EVALUATION OF RED CELL INDICES AND RETICULOCYTE MATURITY INDICES INCLUDING RETICULOCYTE HAEMOGLOBIN CONCENTRATION IN IRON DEFICIENCY ANAEMIA IN ADULT FEMALE POPULATION
Anitha Sunkara¹, Devender Reddy Kotta²

¹Assistant Professor, Department of Pathology, Mallareddy Medical College for Women, Suraram.
²Associate Professor, Department of Pathology, Osmania Medical College, Hyderabad.

ABSTRACT

BACKGROUND
Prevalence of iron deficiency anaemia is higher in both developed, underdeveloped countries and in developing countries particularly in toddlers, adolescent girls and women of childbearing age. Diagnosis of iron deficiency is made by biochemical investigations along with the routine haemogram. Automated analysers in the recent years provide many reticulocyte parameters like Low Fluorescence Ratio (LFR), Medium Fluorescence Ratio (MFR) and High Fluorescence Ratio (HFR), Reticulocyte Haemoglobin Concentration-haemoglobin (Ret-He), which not only aid in the diagnosis of Iron Deficiency Anaemia (IDA) without the necessity of biochemical investigations, but also help in the follow up of these patients for bone marrow response accurately. The aim of the present study is to evaluate the effectiveness of these parameters in the diagnosis of iron deficiency anaemia in the female population in reproductive age group.

MATERIALS AND METHODS
The present study included peripheral blood samples from fifty eight women aged between 18-45 years from routine workload including 20 normal (Hb >12.0 g/dL with normal serum ferritin and serum iron levels) and 38 iron deficiency anaemia samples (Hb <12.0 g/dL and with low serum ferritin and serum iron levels).

RESULTS
There was significant differences in the means between the subjects from the control group and IDA group in red cell distribution width, RDW CV (%) (13.26±1.07 vs. 18.22±3.34 p-value <0.00001) and immature reticulocyte fraction (%) (9.87±5.68 vs. 17.89±9.00 p-value=.000646), reticulocyte haemoglobin concentration (pg) (33.34±2.92 vs. 22.0±4.13, p-value=<0.00001). Serum iron and serum ferritin showed statistically significant difference between two groups.

CONCLUSIONS
The reticulocyte parameters including Ret-He provide an important information in suspected cases of iron deficiency. Routine haemogram can thus be more helpful not only on morphological categorisation of anaemia, but also in knowing the underlying pathology.

KEYWORDS
Anaemia, Iron Deficiency Anaemia (IDA) Reticulocyte Haemoglobin Concentration (Ret-He), Low Fluorescence Ratio (LFR), Medium Fluorescence Ratio (MFR) and High Fluorescence Ratio (HFR).

HOW TO CITE THIS ARTICLE: Sunkara A, Kotta DR. Evaluation of red cell indices and reticulocyte maturity indices including reticulocyte haemoglobin concentration in iron deficiency anaemia in adult female population. J. Evid. Based Med. Healthc. 2016; 3(97), 5315-5318. DOI: 10.18410/jebmh/2016/1105

BACKGROUND
Anaemia is functionally defined as an insufficient red blood cell mass to adequately deliver oxygen to peripheral tissues. Any of the three measurements, the Haemoglobin (Hb) concentration typically expressed as grams per decilitre (g/dL), the Haematocrit (Hct) also called the Packed Cell Volume (PCV) and the RBC concentration expressed in cells per microliter (10⁹/μL) or cells per litre (10¹²/L) can be used to establish the presence of anaemia for practical purposes.¹ The mean normal Hb and the lower limits of the normal ranges of these parameters depend on the age and gender of the subjects as well as their altitude of residence. Normal haemoglobin range in healthy reproductive age women is 12-15 g/dL.² Anaemia in this group is most commonly due to deficiency of iron. Prevalence of iron deficiency anaemia is higher in both developed, underdeveloped countries and in developing countries particularly in toddlers, adolescent girls and women of childbearing age. Today, menstruating women continue to be among the most likely individuals to develop iron deficiency along with young children whose growth outstrips their iron supply.
There are three recognised stages in the progression of iron deficiency anaemia, i.e. A) Pre-latent phase where there is iron depletion/deficiency identified by reduction in iron stores, i.e. low serum ferritin without reduced serum iron levels. B) Second stage of iron-limited erythropoiesis. There will be reduced transferrin saturation, increased Total Iron Binding Capacity (TIBC), increased free erythrocyte protoporphyrin, increased zinc protoporphyrin. The mean corpuscular volume usually remains within normal limits, but a few microcytes may be detected on a blood smear. C) The third stage of iron deficiency anaemia where the blood haemoglobin concentration falls below the lower limit of normal.

