"Comparative study of new reticulocyte and erythrocyte parameters with serum iron parameters for the early diagnosis of Iron deficiency in pregnant women using Sysmex XN1000"

By

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Dissertation submitted to the

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DOCTOR OF MEDICINE

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Under the Guidance of

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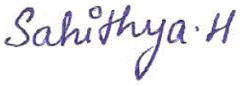

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ABSTRACT

Introduction

Iron deficiency (ID) is the most common anemia in pregnancy. It is one of the major causes of morbidity in pregnancy. Hemoglobin (Hb) estimation is the routine investigation employed in the antenatal screening where 11g/dl is taken as a cut-off for making a diagnosis of anemia. Different Iron studies like Serum iron, transferrin, and transferrin saturation are the additional investigation parameters, currently being used in the diagnostic algorithm of IDA. Serum iron, transferrin saturation shows diurnal variations and serum ferritin values show false increase in the presence of infection or inflammation.With the advancement of technology, new reticulocyte and erythrocyte parameters such as Ret-He, %Micro-R and %Hypo-He are available on automated hematology analyzers for the assessment of iron status. These parameters are simple and cost-effective alternative to serum biochemical tests, and are not influenced by infection or inflammation. The added advantage being the ability to obtain these parameters on the same sample used for complete blood count (CBC) analysis.

Aims and Objectives

To compare the utility of Ret-He, %Micro- R, and %Hypo-He with serum iron parameters and ferritin levels in the diagnosis of latent IDA in first-trimester pregnant women.

Materials and Methods

A hospital-based prospective cross-sectional study was conducted on blood samples of nonanemic pregnant women of first trimester presenting to the antenatal clinic(ANC) for the first time in the Department of Obstetrics and Gynecology(OBG) were sent to the Hematology section of the Department of Pathology of BLDE (Deemed to be University), Shri B. M. Patil Medical College, Vijayapura.

Study period: 1st November 2018 to 30th May 2020

Results

Among the 280 cases included in the study 151cases were ID and 129 were non-ID. Comparison of the new reticulocyte parameter Ret-He and the new erythrocyte parameters %Micro-R and % Hypo-he with Serum Ferritin showed that the sensitivity and specificity of Ret He was similar to Serum Ferritin. At the optimal cut-off value of 32.25, the sensitivity and specificity was 98.0% and 97.0% respectively. The AUC for Ret-He (0.999, 95% CI 0.998-1.000) indicates that Ret-He is the best discriminator of ID with a p value <0.001. %Micro-R at a cut-off of 1.55 showed sensitivity and specificity of 76.9% and 69.9% respectively and was statistically significant. % Hypo-He did not show statistically significant difference between ID and N-ID

Conclusion

Ret-he can be used as an alternative hematological investigation to the traditionally used biochemical parameters such as serum ferritin for the early diagnosis of ID in pregnant women as it is cost effective and can be done along with hematological investigation such as CBC.

Keywords -Ret-He, %Micro-R, %Hypo-He, Iron deficiency anemia, Serum Ferritin

LIST OF ABBREVATIONS USED

Ret- He	Reticulocyte Hemoglobin
IDA	Iron deficiency anemia
ID	Iron deficiency
N-ID	Non Iron Deficient
%Micro- R	Percentage of Microcytic Red Cells
%Hypo- He	Percentage of Hypochromic Red Cells
CBC	Complete Blood Count
WHO	World health organisation
NFHS	National Family Health Survey
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red Cell Distribution Width
TIBC	Total Iron Binding Capacity
sTfR	Soluble Transferrin Receptor
PCV	Packed cell volume
TSAT	Transferrin Saturation
EP	Erythropoietin
ACD	Anemia of chronic disease
CKD	Chronic kidney disease
ESRD	End stage renal disease
Hb	Hemoglobin
RNA	Ribonucleic acid
CHret	Reticulocyte hemoglobin content

DMT 1	Divalent metal transporter 1
EDTA	Ethylene diaminetetra acetic acid
K2 EDTA	Dipotassium ethylene diaminetetra acetic acid
FEP	Free Erythrocyte Porphyrin

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INTRODUCTION

Iron deficiency (ID) is the most common anemia in pregnancy. It is one of the major causes of morbidity in pregnancy.[1] In 2002, Iron deficiency anemia (IDA) was considered as one of the important contributing factors to the global burden of the disease. According to 2010 WHO report, anemia accounts for 8.8% of total disability, of which iron deficiency anemia was the most common etiology worldwide.[2] Main etiological factors for IDA are low intake of iron, low socioeconomic status, malabsorption and increased demand of iron in physiological conditions like pregnancy, adolescence where iron requirement is high.[3]

Maternal complications like postpartum infections and neonatal complications like premature delivery, low birth weight and poor growth in infancy which can occur due to iron deficiency. These complications can be prevented by early diagnosis and treatment.[1]

Hemoglobin (Hb) estimation is the routine investigation employed in the antenatal screening where 11g/dl is taken as a cut-off for making a diagnosis of anemia. However, in case of IDA decrease in the Hb level occurs late in the course of the disease. Thus, the use of the Hb as a screening test delays the diagnosis of IDA. Hence in pregnant women who are not yet anemic diagnosis of ID may be missed in screening test done by Hb estimation.[3]

Different Iron studies like Serum iron, transferrin, and transferrin saturation are the additional investigation parameters, currently being used in the diagnostic algorithm of IDA. Advanced parameters like soluble transferrin receptor and Hepcidin are few upcoming parameters in the diagnosis of ID. However, each one of them has its significant limitations. [1]

A ferritin level of <30 μg/L is a highly sensitive parameter for diagnosing ID in pregnancy. As ferritin is an acute-phase reactant, which also increases in the presence of infection or inflammation thereby, masking the iron deficiency. Further serum iron, transferrin saturation shows diurnal variations and fluctuations with the intake of diet. The soluble transferrin receptor is a relatively expensive and not routinely available investigation in most institutions.[1]

With the advancement of technology, new reticulocyte and erythrocyte parameters are available on automated hematology analyzers for the assessment of iron status. These parameters include the percentage of microcytic red cells (%Micro-R), the percentage of hypochromic red cells (%Hypo- He), and the reticulocyte hemoglobin content (Ret- He). These parameters provide information at a cellular level about iron availability for erythropoiesis in individual reticulocytes and red blood cell (RBC) subsets.

These parameters are simple and cost-effective alternative to serum biochemical tests, and are not influenced by infection or inflammation. The added advantage being the ability to obtain these parameters on the same sample used for complete blood count (CBC) analysis. Amongst these parameters the most widely established is the Ret- He, which allows for early detection of ID. These parameters being not only rapid they are cost-effective as well.[1]

The role of these parameters in the detection of latent iron deficiency anemia in pregnant women in the Indian population has not been explored by much. Analysis of these parameters in pregnant women addresses the dual issue of filling the gap between local context and Indian data. Hence this study was being taken up.

AIMS AND OBJECTIVES

To compare the utility of RET-He, %Micro- R, and %HYPO-He with serum iron parameters and ferritin levels in the diagnosis of latent IDA in first-trimester pregnant women.

REVIEW OF LITERATURE

Anemia is a severe public health issue that disproportionately affects young children and pregnant women around the world. It affects 42 percent of children under the age of five years and 40 percent of pregnant women globally, according to the WHO.[4]

India has the highest prevalence rate of IDA as compared to prevalence rate of many other countries. According to the latest National Family Health Survey (NFHS - 5) 68.4% children and 66.4% women were suffering from anemia. [5]

Iron deficiency anemia was estimated to affect 20% of the global population and was among the five greatest causes of years lived with disability. It was one of the leading cause of years lived with disability in low-income and middle-income countries (LMICs) and was the leading cause of years lived with disability among women across 35 countries.[6]

Menstruation, pregnancy, parturition, and lactation all considerably increase the physiological requirements for iron during a woman's reproductive life.[7,8]Pre-existing iron deficiency from several pregnancies, menstrual blood losses, nutritional deficiencies, helminthiasis, and amebiasis are also significant contributors of IDA in pregnancy. Nutritional anemia affects 60-80 percent of reproductive-age women in India and other developing nations. However nutritional anemia was 10-20 percent in developed countries. [8]

HISTORICAL ASPECTS

Anemia was characterized as a kind of jaundice or Pandu roga by Sushrutha in ancient Indian medicine. The whiteness of the eyes, fingernails, and skin was thought to be caused by a derangement of Kapha (phlegm). Another type of Pandu roga, connected with the consumption of clay, was identified by Charaka. He used iron rust pills to treat the problem. [9]

In an Egyptian manual of therapeutics - Papyrus Erbs, in 1500 BC described Iron deficiency anemia as a disease characterized by pallor, dyspnea, and edema. In the 16th century, all anemias were grouped under a single category, named "Chlorosis" by Varandal. Chlorosis was identified as a disease defined by a reduction in blood iron content around the turn of the twentieth century. The majority of the basic research on iron metabolism and depletion was done in this century.[10]

HEMATOPOIESIS

Hematopoiesis is the process of producing blood cells that is red blood cells (erythropoiesis), white blood cells (granulopoiesis), and platelets (thrombopoiesis) in the bone marrow where the proliferation of their precursors is followed by their maturation and subsequent release of the formed elements in circulation.[11]

Yolk sac is the main site of hematopoiesis in the first few weeks of gestation followed by liver and spleen from 6 weeks to 6-7 months of fetal life. Bone marrow starts contributing to hematopoiesis after 6-7 months and becomes the only source of new blood cells in normal childhood and adult life. However, the liver and spleen can serve as sites of extramedullary hematopoiesis in conditions like myelofibrosis and severe hemolytic anemia.[11]

ERYTHROPOIESIS

In adults, erythropoiesis is confined to the bone marrow. Red blood cells are formed through the following stages: [12]

Proerythroblast
↓
Basophilic Erythroblast
↓
Polychromatophilic Erythroblast
↓
Acidophilic Erythroblast
↓
Reticulocyte
↓
Erythrocyte

The average life span of red cells is about 120 days after which red blood cells degenerate and hemoglobin is broken down into hemosiderin and bile pigments. For proper erythropoiesis, adequate nutrients like minerals, vitamins, proteins, and hormones are essential. Inadequate reserve or increased demand or deficient supply of any of the above constituents interferes with the normal erythropoiesis.[12]

DEFINITION OF ANEMIA

The term anemia is defined as a "condition in which the number of red blood cells or the hemoglobin concentration within them is lower than normal" [4]

Normal range of hemoglobin in non-pregnant females is 12-15g/dl and in pregnant females is 11-12g/dl. In males the reference range is 13.5-18g/dl and in infants (6m-1year) it is 10.4-15.6g/dl . Children(1-7yrs) the range is 10.2-15.2 and children between age group 8-17, the normal range is 12-15g/dl.[31]

CLASSIFICATION OF ANEMIA

In 1983 Bessman DJ et al [13] proposed an "Improved Classification of Anemia" based on mean corpuscular volume(MCV) and red cell distribution width(RDW). This classification has 6 categories and they are:

1. Microcytic Homogenous: having decreased MCV and normal RDW.
2. Microcytic Heterogenous: having decreased MCV and increased RDW
3. Normocytic Homogenous: having normal MCV and RDW
4. Normocytic Heterogenous: having normal MCV and increased RDW
5. Macrocytic Homogenous: having increased MCV and normal RDW
6. Macrocytic Heterogenous: having increased MCV and increased RDW

But currently, two classifications are followed, which are classification of anemia according to underlying mechanism of anemia and classification based on morphology.[14,15]

I. **Classification according to underlying etiological mechanism [14]**

Underlying etiological conditions are broadly categorized as blood loss, increased destruction of RBCs and impaired red cell production.

A. Blood loss can be acute like in trauma or it could be chronic as seen in gynecological or gastrointestinal lesions.

B. Increased destruction of RBCs otherwise called as hemolytic anemia is further subclassified as intrinsic or intracorpuscular abnormalities and extrinsic or extracorpuscular abnormalities. Under intrinsic abnormalities, it can be hereditary causes like membrane abnormality (eg – Spherocytosis, Elliptocytosis), enzyme deficiency (eg – Glucose 6 phosphate dehydrogenase and hexokinase deficiency), hemoglobinopathies such as sickle cell anemia and thalassemia or it can

be acquired like the membrane defects seen in Paroxysmal nocturnal hemoglobinuria. Extrinsic abnormalities include Antibody-mediated hemolysis as seen in transfusion reactions, immune hydrops, SLE, and those associated with drugs. Also includes infections like malaria and mechanical trauma to the red blood cells seen in thrombotic thrombocytopenic purpura and disseminated intravascular hemolysis

C. Impaired red cell production is seen in aplastic anemia and pure red cell aplasia due to disturbed proliferation and differentiation of stem cells. It could also be due to disturbed maturation and proliferation as seen in megaloblastic anemia due to vitamin B12 deficiency, renal failure due to erythropoietin deficiency, anemia of chronic disease, endocrine disorders and defective hemoglobin synthesis. Marrow replacement seen in hematopoietic neoplasms such as acute leukemia and myelodysplastic syndrome and marrow infiltration seen in metastatic neoplasms are also causes for impaired production.

II. **Morphological classification** [14]

Morphology and chromasia of red blood cells give us a clue towards the etiology and probable diagnosis. Morphology is assessed in peripheral smear and is subjective to some extent. Hence morphology can be assessed quantitatively using indices like MCV, mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration(MCHC) and RDW. This classification includes 3 categories:

A. Normocytic Normochromic

B. Microcytic Hypochromic

C. Macrocytic.

IRON METABOLISM:

Total iron content of the body is around 2.5g in women and 3.5g in men. Iron content is more in men as compared to women, probably because of more muscle mass in men .[15]

Distribution:

Most of the body iron (80%) is bound to hemoglobin within the erythrocyte. The remaining iron is located in myoglobin and enzymes like catalase, cytochromes .[16] Hemoglobin Iron is produced by the destruction of red cells. Red cells are phagocytosed by the reticuloendothelial cells and there is a release of hemoglobin. Hemoglobin is broken down and the iron bound to transferrin is released into circulation. Released iron is reutilized by marrow erythroblasts for erythropoiesis. The absolute amount of hemoglobin iron varies from 2-3g. Non-Available Tissue Iron includes myoglobin and enzymes which constitutes about 0.5g. Storage Iron consists of hemosiderin and ferritin. It is around 1-2gm in healthy adults. Hemosiderin is the insoluble form of storage iron that appears as golden yellow to golden brown granules in unstained sections and H & E sections. Hemosiderin contains more iron than ferritin and is present in abundance in the liver. Ferritin is the soluble form of storage iron and is distributed in the tissue, but not visible in unstained sections. It has an outer shell of iron-free protein, apoferritin, and an inner core of trivalent iron. In plasma, approximately 3-4 mg of transport iron is present bound to a specific protein called Transferrin. It is a β globulin molecule. Transferrin is present in a concentration that enables it to combine with 40- 80 μmol of iron /L, this is called the Total iron-binding capacity of serum (TIBC).[16]

Absorption:

Absorption of iron takes place in duodenum and jejunum. A normal regular diet contains 10-20 mg of iron per day. Dietary iron is found in two forms: Heme iron and Nonheme iron. Heme iron is readily absorbed and utilized by the body. It is found in animal based foods like poultry and meat. Nonheme iron needs to be converted from ferric form (Fe+++) to ferrous form (Fe++) for it to be absorbed and this is done by the hydrochloric acid present in the gastric juice. Calcium, tannates and phytic acid inhibit the absorption whereas vitamin C increases the absorption. This form of iron is present in all plant based foods.[17]

The absorption of iron is biphasic. The first phase is the uptake of iron from the lumen into the mucosal cells & the second phase is the transport of iron across the cell into the circulation.[14]

First phase – Ferrous form of iron enters the enterocyte via a transporter protein located at the apical brush border named as a divalent metal transporter 1(DMT1). The transporter protein DMT1 is not specific for iron transport. It also plays an important role in the transport of other divalent ions like manganese, cobalt, copper, zinc, calcium, etc. Within the enterocyte, the iron is stored as ferritin if it is not utilized by the body immediately. Eventually when the cell is shed this stored form of iron is excreted in the feces.[14]

Second phase – The ferrous iron present within the enterocyte is transported into the plasma across the basolateral membrane through a basolateral transporter known as ferroportin 1. Hephaestin converts the ferrous iron to ferric form by its ferroxidase activity. The ferric iron then combines with apotransferrin to form transferrin and is circulated in the blood.[14]

Iron balance is achieved mainly by controlling absorption. Hepcidin is considered the master in regulating iron absorption. It is a peptide hormone produced by the liver. Hepcidin controls the release of iron into the plasma by inhibiting ferroportin 1present over the basolateral membrane. Thus the iron remains within the cell in the stored form or is lost when the cell is shed, therefore maintaining the iron levels in the body. Iron stores, IL-6 and oxygenation upregulate the secretion of hepcidin.[18,19]

During absolute ID or when there is increased iron demand, hepcidin levels are suppressed. This results in increased iron absorption and recycling to optimize the iron supply. During inflammation, the concentration of hepcidin is increased and ferroportin transcription is decreased which limits iron supply to plasma leading to functional ID. The control process of iron has three dimensions, which are independent of each other. The first control process is influenced by recent dietary iron intake. This is called "Dietary regulator". With this process in operation, absorptive cells are resistant to iron uptake for several days after a large dietary bolus of iron. This was called "Mucosal block" by scientists of an earlier era. In the second process, iron absorption can be modulated considerably in response to body iron stores. This is called "Stores regulator". This regulator senses total body stores and not dietary intake. Third control process in which an unidentified signal communicates the state of bone marrow erythropoiesis to the intestine. This is called the "Erythroid regulator". The exact mechanism of this regulator is not known, but it is believed to be carried from the bone marrow to the intestinal cells.[17, 20]

Excretion

The amount of iron lost from the body per day is between 0.5 to 1.0 mg under physiological conditions. In women, the menstrual loss of blood brings the iron loss to an average amount of about 1-2 mg/day. The rate of loss is relatively constant and independent of intake occurring because of the desquamation of epithelial cells.[7,16]

REQUIREMENT OF IRON

Table 1: Daily requirement of Iron [15]

	Recommended Dietary Allowance(RDA)(mg/day)
Adult (male)	8
Adult (female)	8
Adolescent (female)	15
Mid to late pregnancy	27
Infant (7-12months)	11

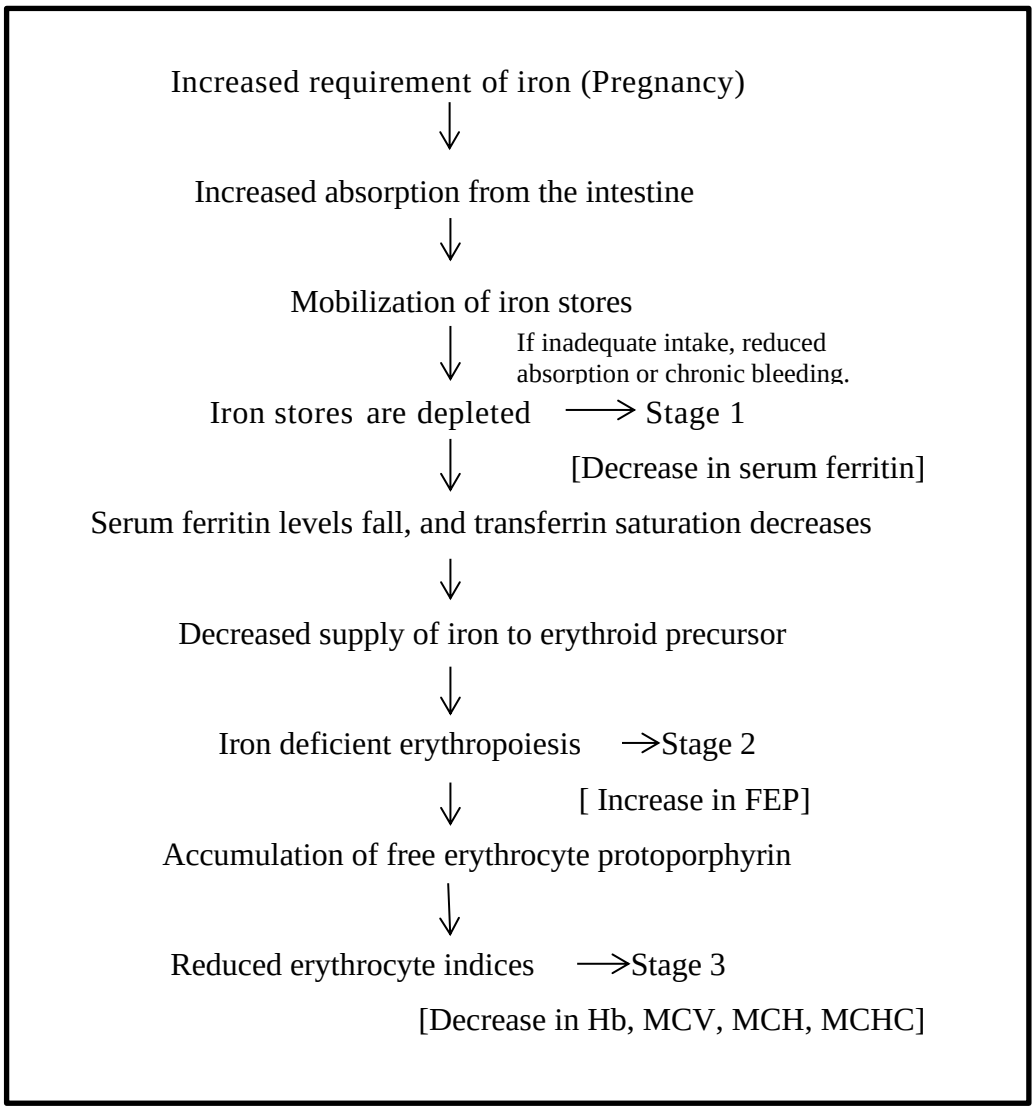
IRON DEFICIENCY ANEMIA

Iron deficiency(ID) is a major contributor to the global burden of disease, particularly affecting children, women mostly in the reproductive age group and people in low-income and middle-income countries. There can be just clinical and functional impairments initially before the anemia manifests.

