritin level in IDA is essential and should not always be associated with IDA. Again, because it is an acute phase reactant, its normal condition does not exclude IDA; the underlying etiology must be defined and regulated (14). In contrast to all these conditions, iron overload reduces the efficiency of iron utilization and induces oxidative stress formation. In addition to these, free erythrocyte zinc protoporphyrin (ER-ZPP), soluble transferrin receptor (sTfR), and reticulocyte hemoglobin content (CHr or Ret-He) are among the reliable laboratory test parameters used to describe IDA. Soluble transferrin receptor with increasing erythrocyte ERZPP value causes early deterioration of iron condition and emergence of IDA (15).

Bone marrow erythropoietic activity and intracellular iron requirement are important criteria in determining sTfR level. Therefore, in conditions associated with iron deficiency and induced erythropoiesis (sickle cell anemia, megaloblastic anemia, thalassemia, polycythemia, etc.), sTfR concentration increases, while aplastic anemia decreases (9). Normal serum sTfR level is 3.5–8.5 mg/L. It is known that ahigh sTfR (>8.5 mg/L) level is an early and sensitive biomarker for the diagnosis of IDA. The ratio of sTfR concentration to logarithmic ferritin level is also determinant in the differential diagnosis of IDA. A ratio of less than 1 is associated with chronic disease anemia, while the ratio higher than 2 is evaluated in favour of IDA (10). The decrease in iron concentration increases zinc transport in the intestines, and therefore the increased concentration of ER-ZPP (80 g/dL) in erythrocytes is associated with iron deficiency. However, routine use of ER-ZPP measurements is difficult and time-consuming due to automation difficulties (12).

CHr, also known as Ret-He, measures the amount of hemoglobin in reticulocytes and is an indicator of cell hemoglobination, reflecting the quality of newly produced reticulocytes. Microcytic, hypochromic red blood cell (RBC) is formed due to ongoing reticulocyte production when there is not enough iron. Thus, RET-He reflects an earlier measure of reduced hemoglobin status compared to hemoglobin and hematocrit (14). Reticulocytes are separated from the erythroblasts after Hb synthesis, pass into the peripheral blood and turn into mature erythrocytes within a few days. Therefore, CHr is the ideal parameter to be considered for real-time Hb synthesis. Reticulocyte hemoglobin content is affected only by the amount of iron unless there are hematopoietic disorders (4). Determination of iron status is possible with RET-He measurement. RET-He is determined by automated fluorescence flow cytometry, which measures the mean values of the forward light scattering intensity of mature red blood cells and reticulocytes using a polymethine dye. The values obtained reflect the reticulocyte hemoglobin content (11). Reticulocyte hemoglobin content is more effective in diagnosing iron deficiency, determining early iron deficiency anemia, differentiation of beta-thalassemia feature, and more effective than the other parameters involved in iron metabolism. CHr is a less variable parameter that performs better than ferritin in response to intravenous (IV) iron therapy, providing better diagnostic accuracy for iron (15).

Conclusion and recommendations:

We came to the conclusion that CHr can be a valid indicator of IDA based on the correlations between the CHr and standard haematological and biochemical indicators of iron deficiency anaemia. We also concluded that, with the routine FBC analysis performed on the same haematology analyzer and the CHr tests, an accurate diagnosis of IDA could be made quickly. We advise taking into account the IgA evaluation in CHr assessment as a strategy to accomplish early detection of IDA and keeping this fact in mind while detailing the most recent criteria for diagnosing IDA. Additionally, additional research must be conducted to examine this problem from every angle.

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References:

1) Seyid Mohamed Moulana, S. A., Muahmed Musthafa, M. M., Sanjeev, R., Seyid Mohamed Moulana, S. H., & Mohamed Thassim Marikar, F. M. (2023). A comparative review on iron deficiency anemia among children and long-term strategy.
2) Sungkar, A., Bardosono, S., Irwinda, R., Manikam, N. R., Sekartini, R., Medise, B. E., ... & Vandenplas, Y. (2022). A Life Course Approach to the Prevention of Iron Deficiency Anemia in Indonesia. Nutrients, 14(2), 277.
3) McEvoy, M. T., Stuckert, A. J., Castellanos, M. I., Gilbert, M. M., Pfeiffer, T., Prudowsky, Z., ... & Powers, J. M. (2023). Management of nutritional iron deficiency anemia for young children in the emergency department. Pediatric Blood & Cancer, 70(3), e30181.
4) Kiliç, M., Özpınar, A., Serteser, M., Kilercik, M., & Serdar, M. (2022). The effect of reticulocyte hemoglobin content on the diagnosis of iron deficiency anemia: A meta-analysis study. Journal of Medical Biochemistry, 41(1), 1-13.
5) Moscheo, C., Licciardello, M., Samperi, P., La Spina, M., Di Cataldo, A., & Russo, G. (2022). New Insights into Iron Deficiency Anemia in Children: A Practical Review. Metabolites, 12(4), 289.
6) Deloughery, T. G. (2017). Iron deficiency anemia. Medical Clinics, 101(2), 319-332.
7) Auerbach, M., & Adamson, J. W. (2016). How we diagnose and treat iron deficiency anemia. American journal of hematology, 91(1), 31-38.
8) Means, R. T. (2020). Iron deficiency and iron deficiency anemia: implications and impact in pregnancy, fetal development, and early childhood parameters. Nutrients, 12(2), 447.
9) Ogawa, C., Tsuchiya, K., & Maeda, K. (2020). Reticulocyte hemoglobin content. Clinica Chimica Acta, 504, 138-145.
10) Gelaw, Y., Woldu, B., & Melku, M. (2019). The Role of Reticulocyte Hemoglobin Content for Diagnosis of Iron Deficiency and Iron Deficiency Anemia, and Monitoring of Iron Therapy: a Literature Review. Clinical Laboratory, 65(12).
11) Cai, J., Wu, M., Ren, J., Du, Y., Long, Z., Li, G., ... & Yang, L. (2017). Evaluation of the efficiency of the reticulocyte hemoglobin content on diagnosis for iron deficiency anemia in Chinese adults. Nutrients, 9(5), 450.
12) Igartua, E. U., Hoffmann, J. J., Izquierdo-Álvarez, S., & Escanero, J. F. (2017). Reticulocyte hemoglobin content (MCHr) in the detection of iron deficiency. Journal of Trace Elements in Medicine and Biology, 43, 29-32.
13) Lofving, A., Domellöf, M., Hellström-Westas, L., & Andersson, O. (2018). Reference intervals for reticulocyte hemoglobin content in healthy infants. Pediatric Research, 84(5), 657-661.
14) Merve, K., Aysel, Ö., Mustafa, S., Meltem, K., & Muhittin, S. (2022). The effect of reticulocyte hemoglobin content on the diagnosis of iron deficiency anemia: A meta-analysis study.
15) Aedh, A. I., Khalil, M. S., Abd-Elkader, A. S., El-Khawanky, M. M., Alshehri, H. M., Hussein, A., ... & Hasan, A. (2023). Reticulocyte Hemoglobin as a Screening Test for Iron Deficiency Anemia: A New Cut-Off. Hematology Reports, 15(1), 201-211.