The laboratory findings in iron deficiency and iron deficiency anaemia are as follows- The important findings in iron deficiency are decreased MCV, MCH and increased RDW-CV measured by automated analysers, which corresponds to microcytosis, hypochromia and anisocytosis on peripheral smear examination. Anisocytosis is an important early sign in iron deficiency anaemia. Though microcytic picture is evident, but a variable number of normocytic normochromic cells, some macrocytes, often polychromatophilic RBC can be present, which corresponds to the reticulocyte count traditionally measured by microscopic examination of a smear prepared from fresh blood stained with a supravital stain. The normal reticulocyte count by light microscopy is 0.5 to 1.5% of the total red cells. Automated reticulocyte analysis by flow cytometry has been incorporated into the laboratory routine in the recent years as an alternative to the manual method. It is rapid, more accurate and easy to perform and provide both the number of reticulocytes and several indices that can be helpful in the diagnosis of underlying pathologies and in monitoring bone marrow recovery. These indices, which require standardisation and definition of reference values are yet to be used in the clinical practice.

The reticulocytes can be further classified into three groups according to the maturity of reticulocytes based on intracellular RNA levels measured by fluorescence intensity, Low Fluorescence Ratio (LFR), Medium Fluorescence Ratio (MFR) and High Fluorescence Ratio (HFR). In more severe anaemia, the maturation time of reticulocytes in the medulla decreases and an increased number of immature reticulocytes are released into the peripheral blood, which remain more than 48 hours in the peripheral blood until they turn into red blood cells. Therefore, the immature reticulocyte count in the peripheral blood will be higher in more severe anaemias.

Recently, the Reticulocyte Haemoglobin content (RET-He) is identified as an early sensitive indicator of iron deficiency. The reticulocyte haemoglobin content directly examines the haemoglobin endowment of the youngest circulating erythrocytes allowing detection of iron deficiency before the bulk population shows evidence of an effect. Similarly, the reticulocyte haemoglobin measurement can show a response to iron therapy approximately 4 days after it has begun, which is much earlier than other haematologic measurements.

Biochemical parameters including serum iron concentration, serum ferritin are decreased, TIBC is often increased. However, serum ferritin lacks its importance especially in the context of associated chronic inflammation making the diagnosis of IDA difficult.

Considering these factors, diagnosis of iron deficiency anaemia can be done by morphological and biochemical parameters of the blood. Recent automated blood analysers not only help in diagnosing and categorising anaemia morphologically on the basis of MCV, MCH, MCHC and RDW-CV, but also help out in reaching the cause of anaemia accomplished by reticulocyte parameters like reticulocyte %, IRF, LFR, MFR, HFR and Ret-He without the aid of supportive biochemical investigation in many situations as routine.

AIMS AND OBJECTIVES
The aim of the present study is to evaluate the red cell indices, reticulocyte maturity indices, reticulocyte haemoglobin concentration, serum ferritin and serum iron concentrations among normal subjects and iron deficiency anaemia in adult female population.