Stages of Iron Deficiency: There are 3 stages

1. The negative iron balance stage shows decreased iron stores with a decrease in serum ferritin levels (<20µg/l) and an increase in total iron binding capacity(TIBC)(> 360µg/dl). In this stage hemoglobin is normal with a normocytic normochromic picture on peripheral smear.

2. In Iron deficient erythropoiesis stage the iron stores are exhausted with a further decrease in serum ferritin levels ((<15µg/l), decrease in transferrin saturation (<20%) and increase in TIBC and soluble transferrin receptor (sTfR).At this stage hemoglobin level is reduced but the blood picture in peripheral smear still shows a normocytic normochromic morphology.
3. Iron deficiency anemia stage is the last stage with no iron stores and reduced serum ferritin (<12µg/l), transferrin saturation (<16%) and hemoglobin. Increase in TIBC (>400µg/dl) and sTfR. The peripheral blood smear in this stage shows microcytic hypochromic morphology of red cells.[14,17]



Etiologic factors in iron deficiency

It is classified under the following categories: Inadequate iron uptake, increased iron requirements and blood loss.

1. Inadequate uptake of iron could be due to insufficient intake as seen in individuals having a vegetarian or vegan diet that has a low iron content, due to low socio-economic status or prolonged breastfeeding. Another cause could be decreased absorption of iron due to intake of iron absorption inhibitors like tea or calcium or in conditions like atrophic gastritis, use of proton pump inhibitors, H.Pylori infection wherein there is inadequate acidification in the stomach. Another cause for inadequate absorption can be due to increased hepcidin levels seen in chronic inflammation or iron refractory iron deficiency anemia caused by TMPRSS mutation.
2. The requirement for iron is increased during pregnancy, lactation and phases of a growth spurt in children and adolescents. Also seen in patients taking erythropoietin stimulating agents.
3. Blood loss is another major causative factor and it can involve any organ in the body. Most common being intestinal bleeding seen in conditions like oesophageal varices, gastric or duodenal ulcers, polyps, inflammatory bowel disease, hereditary hemorrhagic telangiectasia and malignancies. Excessive menstrual bleeding is seen associated with fibroid uterus, intrauterine device, or malignancy. [16]

Clinical Presentation

Symptoms can be there both in the presence and absence of anemia. Some patients can be asymptomatic as well.

Symptoms and signs commonly present in anemia are fatigue, lethargy, reduced concentration, dizziness, tinnitus, pallor, and headache. Other possible presentations could be alopecia, dry skin or hair, pica, atrophic glossitis, and koilonychia. Infants with ID can present with complaints of poor feeding and irritability.

IDA can aggravate symptoms and worsen the prognosis of associated medical conditions. In pregnancy ID affects the outcome of pregnancy and child's neurocognitive development may be affected.[16,17]

INVESTIGATIONS

Various investigations done for the detection of iron deficiency anemia are [21]

1. Erythrocyte parameters
2. Examination of peripheral blood smear
3. Biochemical investigations
4. Bone marrow aspiration/biopsy
5. Analysis of red cell distribution width
6. Reticulocyte parameters

Each of the above methods has its advantage and disadvantages as well as its unique source of error. Routine hematological investigations like the erythrocyte parameters, red cell distribution width, and reticulocyte parameters are obtained by an automated hematology analyzer. [21]

A variety of automated instruments for performing blood counts are currently used. Semi-automated instruments require some steps like dilution of the blood

sample to be carried out by the operator. But fully automated instruments require an optimal amount of blood sample to be presented to the instrument. They have high precision and accuracy for cell counting and cell sizing when compared to manual techniques. The Principle of the automated analyzer depends on impedance or light scattering technology. [22]

The impedance principle depends on the increased resistance that occurs when a blood cell with poor conductivity passes through an electrical field. The number of pulses indicates the blood cell count and the amplitude of each pulse is proportional to the volume of the cell. Whereas optical light scattering is based on light scattering measurements obtained as a single blood cell passes through a beam of light(optical or laser). Blood cells create forward scatter and side scatter that are detected by photodetectors. The degree of forward scattering is a measurement of cell size, while the degree of side scatter is a measurement of cell complexity of granularity. [22]

1. **Erythrocyte Parameters**: Includes the following parameters

a) Hemoglobin concentration

b) Packed Cell Volume(PCV)

c) Mean corpuscular volume(MCV)

d) Mean Corpuscular Hemoglobin (MCH)

e) Mean Corpuscular Hemoglobin Concentration(MCHC)

f) Red cell count

g) Percentage of microcytic red cells (%Micro- R)

h) Percentage of hypochromic red cells (%Hypo- He)

a) Hemoglobin (Hb)concentration: In the early stage of iron deficiency Hb concentration will be normal, while in advanced cases a definite reduction in the Hb concentration is observed. In pregnant women, Hb level less than 11g/dL is taken as anemic. Anemia is graded arbitrarily as Mild, Moderate, and severe based on Hb value [22,23]

Table 2: Anemia grading in pregnant and nonpregnant women [23]

	Non pregnant women of reproductive age 15- 49 years	**Pregnant women**
Non-anemic	≥12 g/dL	≥11 g/dL
Mild anemia	10-11.9 g/dL	10-10.9 g/dL
Moderate anemia	7-9.9 g/dL	7-9.9 g/dL
Severe anemia	<7 g/dL	<7 g/dL

b) MCV, MCH, MCHC & PCV: MCV tends to be the single most useful index. When MCV is less than 80fL microcytosis is considered. MCH less than 27 pg is significant and MCHC when less than 31.5% suggests hypochromia. A decrease in MCHC occurs late in the disease process indicating the advanced stage of the disease. Hematocrit reduction is directly proportional to the fall in Hb.[21]

c) % HYPO-He The percentage of RBCs with Hb less than 17pg. It is derived from the hemoglobin content of all mature RBC and is analyzed in the reticulocyte channel. The percentage of hypochromic cells is calculated based

on the high angle forward scatter. This is one of the parameters used to distinguish between Iron deficiency anemia and thalassemia. [24]

The utility of Ret-he, Micro-R, Hypo-He and RBC-He in early diagnosis of IDA was studied by Agarwal C *et al*.[25] Hypo-he of more than 6% showed to be comparable with serum transferrin receptor (sTfR), serum ferritin and TIBC to differentiate between ID and non-ID groups but did not show statistical significance.

Another study was by Urrechaga E *et al*.[26] mentioned that hypochromic markers like Ret-he, %Hypo-He and %LHD can be used to assess the presence of latent ID in premenopausal women. There authors also studied comparison of these parameters with serum ferritin. They also explained that this maybe because these hypochromic markers assess the functional iron availability and not the stored iron. Despite low concordance with the standard biochemical test, they concluded that %Hypo-He is a reliable test but %LHD and Ret-He proved to be more accurate.

Dinh N *et al*.[27]studied the utility of %Hypo-He in end-stage renal disease patients and a cutoff value of more than 10pg was recommended to identify the presence of ID and can be used for screening purpose in ESRD patients.

In a study conducted by Torino ABB *et al*.[28] with a sample size of 117 anemic patients were classified into groups namely: IDA, ACD, ACD with IDA and beta-thalassemia. The new erythrocyte and reticulocyte parameters were studied in each of these groups and they concluded that %Hypo-he showed the best sensitivity and specificity of 72.7% and 70.4% respectively.

A combination of increased %Hypo-he and decreased Ret-he was concluded as the best parameters by Urrechaga E *et al*.[29] in their study when compared with the other hematological parameters to identify ID.

d) % Micro-R : The values are obtained from the RBC histogram. The histograms of samples with microcytic RBCs are shifted to the left. Two discriminators are applied one at the upper and lower area of the histogram to determine the microcytic and macrocytic population of red cells and the resulting parameters reflect the microcytic (Micro-R) and macrocytic RBC (Macro-R) as a percentage of all red blood cells.[24]

Urrechaga E *et al.*[30] in their study wanted to formulate a screening test for thalassemia and have stated that % MicroR – % Hypo-He (M-H) index at a cut off of 7.3 can be used to differentiate microcytosis due to thalassemia from other causes. But the sensitivity and specificity were low. To improve the efficacy of the test, they added another parameter that is RDW and together at a cutoff of 7.6 the sensitivity was 100% with no false negatives. Any value above 7.6 is highly suspicious for β-thalassemia trait and needs to be investigated further.

The European Best Practice Guidelines and National Kidney Foundation Kidney Disease Outcome Quality Initiative guidelines suggest the use of %Hypo-he and Micro-R as a part of routine investigations for diagnosing ID.[24]

Both % Micro-R and % HYPO-He increases in iron deficient erythropoiesis. Small changes in the number and hemoglobinization can be picked by these parameters. But these parameters reflect only the functional iron availability and not the availability of iron stores. Another disadvantage is that they are expressed as a single mean value of all the hypochromic and microcytic cells and other morphological variations are not taken into consideration.[24]

2. Peripheral blood smear examination:

On peripheral smear, RBCs are predominantly microcytic hypochromic showing moderate to severe anisopoikilocytosis with few pencil shaped cells and teardrop cells. Microcytic cells are RBCs having size less than the size of small lymphocyte. If RBCs show central pallor more than 2/3rd the diameter of the RBC or only a narrow rim of chromasia, then such cells are called microcytic hypochromic cells. The differential diagnosis will for microcytic hypochromic anemia includes IDA, anemia of chronic disease, Beta thalassemia trait and sideroblastic anemia. All these conditions will have a low MCV. In beta thalassemia trait there will be mild anisopoikilocytosis with target cells and basophilic stippling .In sideroblastic anemia the picture will be dimorphic. These clues can only raise suspicion towards the possible diagnosis but biochemical investigations are needed to confirm the diagnosis.[21,22]

3. Biochemical Investigations

a) **Serum iron** – It is the iron in the circulation that is bound to transferrin. The values show diurnal variation and fluctuate with the absorption of dietary iron. Hence overnight fast is advised before collecting the sample. Serum iron is low in IDA and ACD, increased in sideroblastic anemia and is normal in beta thalassemia. Serum iron by itself is not a diagnostic test, hence additional investigations are recommended.[31]

b) **Total iron-binding capacity (TIBC):** It is the measure of iron binding capacity of transferrin in the circulation. When there are depleted iron stores the transferrin levels increase so does the binding capacity.

Therefore in iron deficiency anemia the TIBC values increase. The normal reference range is 240 mcg/dL to 450 mcg/dL. The test results are altered by diurnal variation, hence a morning sample is preferred. Also interfered by repeated blood transfusion and prolonged usage of OCPs. A relevant history is to be taken before processing the sample to avoid false positives. TIBC is elevated in all the stages of iron deficiency and this parameter can be used to differentiate from the other conditions presenting with microcytic hypochromic picture. Beta thalassemia and sideroblastic anemia show a normal TIBC but ACD shows low TIBC. [32]

c) **Transferrin saturation (TSAT):** Assesses the iron content both in peripheral blood and the bone marrow. It is calculated from the measurement of serum iron and TIBC and is expressed in percentage. (Serum iron/TIBC) X 100 = % Transferrin saturation. The normal reference range is 25-45 percent. A cutoff of 19% is used for IDA screening and a value less than 10% is seen in all IDA patients. AS TSAT is derived from serum iron and TIBC, the factors affecting are also the same.[31]

d) **Free Erythrocyte Porphyrin (FEP):** FEP like transferrin, is a functional index of the adequacy of iron delivery to the erythroid marrow.

In developing red blood cells, the porphyrin ring is formed before the iron is inserted to form heme. When iron supply is inadequate, porphyrin accumulates within the cells. Values above 3 microgram/gm hemoglobin are considered abnormal. The rise in the concentration of EP is proportional to the relative deficit in iron.There are two methods to estimate the concentration of EP in blood samples.

1. Chemical extraction method
2. Hematofluorimeter method

Amongst the two methods, the newer hematofluorimeter is the method of choice as it requires little expertise, <20 µl blood, and can be reported in few minutes.

Two factors can affect the results:

1. The sample needs to be oxygenated to get better reproducibility
2. Before measurement, the red blood cells need to be washed in saline as it lowers the falsely high results and improves reproducibility.[33,34]

e) Serum Ferritin: Serum ferritin concentration is directly related to macrophage iron stores. A serum ferritin concentration less than 12 -15 µg/L is diagnostic of an iron deficiency state. A systematic overview of 55 studies relevant to laboratory tests for diagnosis of iron deficiency anemia in variable patient populations found serum ferritin radioimmunoassay to be the most powerful test. Ferritin levels are considered the gold standard for the diagnosis of iron-deficiency anemia in pregnancy [34]

In the study conducted by Guyatt GH *et al.*[35]a systematic overview of the diagnostic values used in the evaluation of iron deficiency anemia showed that serum ferritin was by far the most powerful test for the diagnosis of iron deficiency, outperforming red cell protoporphyrin, transferrin saturation, mean cell volume, or red cell distribution, with an area under the receiving operating characteristic curve of 0.95. Thus authors concluded that serum ferritin concentration should be the only blood test ordered to evaluate suspected iron deficiency anemia and that the traditional cutoff point dividing normal and abnormal (typically between 12 and 20 ng/ml) was considered too low to detect iron deficiency anemia in the general population but especially for those with inflammatory or liver disease. Using pretest

probabilities and likelihood ratios, they suggested that a level is higher than approximately 40ng/ml should be used to exclude iron deficiency in most patients, whereas a level higher than 70ng/ml was more appropriate to exclude iron deficiency in patients with inflammation or liver disease.

Hallberg L *et al*.[36] in their study mentioned that 25% of women with no stainable bone marrow iron (the gold standard test for diagnosis of iron deficiency) had serum ferritin levels greater than 15 ng/ml (previously considered the low end of the normal range of ferritin), confirming that iron deficiency can exist even with ferritin levels within the normal range.

4. **Bone marrow aspiration/biopsy** : The bone marrow characteristically shows erythroid hyperplasia with micronormoblastic maturation. The morphological features are small sized normoblasts and persistent basophilia with irregular and ragged cytoplasmic edges in the late normoblasts indicating incomplete hemoglobinization. Dyserythropoiesis in the form of karyorrhexis, nuclear budding, nuclear fragmentation, and intranuclear bridges may be observed. All the other lineages are normal. Bone marrow aspiration and biopsy are the gold standard to evaluate iron stores. The smears are stained with Prussian blue and the iron stores are assessed. The iron stores are graded from 1 to 6 wherein grade 1 is absent iron granules and grade 6 is very large deposits almost obscurin the marrow particles. IDA normally has no iron stores and falls in grade 1, beta thalassemia and sideroblastic anemia have normal stores with a grading between 1 to 3. In ACD the stores can be normal or increased(Grade 4 to 6) [21,22]

5. **Red Cell Distribution Width(RDW)** The size variation of the RBCs is quantitatively measured by RDW. Initially, it was measured manually using "Prince Jones curves" but was time consuming and was not practical for day-to-day application. With the advent of automated hematology analyzers, quick construction of frequency distribution curves of red cell volume has become possible. This value obtained is equivalent to the degree of anisopoikilocytosis seen on the peripheral smear. Normal RDW is 11.5 – 14.5. The value increases considerably in IDA (stage 3), which implies severe anisopoikilocytosis. RDW is either normal or low in thalassemia and normal in ACD.[21,38]

6. **Reticulocyte Parameters**

- **Reticulocyte count**: It is a quantitative assessment of erythropoietic activity in the bone marrow. It can be measured by manual method or by an automated hematology analyzer. For manual assessment, the blood collected in EDTA tube is taken and smears are prepared. It is then stained using supravital stain and the residual ribosomes within the cell take up the blue stain. On microscopy, any RBC with 2 or more blue stained particles is considered as reticulocytes. The number of reticulocytes for 1000 RBCs is counted under oil immersion and the reticulocyte count is given based on the formula: Number of reticulocytes/1000 RBCs counted X 100. Reticulocytes are often confused with Heinz bodies, howell jolly bodies, and pappenheimer bodies. Other sources of error depend on the moisture in air or poor drying of smear making it difficult to identify the remnants as they are not refractile as seen in well dried smears. [21]

Automated reticulocyte counts are obtained by using different dyes and fluorochromes which combine with the RNA of reticulocytes. Following the binding of the dye, fluorescent cells can be enumerated using a flow cytometer. The dyes used in Sysmex include auramine O or polymethine with oxazine.The normal reticulocyte count in men or women is: 0.5–2.5% [22]

- **Reticulocyte Hemoglobin Equivalent (Ret-He)** Iron deficiency anemia is one of the leading causes of anemia worldwide and the many adverse effects it has on the patient makes it necessary to have an investigation that can identify ID at the early stage. Bone marrow is the gold standard for assessing iron stores but is an invasive and very painful procedure. Another investigation that is considered close to bone marrow study is serum ferritin levels but it is an acute phase reactant and shows increase in values when there is infection or inflammation masking the underlying iron deficiency. Therefore the need for a reliable test that is not invasive, convenient, cost-effective, not affected by infection/inflammation and not affected by diurnal variation. Ret-He was first studied in 2005 and many studies have been conducted since to evaluate its efficacy in the early detection of ID in various study groups. Ret-he is the measure of hemoglobin content of reticulocytes. Reticulocytes released into peripheral blood take 1- 2 days to form mature RBCs. Thus estimated hemoglobin in these reticulocytes reflects the iron availability in the bone marrow for hemoglobinization.[38,22]

Ret-He is a measure of the forward scatter of stained reticulocytes. The reticulocyte hemoglobin content provides an indirect measure of the functional iron available for new red blood cell production over the previous 3–4 days. Hence a

useful parameter to diagnose anemia, iron restricted erythropoiesis, functional iron deficiency, and response to therapy. A Ret-He value < 25 pg is suggestive of classical iron deficiency. A Ret-He value <30.6 pg appears to have the best predictive value for the likelihood of response to IV iron therapy in chronic kidney disease (CKD) patients on hemodialysis. 29pg is the cutoff value that defines deficient erythropoiesis.[24]

Brugnara C *et al*.[39] conducted a study to measure Ret-He and RBC-he for diagnosis and treatment of ID. The study population included patients with end-stage renal disease. Ret He was compared with the traditional parameters and a cut-off of 27.2pg with a sensitivity of 93.3% and specificity of 83.2% was used to diagnose ID. Hence concluded that Ret He is a reliable marker of cellular hemoglobin content and can be used to diagnose ID state.

