

Reticulocyte haemoglobin equivalent (ret-he) as a marker of bioavailability of iron in pregnant female- a study of 75 cases in a tertiary care centre

Dr.Dhara P. Trivedi¹, Dr. Alpesh Chavda², Dr.Divyesh Savjiyani^{3*}

¹Associate Professor, Department of Pathology, Shri M. P. Shah Govt. Medical College, Jamnagar, Gujarat, India.

²Assistant Professor, Department of Pathology, Shri M. P. Shah Govt. Medical College, Jamnagar, Gujarat, India.

³Senior Resident, Department of Pathology, Shri M. P. Shah Govt. Medical College, Jamnagar, Gujarat, India.

*Corresponding Author: Dr Divyesh D. Savjiyani,

E-mail:divyeshsavjiyani@gmail.com

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Abstract

Background: Anaemia is the most frequent derailment of physiology in the world throughout the life of a woman. Women in the gestational period have a higher risk of presenting with iron-deficiency anaemia. The anaemia may occur due to inadequate intake of dietary iron, greater foetal demand and haemodilution, a physiological anaemia common in pregnant women in which changes in the maternal organism, such as changes in blood volume and factors associated with haemostasis, decrease haemoglobin concentration. The aim of study is to utilize Ret-He as a marker of bioavailability of iron in pregnant female. **Material and Methods:** This was a retrospective and prospective study carried out for a duration of 1 years and included total of 75 cases referred to Pathology Department, M. P. Shah Govt. Medical College and Guru Gobind Singh Hospital, Jamnagar- Gujarat. Clinical history, RET-He and other important parameters as well as findings of all the concerned pregnant females were collected. **Results:** Age of pregnant females varied from 19 years to 42 years. Mean age being 25.2 years. The reference ranges of RET-Hewas between 32.1 and 38.8 pg with a median of 34.17 pg. Hb and/or ferritin level were low in 3 pregnant women in 1st trimester with anaemia, 7 in 2nd and in 16 patients in 3rd trimester. **Conclusions:** Early diagnosis of iron deficiency anaemia in pregnancy is essential to prevent damage to both maternal and foetal health. In this study, we showed that the RET-He presents an excellent supplementary tool for the diagnosis of iron deficiency in pregnant women.

Keywords: Anaemia, Iron deficiency, Pregnancy diagnosis, Reticulocytes, RET-He.

Introduction

Anaemia is a condition in which red blood cell numbers, or the haemoglobin concentration inside these cells, is lower than normal. Approximately 30% of the world population is estimated to have anaemia, with most of these cases being caused by iron deficiency¹. Women in the gestational period have a higher risk of presenting with iron-deficiency anemia². The anaemia may occur due to are inadequate intake of dietary iron, greater foetal demand and haemodilution, a physiological anaemia common in pregnant women in which changes in the maternal organism, such as changes in blood volume and factors associated with haemostasis, decrease haemoglobin concentration.

Iron deficiency is associated with a higher rate of mother/foetus morbidity and mortality and the most common complications are early labour, low birth weight, preeclampsia and a higher risk for miscarriage³. According to the World Health Organization (WHO), the reference value for haemoglobin for non-pregnant women is 12 g/dL or higher, while for normal pregnant women is 11 g/dL⁴. The clinical routine for assessing anaemia in pregnant women includes measuring the haemoglobin (Hb) concentration⁵, mean corpuscular volume (MCV), mean corpuscular haemoglobin

concentration (MCHC), reticulocyte count⁶ and other biomarkers for determining iron status, such as serum ferritin (SF), soluble transferrin receptor (sTfR), serum iron^{7,8}. Serum ferritin is the most clinically applicable parameter in pregnancy^{2,6}, however serum ferritin values can be affected by other non-physiological changes, such as the presence of inflammatory and infectious processes, which increase ferritin levels^{10,11}, potentially limiting this parameter in certain cases.

The new reticulocyte parameters provided by some equipment, allow for the earliest possible diagnosis of anaemia. The reticulocyte haemoglobin equivalent (RET-He) is a parameter that reflects the haemoglobin content in reticulocytes. Since the normal lifespan of these cells is between one and two days in the peripheral blood, RET-He is a good indicator of the availability of iron and can point to early iron-deficient erythropoiesis^{12,13,14}.

Material and Methods

This was a retrospective and prospective study carried out at the department of pathology in CCL and OPD 18 section of G.G.G.H hospital, Jamnagar for a duration of 1 year (from October 2021 to September 2022) and included total of 75 pregnant female cases (25 in each gestational period) were studied with aged 19 to 42 years (median age of 25.2 years). Blood samples from pregnant females were collected (in K3-EDTA) from prenatal examination and proceed further for complete blood count and automated reticulocyte count and parameters. Ferritin level was also measured. CBC were assessed by Horiba Pentra XLR and reticulocyte count is done by fluorescence flow cytometry technology and Ret-He was measured. Ferritin level was measured in Cobas equipment and electrochemiluminescence method. The RET-He value were assessed and compared with ferritin (normal range 13 – 150 ng/ml), the gold standard for the diagnosis of anaemia.