MATERIALS AND METHODS
The present study comprised peripheral blood samples from fifty eight women aged between 18-45 years from routine workload including 20 normal (Hb >12.0 g/dL) and 38 iron deficiency anaemia samples (Hb <12.0 g/dL). Those who are on iron supplementation suffering from chronic diseases are excluded. The samples were collected in EDTA tubes (for haematology analysis) and tubes without EDTA (for biochemical investigations).

The haematological parameters including Haemoglobin (Hb), Red Blood Cell count (RBC count), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), reticulocyte %, Absolute Reticulocyte Count (ARC), Low Fluorescence Ratio (LFR), Medium Fluorescence Ratio (MFR), High Fluorescence Ratio (HFR) and Immature Reticulocyte Fractions (IRF). The reticulocyte haemoglobin content (RET-He) were measured using Sysmex-XN1000, automated analyser using flow cytometry and hydrodynamic focusing and biochemical parameters including serum ferritin and iron were measured by Architect Plus I 1000 SR using chemiluminescent microparticle immunoassay and Dimension RxL using Ferene bichromatic endpoint, respectively. The samples were divided into anaemic and non-anaemic groups based on haemoglobin values with cutoff of 12 g/dL. Anaemic cases confirmed as iron deficient by biochemical investigations were considered. Data are reported as means and Standard Deviation (SD).

For the statistical analysis, the results were analysed by Student’s t-test for comparison of means. Differences were considered significant when the two tailed p-value <0.05.

RESULTS
The study comprised of fifty eight females (20 normal and 38 iron-deficient anaemia). The demographic and laboratory results are shown in Table 1. P value is insignificant in age
characteristics between both groups. When the means were compared between two groups with respect to all haematological and biochemical parameters, statistically significant differences were found with red cell parameters including haemoglobin, MCV, MCH, MCHC, RDW-CV. Reticulocyte parameters like reticulocyte %, IRF, LFR, MFR, HFR, RET-He showed a statistically significant difference. Biochemical parameters including serum iron and serum ferritin showed statistically significant difference between two groups. RBC count and absolute reticulocyte count showed no statistically significant difference.

**DISCUSSION**

Iron deficiency anaemia is caused by negative iron balance in the body and is the most common scenario in the female population in the reproductive age group. Among these individuals, iron deficiency is landed up either due to dietary deficiency by inadequate intake/absorption or excess loss of blood. Iron deficiency is not only linked to anaemia causing weakness and fatigue, but also irritability, behaviour abnormalities and epithelial abnormalities.

Therefore, identification of iron deficiency and differentiation from other causes of anaemia like anaemia of chronic disease, haemoglobinopathies and haemolytic anaemia, etc., is essential for proper management of this entity. The roots of the present study started from the idea of believing on a single, simple, cost-effective test, which can give multiple clues for the identification of aetiology of anaemia. In the present era of evidence based medicine, this approach reduces the cost of bearing for other additional tests and also avoids unnecessary repeated sampling for further biochemical investigations. So, in this study, the effectiveness of the RBC and reticulocyte parameters, which can be derived from a simple haemogram done in recent automated cell counters is compared with serum iron and serum ferritin in the diagnosis of iron deficiency. In our sample control group, mean haemoglobin level is 13.3 g/dL. Mean MCV, MCH, MCHC, RDW-CV are 85.02 fl, 28.18 pg, 33.12 gm/dL, 13.26%, respectively. Mean haemoglobin is 9.02 g/dL. RBC parameters, i.e. MCV, MCH, MCHC, RDW-CV are 67.93 fl, 20.19 pg, 29.29 gm/dL, 18.22%, respectively, in IDA group. All these parameters showed statistically significant difference with p value <0.00001. The RDW-CV range in the sample control group is 11.12% to 15.30%. The RDW-CV range in the IDA group is 14±6.72 suggesting that iron deficiency can be seen in some cases even with absence of marked anisocytosis. However, other parameters like Ret-He was less than 26 pg in these cases of IDA with normal RDW-CV. Many studies have found that RDW, MCV and MCH are important parameters for screening and detecting IDA. Sensitivity of RDW was the highest (89%) followed by MCH and MCV (84%) compared to only 63% in MCHC. In iron deficiency anaemia, earliest change will take place in MCV (21 days) followed by RDW (30 days) and Hb may take up to 60 days to become low. Although, there is relative erythroid hyperplasia in the bone marrow, both the degree of erythroid hyperplasia and the reticulocyte count are low for the degree of anaemia in IDA. There is a significant component of “ineffective erythropoiesis” in iron deficiency, and a proportion of immature erythroid cells in iron-deficient subjects are so defective that they are rapidly destroyed. Both the percentage and the absolute number of reticulocytes may be normal or slightly increased except for the absolute reticulocyte count. All other reticulocyte parameters showed significant difference between the two groups with p value <0.0001. Immature Reticulocyte Fraction (IRF) is a quantitative measurement of the RNA content of the reticulocytes. Immature (younger) reticulocytes contain a higher RNA content than more mature reticulocytes. The values were significant between two groups. IRF was quite high compared to normal controls in IDA group. Mean is (9.87±5.68 in normal; 17.89±9.00 in IDA).