Assessment of the clinical usefulness of biochemical and cellular parameters as predictors of ID inpatient undergoing long term hemodialysis was studied by Buttarello *et a*[40]. The reticulocyte parameters showed better ability to predict the iron responsiveness and they concluded that the new reticulocyte parameter Ret-He is reliable and equivalent to CHret and HYPO%.

A study to assess the utility of reticulocyte and erythrocyte indices in assessing erythropoiesis and iron availability was done by Urrechaga. C *et al* [41] in 2012. Chronic kidney disease is a condition where there is a decrease in erythropoietin production and iron availability leading to impaired erythropoiesis. Ret-He decreases rapidly in patients with iron deficient erythropoiesis with a cut-off value of 29.8pg (currently accepted value for functional iron deficiency anemia). %Hypo-He in the detection of iron deficiency was better than MCH and MCV.

Peerschke *et al*[42] conducted a study using Ret-He to evaluate IDA in patients with cancer. Evaluation of anemia in cancer patients is difficult but is very important in patients being considered for therapy with erythropoiesis stimulating agents. This study supports the use of Ret-He to rule out IDA.

Levy S *et al* [1] in their study on the utility of new reticulocyte and erythrocyte parameters to diagnose latent anemia in pregnant women in the first trimester observed that Ret He at a cut-off value <31.2pg showed optimal sensitivity and specificity to distinguish ID from Non-ID. and combination of Ret-He, %Hypo-He and Micro-R improved the sensitivity. Thus proving that the new reticulocyte and erythrocyte parameters are reliable tests for the diagnosis of subclinical/latent ID in pregnant women.

Kumar U *et al* [43]also studied the utility of Ret-He in pregnant women in their first trimester. But found the cut-off of less than 27.2pg to have optimal sensitivity and specificity to diagnose IDA.

Several findings in the field of iron metabolism and erythropoiesis are modifying the traditional concepts on anemia. An appropriate combination of laboratory tests gives evidence of iron depletion, reflects iron restricted red cells production, and so will help to establish a correct assessment of the iron status and thus the appropriate treatment [24]

NORMAL HAEMATOLOGICAL CHANGES IN PREGNANCY:

The maternal blood volume during pregnancy increase to the extent of 50 percent above the non-pregnant volume. However, the degree of expansion varies to a considerable extent. The increase in blood volume results from an increase in both plasma and erythrocytes. Since the increase in plasma volume is much greater (35%)

than the increase in red cell volume and hemoglobin mass (15%), there is a positive haemodilution, despite augmentation in erythropoiesis which results in decrease in hemoglobin and hematocrit value. The resulting change in red cell mass and hematocrit values had led to the term physiological anemia of pregnancy. The increase in blood volume starts very early in pregnancy, reaches a peak around 28-32 weeks, and thereafter stabilizes. The increase in erythropoiesis during the pregnancy is controlled by various factors such as the increase in erythropoietin levels, placental lactogen, and estrogen levels.[45]

Due to the disproportionate increase in plasma volume, the determination of hematological parameters is altered during pregnancy. The normality of hematological parameters in pregnancy cannot be judged by reference to non-pregnant standards. However, it is generally agreed that if levels of hemoglobin drop below 11gm %, it certainly is pathological. Many studies have pointed out that supplementation of iron during pregnancy can keep hemoglobin levels above 11gm% despite the changes in plasma volume.[46]

- Plasma volume gradually increases until term. It begins approximately halfway through the first trimester and peaks about the 28th to 30th week. After that, it slowly rises until term, but at a very slow rate that simulates a plateau effect.
- The volume of red blood cells begins to rise late in the first trimester and continues to rise throughout the pregnancy, following a different curve than plasma volume. The total increase in red cell mass during pregnancy approximates 20 to 40%.

Pregnancy causes "anemia of dilution" because the rise in red cell mass is of lesser magnitude than the increase in plasma volume. Plasma volume growth reaches

a halt around the 30th week of pregnancy, and the steady fall in hemoglobin content comes to an end. A small rise in Hb concentration towards the pre-pregnancy levels may occur during the third trimester. Increased blood volume associated with pregnancy is lost after birth, typically 500 ml for a normal vaginal birth, and greater amounts if delivery is by cesarean section. This expected loss of blood after birth leaves the women in a relatively hypovolemic state. During the puerperium, prepregnant blood, plasma, and red cell volume levels are rapidly restored within one week.[45,46]

Table 3: Blood and plasma volume changes during pregnancy are as mentioned in the table.[46]

Parameters	Non-pregnant	Pregnancy Near Term	Total Increment	Change
Blood volume(ml)	4000	5500	1500	+30–40%
Plasma volume (ml)	2500	3750	1250	+ 40–50%
Red Cell Volume (mL)	1400	1750	350	+ 20–30%
Total Hb (g)	475	560	85	+ 18–20%
Hematocrit	38%	32%		Diminished

Effect Of Anemia On Pregnancy

Maternal effects: Mild anemia will not have any effect on present pregnancy but if not corrected can lead to moderate to severe anemia in subsequent pregnancies. In moderate and severe anemia the women experience excessive weakness, easy fatiguability, breathlessness, frequent urinary tract infections, and pre-term labour. During labour the woman is at a high risk to develop antepartum hemorrhage or postpartum hemorrhage which can eventually lead to collapse and death. In the

postpartum period there is an increased risk to develop puerperal sepsis, delayed wound healing and can cause failure of lactation. 2% of maternal mortality is due to anemia.[45]

Effects on the fetus are low birth weight, birth asphyxia, failure to thrive and increased perinatal infant mortality. The poor iron stores in the infant lead to the development of anemia by the age of 10months.[45]

MATERIALS AND METHODS

Study Design: Hospital-based Prospective Cross-sectional study.

Source of Data: Blood samples of nonanemic pregnant women of first trimester presenting to the antenatal clinic for the first time in the Department of Obstetrics and Gynecology(OBG) were sent to the Hematology section of the Department of Pathology of Shri B. M. Patil Medical College, BLDE (Deemed to be University), Vijayapura.

Study period: 1st November 2018 to 30th May 2020

Inclusion criteria:

- Healthy pregnant women in the first trimester of pregnancy presenting for the first time to the ANC at BLDE (Deemed to be University) ,SHRI B.M.Patil Medical College, Vijayapura

Exclusion criteria:

- Patients on iron supplementation or with a history of blood transfusion in the last 3 months.
- Hemoglobin <11g/dl or MCV > 100fL or a diagnosis of hemoglobinopathy.
- Serum ferritin level above the normal laboratory reference range.
- Clinical symptoms of the disease such as pallor, easy fatigability.
- Samples with inadequate volume (<2ml).

METHODS OF COLLECTION OF DATA

Blood samples of all pregnant women attending the ANC of the OBG Department from 1st November 2018 to 30th May 2020 were included in the study. 2ml of blood in K2 EDTA vacutainer was collected and processed in Sysmex XN 1000 5-part fully automated hematology analyzer. Pregnant women with Hb more than 11g/dl were included in the study. 2ml blood sample was additionally collected in a plain tube for biochemical investigations and was processed in the VITROS5,1 FS Chemistry System. Clinical details regarding the present pregnancy, intake of any supplements, or any other additional presenting complaints were recorded. Those with signs and symptoms of anemia were excluded from the study.

Figure 1: Sysmex XN 1000 5-part fully automated hematology analyzer

Figure 2: VITROS 5,1 FS Chemistry System

SAMPLE SIZE

With a 95% confidence level and margin of error of ±5%, a sample size of **278** subjects with finite population correction (1000) will allow the study to determine the clinical utility of Ret-He, %Micro-R and %Hypo-He in comparison with serum iron studies and ferritin levels.

By using the formula:

n = z 2 p (1-p)

d 2

Where

Z= z statistic at 5% level of significance

d is margin of error

p is anticipated prevalence rate (50%)

STATISTICAL ANALYSIS

Statistical methods used

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean±standard deviation (SD) were used.

The difference in the means of analysis variables between two independent groups was tested by an unpaired t-test.

The t statistic to test whether the means are different was calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

s_1^2 = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

s_2^2 = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

ROCanalysis for Sensitivity- specificity was done to check relative efficiency.

Sensitivity or True Positive Rate (TPR)

TPR = TP/P = TP/(TP+FN)

Specificity(SPC) or True Negative Rate

SPC = TN/N = TN/(FP+TN)

Precision or Positive Predictive Value(PPV)

PPV = TP/(TP+FP)

Negative Predictive Value(NPV)

NPV = TN/(TN + FN)

If the p-value was < 0.05, then the results were considered as statistically significant. Data were analyzed using SPSS software v.23(IBM Statistics, Chicago, USA)and Microsoft office 2007.

RESULTS

Total number of cases included in the present study was 280 pregnant women attending the ANC clinic in the first trimester and having Hb more than 11g/dl.

Table 4: Age and gravida status distribution.

Age(yrs)	Gravida Status									
	G1		G2		G3		G4		G5	
	N	%	N	%	N	%	N	%	N	%
18-22 (n-109)	65	23.2	37	13.2	7	2.5	0	0	0	0
23-27 (n-137)	51	18.2	62	22.1	18	6.4	6	2.1	0	0
28-32 (n-21)	1	0.3	9	3.21	6	2.1	4	1.5	1	0.35
33-37 (n-11)	0	0	5	1.8	4	1.5	1	0.3	1	0.35
38-42 (n-2)	0	0	0	0	1	0.3	1	0.3	0	0
Total	117	41.7	113	40.3	36	12.8	12	4.2	2	0.7

In the present study majority of the cases were in the age group of 23-27yrs amounting to 48.9% followed by 18-22 yrs amounting to 38.9%. Out of 280 pregnant women studied majority belonged to gravida 1(G1) and gravida 2(G2) groups

amounting to 117(41.7%) cases and 113(40.3) cases respectively. Most of the G1 and G2 cases belonged to the age group of 18-27yrs amounting to 87.8%. Only two cases were gravida 5(G5). In one case age was 29yrs and another case age was 35yrs.

Figure 3: Age and gravida status distribution.

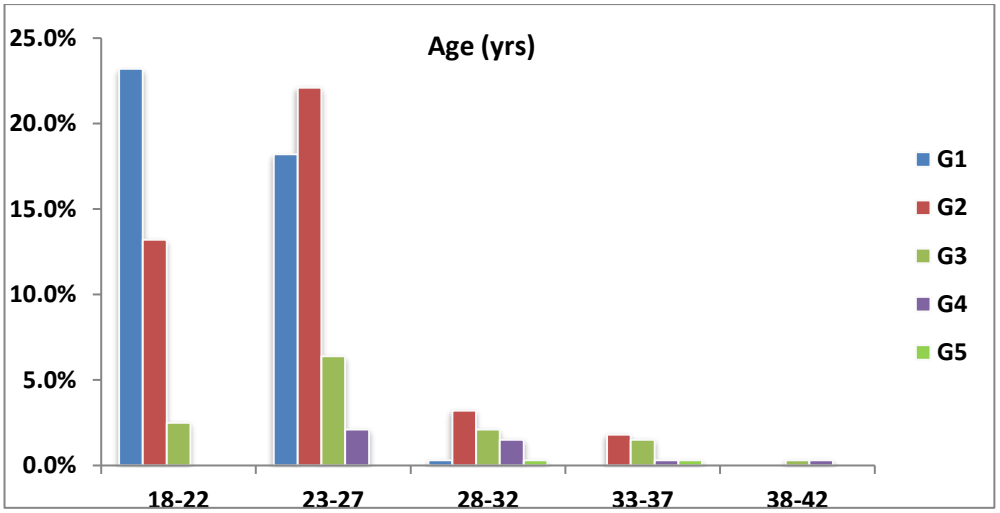


Table 5 : Age and Serum iron parameters distribution.

Age (yrs)	Serum Iron# (40-177µg/dl)				TIBC# (250-400µg/dl)				Serum Ferritin# (20-137ng/ml)			
	ID		N-ID		ID		N-ID		ID		N-ID	
	N	%	N	%	N	%	N	%	N	%	N	%
18-22	3	1.1	106	37.9	37	13.2	72	25.7	55	19.6	54	19.3
23-27	13	4.7	124	44.3	41	14.6	96	34.4	78	27.9	59	21.1
28-32	1	0.3	20	7.2	8	2.9	13	4.6	12	4.3	9	3.3
33-37	0	0	11	3.9	3	1.1	8	2.9	5	1.8	6	2.1
38-42	1	0.3	1	0.3	1	0.3	1	0.3	1	0.3	1	0.3
Total	18	6.4	262	93.6	90	32.1	190	67.9	151	53.9	129	46.1

*ID – Iron deficient group, N-ID – Non Iron deficient group

#TIBC value >400µg/dl is considered ID and <400µg/dl as N-ID, Serum iron <40 µg/dl is considered ID and >40 µg/dl as N-ID, Serum ferritin <20ng/ml is considered ID and >20ng/ml as N-ID

Serum iron was less than 40µg/dl in 18 (6.4%) cases and within normal range in 262(93.6%) cases. Out of 18 ID cases 13 were in the age group of 23-27(72.2%)yrs. TIBC was increased in 90(32.1%) cases .Based on these findings this group was categorized as ID group. Amongst the 90 ID cases, majority were seen in the age group of 23-27yrs and 18-22yrs amounting to 45.5% and 41.1% respectively. Serum ferritin less than 20ng/ml in 151(53.9%) cases and was within normal range in 129(46.1%) cases. Highest number of ID cases were seen in age group of 23-27yrs followed by 18-22yrs with 78(51.6%) cases and 55(36.4%)cases respectively. Of the three biochemical parameters serum ferritin has 53.9% cases are grouped under ID as compared to the other two parameters. The mean age group of ID and N-ID was 23.9 and 23.7 respectively.

Table 6: Gravida status and serum iron parameters distribution.

GRAVIDA	TOTAL CASES	Serum Iron (40-177µg/dl)				TIBC (265-400µg/dl)				Serum Ferritin (20-137ng/ml)			
		ID		N-ID		ID		N-ID		ID		N-ID	
		N	%	N	%	N	%	N	%	N	%	N	%
G1	117	7	6	110	94	32	27.4	85	72.6	52	44.4	65	55.6
G2	113	6	5.3	107	94.7	36	31.9	77	68.1	58	51.3	55	48.7
G3	36	3	8.3	33	91.7	17	47.2	19	52.8	27	75	9	25
G4	12	2	16.7	10	83.3	4	33.3	8	66.7	12	100	0	0
G5	2	0	0	2	100	1	50	1	50	2	100	0	0

*ID – Iron deficient group, N-ID – Non Iron deficient group

#TIBC value >400µg/dl is considered ID and <400µg/dl as N-ID, Serum iron <40 µg/dl is considered ID and >40 µg/dl as N-ID, Serum ferritin <20ng/ml is considered ID and >20ng/ml as N-ID

Out of 280 G1 included 117 cases of which serum iron was less than 40µg/dl in 7(6%)cases, TIBC was above normal limit in 32(27.4%) cases and serum ferritin was less than 20ng/ml in 52(44.4%) cases. Of the 113 gravida 2 cases serum iron grouped 6(5.3%) cases, TIBC grouped 36(31.9%) cases and serum ferritin grouped 58(51.3%) cases under ID. G1 and G2 showed almost equal distribution between ID and N-ID based on serum ferritin values. Gravida 3 includes 36 cases of which 3(8.3%) cases had low serum iron, 17(47.2%) had high TIBC and 27(75%) showed low serum ferritin. All the cases of G4 and G5 based on low serum ferritin values were grouped under ID.

Table 7: Age and Erythrocyte parameters distribution.

AGE (yrs)	MCV (80-100 fL)				%Micro-R (0.3%-2.8%)				%Hypo-He (0.1%-1.1%)			
	ID		N-ID		ID		N-ID		ID		N-ID	
	N	%	N	%	N	%	N	%	N	%	N	%
18-22	9	3.2	100	35.7	27	9.6	82	29.3	10	3.5	99	35.4
23-27	22	7.9	115	41.1	37	13.2	100	35.7	16	5.7	121	43.2
28-32	0	0	21	7.5	6	2.1	15	5.4	1	0.4	20	7.1
33-37	1	0.3	10	3.6	5	1.8	6	2.1	1	0.4	10	3.6
38-42	0	0	2	0.7	1	0.4	1	0.4	0	0	2	0.7
Total	32	11.4	248	88.6	76	27.1	204	72.9	28	10	252	90

*MCV <80fl is ID and >80fl is N-ID, %Micro-R >2.8% is ID and <2.8% is N-ID, %Hypo-He >1.1% is ID and <1.1% is N-ID.

Out of 280 cases 32(11.4%)cases showed MCV less than 80fL and 248(88.6%) cases showed normal MCV. %Micro-R was more than 2.8% in 76(27.1%) cases and within range in 204(72.9%) cases. %Hypo-he of more than 1.1% was seen in 28(10%) cases was grouped under ID and the remaining 252(90%) cases were grouped under non ID.

Table 8: Gravida and erythrocyte parameters distribution.

GRAVIDA	TOTAL CASES	MCV (80-100 fL)				%Micro-R (0.3%-2.8%)				%Hypo-He (0.1%-1.1%)			
		ID		N-ID		ID		N-ID		ID		N-ID	
		N	%	N	%	N	%	N	%	N	%	N	%
G1	117	5	4.3	112	95.7	22	18.8	95	81.2	9	7.7	108	92.3
G2	113	21	18.6	92	81.4	33	29.2	80	70.8	11	9.7	102	90.3
G3	36	4	11.1	32	88.9	15	41.7	21	58.3	5	13.9	31	86.1
G4	12	1	8.3	11	91.7	4	33.3	8	66.6	2	16.7	10	83.3
G5	2	1	50	1	50	2	100	0	0	1	50	1	50

*MCV <80fl is ID and >80fl is N-ID, %Micro-R >2.8% is ID and <2.8% is N-ID, %Hypo-He >1.1% is ID and <1.1% is N-ID.

Out of 117 G1 cases, low MCV was seen in 5(4.3%) cases, increased %Micro-R and %Hypo-He was seen in 22(18.8%) cases and 9(7.7%)cases respectively. G2 included 113 cases of which 21(18.6%) cases showed low MCV, 33(29.2%)cases showed increased %Micro-R and 11(9.7%)cases showed increase in %Hypo-He. In G3 out of 36 cases, 4(11.1%) cases showed low MCV, 15(41.7%)cases were with high %Micro-R and 5(13.9%)cases with high %Hypo-He. G4 includes 12 cases of which 1(8.3%)case showed low MCV, 4(33.3%)cases showed increased %Micro-R and 2(16.7%)cases showed increased %Hypo-He. Out of the 2 cases of G5 one case showed low MCV and High %Hypo-He and both cases showed increased %Micro-R.

Table 9: Age and Reticulocyte parameter distribution

Age	Ret-He (29-36 pg)			
	ID		N-ID	
	N	%	N	%
18-22	47	16.8	62	22.1
23-27	60	21.4	77	27.6
28-32	10	3.6	11	3.9
33-37	3	1.1	8	2.9
38-42	1	0.3	1	0.3
Total	121	43.2	159	56.8

*****Ret-He <29pg is ID and >29pg is N-ID

Reticulocyte count was within normal range in all the 280 cases. Ret-He of less than 29pg was seen in 121(43.2%) cases out of which 60(21.4%) were between age group of 23-27yrs and 47(16.8%)cases were between age group of 18-22yrs.

Table 10: Gravida and Reticulocyte parameter distribution

Gravida	Total	Ret-He (29-36 pg)			
		ID		N-ID	
		N	%	N	%
G1	117	39	33.3	78	66.7
G2	113	50	44.2	63	55.8
G3	36	22	61.1	14	38.8
G4	12	8	66.7	4	33.3
G5	2	2	100	0	0

*****Ret-He <29pg is ID and >29pg is N-ID

G1 and G2 showed increase in Ret-He in 39(33.3%) and 50(44.2%) respectively. Out of 36 cases under G3, 22(61.1%)cases showed increase in Ret-H and out of the 12 cases in G4, 8(66.7%) showed increase. All the cases under G5 showed increase in Ret-He.

