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Short communications
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DIAGNOSING ANEMIA AND IRON DEFICIENCY IN PREGNANCY BEYOND FERRITIN: A SYSTEMATIC NARRATIVE REVIEW OF RETICULOCYTE AND FUNCTIONAL IRON BIOMARKERS

DIJAGNOSTIKOVANJE ANEMIJE I NEDOSTATKA GVOŽĐA U TRUDNOĆI – NE SAMO NA OSNOVU FERITINA: SISTEMATSKI NARATIVNI PREGLED RETIKULOCITNIH I FUNKCIONALNIH BIOMARKERA STATUSA GVOŽĐA

Berrin Gunaydin[1], Snežana Jovičić[2, 3], Neda Milinković[3]

[1]Department of Anaesthesiology and Reanimation, Gazi University School of Medicine, Ankara, Turkey
[2]Department of Global Medical and Clinical Affairs, Business Unit for Preanalytics, Greiner Bio-One GmbH, Kremsmünster, Austria
[3]Department for Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Summary

Anaemia in pregnancy is common and is most frequently attributable to iron deficiency. However, physiological hemodilution, inflammation, hemoglobinopathies, folate or vitamin B12 deficiency, and chronic disease may contribute to reduced haemoglobin concentration. Current clinical practice remains largely centred on haemoglobin, red cell indices, and serum ferritin. Yet, ferritin interpretation in pregnancy is complicated by gestational changes, inflammation, obesity, infection, and the absence of universally accepted trimester-specific thresholds. This narrative systematic review summarises evidence for laboratory parameters beyond ferritin, including reticulocyte count, immature reticulocyte fraction, reticulocyte haemoglobin content, soluble transferrin receptor, soluble transferrin receptor–ferritin indices, mean reticulocyte volume, and percentage of hypochromic erythrocytes. Available data suggest that reticulocyte haemoglobin content and soluble transferrin receptor-based indices are the most clinically promising adjuncts for identifying iron-restricted erythropoiesis in pregnancy, whereas immature reticulocyte fraction and hypochromic erythrocytes remain insufficiently validated in pregnancy-specific diagnostic pathways. Diagnostic algorithms should integrate haemoglobin, red cell indices, inflammation assessment, ferritin, and functional biomarkers, while accounting for trimester, analytical platform, and local assay standardisation.

Keywords: pregnancy anaemia, iron deficiency, reticulocyte haemoglobin content, soluble transferrin receptor, iron-restricted erythropoiesis

Kratak sadržaj

Anemija u trudnoći je česta i najčešće je posledica nedostatka gvožđa. Međutim, fiziološka hemodilucija, inflamacija, hemoglobinopatije, nedostatak folata ili vitamina B12 i hronične bolesti mogu doprineti smanjenju koncentracije hemoglobina. Trenutna klinička praksa i dalje je u velikoj meri usmerena na hemoglobin, eritrocitne indekse i serumski feritin. Međutim, tumačenje vrednosti feritina u trudnoć i otežavaju promene povezane sa gestacijom, inflamacija, gojaznost, infekcije i nepostojanje univerzalno prihvaćenih graničnih vrednosti specifičnih za pojedina tromesečja trudnoće. U ovom sistematskom narativnom pregledu sumirani su dokazi o laboratorijskim parametrima koji se koriste pored feritina, uključujući broj retikulocita, frakciju nezrelih retikulocita, sadržaj hemoglobina u retikulocitima, rastvorljivi receptor za transferin, indekse rastvorljivog receptora za transferin i feritina, srednju zapreminu retikulocita i procenat hipohromnih eritrocita. Dostupni podaci ukazuju na to da sadržaj hemoglobina u retikulocitima i indeksi zasnovani na rastvorljivom receptoru za transferin predstavljaju klinički najperspektivnije dodatne parametre za utvrđivanje eritropoeze ograničene dostupnošću gvožđa u trudnoći, dok frakcija nezrelih retikulocita i hipohromni eritrociti još uvek nisu dovoljno validirani u dijagnostičkim algoritmima specifičnim za trudnoću. Dijagnostički algoritmi treba da obuhvate hemoglobin, eritrocitne indekse, procenu inflamacije, feritin i funkcionalne biomarkere, uz uzimanje u obzir tromesečja trudnoće, analitičke platforme i lokalne standardizacije laboratorijskih metoda.