Mean reticulocyte haemoglobin content (CHR) is measured by stained reticulocytes using two angle light scatter and reticulocyte haemoglobin concentration (Ret-He) is a measure of the forward scatter of stained reticulocytes and has a curvilinear relationship with CHR. Ret-He was significantly different between two groups. However, the lower limit of this parameter is 27.5 pg in non-anaemic and upper limit in anaemic is 26.16 pg.

Measurement of Ret-He provides an indirect measure of the functional iron available for new RBC production. Mest et al reported that CHR of <28 pg had an optimal sensitivity (74%) and specificity (73%) for diagnosis of iron deficiency using Prussian blue staining of the bone marrow aspirate to define iron deficiency. In this study, the area under the curve of CHR exceeded that of ferritin, Transferrin Saturation(TS) and MCV showing that CHR is a useful marker for diagnosis of iron deficiency in adults. Several studies have assessed the value of CHR as an indicator of iron deficiency in dialysis patients. Fishbane et al also reported that CHR of <28 pg predicted iron deficiency more accurately than did serum ferritin and TS in dialysis patients receiving erythropoietin. In the study by Thomas et al, functional iron deficiency was defined as CHR <28 pg. Mitsuiki et al reported that a CHR index for iron deficiency with 100% high sensitivity was 32 pg.

Since serum ferritin can be falsely elevated as an acute phase reactant despite low body iron storage combined with the physiologic variation of serum iron and total iron-binding capacity in chronic inflammation and chronic renal disease, Ret-He may be a better predictor of marrow iron stores than traditional serum iron parameters. It is more sensitive predictor of iron deficiency than haemoglobin for screening infants and adolescents for iron deficiency prior to the development of anaemia.

An increased IRF reflects early marrow recovery from the conditioning regiments of stem cell transplantation, cancer chemotherapy or treatment for nutritional anaemias, which usually precedes the increase in absolute reticulocyte count. IRF has also been used to evaluate ineffective erythropoiesis and to differentiate megaloblastic anaemia or myelodysplasia (increased IRF) from other causes.
In our study, the Ret-He and reticulocyte maturity indices showed significant differences with control and anaemic group.

Reticulocyte haemoglobin concentration is equally helpful as serum ferritin and serum iron levels in diagnosing iron deficiency. When there are limitations with usefulness of serum ferritin in anaemia associated with chronic disorders, Ret-He can be helpful in identifying iron deficiency in these settings.

CONCLUSION
The diagnosis of iron deficiency anaemia can be made based on the reticulocyte parameters provided by recent automated analysers without the requirement of additional biochemical investigations. Especially, Reticulocyte Haemoglobin Concentration-Haemoglobin (Ret-He) is significantly decreased among the iron deficient population, which can also be used for monitoring subsequent iron therapy along with other reticulocyte indices.

REFERENCES