Table 11: Comparison of new reticulocyte and erythrocyte parameters with serum iron parameters

Parameters	Reference interval	Iron deficient		Non Iron deficient	
		N	%	N	%
RBC (× 10^{12}/L)	3.8 – 5.2	48	17.1	232	82.9
Hb (g/dL)	11.0 - 15	0	0	280	100
MCV (fL)	80 - 100	32	11.4	248	88.6
RDW (%)	11.5-14.5	84	30	196	70
Retic count (%)	0.5 – 2.5	0	0	280	100
Ret-He (pg)	29 - 36	121	43.2	159	56.8
% Micro-R (%)	0.3 – 2.8	76	27.1	204	72.9
%Hypo-He (%)	0.1 – 1.1	28	10	252	90
TIBC (mugm/dl)	265 – 450	90	32.1	190	67.9
Fe (mugm/dl)	40 – 177	18	6.4	262	93.6
Ferritin (ng/ml)	20 - 137	151	53.9	129	46.1

Table 12: Comparison of new reticulocyte and erythrocyte parameters with serum iron parameters

Parameters	Reference interval	Iron deficient		Non Iron deficient		p value
		Mean	SD	Mean	SD	
RBC (× 10 ¹²/L)	3.8 – 4.8	4.3	0.5	4.3	0.5	0.162
Hb (g/dL)	11.0 - 15	12.3	1.1	12.0	1.7	0.098
MCV (fL)	78.9 - 98.5	84.3	5.0	85.7	3.1	0.006*
RDW (%)	12.4 – 17.3	14.1	1.7	13.9	1.2	0.266
Retic count (%)	0.5 – 2.5	1.4	0.4	1.5	1.0	0.207
Ret-He (pg)	29 - 36	28.3	1.6	33.8	1.6	<0.001*
% Micro-R (%)	0.3 – 2.8	3.9	3.4	1.1	0.6	<0.001*
%Hypo-He (%)	0.1 – 1.1	0.9	2.2	0.4	0.3	0.018*
TIBC (mugm/dl)	265 – 450	391.0	82.9	310.4	71.0	<0.001*
Fe (mugm/dl)	40 – 177	71.5	24.9	70.7	28.2	0.814
Ferritin (ng/ml)	20 - 137	14.3	1.5	69.1	23.9	<0.001*

Note: p value* significant at 5% level of significance (p<0.05)

The values of RBC count was less than normal in 48(17.1%) cases and within normal range in 232(82.9%)cases. The mean ± SD values of both ID and N-ID were 4.3 ±0.5. Haemoglobin showed little variation between ID and N-ID groups with mean ± SD of 12.3±1.1 and 12±1.7 respectively. The mean ± SD of MCV showed statistically significant variation between the two groups. Ret-He values were normally distributed with mean ± SD values of 28.3 ± 1.6 and 33.8 ± 1.6 pg in ID and non-ID groups, respectively with P < 0.001. Similarly, %Micro-R values showed mean ± SD values of 3.9 ± 3.4% in the ID group and 1.1± 0.6% in the non-ID group (P < 0.001) and %Hypo-He showed mean ± SD values of 0.9 ± 2.2% in the ID group and 0.4± 0.3% in the non-ID group with P value 0.018 .

Figure 4: Distribution of Ret-He between ID and N-ID

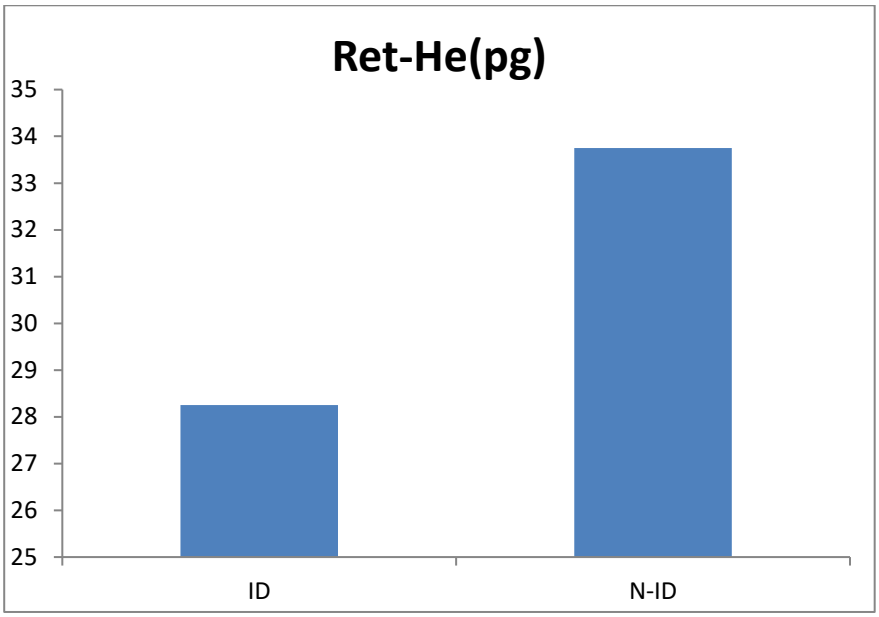


Figure 5: Distribution of % Micro-R (%) between ID and N-ID

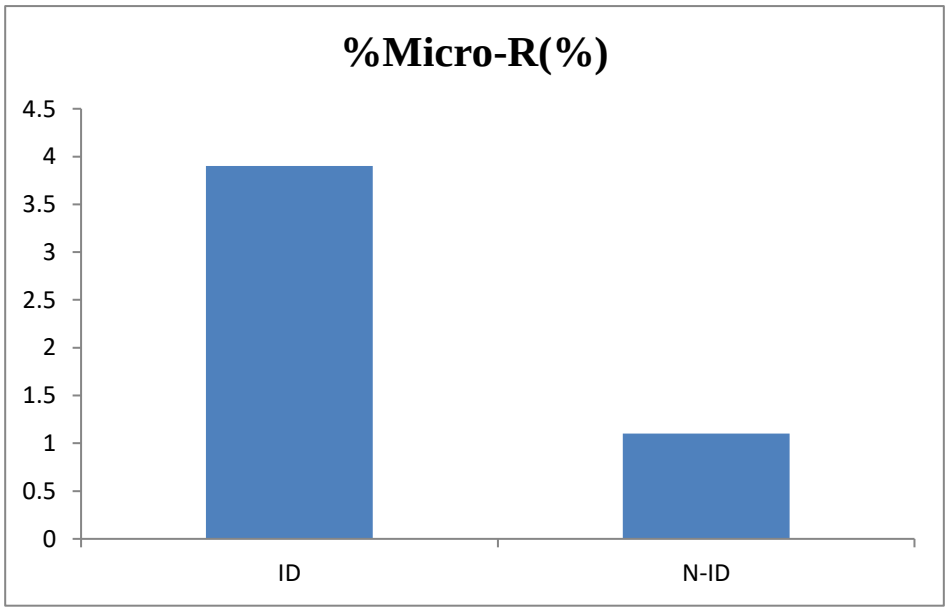
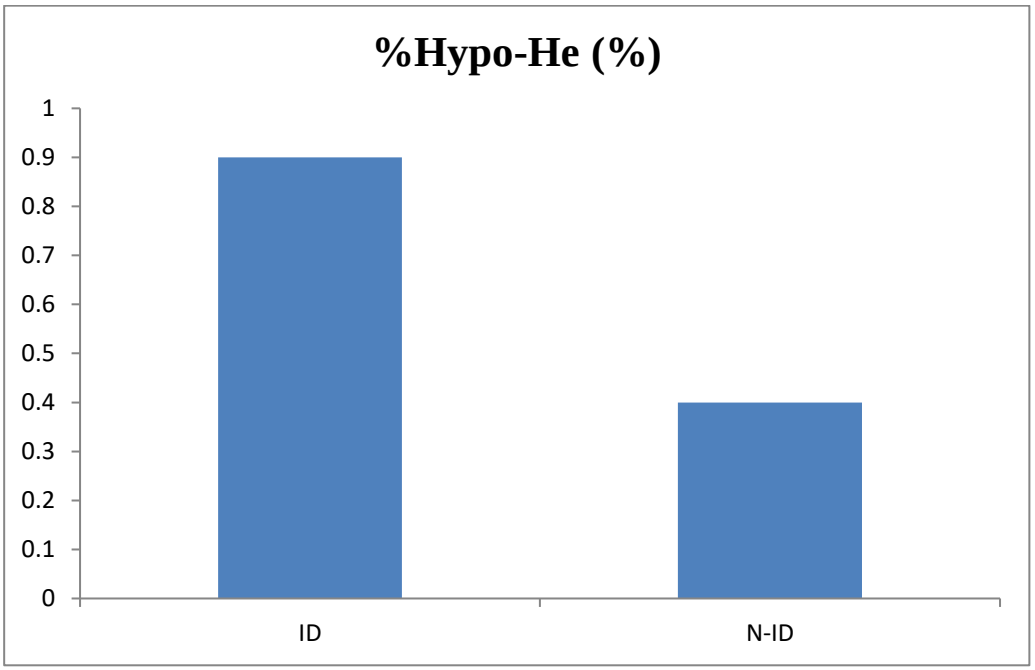


Figure 6: Distribution of %Hypo-He (%) between ID and N-ID



Amongst the biochemical parameters considered in the study only TIBC and Serum Ferritin showed significant difference in the mean values of ID and non-ID groups with a P value < 0.001. Whereas Serum Iron values showed overlap and a narrow difference in the mean values with no statistical significance in differentiating the two study groups.

Figure 7: Distribution of TIBC between ID and N-ID

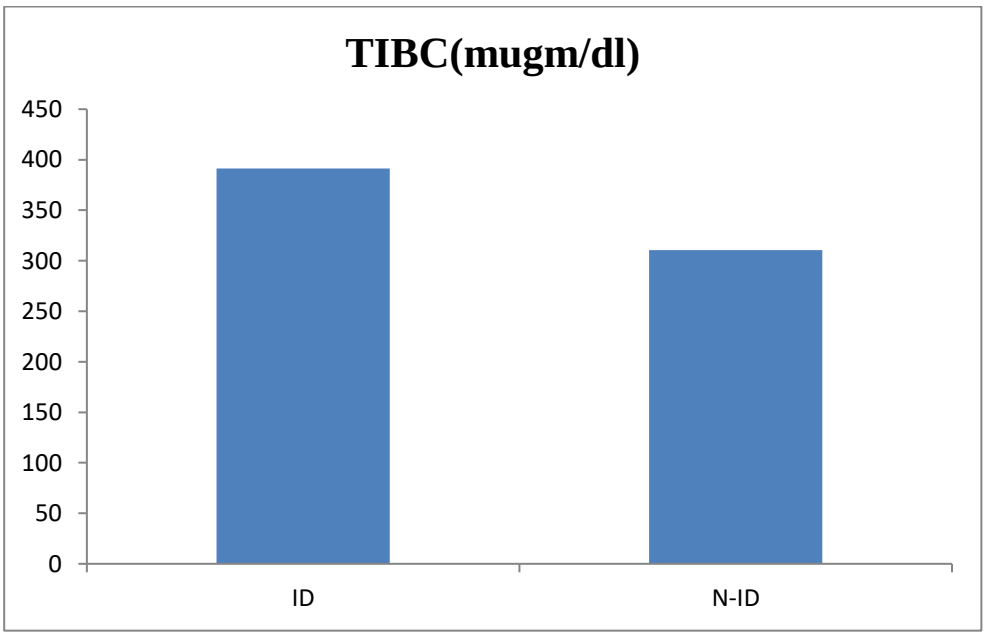


Figure 8: Distribution of Ferritin between ID and N-ID

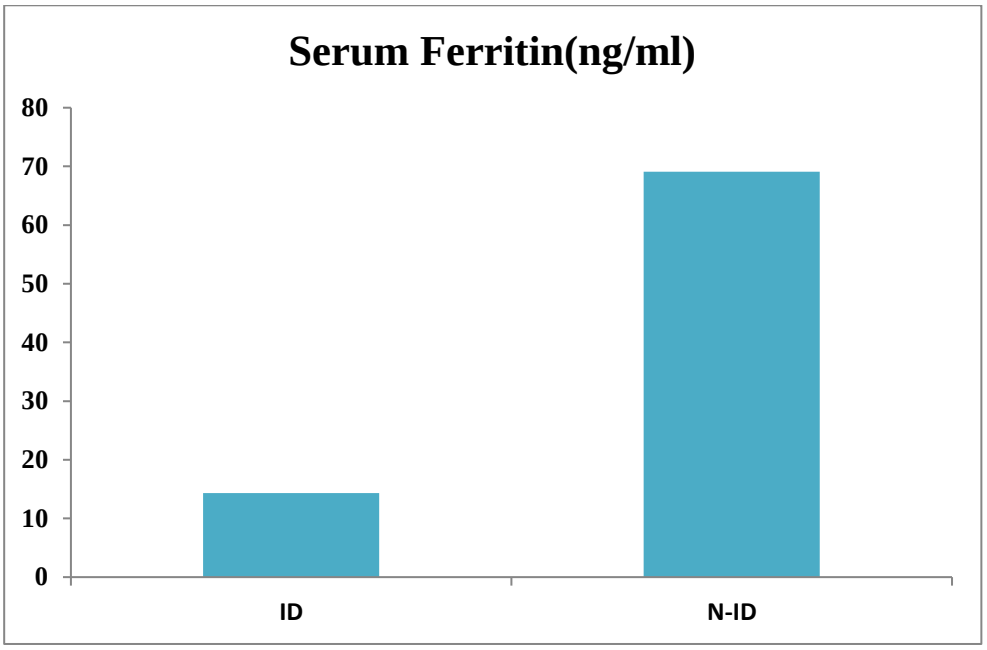
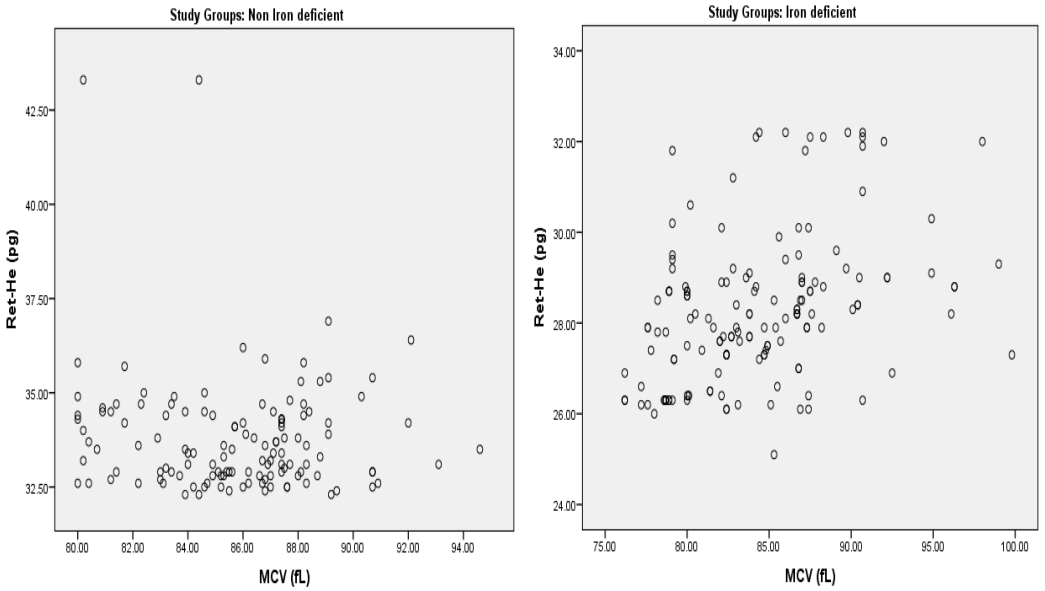


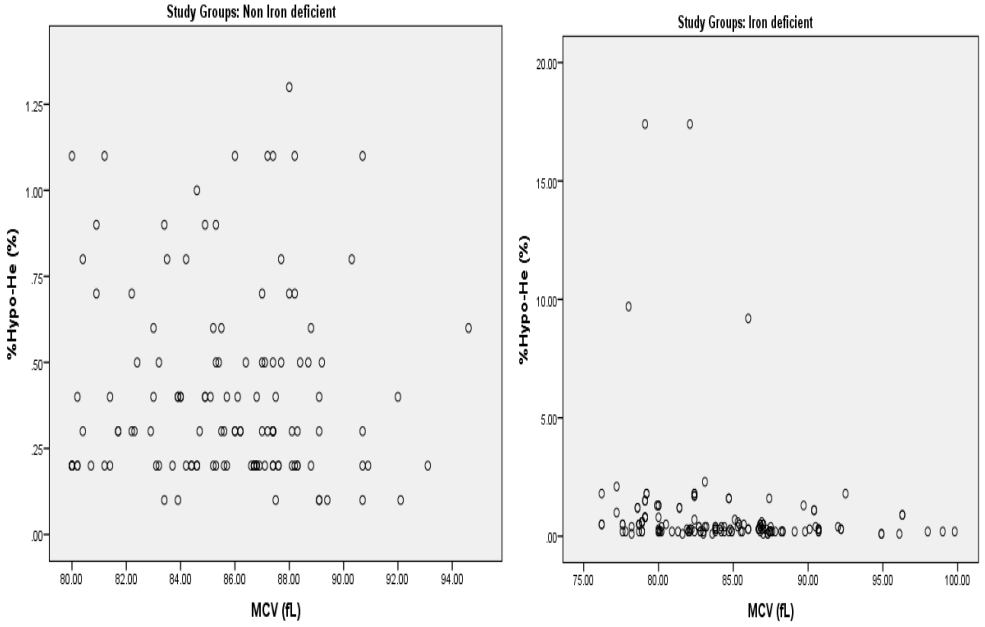
Table 13: Correlation of MCV with % Micro-R, Ret He and %Hypo-He (%)

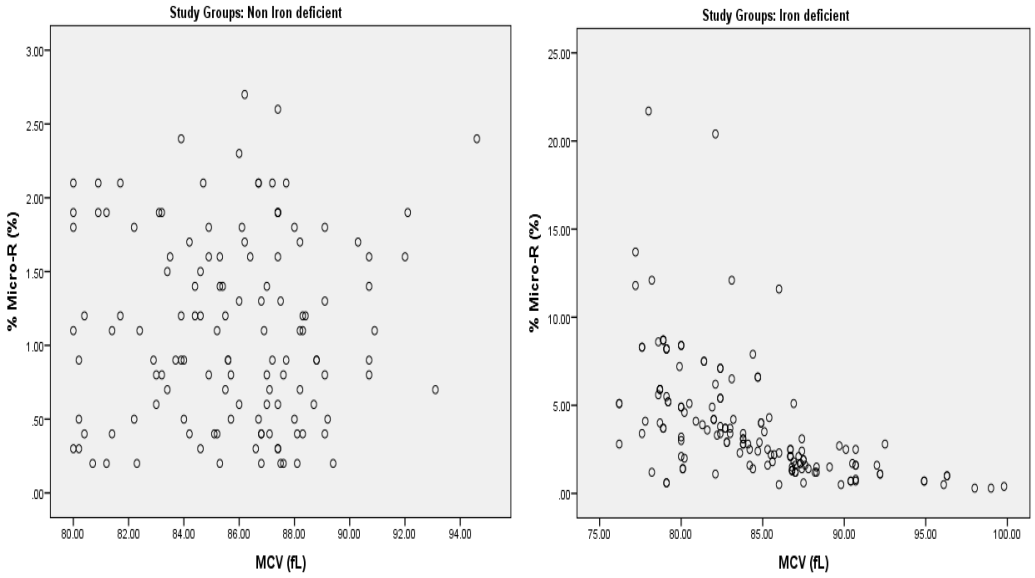
Correlation of MCV with	% Micro-R (%)		Ret-He (pg)		%Hypo-He (%)	
	r value	p value	r value	p value	r value	p value
Iron deficient	-0.551	<0.001*	0.394	<0.001*	-0.154	0.059
Non Iron deficient	0.025	0.778	-0.104	0.240	-0.038	0.666