Ključne reči: anemija u trudnoći, nedostatak gvožđa, sadržaj hemoglobina u retikulocitima, rastvorljivi receptor za transferin, eritropoeza ograničena dostupnošću gvožđa

Address for correspondence:
Shiqiong Niu
e-mail: niushiqiong@sysush.com

Introduction

Anaemia during pregnancy remains a major global health concern. It continues to present diagnostic challenges for both clinicians and laboratory specialists because of the physiological haematological changes that occur throughout gestation. According to the World Health Organisation (WHO), approximately 40.1% of pregnant women worldwide are affected by anaemia, with nearly half of all cases attributable to iron deficiency (1). The burden is particularly high in low- and middle-income countries, especially in Southeast Asia and sub-Saharan Africa, where maternal anaemia contributes substantially to maternal morbidity and mortality (1).

Early identification of iron deficiency anaemia (IDA) during pregnancy is essential to prevent adverse maternal, foetal, and neonatal outcomes. Accurate differential diagnosis requires a combination of clinical assessment and appropriate laboratory investigations that are reliable, reproducible, and simple, accessible, and cost-effective. Although assessment of iron stores using serum ferritin is currently regarded as the recommended laboratory test for diagnosing iron deficiency, its interpretation during pregnancy has important limitations. Serum ferritin is an acute-phase reactant and may be elevated in the presence of inflammation or infection, potentially masking underlying iron deficiency. Furthermore, physiological changes during pregnancy and variations in recommended diagnostic thresholds complicate its interpretation (2, 3). Nevertheless, serum ferritin remains the most widely used biomarker, with concentrations below 15 ng/mL traditionally considered indicative of depleted iron stores. However, higher pregnancy-specific cut-off values have also been proposed (4, 5).

In recent years, reticulocyte parameters including reticulocyte haemoglobin (Ret-Hb) content, immature reticulocyte fraction (IRF), and reticulocyte count (RC) have emerged as promising biomarkers for the early detection of iron-restricted erythropoiesis in both paediatric and adult populations, including pregnant women (6, 7). Among these, Ret-Hb has attracted particular attention because it reflects the amount of iron immediately available for erythropoiesis and responds rapidly to changes in iron status. Recent studies have demonstrated its excellent diagnostic performance during the first trimester of pregnancy and its ability to distinguish absolute iron deficiency from functional iron deficiency (8, 9).

This review aims to provide an integrated clinical and laboratory perspective on the role of reticulocyte parameters in the early diagnosis and differential diagnosis of iron deficiency anaemia and other causes of anaemia during pregnancy. We also discuss their potential contribution to timely clinical decision-making, optimisation of patient blood management strategies, and ultimately the prevention of adverse pregnancy outcomes.

Epidemiology of anaemia

Anaemia during pregnancy remains a major global health problem with considerable clinical significance. The prevalence is approximately 18% in developed countries, whereas it ranges from 31% to 75% in developing countries, including 53% in Sudan (10), 49% in Egypt (11), 31.6% in Ethiopia (12), and 31.3% in South Africa (13). In Ankara, Türkiye, the reported prevalence is approximately 20% (14).

Impact of anaemia on pregnancy outcomes

According to a recent meta-analysis including 31 cohort studies, maternal anaemia is associated with significantly worse maternal and neonatal outcomes. Compared with non-anaemic pregnant women, maternal anaemia is associated with an increased risk of the following adverse outcomes (15):

1. Postpartum haemorrhage (PPH) [OR 2.76 (95% CI: 1.63–4.66)]
2. Premature rupture of membranes [OR 1.94 (95% CI: 1.26–3.00)]
3. Preterm delivery [OR 1.51 (95% CI: 1.33–1.72)]
4. Low birth weight [OR 1.40 (95% CI: 1.19–1.63)]
5. Caesarean delivery [OR 1.33 (95% CI: 1.02–1.74)]
6. Gestational hypertension [OR 1.28 (95% CI: 1.14–1.44)]
7. Neonatal asphyxia [OR 1.21 (95% CI: 1.07–1.37)]

Definition and types of anaemia

According to the WHO, anaemia during pregnancy is defined as a haemoglobin (Hb) concentration below 11 g/dL. However, trimester-specific Hb thresholds have also been proposed, with cut-off values of 11.0 g/dL, 10.5 g/dL, and 11.0 g/dL during the first, second, and third trimesters, respectively (16, 17).