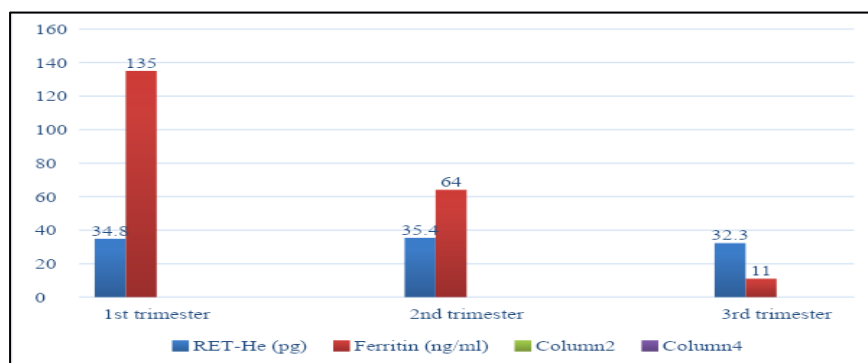
Results

The present study was conducted from October 2021 to September 2022 in the Department of Pathology, at tertiary care hospital, 75 cases (25 in each gestational period) were studied with median age of 25.2 years (19-42 years) and following observations were made. Table 1, table 2 and table 3 shows comparison of various haematological parameters and ferritin level in first, second and third trimester of pregnant female respectively. Hb and/or ferritin level were low in 3 pregnant women in 1st trimester with anaemia, 7 in 2nd and in 16 patients in 3rd trimester. Figure 1 shows graphical representation of comparison of mainly RET-He and ferritin level in first, second and third trimester of pregnant female. The reference ranges of RET-He are 32.1 – 38.8 pg^[15].

Table- 1 Average haematological parameter and ferritin in 1st trimester of pregnant female

Parameter	1 st trimester - No of cases(n=25)Average
Hb (g/dl)	12.7
RBC (million/mm ³)	4.30
MCV (fL)	89
Retic %	1.77
Absolute retic count (/cumm)	81000
IRF	9.6
Ret-He (pg)	34.8
Ferritin (ng/ml)	135

Hb: Haemoglobin; RBC: Red blood cell count; MCV: Mean corpuscular volume; Retic%: Reticulocyte count; Absolute retic count: Absolute reticulocyte count; IRF: Immature reticulocyte fraction; Ret-He: Haemoglobin content in reticulocytes.

Figure 1. Comparison of RET-He and ferritin values in 1st, 2nd and 3rd trimester of pregnant female**Table- 2 Average haematological parameter and ferritin in 2nd trimester of pregnant female**

Parameter	2 nd trimester - No of cases(n=25)Average
Hb (g/dl)	11.2
RBC (million/mm ³)	3.98
MCV (fL)	87
Retic %	1.6
Absolute retic count (/cumm)	72000
IRF	14.5
Ret-He (pg)	35.4
Ferritin (ng/ml)	64

Table- 3 Average haematological parameter and ferritin in 3rd trimester of pregnant female

Parameter	3 rd trimester - No of cases(n=25)Average
Hb (g/dl)	11
RBC (million/mm ³)	3.75
MCV (fL)	86
Retic %	1.82
Absolute retic count (/cumm)	85000
IRF	20.4
Ret-He (pg)	32.3
Ferritin (ng/ml)	11

Discussion

Ret-He has been shown to have great potential as a test for detecting the early stages of iron-deficiency anaemia^{10,13,14} and for the follow-up of replacement therapy^{16,17,18}. Data in table 1 indicate that the RET-He showed a pattern similar to that of serum ferritin, with a significant difference in the 3rd trimester of pregnancy, in relation to the 1st and 2nd trimesters. It was expected, as the number of women with ferritin below the normal range is higher in the second and particularly in third semester

¹⁹. When pregnant women with evidence of anaemia or iron deficiency were excluded, there were no significant differences in the level of serum ferritin between the first and the third trimesters. In this study, the RET-He was found to be the best marker for detecting anaemia in pregnant women, when compared to the other parameters determined (Hb, MCV), findings are coincided with study by GilangNugraha⁹. Similarly, Kumar U²⁰ showed that RET-He found to be quite useful for early detection of anaemia in first trimester pregnancy and to prevent maternal and foetal complications. Results of our study are comparable with the study carried by Ervasti M²¹. They observed that with the use of RET-He, it will improve the diagnostic accuracy for the detection of anaemia at term pregnancy.

Of the 75 pregnant women evaluated in the present study, 35% reported having used ferrous sulphate at some point in the gestational period, which may have interfered with the observation of the development of iron-deficiency anaemia in this population. Another potentially interfering factor is the fact that ferritin acts as an acute-phase reagent¹⁰ and is increased in the presence of inflammatory and infectious processes. As such, there is an increase in the levels of this marker in the month before childbirth²², which may mask the presence of iron-deficiency anaemia during this period.

In addition to demonstrating a better performance than the traditional markers for the diagnosis of iron-deficiency anaemia^{10,13,14,17,18} and efficiency for the follow-up of patients undergoing iron supplementation treatment^{16,17,18} the RET-He has great potential as an auxiliary tool during the follow-up of pregnant women in prenatal care. The progressive decrease of a patient's RET-He values may indicate the onset of iron deficiency, indicating a need for further investigation, using traditional iron dosages. As such, since the RET-He is included in the complete blood count analysis of the equipment that performs reticulocyte testing, this is a low-cost alternative that does not require additional sample collection, representing an advantage over other traditional markers.

Conclusion

The early diagnosis of iron deficiency anaemia in pregnancy is essential to prevent damage to both maternal and foetal health. The RET-He presents as a useful supplementary parameter for the diagnosis of iron deficiency in pregnant women, especially during prenatal care. Integrating periodic observations of RET-He values into routine blood counts may facilitate monitoring for the early development of anaemia, where a progressive decrease in RET-He values could indicate the need for further investigation, using conventional iron dosages.

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