Note: p value* significant at 5% level of significance (p<0.05)

Figure 9: Correlation of MCV with % Micro-R, Ret He and %Hypo-He (%)







A strong correlation between %Micro-R and the MCV was observed having (r = −0.551, 95% confidence interval (CI) 0.796 to 0.887, P < 0.001). Also noted a strong correlation between MCV and Ret-He (r= 0.394, 95% confidence interval (CI) 0.998 to 1.00, P < 0.001) . But %Hypo-He did not show a strong correlation with MCV(P-0.059). However, %Hypo-He was significantly higher in the ID group (0.90 ± 2.2) as compared to the non-ID group with no overlap (0.4 ± 0.3) (P < 0.018)

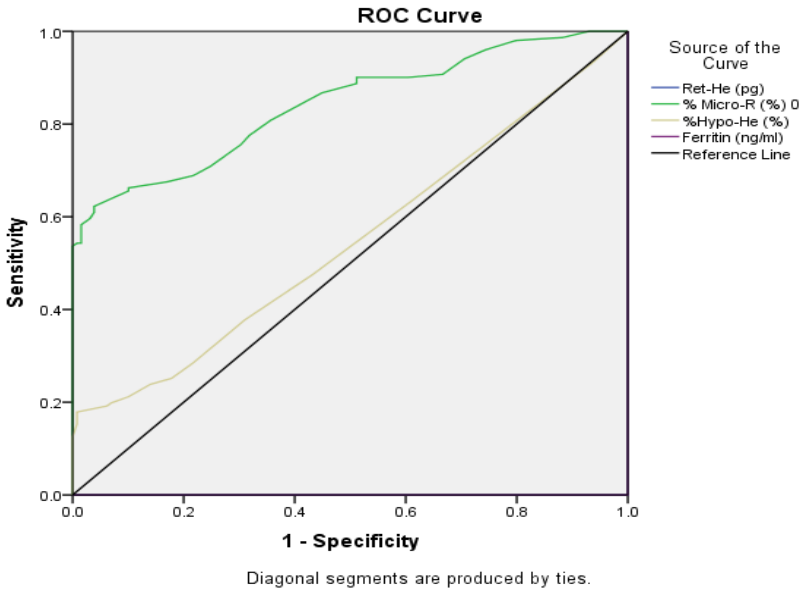
Table 14: ROC Analysis of Parameters in Predicting Iron Deficiency

Parameters	Area Under the Curve	Std. Error	p value	95% CI	
				Lower	Upper
Ret-He (pg)	0.999	0.001	<0.001*	0.998	1.000
% Micro-R (%)	0.841	0.023	<0.001*	0.796	0.887
%Hypo-He (%)	0.546	0.034	0.184	0.479	0.613
Ferritin (ng/ml)	0.989	0.007	<0.001*	0.975	1.000

Note: p value* significant at 5% level of significance (p<0.05)

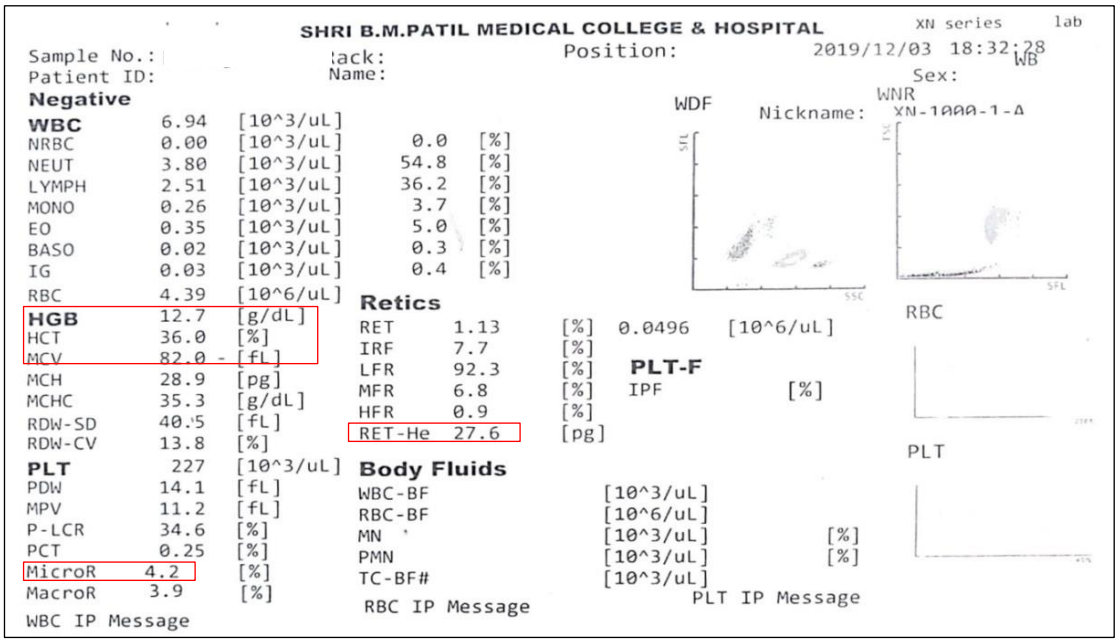
Parameters	cutoff value	Sensitivity	Specificity
Ret-He (pg)	32.25	98.0%	97.0%
% Micro-R (%)	1.55	76.9%	69.9%
%Hypo-He (%)	0.25	63.9%	39.1%
Ferritin (ng/ml)	15.1	99.3%	97.7%

Figure 10: ROC curves of Parameters in Predicting Iron Deficiency



The Receiver operating characteristic (ROC) analysis of the parameters showed cutoff values for diagnosing ID as mentioned in the table 10. Amongst the biochemical parameters Serum Ferritin showed sensitivity of 99.3% and specificity of 97.7% for a cut off value of 15.1 for identifying ID (P <0.001). Comparison of the new reticulocyte parameter Ret-He and the new erythrocyte parameters %Micro-R and % Hypo-he with Serum Ferritin showed that the sensitivity and specificity of Ret He was similar to Serum Ferritin. At the optimal cut-off value of 32.25, the sensitivity and specificity was 98.0% and 97.0% respectively. The AUC for Ret-He (0.999, 95% CI 0.998-1.000) indicates that Ret-He is the best discriminator of ID with a p value <0.001.

Figure 11: Hematology analyzer report.



SHRI B.M.PATIL MEDICAL COLLEGE & HOSPITAL

XN series lab

Sample No.: Rack: Position: 2019/12/03 18:32:28

Patient ID: Name: Sex:

Negative

WBC	6.94	[10^3/uL]		
NRBC	0.00	[10^3/uL]	0.0	[%]
NEUT	3.80	[10^3/uL]	54.8	[%]
LYMPH	2.51	[10^3/uL]	36.2	[%]
MONO	0.26	[10^3/uL]	3.7	[%]
EO	0.35	[10^3/uL]	5.0	[%]
BASO	0.02	[10^3/uL]	0.3	[%]
IG	0.03	[10^3/uL]	0.4	[%]
RBC	4.39	[10^6/uL]		
HGB	12.7	[g/dL]		
HCT	36.0	[%]		
MCV	82.0	[fL]		
MCH	28.9	[pg]		
MCHC	35.3	[g/dL]		
RDW-SD	40.5	[fL]		
RDW-CV	13.8	[%]		
PLT	227	[10^3/uL]		
PDW	14.1	[fL]		
MPV	11.2	[fL]		
P-LCR	34.6	[%]		
PCT	0.25	[%]		
MicroR	4.2	[%]		
MacroR	3.9	[%]		

WBC IP Message

Retics

RET	1.13	[%]	0.0496	[10^6/uL]
IRF	7.7	[%]		
LFR	92.3	[%]	**PLT-F**	
MFR	6.8	[%]	IPF	[%]
HFR	0.9	[%]		
RET-He	27.6	[pg]		

Body Fluids

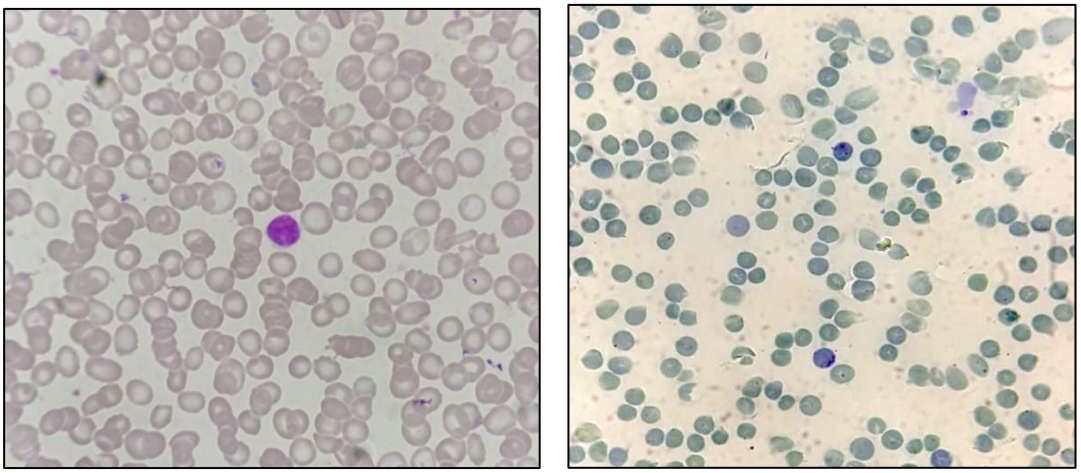
WBC-BF	[10^3/uL]	
RBC-BF	[10^6/uL]	
MN	[10^3/uL]	[%]
PMN	[10^3/uL]	[%]
TC-BF#	[10^3/uL]	

RBC IP Message

PLT IP Message

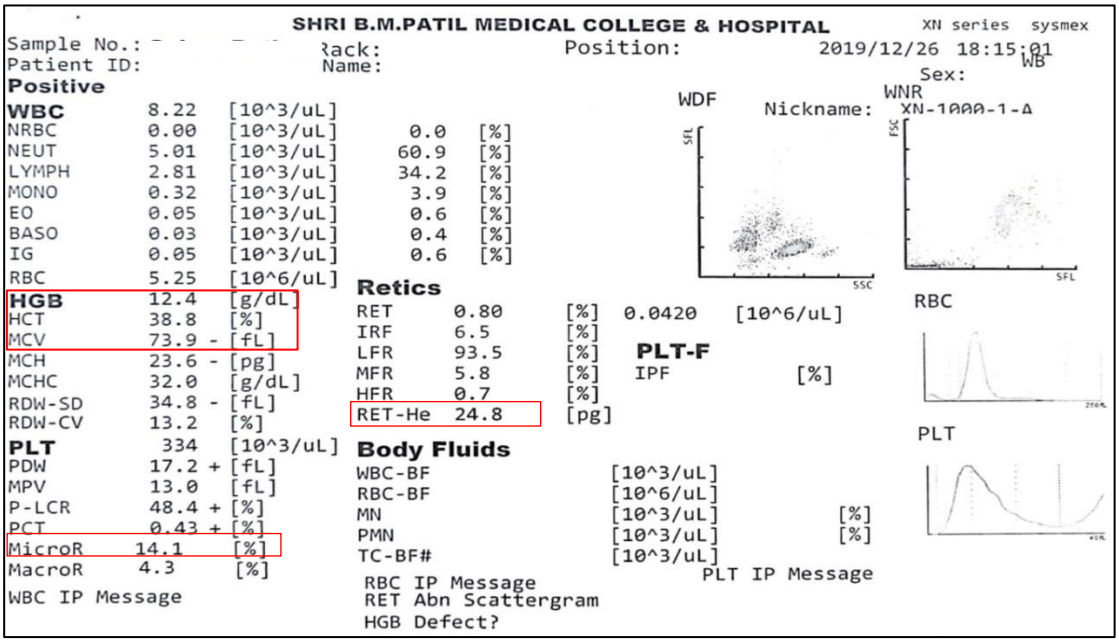
WDF Nickname: XN-1000-1-A WNR WB RBC PLT

Figure 12: A-Peripheral smear showing normocytic normochromic blood picture (1000X,Leishman stain). B-Peripheral smear showing reticulocytes (1000X, New methylene blue stain)



A B

Figure 13: Hematology analyzer report.



SHRI B.M.PATIL MEDICAL COLLEGE & HOSPITAL

XN series sysmex

Sample No.:
Patient ID:
Rack:
Name:
Position:
2019/12/26 18:15:01
Sex:
Nickname:

Positive

WBC	8.22	[10^3/uL]		
NRBC	0.00	[10^3/uL]	0.0	[%]
NEUT	5.01	[10^3/uL]	60.9	[%]
LYMPH	2.81	[10^3/uL]	34.2	[%]
MONO	0.32	[10^3/uL]	3.9	[%]
EO	0.05	[10^3/uL]	0.6	[%]
BASO	0.03	[10^3/uL]	0.4	[%]
IG	0.05	[10^3/uL]	0.6	[%]
RBC	5.25	[10^6/uL]		
HGB	12.4	[g/dL]		
HCT	38.8	[%]		
MCV	73.9 -	[fL]		
MCH	23.6 -	[pg]		
MCHC	32.0	[g/dL]		
RDW-SD	34.8 -	[fL]		
RDW-CV	13.2	[%]		
PLT	334	[10^3/uL]		
PDW	17.2 +	[fL]		
MPV	13.0	[fL]		
P-LCR	48.4 +	[%]		
PCT	0.43 +	[%]		
MicroR	14.1	[%]		
MacroR	4.3	[%]		

WBC IP Message

Retics

RET	0.80	[%]	0.0420	[10^6/uL]
IRF	6.5	[%]		
LFR	93.5	[%]	**PLT-F**	
MFR	5.8	[%]	IPF	[%]
HFR	0.7	[%]		
RET-He	24.8	[pg]		

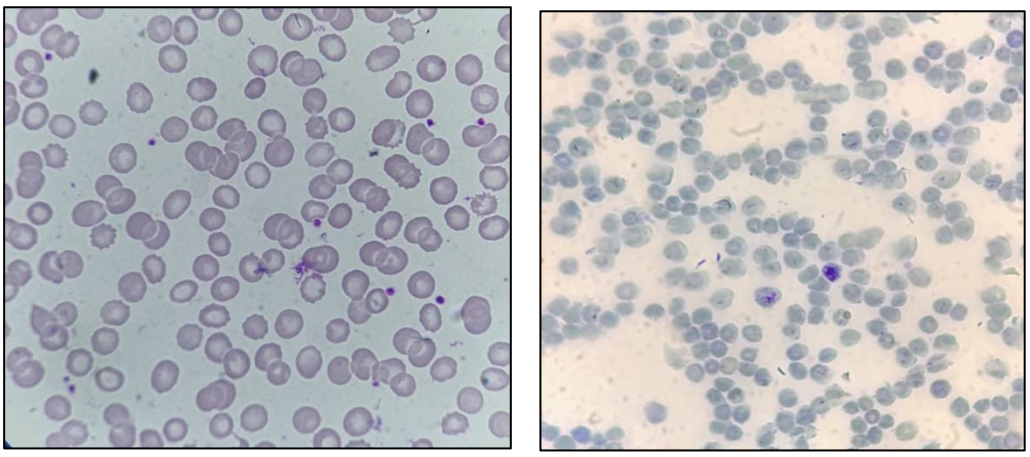
Body Fluids

WBC-BF	[10^3/uL]	
RBC-BF	[10^6/uL]	
MN	[10^3/uL]	[%]
PMN	[10^3/uL]	[%]
TC-BF#	[10^3/uL]	

RBC IP Message
RET Abn Scattergram
HGB Defect?
PLT IP Message

WDF
WNR
Nickname: XN-1000-1-A
WB
RBC
PLT

Figure 14 A-Peripheral smear showing normocytic normochromic blood picture (1000X,Leishman stain). B-Peripheral smear showing reticulocytes (1000X, New methylene blue stain)



A

B

DISCUSSION

One of the routine investigations done in pregnant women visiting ANC is CBC. Amongst the parameters included in CBC - Hb, MCV and RDW values are considered to screen for IDA. But these parameters do not help in identifying the patients with subclinical anemia. And if there is a serum suspicion of anemia the next investigation of choice are the biochemical tests to assess the serum iron status. The most commonly used parameters to rule out anemia is serum iron, TIBC and ferritin. Serum iron and TIBC do not show changes until the advanced stages of the disease. Serum ferritin which is considered as the gold standard is affected by ongoing inflammation/infection and gives falsely high results. Serum transferrin saturation though a reliable test, it is expensive. Hence there has been a need for reliable parameters to identify IDA in early stages of the disease. The newer parameters introduced in the automated hematology analyzers like Ret-He, %Micro-R and %Hypo-He have been studied extensively to evaluate its efficacy when compared to the already existing gold standard parameters.[1,46]

In contrast to biochemical tests, the values of new reticulocyte and erythrocyte parameters are not affected by infection or inflammation and can be performed on the same sample utilized for complete blood count. These additional tests can be performed on the same sample collected in EDTA tube for CBC with the same volume of blood sample. A study conducted by Schapkaitz E [47], stated that Ret-He can be measured on K2 EDTA specimens maintained at 4° to 8°C for up to 72 hours .Storage at the above mentioned temperature causes swelling of RBCs hence not a viable option for the estimation of erythrocyte parameters such as %Hypo-He and % Micro-R . The erythrocyte parameters should be estimated within 12hrs for reliable results. [47]

Women in reproductive age group are likely to develop IDA due to pregnancy, menstruation and lack of nutritional intake.[46] The relation between parity and IDA was studied by Prasad SG [48] with a sample size of 200 pregnant women of which 108 were primigravida and 92 were multigravida. Of the multi gravida group 40.2% had mild anemia, 30.5% had moderate anemia and 7.6% had severe anemia. Whereas in primigravida group there were 54.6% with no anemia. Based on these findings the author concluded that with increase in parity both in urban and rural population there is increased risk of IDA. Similarly a study by Al-Farsi Y *et al*[49] also showed an increase in anemia with increase in parity. However they also noted considerably high ID cases in primigravida as well. In the present study out of 280 cases, 117 were primigravida.113 were second gravida and 52(44.4%) and 58(51.3%) were ID respectively. Women with gravida 3 were 36 cases of which 27(75%) were ID. All the cases belonging to gravida 4 and 5 were ID. Though the number of cases considered in gravida 3,4 and 5 are less and is not statistically significant, still it showed an upward trend with most of the cases classified under ID group unlike the primigravida having almost equal distribution between the groups.

Bone marrow study is the gold standard for iron store estimation but is not practical to perform in all patients and more so in pregnancy when they do not have any clinical presentation. Back in 1993 and 1998 two studies were conducted by Broek VD *et al*[50] and Hallberg L*et al* [51]compared the bone marrow iron stores with the serum iron, TIBC and serum ferritin levels and found serum ferritin to be the best discriminator amongst the 3 parameters. Broek VD *et al* [50] stated that a cutoff of <30µg/dl was significant whereas Hallberg L *et al*[51] found the sensitivity and specificity to be highest at less than 16µg/dl, with start of the disease process at a a cut off of less than 25µg/dl. Many studies have proved the accuracy of serum ferritin

to differentiate ID from non ID groups. In a recent study done by Daru J *et al* [52]stated that there are very few studies conducted in pregnant women to determine a appropriate cut-off value. In their study they found that a cutoff of less than 15µg/dl was specific but not sensitive. In the present study a cut off value of less than 20µg/dl was considered for IDA and based on this cutoff, out of 280 pregnant women, 151 were classified as ID (cases) and 129 as non-ID (controls). Based on the ROC analysis, serum ferritin at a cutoff value of 15.1µg/dl showed a sensitivity of 99.3% and specificity of 97.7% with AUC 0.989.