Regarding the type of anaemia, iron deficiency is by far the most common cause of anaemia during pregnancy; other aetiologies include anaemia of

chronic disease, infections, malignancies, vitamin B12 or folate deficiency, thalassemia, and acute or chronic blood loss. Therefore, clinicians should always consider the full spectrum of possible underlying causes when evaluating pregnant women with anaemia (18).

Diagnosis of anaemia

Anaemia in pregnancy is generally defined as Hb <11 g/dL; the serum ferritin threshold used to diagnose iron deficiency varies among international guidelines. In current obstetric and haematology practice, a ferritin concentration <30 μg/L is commonly accepted. In addition, low transferrin saturation, elevated total iron-binding capacity, and microcytosis should be interpreted collectively. Iron status reflects the balance between erythropoietic demand and available iron stores. When iron requirements exceed iron supply, the haemoglobin content of newly produced erythrocytes decreases, resulting in hypochromic mature red blood cells and reticulocytes.

Initial evaluation is usually based on conventional laboratory investigations (19):

- Complete blood count (CBC) indices including MCV(Mean Corpuscular Volume) and MCH (Mean Corpuscular Haemoglobin)
- Serum ferritin <30 ng/mL (<10–15 ng/mL is highly specific)
- Transferrin saturation <20%
- C-reactive protein (CRP)
- Thyroid, liver, and renal function tests, when clinically indicated

Diagnosing iron deficiency may become particularly challenging in acute or chronic inflammatory conditions because conventional biochemical markers of iron metabolism, such as serum ferritin and transferrin, are influenced by the acute-phase response. For this reason, simultaneous evaluation of erythrocyte and reticulocyte parameters generated automatically by haematology analysers may facilitate the early identification of iron deficiency (absolute or functional), iron-restricted erythropoiesis, ineffective erythropoiesis, and thalassemia without requiring additional laboratory investigations or increasing healthcare costs (20).

β-Thalassemia trait, characterised by reduced β-globin synthesis and mild microcytic anaemia, deserves particular attention during pregnancy because physiological haemodilution and increased maternal-foetal iron requirements may complicate haematological interpretation (21). Despite the reported prevalence of iron deficiency anaemia among pregnant women with different β-thalassemia genotypes being relatively low (<10%) (22), distinguishing isolated β-thalassemia trait from concomitant iron-restricted erythropoiesis is clinically important to avoid both undertreatment and overtreatment with iron. Although conventional haematological indices, including Hb, MCV, and MCH, remain useful for characterising anaemia, they are less reliable for differentiating inherited microcytosis from iron-restricted erythropoiesis (19, 20). Ret-Hb, which reflects the iron immediately available for erythropoiesis and is associated with conventional markers of iron status, including serum ferritin, has emerged as a valuable parameter of the reticulocyte panel (23, 24). Kumar et al. (8) demonstrated that Ret-Hb outperformed serum ferritin, MCV, and MCH in diagnosing iron deficiency anaemia during the first trimester of pregnancy. Ret-Hb differed significantly across all Hb and MCV categories ($p < 0.05$). ROC curve analysis using MCV <83 fL and MCH <27 pg demonstrated an area under the curve (AUC) of 0.93 (95% CI: 0.90–0.98), with a Ret-Hb cut-off value of 27.8 pg yielding a sensitivity of 93% and specificity of 83%. Likewise, a Ret-Hb threshold of 27.2 pg demonstrated 71% sensitivity, 86% specificity, a positive predictive value of 0.39, and a likelihood ratio of 5.1 for diagnosing iron deficiency anaemia.

More recently, we reported the clinical utility of Ret-Hb in a 29-year-old primiparous woman with β-thalassemia trait undergoing elective caesarean delivery following IVF pregnancy (25). Preoperative laboratory evaluation demonstrated hypochromic microcytic anaemia (Hb 8.9 g/dL, Hct 27.3%, MCV 67.7 fL, MCH 22.1 pg), normal serum ferritin (35.7 μg/L) and serum iron (78 μg/dL), elevated total iron-binding capacity (460 μg/dL), and reduced transferrin saturation (16%). The reticulocyte panel demonstrated an increased RC (2.5%), elevated IRF (25.3%), and a markedly decreased Ret-Hb (22.6 pg), despite a ferritin concentration within the laboratory reference interval (*Table I*). Collectively, these findings suggested functional iron deficiency with iron-restricted erythropoiesis.