The new erythrocyte parameters %Micro-R and %Hypo-He have been considered in the present study to identify subclinical ID. %Micro-R was statistically significant($p<0.001$) whereas %Hypo-He was not. The new reticulocyte parameter, Ret-He showed statistical significance ($p<0.001$). The highest sensitivity and specificity of 98% and 97% respectively was seen at a cutoff value of 32.25pg and was comparable to the sensitivity and specificity of serum ferritin which was 99.3% and 97.7% at a cut0ff of 15.1µg/dl. Statistically significant correlation of Ret-he and %Micro-R with MCV was also observed.

Levy S *et al* [1] in their study titled "The clinical utility of new reticulocyte and erythrocyte parameters on the Sysmex XN 9000 for iron deficiency in pregnant patients " showed statistically significant difference in Ret-He, %Micro-R and %Hypo-He to discriminate ID from non-ID. Ret-He at a cut-off of less than 31.2pg was considered as the best discriminator with a sensitivity of 62.5% and specificity of 86.44%. At a cut-off of less than 32pg the sensitivity was better but the specificity came down to 67.8% .However in present study high sensitivity and specificity was noted at a similar cut-off value of 32.25pg. The sensitivity and specificity was best at a cut-off more than 0.2% and more than 1.4% for %Hypo-he and %Micro-R and was

significant in their study. The present study showed a sensitivity of 76.9% and specificity of 69.9% at a cut off of more than 1.55% and was statistically significant. Whereas %Hypo-He at a cut off value of more than 0.25 did not show statistical significance.

Urrechaga E *et al* [29] conducted a study on healthy premenopausal women to identify latent ID. Ret-He and %Hypo-He were the two parameters considered. Ret-he at a cut off of less than 29.9pg showed a good sensitivity (86.8%)and specificity(85.7%) with AUC of 0.914 at a 95% CI of 0.824-1.000 and %Hypo-He at a cut-off value of more than 1.6 % showed highest sensitivity and specificity. When compared with the present study the Ret-he cut off is much lower but the present study showed better sensitivity and specificity at a cutoff of 32.25pg. These authors also studied the same parameters in CKD patients and patients on hemodialysis. According to their study Ret-He at a similar cutoff of less than 29.8pg as considered in the above mentioned study showed better sensitivity(90.7%) but lesser specificity(83.1) with area under curve (AUC) 0.935 . % Hypo-He with AUC 0.925 a cutoff 3.5% showed sensitivity 87.3%, specificity 88.0%.[8]

Table 15: Comparison of sensitivity and specificity of the new reticulocyte and erythrocyte parameters.

Parameters	Present study			Levy S *et al*[1]			Urrechaga E *et al*[29]		
	Cut-off	Sn(%)	Sp(%)	Cut-off	Sn(%)	Sp(%)	Cut-off	Sn(%)	Sp(%)
Ret-He(pg)	<32.25	98	97	<31.2	62.50	86.44	<29.8	90.7	83.1
%Micro-R(%)	>1.55	76.9	69.9	>1.4	78.00	75.00	>5.6	86.1	85.2
%Hypo-He(%)	>0.25	63.9	97.7	>0.2	58.00	88.46	>3.5	87.3	88.0

*Sn- sensitivity, Sp-Specificity.

The present study showed maximum sensitivity and specificity at higher cut off value of Ret-He . The sensitivity, specificity and AUC of Ret-he was highest in the present study when compared to other studies.

Table 16: Comparison of Sensitivity and specificity of Ret-He.

Other studies	Ret-He(pg)			
	Cut off(pg)	**Sn(%)**	**Sp(%)**	**AUC**
Brugnara C et al[39]	27.2	93.3	83.2	0.913
Kumar U et al [43]	27.8	93	83	0.93
Buttarello [40]	30.6	45	83	0.72
Cai J et al [54]	27.2	87.5	92.9	0.92
Joosten E et al [55]	26	85	69	0.75
Present Study	32.25	98	97	0.99

*Sn- sensitivity, Sp-Specificity.

SUMMARY

A hospital-based prospective cross-sectional study was conducted. The study group included nonanemic pregnant women in first trimester visiting to the ANC for the first time in Department of OBG from 1st December 2018 to 30th May 2020. Blood samples of these cases were sent to hematology section of Department of Pathology and those having Hb more than 11g/dl were included in the study. The new reticulocyte parameter - Ret-He and new erythrocyte parameters- %Micro-R and %Hypo-He were studied in comparison with serum iron parameters – serum iron, TIBC and serum ferritin to identify iron deficient cases in the latent stage of the disease.

A total of 280 pregnant women were included in the study. Blood samples were analyzed using automated hematology analyzer (Sysmex XN-1000) for the reticulocyte and erythrocyte parameters. VITROS5,1 FS Chemistry System was used to analyse serum iron parameters.

Majority of the cases included in the study were between age group of 18-27yrs. The youngest patient in the study group was 18yrs and the oldest was 37yrs. Gravida status of most of the cases was G1 and G2. Only 2 cases were G5 with their age being 29yrs and 35yrs.

Ret-He and %Micro-R showed statistically significant difference between ID and N-ID groups. These parameters also showed strong correlation with MCV and serum ferritin. Ret-He at a cutoff value of 32.25pg showed a sensitivity and specificity of 98% and 97% respectively and was comparable with the sensitivity (99.3%) and specificity (97.7%) of serum ferritin. %Micro-R at a cutoff value of 1.55% showed a sensitivity 0f 76.9% and specificity of 69.9%.

%Hypo-He did not show statistically significant difference between study groups. At a cut off value of 0.25, sensitivity of 63.9% and specificity of 39.1% was seen.

CONCLUSION

In developing countries like India where the prevalence of ID is high, early identification is critical. Pregnant women visiting the hospital for routine antenatal checkup should be examined thoroughly for the timely detection of anemia in the subclinical stage so as to prevent any untoward complications in the later stages of pregnancy and fetal outcome.

In the present study sensitivity and specificity of Ret-He is 98% and 97% respectively with a cut off value of 32.2pg .Sensitivity and specificity of %Micro-R was 76.9% and 69.9% respectively. Based on these findings we conclude that Ret-he can be used as an alternative hematological investigation to the traditionally used biochemical parameters such as serum ferritin for the early diagnosis of ID in pregnant women as it is cost effective and can be done along with hematological investigation such as CBC.

This study adds to our understanding of the clinical efficacy of the newer parameters that is Ret-He, % Hypo-He and % Micro-R in comparison with the traditional parameters used for detecting subclinical ID in pregnant women.

As the sample size in present study is less, further extensive study is needed to prove the efficacy of %Micro-R and %Hypo-He.

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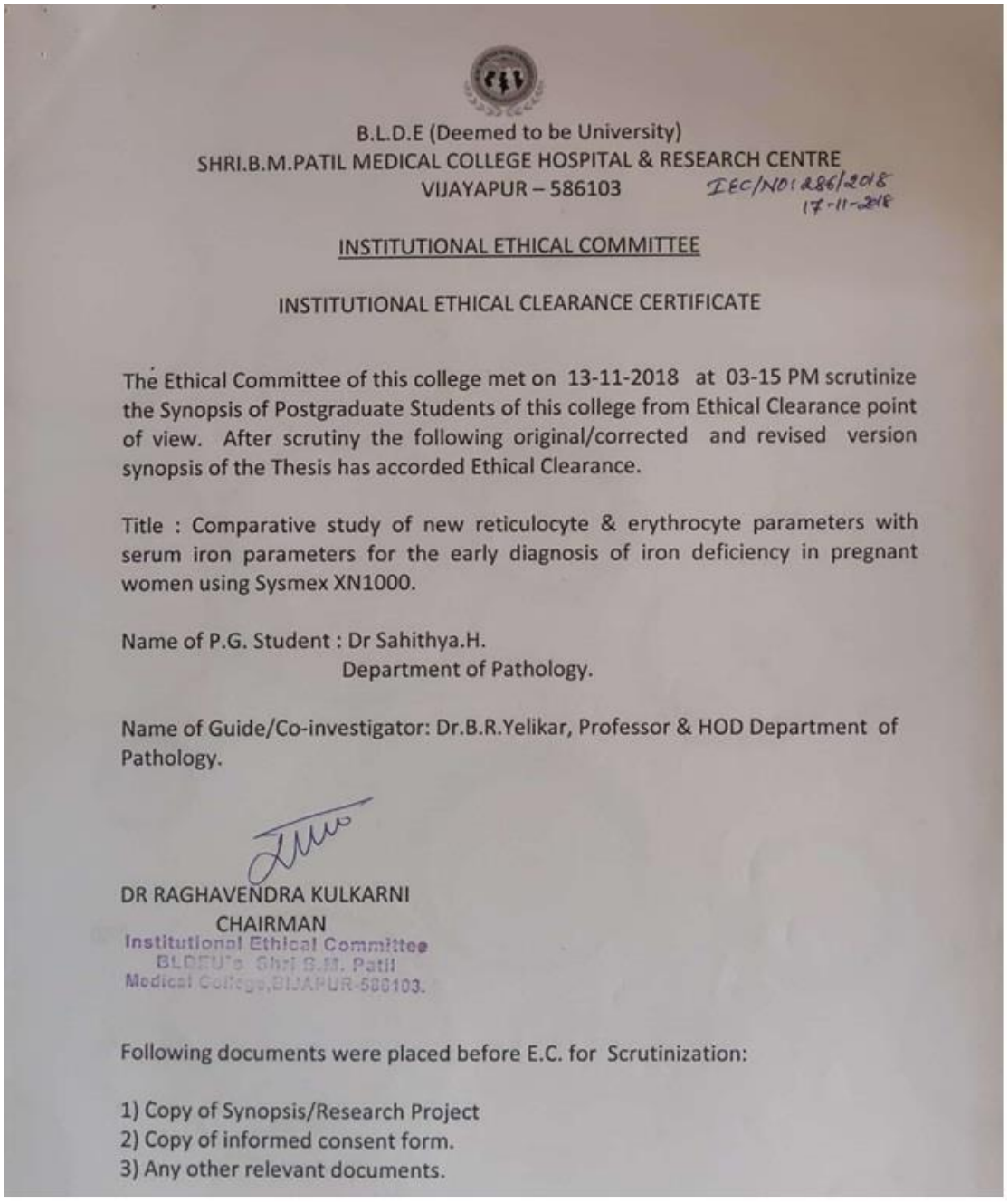
ANNEXURE-I



B.L.D.E (Deemed to be University)
SHRI.B.M.PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE
VIJAYAPUR – 586103

IEC/NO:1286/2018
17-11-2018

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2018 at 03-15 PM scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title : Comparative study of new reticulocyte & erythrocyte parameters with serum iron parameters for the early diagnosis of iron deficiency in pregnant women using Sysmex XN1000.

Name of P.G. Student : Dr Sahithya.H.
Department of Pathology.

Name of Guide/Co-investigator: Dr.B.R.Yelikar, Professor & HOD Department of Pathology.

DR RAGHAVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization:

1) Copy of Synopsis/Research Project
2) Copy of informed consent form.
3) Any other relevant documents.

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE(DU)/REG/PG-Guide/2019-20/994 July 23, 2019

To,
The Professor and HOD
Department of Pathology,
BLDE (DU)'s Shri B. M. Patil Medical College,
Hospital and Research Centre,
Vijayapura

Madam,

Sub: Regarding change of PG Guide.
Ref: Your letter no. Path/2019/637/19 dated 8th July, 2019.

With reference to the subject and letter cited above, on approval of the Hon'ble Vice-Chancellor, the change of PG Guide is permitted in respect of PG Student of your department:

Sl. No.	Name of the Student	Previous Guide	New Guide
1.	Dr. Shashikala H. M.	Dr. B. R. Yelikar	Dr. Surekha B. Hipparagi
2.	Dr. Sahithya H.	Dr. B. R. Yelikar	Dr. R. M. Potekar
3.	Dr. Shubham Chourashi	Dr. Mahesh Karigoudar	Dr. Vijayalaxmi S. Patil

This is for your information and needful.

REGISTRAR

REGISTRAR
BLDE (Deemed to be University)
Vijayapura-586103, Karnataka

Copy to:
- The Dean, Faculty of Medicine and Principal
- The Controller of Examinations
- The Concerned PG Teachers

Noted
Circulate to concerned P.G. students & guides.

Smt. Bangaramma Sajjan Campus, Sholapur Road, Vijayapura – 586103, Karnataka, India.
University: Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail: office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldedu.ac.in, E-mail: bmpmc.principal@bldedu.ac.in

Inward No : 2...
Date : 17/6/2021

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(DEEMED TO BE UNIVERSITY)
Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE(DU)/REG/PG-Guide/2021-22/518 June 16, 2021

To,
The Professor and HOD
Department of Pathology,
BLDE (DU)'s Shri B. M. Patil Medical College,
Hospital and Research Centre,
Vijayapura

Sir,

Sub: Regarding change of PG Guide.
Ref: Your letter no. Path/2021/420 dated 1st June, 2021.

With reference to the subject and letter cited above, on approval of the Hon'ble Vice-Chancellor, the change of PG Guide is permitted in respect of PG Student of your department as per below:

Sl. No.	Name of the Student	Previous Guide	New Guide	Batch/ Year
1.	Dr. Sahitya H.	Dr. R. M. Potekar	Dr. S. U. Arakeri	2018 - 19
2.	Dr. Saswati Subhadarshini		Dr. Vijayalaxmi Patil	2019 - 20
3.	Dr. Sultana Shahnaz		Dr. S. B. Hipparagi	2020 - 21
4.	Dr. Anin Prakash		Dr. Savitri Nerune	2020 - 21

This is for your information and needful.

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Vijayapura-586103. Karnataka

Copy to:
- The Dean, Faculty of Medicine and Principal
- The Controller of Examinations
- The Concerned PG Teacher

Circulate to concerned PG guides & P.G. Students.

Prof. & HOD
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B.L.D.E (DEEMED TO BE) UNIVERSITY,

SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURAA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, , S/O D/O W/O , aged years, ordinarily resident of _ do hereby state/declare that Dr of Hospital has examined me thoroughly on at (place) and it has been explained to me in my own language that I am suffering from disease (condition) and this disease/condition mimic following diseases. Further Doctor informed me that he/she is conducting dissertation/research titled under the guidance of Dr requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for

academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor: Witness:

1.

2.

Date:

Place:

ANNEXURE-III

PROFORMA

NAME	:	OP/IP No. :
AGE	:	
SEX	:	
RELIGION	:	
OCCUPATION	:	
RESIDENCE	:	
Presenting Complaints	:	
Past history	:	
Personal history	:	
Family history	:	
Treatment history	:	
General physical examination	:	
Pallor		present/absent
Icterus		present/absent
Clubbing		present/absent
Lymphadenopathy		present/absent
Edema		present/absent
Built		poor/average/well
VITALS:		PR: RR:
BP:		TEMPERATURE:
WEIGHT:		

SYSTEMIC EXAMINATION:

Cardiovascular system: Respiratory system: Per Abdomen:

Central nervous system: Clinical Diagnosis:

INVESTIGATIONS:

Red blood cell count (RBC count):

Haemoglobin (Hb):

Haematocrit / Packed cell volume (HCT/PCV):

Mean corpuscular volume (MCV):

Mean corpuscular haemoglobin (MCH):

Mean corpuscular haemoglobin concentration (MCHC):

Red cell distribution width (RDW):

Reticulocyte count:

Reticulocyte Haemoglobin equivalent (RET-He):

Percentage of microcytic red cells (%Micro- R)

Percentage of hypochromic red cells (%Hypo- He)

Total iron binding capacity (TIBC):

Serum Iron (Fe):

Serum Ferritin:

Key to Master Chart

1.	Sl. No.	Serial Number
2.	OP no	Out Patient number
3.	RBC	Red Blood Cell
4.	Hb	Hemoglobin
5.	PCV	Packed cell volume
6.	MCV	Mean corpuscular volume
7.	MCH	Mean corpuscular hemoglobin
8.	MCHC	Mean corpuscular hemoglobin concentration
9.	RDW	Red cell distribution width
10.	Retic	Count Reticulocyte Count
11.	Ret-He	Reticulocyte Hemoglobin
12.	%Micro-R	Percentage of Microcytic red cells
13.	%Hypo-He	Percentage of Hypochromic red cells
14.	TIBC	Total Iron Binding Capacity
15.	S. Iron	Serum Iron
16.	S. Ferritin	Serum Ferritin

MASTER CHART

Sl.no	OP no	Age	Sex	Gravida	RBC (× 10 ^{12}/L)	Hb (g/dL)	PCV (L/L)	MCV (fL)	MCH (pg	MCHC (g/L)	RDW (%)	Retic count (%)	Ret-He (pg)	% Micro-R (%) 0	%Hypo-He (%)	TIBC (mugm/dl)	S.Fe (mugm/dl)	S.Ferritin (ng/ml)
1	227902/19	20	F	G2	4.26	11.5	34.7	82.2	27.5	30.7	13.6	1.12	32.6	0.5	0.7	376	86	58.9
2	372783/19	26	F	G3	4.36	11.01	33.2	80.2	26	32.5	15	1.12	28.1	2	0.4	269	127	14.4
3	371688/19	21	F	G1	5.3	11.9	34.1	87	29.1	33.5	13.7	1.43	32.8	1.4	0.7	234	42	61.9
4	373003/19	23	F	G2	4.67	11.02	30.7	79.1	30.4	34.1	12	1.52	29.2	0.6	1.5	383	88	13.3
5	373287/19	22	F	G1	4.32	12.6	36.3	84	29.2	34.7	14.5	1.6	33.1	0.5	0.4	332	95	73.2
6	385147/19	29	F	G4	4.81	14.3	46.3	96.3	29.7	30.9	13.2	0.9	28.8	1	0.9	385	85	18.7
7	385146/19	23	F	G2	4.01	11.6	35.1	87.5	28.9	33	14	1.66	28.7	1.9	0.2	232	95	19.9
8	385144/19	25	F	G1	4.23	13.1	37.7	89.1	31	34.7	12.1	2.34	36.9	0.8	0.1	387	109	31.8
9	385143/19	19	F	G1	4.32	11.9	35.6	82.4	27.5	33.4	14.6	1.14	27.3	5.4	1.7	456	47	14.5
10	385145/19	24	F	G2	4.07	11.1	30.5	83.9	24.1	32.1	13.4	1.58	32.3	1.2	0.4	211	62	65.2
11	385141/19	24	F	G1	3.97	11.2	32.3	81.4	25.7	31.6	12.2	1.87	32.9	0.4	0.2	277	92	46.2
12	325829/19	24	F	G1	3.94	12	34.8	88.3	30.5	34.5	12	1.17	32.1	1.2	0.2	234	91	13.1
13	411266/19	22	F	G2	4.05	11.3	33.5	82.7	27.9	33.7	13.7	1.35	27.7	3.7	0.4	406	86	13.8
14	411269/19	24	F	G2	5.53	11.8	48.3	87.3	28.6	32.7	12.8	1.88	27.9	1.7	0.1	390	68	15.4
15	411268/19	21	F	G1	5.23	13.6	41.1	78.6	26	33.1	14.4	1.28	26.3	5.6	1.2	412	108	14.8
16	411267/19	28	F	G2	5.1	11.6	35.4	80.4	20.8	29.9	14.2	1.79	33.7	1.2	0.8	356	50	45.1
17	371861/19	23	F	G1	4.46	11.2	34.5	87.4	25.1	32.5	15.7	1.52	34.2	0.6	0.2	438	50	61.2
18	376200/19	29	F	G5	4.19	11.3	35.5	84.7	27	31.8	17.6	1.79	27.3	6.6	1.6	445	53	15
19	374738/19	26	F	G4	4.51	13.3	39.6	87.8	29.5	33.6	12.7	1.73	28.9	1.4	0.2	390	96	14.9
20	376478/19	25	F	G2	3.66	11.5	32.2	88	28.7	32.6	17.3	0.61	33.8	0.5	1.3	321	148	34.2
21	371253/19	24	F	G1	3.76	11	34.1	90.7	29.3	32.3	12.1	1.19	26.3	0.7	0.2	371	76	15.3
22	374600/19	20	F	G1	3.9	11.8	34.1	87.4	27.7	31.7	14.9	1.46	26.1	3.1	1.6	459	61	12.1
23	371172/19	23	F	G3	3.84	11.7	36.9	96.1	30.5	31.7	13.2	1.42	28.2	0.5	0.1	398	166	14
24	371699/19	22	F	G2	4.56	11.8	36.5	80	23.7	29.6	12.3	1.12	34.4	0.3	0.2	312	72	67.6
25	371440/19	25	F	G2	3.7	11.8	33.4	90.3	29.2	32.3	14.8	2.37	34.9	1.7	0.8	230	81	75.9
26	276836/19	22	F	G1	3.95	11.7	35.9	90.9	29.6	32.6	13.1	2.48	32.6	1.1	0.2	200	89	24.5

27	373474/19	21	F	G1	3.8	12.7	32.9	80.9	28.2	32.5	13.8	1.69	34.6	1.9	0.7	303	49	58.5
28	350694/19	25	F	G2	4.25	11.8	36.3	85.4	27.8	32.5	15.9	2.23	27.9	4.3	0.5	403	68	14.1
29	350954/19	22	F	G2	3.93	12.4	36.2	92.1	31.6	34.3	13.6	1.94	36.4	1.9	0.1	366	115	33.1
30	350676/19	25	F	G1	4.31	11.7	37.8	87.7	27.1	31	15.9	1.35	33.1	2.1	0.8	300	129	38.2
31	351040/19	24	F	G1	4.33	12.5	35.2	81.3	28.9	35.5	13	1.35	28.1	3.9	0.2	493	45	14.8
32	304933/19	23	F	G1	4.6	13	37	80.4	28.3	35.1	13.4	0.9	32.6	0.4	0.3	311	37	85.1
33	304630/19	22	F	G2	3.94	11.9	33.9	86	30.2	35.1	13.6	1.63	29.4	2.3	0.3	359	63	14.8
34	303595/19	18	F	G1	4.3	12.9	34.5	80.2	30	37.4	13	2.24	30.6	4.6	0.2	384	54	13.1
35	240842/19	23	F	G1	4.02	11.9	40.1	99.8	29.6	29.7	13.8	0.8	27.3	0.4	0.2	438	32	13.9
36	245562/19	19	F	G1	4.43	12.5	36.5	82.4	28.2	34.2	13	1.74	28.9	3.4	0.2	360	66	13.9
37	228299/19	22	F	G3	4.21	11.6	36.6	86.9	27.6	31.7	14.9	1.4	28.5	5.1	0.6	423	55	14.2
38	50219/19	24	F	G3	5.05	14.2	42.5	84.2	28.1	33.4	12.3	0.86	28.8	2.5	0.2	415	56	13.2
39	48984/19	21	F	G2	4.42	11.6	35.3	88.2	26.2	32.9	13.7	1.29	35.8	0.7	1.1	259	81	121
40	28719/19	21	F	G1	3.99	11.8	36.1	90.5	29.6	32.7	14.1	1.16	29	1.7	0.4	514	75	14.5
41	459993/19	34	F	G2	4.46	11	27.9	82.9	19.5	31.2	12.7	0.85	33.8	0.9	0.3	203	46	66.14
42	459995/19	21	F	G2	4.21	12.2	35.9	85.3	29	34	13.7	1.74	28.5	2.5	0.4	365	101	14
43	459998/19	18	F	G2	4.21	11.8	24.6	83.7	18.5	31.7	13.2	3.81	32.8	0.9	0.2	294	81	117
44	459996/19	37	F	G3	3.57	11	30	80	28	33.3	13.1	1.26	26.3	3	0.8	336	45	14.7
45	459992/19	23	F	G1	3.82	11	31.4	82.2	28.8	35	12.9	1.53	27.7	3.3	0.3	415	66	14.8
46	459997/19	26	F	G3	4.09	12	35	85.6	29.3	34.3	12.3	0.72	29.9	1.8	0.2	411	112	14.4
47	459991/19	25	F	G2	4.79	14.4	40.2	83.9	30.1	35.8	12.7	1.59	34.5	2.4	0.1	420	127	32.1
48	459994/19	20	F	G1	3.96	11.7	28.9	83	22	30.1	12.5	1.16	32.9	0.6	0.4	356	42	61.9
49	354650/19	19	F	G1	4.57	11.8	46	90.7	25.8	25.7	16.1	0.84	32.9	0.9	1.1	380	58	54.9
50	358098/19	23	F	G1	4.48	12.9	34.7	87.5	24.3	31.4	14.6	1.06	33	0.2	0.1	243	56	62.1
51	352426/19	22	F	G2	4.95	13.6	42.1	85.1	27.5	32.3	14.5	1.05	26.2	3.5	0.7	420	76	13.2
52	318060/19	23	F	G1	4.42	11.4	30.3	86.6	22.6	33	15.6	1.13	32.8	0.3	0.2	255	38	48.3
53	242319/19	19	F	G1	4.37	11.3	34.2	88.3	21.3	27.2	12.4	1.08	33.1	1.1	0.3	218	66	75.9
54	351080/19	25	F	G1	4.67	11.7	32.6	89.8	20.8	29.8	12.3	2.19	32.2	0.5	0.2	240	46	14
55	373534/19	23	F	G1	3.98	12.5	41.1	99	31.4	30.4	13.3	1.23	29.3	0.3	0.2	333	72	18.1
56	376439/19	21	F	G2	4.34	11.1	32.4	84.7	23.3	31.2	13.8	1.63	32.6	2.1	0.3	345	72	54.6
57	374242/19	24	F	G2	4.33	11.2	33.9	88.3	23.6	30.1	12.7	1.5	32.6	0.4	0.2	262	58	44.5
58	197/19	24	F	G2	4.91	12.4	37.9	77.2	25.3	32.7	16.6	1.09	26.2	13.7	2.1	456	67	14.7
59	976/19	23	F	G2	4.24	12.3	34.2	80.7	29	36	12.2	2.17	33.5	0.2	0.2	224	49	89
60	198/19	26	F	G2	3.83	11	31.8	83	28.7	34.6	14.3	1.67	28.4	3.7	0.1	439	65	12.8

61	134/19	24	F	G2	3.65	11.4	30.8	84.4	28.5	33.8	17.9	1.82	27.2	7.9	0.4	323	42	14.2
62	305/19	20	F	G1	3.49	11.7	33	94.6	30.7	32.4	13.4	2.38	33.5	2.4	0.6	287	78	101.2
63	4067/19	38	F	G4	5	13.3	41.2	82.4	26.6	32.3	13.4	1.2	27.3	3.8	0.7	520	39	12.8
64	4293/19	24	F	G2	4.52	13.1	37.8	83.6	29	34.7	12.6	1.27	29	2.3	0.1	432	65	13.2
65	350/19	22	F	G1	4.04	12.5	35.3	87.4	30.9	35.4	14.9	1.86	32.9	2.6	0.3	267	87	123.8
66	352424/19	22	F	G2	4.38	11.1	34.2	81.2	25.3	32.5	12.8	1.76	34.5	0.2	0.2	366	56	76.3
67	2369/19	28	F	G4	3.52	11.2	30.6	86.9	29	33.3	13.1	1.23	26.1	1.8	0.3	456	73	14.3
68	619/19	20	F	G1	4.51	11.9	33.8	84.9	24.2	32.2	12.3	0.98	33.1	1.8	0.9	250	92	69.3
69	7910/19	35	F	G2	4.16	11.3	29.2	87.4	21.4	30.5	12.6	2.28	33.1	0.3	0.5	445	72	47.9
70	600/19	24	F	G1	4.09	11.7	33.7	82.4	26.2	31.8	16.8	1.77	26.1	7.1	1.8	321	39	14.8
71	644/19	21	F	G1	3.74	11.2	32	85.6	27.3	31.9	12.9	1.35	32.9	0.9	0.3	343	59	77.2
72	354682/19	24	F	G1	4.45	12.8	37.3	83.8	28.8	34.3	13.1	1.01	27.7	3.1	0.4	382	86	14.2
73	439451/19	25	F	G2	4.7	12.7	37.6	80	27	33.8	13	1.9	28.6	4.9	0.2	252	132	14.8
74	252488/19	18	F	G1	4.05	12.2	35.4	87.4	30.1	34.5	14.5	1.65	34.3	1.9	0.3	405	158	75.2
75	350/19	22	F	G2	3.77	11.6	34.2	90.7	30.8	33.9	15	1.73	32.9	1.6	0.3	243	120	43.2
76	492/19	23	F	G1	3.3	11.1	28.6	86.7	29.1	33.6	13.2	1.55	28.2	2.1	0.3	275	76	16.3
77	719/19	23	F	G2	4.21	11.3	29.6	85.3	22.1	31.4	14.2	1.03	32.8	1.6	0.5	230	67	62.1
78	650/19	35	F	G2	4.26	11.5	33.6	78.9	27	34.2	14.9	1.97	28.7	8.7	0.6	422	49	13.6
79	10118/19	19	F	G1	3.85	11	33.4	86.8	28.6	32.9	16.1	0.74	32.7	0.2	0.2	282	74	93.2
80	10117/19	20	F	G1	4.11	11.2	32.1	88.1	25.5	32.7	12.9	1.55	35.3	0.4	0.2	289	59	59.9
81	10119/19	22	F	G1	4.04	11.3	32.3	80	27	33.7	16	1.63	28.7	8.4	1.3	420	105	14
82	325555/19	23	F	G1	3.81	11.1	29.5	84.4	24.4	31.5	13.2	1.22	32.3	1.4	0.2	161	109	46.6
83	641/19	24	F	G3	4.85	12.9	38.4	79.2	26.6	33.6	12.5	1.45	27.2	5.2	1.8	519	43	12.5
84	327313/19	28	F	G3	3.63	11.3	30.8	84.8	28.4	33.4	13.7	1.2	27.4	2.9	0.3	458	44	12.2
85	417815/19	20	F	G1	5.55	15.6	45.3	81.6	28.1	34.4	12.7	1.53	27.9	3.6	0.1	430	108	15.2
86	417816/19	25	F	G3	5.42	14	43.3	79.9	25.8	32.3	14.4	1.07	28.8	7.2	1.3	307	70	19.3
87	417817/19	19	F	G1	4.29	11.3	33.1	77.2	26.3	34.1	15.4	1.33	26.6	11.8	1	308	64	14.8
88	424038/19	24	F	G1	5.64	13.4	41.4	83.4	23.8	32.4	14.6	0.89	32.9	0.7	0.1	388	84	62.8
89	420915/19	23	F	G1	4.62	12.3	37.2	80.5	26.6	33.1	13.5	1.25	28.2	5.1	0.5	506	67	14.4
90	420914/19	24	F	G1	4.27	12.2	35.9	84.1	28.6	34	13.2	1.54	28.7	2.8	0.3	386	65	17
91	420913/19	19	F	G1	4.9	12.3	36	83.5	25.1	34.2	14.3	0.75	34.9	1.6	0.8	292	64	87.2
92	386577/19	22	F	G2	5.06	12.3	37.9	84.2	24.3	32.5	14.2	0.81	33.4	0.4	0.2	328	50	66
93	386576/19	19	F	G1	4.71	14.3	41.2	87.5	30.4	34.7	13.9	2.48	33.8	1.3	0.4	252	51	26.3
94	439452/19	19	F	G3	4.25	13	39.1	92	30.6	33.2	14.8	1.97	32	1.6	0.4	301	45	13.2

95	439449/19	25	F	G3	4.22	12.8	38.9	92.2	30.3	32.9	12.9	1.31	29	1.1	0.3	342	58	15
96	439447/19	21	F	G1	4.39	12.7	36	82	28.9	35.3	13.8	1.13	27.6	4.2	0.2	338	43	15.1
97	439453/19	19	F	G1	4.38	12.6	38.2	87.2	28.8	33	13.9	1.17	31.8	2.1	0.3	350	86	14
98	439454/19	22	F	G2	4.38	11.9	34	77.6	27.2	35	13.8	2.11	27.9	8.3	0.5	264	61	18.9
99	439456/19	20	F	G1	4.65	11.5	34.7	84.6	24.7	33.1	12.4	0.74	35	0.3	0.2	397	45	78.2
100	475593/19	27	F	G1	4.68	13.4	40.1	85.7	28.6	33.4	13	0.61	27.6	2.2	0.5	442	66	12.9
101	475595/19	23	F	G1	3.59	10.8	31.7	88.3	30.1	34.1	13	2.06	28.8	1.5	0.2	450	86	14.6
102	475594/19	27	F	G2	4.16	11.3	27.9	87.1	21.2	31.5	14	0.67	33.4	0.4	0.5	293	43	53.5
103	475597/19	25	F	G3	5.26	13.7	43.7	83.1	26	31.4	16.4	1	26.2	6.5	2.3	405	65	14.9
104	475596/19	30	F	G2	4.19	11.8	32.5	87.6	25.8	33.2	14.4	1.36	32.5	0.8	0.2	369	61	63.2
105	432492/19	28	F	G3	4.86	13.1	39.3	80.9	27	33.3	12.5	1.63	27.4	4.1	0.2	455	66	15.5
106	432489/19	20	F	G1	4.17	11.6	33.2	86.9	23	28.9	15.7	2.38	33.1	1.1	0.2	313	58	81.8
107	432491/19	22	F	G2	3.45	11	31.9	92.5	29	31.3	16.9	1.12	26.9	2.8	1.8	443	84	13.2
108	432490/19	22	F	G1	4.45	13.2	38.7	87	29.7	34.1	11.9	0.94	28.5	1.2	0.1	412	79	14.9
109	432488/19	27	F	G1	4.38	12.8	39.3	89.7	29.2	32.6	15.4	1.22	29.2	2.7	1.3	359	82	13.4
110	475586/19	18	F	G1	3.39	11.5	34.5	98	33.9	33.3	14.3	2.06	32	0.3	0.2	270	97	16
111	475584/19	25	F	G2	4.28	12.2	37.5	87.6	28.5	32.5	13.1	0.76	28.2	1.6	0.2	401	50	13.8
112	475583/19	25	F	G4	4.37	12.6	38	87	28.8	33.2	11.7	1.15	29	1.6	0.3	385	79	13.5
113	475582/19	19	F	G2	3.87	11	33.1	85.5	28.2	32.9	12.7	1.21	26.6	2.2	0.2	470	110	14.1
114	475585/19	24	F	G1	4.77	12.8	39.7	83.2	26.8	32.2	14	1.49	27.6	4.2	0.4	322	76	14.3
115	475581/19	22	F	G2	4.52	11.1	32.2	78	22.3	31.4	14.3	1.15	26	21.7	9.7	525	45	12.8
116	475580/19	20	F	G2	4.82	13.7	40	83	28.4	34.3	13.2	1.68	27.9	3.4	0.2	292	90	15
117	357014/19	20	F	G2	4.65	13.1	37.9	80	25.7	34	12.5	1.28	27.5	3.2	0.3	321	49	18.4
118	366143/19	24	F	G2	4.16	11.2	35.4	90.7	25.3	34.7	12.5	1.09	32.5	0.8	0.2	276	87	96.5
119	475590/19	24	F	G2	4.02	11.2	37	78.9	24.1	34.3	13.3	1.77	26.3	3.7	0.2	267	36	13.8
120	369746/19	25	F	G2	4.21	11.9	35	80	28.9	33.8	12	1.53	32.6	1.8	0.2	380	62	79
121	386571/19	24	F	G2	5.55	11.9	36.5	76.2	24.8	32.6	21.5	1.07	26.3	5.1	0.5	481	52	15
122	386570/19	20	F	G2	5.64	12.2	35.4	87.4	30.1	34.5	14.5	1.23	26.4	2.4	0.2	429	41	11.7
123	475592/19	25	F	G1	4.62	12.4	37.8	89.1	25.9	32.8	14	1.56	33.9	1.3	0.4	254	38	62.9
124	363159/19	19	F	G1	3.77	13.8	39.9	78.7	27.2	34.6	12.8	1.67	27.8	4	0.2	230	46	13.3
125	356313/19	26	F	G2	3.45	11.2	32.1	88.1	25.5	32.7	14.5	1.32	32.9	0.2	0.3	265	87	56.4
126	367908/19	25	F	G1	5	11.3	32.3	80	27	33.7	16	1.29	26.4	2.1	0.2	346	84	13.2
127	360957/19	20	F	G1	3.9	12.9	38.4	89.2	26.6	33.6	12.5	1.22	32.3	0.5	0.5	259	82	113
128	353908/19	22	F	G2	3.67	13.7	43.7	83.1	26	31.4	16.4	1.67	27.8	12.1	0.4	401	50	14.4

129	346363/19	22	F	G2	3.98	11.3	36.1	77.8	24.4	31.3	20.9	1.63	27.4	4.1	0.2	237	84	14.9
130	475587/19	25	F	G1	3.74	11.8	35	86.4	25.8	33.7	12.6	2.38	33.8	1.6	0.5	254	42	66.9
131	386572/19	22	F	G2	3.94	12	34.8	88.3	30.5	34.5	12	1.17	33.6	1.2	0.2	234	91	29.1
132	353669/19	27	F	G4	4.05	11.3	33.5	82.7	27.9	33.7	13.7	1.35	27.7	3.7	0.4	406	86	13
133	475591/19	25	F	G2	5.23	13.6	41.1	78.6	26	33.1	14.4	1.28	26.3	8.6	1.2	482	108	13.8
134	362579/19	22	F	G1	5.1	11.6	35.4	89.4	20.8	29.9	13.2	1.79	32.4	0.2	0.1	432	40	61.3
135	159269/20	25	F	G2	4.46	11.2	34.5	87.4	25.1	32.5	15.7	1.52	34.1	1.6	1.1	256	50	58
136	358209/19	24	F	G2	4.19	11.3	35.5	84.7	27	31.8	17.6	1.79	27.3	6.6	1.6	345	53	15
137	365477/19	24	F	G2	4.35	12.1	35.4	81.4	27.8	34.2	15.7	1.76	26.5	7.5	1.2	367	42	14.4
138	352124/19	25	F	G3	4.36	12.8	37	84.9	29.4	34.6	14.8	1.45	27.5	4	0.2	389	46	13.9
139	366720/19	24	F	G3	3.67	12	34.9	80.1	30.4	34.9	13	1.34	26.4	1.4	0.2	542	42	13.8
140	356831/19	23	F	G3	4.39	12.7	36	82	28.9	35.3	13.8	1.13	27.6	4.2	0.2	338	43	13.4
141	362924/19	25	F	G2	4.38	11.9	34	77.6	27.2	35	13.8	2.11	27.9	8.3	0.5	364	61	14.8
142	352717/19	24	F	G1	3.77	11.6	34.2	90.7	30.8	33.9	15	1.73	32.2	1.6	0.3	343	120	13.2
143	367841/19	22	F	G2	4.21	11.3	29.6	85.2	22.1	31.4	12.2	1.03	32.5	1.1	0.6	470	43	62.9
144	475589/19	25	F	G2	5.07	13.8	39.9	78.7	27.2	34.6	12.8	1.07	26.3	5.9	0.5	432	64	12.4
145	354967/19	28	F	G2	3.81	11.1	29.5	84.4	24.4	31.5	13.1	1.22	32.2	1.4	0.2	261	42	15.5
146	365901/19	35	F	G3	4.3	12.7	37	86	29.4	34.3	16.1	1.28	32.5	0.6	0.3	325	84	72.5
147	357002/19	22	F	G3	4.23	11.1	33.5	83.2	29.1	31.4	16	1.54	33	1.9	0.2	403	89	67.2
148	360720/19	23	F	G2	4.19	11.4	43.5	85.5	30.6	34.2	14.3	1.32	32.4	1.2	0.3	432	67	62.3
149	386574/19	20	F	G1	4.35	13.5	38.4	86.8	28.3	34.6	12.9	1.09	33.6	1.3	0.4	320	69	46.7
150	386575/19	22	F	G1	5.55	11.9	36.5	76.2	24.8	32.6	21.5	1.07	26.3	5.1	0.5	481	52	14.3
151	358017/19	28	F	G2	3.9	24.1	69.8	86.2	29.8	34.5	14.7	2.07	32.9	1.7	0.3	254	66	56.7
152	352424/19	22	F	G3	4.09	11.7	33.7	82.4	26.2	31.8	16.8	1.77	26.1	7.1	1.8	421	39	13.6
153	360294/19	22	F	G1	4.45	12.8	37.3	83.8	28.8	34.3	13.1	1.01	27.7	3.1	0.4	382	86	14.2
154	420913/19	24	F	G3	4.26	11.5	33.6	78.9	27	34.2	14.9	1.97	28.7	8.7	0.6	322	59	14.9
155	386573/19	25	F	G2	5.07	13.8	39.9	78.7	27.2	34.6	12.8	1.07	26.3	5.9	0.5	332	64	14.2
156	366200/19	18	F	G1	3.85	11	33.4	86.8	28.6	32.9	12.1	0.74	32.4	0.4	0.2	282	74	92.4
157	367213/19	25	F	G3	4.11	11.2	32.1	87	25.5	32.7	13.5	1.55	32.5	0.8	0.5	289	59	48.9
158	37878/20	22	F	G1	3.78	11.9	32	84.7	28.8	34.1	13.3	2.31	27.9	2.4	0.2	498	84	14.9
159	37879/20	24	F	G1	4.84	11.9	35.4	83.1	24.6	33.6	13.8	1.25	32.6	1.9	0.2	390	43	62.3
160	13909/20	27	F	G2	3.51	11.2	28.9	82.3	26.2	31.8	13.8	1.48	34.7	0.2	0.3	298	122	73.2
161	85106/20	23	F	G2	4.5	13.7	40.8	90.7	30.4	33.6	13.4	1.77	35.4	1.4	0.1	254	139	96
162	37880/20	26	F	G1	4.74	11.1	34.9	83	23.4	31.8	13.4	0.82	32.7	0.8	0.6	367	87	76.4