Lately, Jamnok et al. proposed the Ret-Hb Factor (RHF), calculated as (Ret-Hb/RDW-SD) ×10, for populations with a high prevalence of thalassemia (26). This composite index demonstrated superior diagnostic performance compared with Ret-Hb alone, particularly among individuals with microcytosis undergoing thalassemia screening. The authors recommended considering iron supplementation in women with β-thalassemia whose RHF is <6.1 (26). In our patient, the RHF was 6.47 (*Table I*), and iron supplementation was therefore withheld (25). This case illustrates how Ret-Hb and RHF may assist clinical decision-making when conventional iron markers provide

Table I Reticulocyte and hemogram (complete blood count: CBC) indices with iron panel.

Parameter	Result	Reference values
Reticulocyte Panel		
RET percent	2.5%	0.86–1.36
RET count (#)	0.1008	0.039–0.057
IRF percent	25.3%	9.3–17.4
Ret-Hb	22.6 pg	30.2–36.2
RHF	6.47	<6.1
Hemogram (CBC)		
Hb	8.9 g/dL	≥11 g/dL
Htc	%27.3	≥33%
MCV	67.7 fL	82.9–98 fL
MCH	22.1 pg	27–32.3 pg
RDW	15	11.5–14.5%
RDW-SD	34.9	38.2–49.2 fL
Iron panel		
Serum Ferritin	35.7 μg/L	13–150
Serum Iron	78 μg/dL	33–93
TIBC	460 μg/dL	135–392
TSAT	%16	≥20%

Hb, haemoglobin; Htc, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; RET, reticulocyte; Ret-Hb, reticulocyte haemoglobin; IRF, immature reticulocyte fraction; RHF, (Ret-Hb Factor) calculated as (Ret-Hb/RDW-SD) ×10; RDW, red cell distribution width; RDW-SD, red cell distribution width – standard deviation; TSAT, transferrin saturation; TIBC, total iron binding capacity.

inconclusive results. Since Ret-Hb reflects iron incorporation into newly produced erythrocytes over the preceding few days, it can detect iron-restricted erythropoiesis earlier than changes in haemoglobin concentration or conventional iron indices, making it a valuable tool for peripartum assessment (27). In the preoperative setting, Ret-Hb may facilitate multidisciplinary decision-making, optimise patient blood management (PBM) strategies, and support individualised perioperative planning by identifying women with limited hematologic reserve who may poorly tolerate PPH, particularly during caesarean delivery.

Clinical implications of Ret-Hb

- Ret-Hb is a reliable, feasible, and cost-effective marker for the early detection of iron deficiency during pregnancy, as modern haematology analysers automatically generate it during routine complete blood count testing without requiring additional laboratory investigations.
- Ret-Hb may serve as an early warning marker of iron-restricted erythropoiesis before a measurable decline in haemoglobin concentration occurs.
- Early identification of iron deficiency enables timely initiation of iron therapy, thereby reducing the risk of adverse maternal and neonatal outcomes, including low birth weight, preterm delivery, and maternal morbidity.
- In women with β-thalassemia trait or other microcytic disorders, interpretation of Ret-Hb together with ferritin and transferrin saturation may facilitate more appropriate iron supplementation while avoiding unnecessary iron administration.
- Integration of Ret-Hb into routine antenatal and preoperative anaemia screening algorithms has the potential to improve diagnostic accuracy, optimise patient blood management strategies, and support individualised clinical decision-making in pregnant women.

Conclusion

Reticulocyte haemoglobin represents a promising biomarker for the early detection of iron-restricted erythropoiesis during pregnancy. Compared with conventional laboratory markers, it provides a more timely assessment of iron availability for erythropoiesis. It may be particularly valuable in clinical situations in which interpretation of serum ferritin is limited, such as inflammation, anaemia of chronic disease, or coexisting β-thalassemia trait. Therefore, incorporating Ret-Hb into routine obstetric anaemia screening and diagnostic protocols has the potential to improve diagnostic accuracy, facilitate timely therapeutic interventions, and strengthen PBM strategies. Ultimately, wider implementation of Ret-Hb in routine clinical practice may contribute to improved maternal and neonatal outcomes through earlier recognition and more individualised management of anaemia during pregnancy.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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