163	2331/20	30	F	G3	4.32	11.5	30.2	88.2	28	32.1	12.3	1.54	27.9	1.2	0.2	373	64	13.3
164	5468/20	23	F	G3	3.74	11.4	33.7	90.1	30.5	33.8	16.6	1.89	28.3	2.5	0.3	476	87	13.7
165	10627/20	30	F	G3	3.48	11.2	27.7	88.8	26.4	33.2	13.4	1.65	33.3	0.9	0.2	390	39	73
166	10924/20	28	F	G4	4.87	13.7	39.9	81.9	28.1	34.3	14.2	1.45	26.9	4.9	0.3	365	87	17.4
167	945/20	24	F	G1	4.51	13.5	40.2	89.1	29.9	33.6	13.4	2	29.6	1.5	0.2	254	92	13.7
168	951/20	22	F	G1	4.71	11.1	34.1	82.1	23.6	32.6	14.3	1.02	30.1	1.1	0.2	305	42	16.4
169	979/20	24	F	G2	4.85	11.9	36.8	85.2	24.5	32.3	12.2	1.72	32.8	0.4	0.2	382	36	132
170	1130/20	22	F	G1	3.78	11.08	34.9	83.4	27	31.5	16	11.67	34.7	1.5	0.9	232	62	79.3
171	619/20	25	F	G3	3.46	11.5	26.7	81.2	24.6	31.8	15.2	0.9	32.7	1.9	1.1	387	47	62.5
172	37889/20	25	F	G2	3.9	24.1	69.8	86.2	29.8	34.5	16.4	2.07	32.6	2.7	0.3	254	66	69.1
173	37887/20	21	F	G1	4.79	11.9	36.5	80.2	24.8	32.6	14.6	1.34	33.2	0.3	0.2	352	66	100.9
174	829/20	25	F	G2	4.78	12.4	37.8	79.1	25.9	32.8	14.6	1.39	29.5	8.2	0.8	381	129	15.2
175	641/20	24	F	G2	5.07	13.8	39.9	78.7	27.2	34.6	12.8	1.07	26.3	5.9	0.5	252	64	17.2
176	15987/20	25	F	G3	4.3	12.7	37	86	29.4	34.3	14.8	1.28	32.2	0.5	0.3	325	84	12.5
177	18271/20	22	F	G1	4.23	11.1	33.5	83.9	29.1	31.4	12.9	1.54	33.5	0.9	0.4	300	47	61.2
178	18329/20	24	F	G1	3.49	11	30.3	86.8	28.7	33	12.5	1.27	27	1.3	0.5	436	68	14.3
179	22451/20	24	F	G2	3.98	11.8	32.5	81.7	27.1	33.2	13.1	1.09	35.7	2.1	0.3	450	46	85.2
180	16143/20	32	F	G2	3.82	11.2	28.1	93.1	24.1	32.7	15.2	1.98	33.1	0.7	0.2	350	58	47.6
181	18833/20	24	F	G2	3.79	11.3	28.8	86	25.3	33.3	15.6	1.32	34.2	1.3	1.1	339	47	52
182	2049/20	25	F	G1	4.35	11.7	34	88.2	26.9	34.4	13.7	1.77	34.7	1.1	0.7	377	48	88.2
183	2033/20	24	F	G1	4.4	12.6	37.9	86.1	28.6	33.2	15.4	1.23	33.9	1.8	0.4	211	65	58.2
184	2086/20	22	F	G1	4.15	12.6	35.7	86	30.4	35.3	13	1.09	36.2	2.3	0.3	317	83	63.6
185	2140/20	25	F	G2	3.77	11.3	32.7	86.7	30	34.6	14.5	1.34	28.3	2.5	0.3	432	82	13.9
186	24944/20	36	F	G4	4.35	12.1	35.4	81.4	27.8	34.2	15.7	1.76	26.5	7.5	1.2	367	42	14.4
187	25287/20	19	F	G2	3.45	11.7	31.2	90.4	31	34.3	12.7	1.67	28.4	0.7	1.1	329	51	14
188	2253/20	22	F	G1	5	15.8	43.5	87	31.6	36.3	12.5	1.88	28.9	1.2	0.5	457	98	14.4
189	2237/20	28	F	G2	4.36	12.8	37	84.9	29.4	34.6	14.8	1.45	27.5	4	0.2	489	46	13.4
190	28072/20	23	F	G1	3.9	11.1	29.1	84.6	25.4	34	14.8	1.09	32.5	1.5	1	376	76	62.5
191	28356/20	35	F	G3	4.64	14	38.4	82.8	30.2	36.5	12.5	1.29	31.2	2.9	0.2	465	53	12.5
192	2415/20	22	F	G1	3.67	12	34.9	80.1	30.4	34.9	13	1.34	26.4	1.4	0.2	330	42	14.1
193	30017/20	23	F	G3	3.69	12.8	35	94.9	34.7	36.6	14.3	1.26	30.3	0.7	0.1	512	72	12.9
194	29569/20	36	F	G2	4.52	11.6	34.2	85.7	23.5	31	13.3	1.88	34.1	0.8	0.4	365	41	62.3
195	2583/20	29	F	G3	4.02	11.1	33	82.1	27.6	33.6	14.9	1.23	26.4	6.2	0.3	532	45	12.9
196	59021/20	25	F	G2	3.66	11.2	28.7	85.5	25.1	32.1	15.2	1.56	32.9	0.7	0.6	181	52	143

197	59301/20	21	F	G1	4.35	11.2	34.4	84.2	25.7	32.6	12.3	1.98	32.5	1.7	0.8	410	69	78.3
198	59574/20	19	F	G2	4.64	11.3	36.1	87	24.4	31.3	12.9	2.1	33.2	0.6	0.3	367	74	49
199	59491/20	19	F	G1	4.58	11.8	35	85.4	25.8	33.7	14.6	1.22	32.9	1.4	0.5	254	93	52.9
200	4951/20	24	F	G4	4.62	11.7	35.2	84.2	25.3	33.2	13.2	1.51	32.1	1.6	0.4	286	38	14
201	5152/20	24	F	G2	4.56	11.1	34.5	78.2	26.4	32.1	13	1.67	27.8	12.1	0.4	576	58	12.9
202	60288/20	25	F	G2	3.89	11.9	32.6	83.8	30.6	36.5	15	1.22	29.1	3.4	0.2	552	117	12.5
203	60978/20	19	F	G1	3.77	11	28.3	85.1	24.1	32.2	13.2	1.32	32.9	0.4	0.4	330	65	66.3
204	60939/20	28	F	G3	5.18	11.2	35	87.6	21.6	32	14.1	1.29	32.5	0.2	0.2	232	72	48.1
205	61182/20	25	F	G2	4.56	12.9	36.1	80.2	28.3	35.7	13.1	1.14	43.3	0.5	0.4	330	43	53.4
206	83969/20	22	F	G1	4.68	12.5	41.2	87.4	24.4	34.2	14.8	1.54	30.1	1.4	0.2	245	77	13.2
207	82704/20	23	F	G2	3.59	11.1	32.7	89.1	25.8	32.5	14.8	1.27	35.4	1.8	0.3	253	38	76.2
208	54278/20	25	F	G1	5.26	11.5	31.2	86.7	26.4	34.6	13	1.98	32.6	2.1	0.2	329	77	110
209	37872/20	22	F	G2	4.19	11.4	43.5	85.3	30.6	34.2	14.3	1.32	33.3	0.2	0.2	332	89	61.7
210	37881/20	24	F	G2	3.66	13.7	29.1	88.7	21.6	36.3	14.8	1.23	32.8	0.6	0.5	223	39	43.7
211	65737/20	32	F	G4	4.35	13.5	38.4	86.8	28.3	34.6	12.9	1.09	29.5	1.3	0.4	520	69	12.7
212	154790/20	24	F	G1	4.64	11.1	34.9	88.2	23.1	34	14.8	1.2	34.4	1.7	0.2	399	45	53.4
213	156903/20	22	F	G1	5.42	12.8	37.3	83.8	28.8	34.3	13.1	1	28.2	2.8	0.3	410	69	12.2
214	63117/20	28	F	G2	4.29	12.7	37.6	80	27	33.8	13	1.36	34.9	1.1	0.2	267	54	49.3
215	72075/20	19	F	G1	4.27	11.6	34.2	90.7	30.8	33.9	15	1.98	30.9	2.5	0.3	476	58	14.4
216	37885/20	24	F	G2	4.9	11.1	28.6	86.7	29.1	33.6	13.2	2.1	33.2	0.5	0.2	352	77	41.3
217	6038/20	23	F	G1	5.06	11.3	29.6	85.3	22.1	31.4	15.7	1.22	33.6	1.4	0.9	340	37	66.9
218	6089/20	24	F	G2	4.71	11.5	33.6	82.4	27	34.2	13.9	1.51	35	1.1	0.5	367	67	47.2
219	85877/20	22	F	G2	4.35	11	33.4	86.8	28.6	32.9	16.1	1.22	30.1	1.5	0.2	354	66	18.5
220	28038/20	24	F	G1	4.36	11.1	29.5	84.4	24.4	31.5	13.2	1.14	43.3	1.2	0.2	412	79	54.1
221	162610/20	23	F	G1	4.64	12.7	37	86	29.4	34.3	16.1	1.51	28.1	11.6	9.2	470	97	12.7
222	2369/20	25	F	G2	3.69	11.8	32.5	77.6	25.8	33.2	14.4	1.22	26.2	3.4	0.2	485	59	13.9
223	15891/20	22	F	G1	4.52	11.1	33	82.1	27.6	33.6	14.9	1.32	28.9	20.4	17.4	470	110	13
224	152487/20	21	F	G1	4.25	11.2	28.7	88.4	25.1	32.1	15.2	1.29	34.5	1.2	0.5	382	44	63.4
225	6034/20	25	F	G2	4.32	11.2	34.4	79.1	25.7	32.6	18	1.14	26.3	5.5	17.4	365	62	14.2
226	37884/20	24	F	G2	4.77	11.7	35.2	76.2	25.3	33.2	14.6	1.12	26.9	2.8	1.8	420	69	14.4
227	156830/20	22	F	G2	5.23	11.1	34.5	78.2	26.4	32.1	13	0.94	28.5	1.2	0.1	418	83	14.3
228	6663/20	24	F	G1	4.26	11.5	34.7	88.8	27.5	30.7	13.6	1.12	35.3	0.9	0.6	276	127	143
229	162105/20	21	F	G1	4.36	11.01	33.2	80.2	26	32.5	12.6	1.12	34	0.9	0.2	269	127	132.9
230	37882/20	24	F	G2	5.3	11.9	34.1	88	29.1	33.5	13.7	1.43	32.8	1.8	0.7	424	54	53.8

231	66577/20	27	F	G4	4.67	11.02	30.7	79.1	30.4	34.1	12	1.52	29.4	0.6	1.5	383	88	15
232	37876/20	18	F	G1	4.32	12.6	36.3	84	29.2	34.7	14.5	1.6	33.4	0.9	0.4	365	95	73.2
233	153151/20	25	F	G1	4.81	14.3	46.3	96.3	29.7	30.9	13.2	0.9	28.8	1	0.9	485	85	14.3
234	155222/20	22	F	G3	4.01	11.6	35.1	87.5	28.9	33	14	1.66	28.7	1.9	0.2	432	95	16.3
235	158329/20	26	F	G2	4.23	13.1	37.7	89.1	31	34.7	13.5	2.34	34.2	0.4	0.1	287	109	31.8
236	37873/20	18	F	G1	4.32	11.9	35.6	82.4	27.5	33.4	14.6	1.14	27.3	5.4	1.7	450	53	12.5
237	37883/20	23	F	G1	4.07	11.1	30.5	84.9	24.1	32.1	12.4	1.58	32.8	1.6	0.4	311	62	74.3
238	15842/20	24	F	G1	3.97	11.2	32.3	81.4	25.7	31.6	12.7	1.87	34.7	1.1	0.4	276	98	74.3
239	37875/20	25	F	G2	5.53	15.8	48.3	87.3	28.6	32.7	12.8	1.88	27.9	1.7	0.1	390	68	14.9
240	16017/20	22	F	G1	5.06	12.3	37.9	84.9	24.3	32.5	14.8	0.81	34.4	0.8	0.4	328	50	57.2
241	36258/20	24	F	G2	4.71	14.3	41.2	87.5	30.4	34.7	12.2	2.48	32.1	0.6	0.4	252	51	15.3
242	157928/20	21	F	G2	3.77	11.3	32.7	86.7	30	34.6	14.5	1.34	28.3	2.5	0.3	335	82	15.4
243	157367/20	27	F	G4	3.45	11.7	31.2	90.4	31	34.3	12.7	1.67	28.4	0.7	1.1	229	51	19.2
244	152890/20	18	F	G2	5	15.8	43.5	87	31.6	36.3	12.5	1.88	28.9	1.2	0.5	357	98	12.9
245	152951/20	25	F	G2	3.9	11.1	29.1	84.6	25.4	34	14.8	1.09	34.5	1.2	0.2	376	53	67.3
246	66981/20	19	F	G2	4.64	14	38.4	82.8	30.2	36.5	12.5	1.29	29.2	2.9	0.2	465	53	14.4
247	61187/20	22	F	G1	3.69	12.8	35	94.9	34.7	36.6	14.3	1.26	29.1	0.7	0.1	412	72	14
248	154181/20	20	F	G1	4.52	11.6	34.2	85.7	23.5	31	13.3	1.88	34.1	0.5	0.2	256	122	62.5
249	158062/20	20	F	G1	4.25	13	39.1	92	30.6	33.2	14.8	1.97	34.2	1.6	0.4	301	45	92.6
250	152547/20	22	F	G1	4.22	12.8	38.9	92.2	30.3	32.9	12.9	1.31	29	1.1	0.3	242	58	17.3
251	6037/20	24	F	G1	4.38	12.6	38.2	87.2	28.8	33	13.9	1.17	33.7	2.1	0.3	240	86	127
252	16310/20	22	F	G2	4.05	12.2	35.4	87.4	30.1	34.5	14.5	1.65	34.3	1.9	0.3	405	158	83.2
253	80701/20	24	F	G1	4.78	12.4	37.8	79.1	25.9	32.8	14.6	1.39	30.2	8.2	0.8	489	129	12.7
254	17043/20	32	F	G2	3.3	11.1	28.6	86.7	29.1	33.6	13.2	1.55	28.2	2.1	0.3	475	76	12.6
255	15899/20	19	F	G2	4.26	11.5	33.6	78.9	27	34.2	14.9	1.97	28.7	8.7	0.6	422	39	12.9
256	67110/20	36	F	G3	3.85	11	33.4	86.8	28.6	32.9	16.1	0.74	35.9	0.4	0.2	282	74	96.3
257	152847/20	19	F	G1	4.11	11.2	32.1	83.2	25.5	32.7	13.5	1.55	34.4	0.8	0.5	289	59	108.2
258	154803/20	22	F	G3	4.04	11.3	32.3	80	27	33.7	16	1.63	28.7	8.4	1.3	420	105	13.8
259	37888/20	23	F	G2	4.85	12.9	38.4	79.2	26.6	33.6	12.5	1.45	27.2	5.2	1.8	509	43	12.5
260	37886/20	23	F	G1	3.49	11	30.3	86.8	28.7	33	12.5	1.27	27	1.3	0.5	236	68	14.9
261	157897/20	36	F	G2	3.98	11.8	32.5	81.7	27.1	33.2	13.1	1.09	34.2	1.2	0.3	250	46	82.1
262	6036/20	19	F	G2	3.59	11.1	32.7	82.2	25.8	32.5	13.1	1.27	33.6	1.8	0.3	300	38	72.4
263	15734/20	25	F	G2	4.16	11.2	35.4	90.7	25.3	34.7	12.5	1.09	32.1	0.8	0.2	276	87	12.5
264	40110/20	21	F	G3	5.26	11.5	31.2	86.7	26.4	34.6	13	1.98	34.7	2.1	0.2	229	77	109

265	6035/20	26	F	G3	4.02	11.2	37	78.9	24.1	34.3	13.3	1.77	26.3	3.7	0.2	567	36	13.9
266	62737/20	24	F	G1	3.66	13.7	29.1	87.7	21.6	36.3	14.8	1.23	34.8	0.9	0.5	298	39	59.1
267	15909/20	38	F	G3	4.64	11.1	34.9	87.1	23.1	34	13.8	1.2	34.5	0.7	0.2	399	54	54.1
268	85788/20	24	F	G2	4.21	11.9	35	80	28.9	33.8	12	1.53	35.8	1.9	0.2	131	62	79.9
269	157230/20	22	F	G2	5.42	12.8	37.3	83.8	28.8	34.3	13.1	1	28.2	2.8	0.3	310	69	14.9
270	156946/20	28	F	G1	4.29	12.7	37.6	80	27	33.8	14.2	1.36	34.3	2.1	1.1	165	65	49.9
271	95/20	30	F	G2	3.46	11.5	26.7	87.2	24.6	31.8	13.8	0.9	33.7	0.9	1.1	387	73	67.3
272	37874/20	24	F	G2	4.16	11.3	29.2	87.4	21.4	30.5	12.6	2.28	33.4	0.3	0.2	154	45	47.3
273	8019/20	20	F	G1	3.74	11.2	32	85.6	27.3	31.9	13.2	1.35	33.5	0.9	0.2	243	59	77.2
274	15882/20	24	F	G1	4.79	11.9	36.5	80.9	24.8	32.6	15.2	1.34	34.5	2.1	0.9	252	75	87.4
275	16425/20	25	F	G2	4.7	12.7	37.6	80	27	33.8	13	1.9	28.6	4.9	0.2	452	132	14.6
276	154865/20	25	F	G1	4.05	12.2	35.4	87.4	30.1	34.5	14.5	1.65	34.3	1.9	0.3	405	158	67.3
277	64452/20	35	F	G5	4.78	12.4	37.8	79.1	25.9	32.8	14.6	1.39	31.8	8.2	0.8	381	129	14.9
278	62861/20	24	F	G2	3.77	11.6	34.2	90.7	30.8	33.9	15	1.73	31.9	1.6	0.3	245	120	14.2
279	158615/20	21	F	G1	3.3	11.1	28.6	86.7	29.1	33.6	13.2	1.55	28.2	2.1	0.3	479	76	12.4
280	40339/20	21	F	G2	4.21	11.3	29.6	85.3	22.1	31.4	13.2	1.03	25.1	1.6	0.6	429	56	12.2