“COMPARATIVE STUDY OF PERIPHERAL SMEAR WITH RBC INDICES AND RBC HISTOGRAMS IN CASES OF ANEMIA IN ADULTS”

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Reg. No. BN0121005

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DR. Vijayalaxmi Dhorigol MD.
Professor and HOD
Department of Pathology,
J. N. Medical College,
Belagavi, Karnataka

Date: 28/6/24.
Place: Belagavi.

DR.(Mrs) N. S. Mahantashetti MD (Paed).
Principal
J. N. Medical College,
Belagavi, Karnataka.

Date: 28/06/2024
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Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350 0831 - 2470759 www.jnmc.edu principal@jnmc.edu

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To,
Reg. No. BN0121005
Postgraduate Student,
2021-22 Batch,
Department of Pathology
J. N. Medical College, Belagavi.

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(Deemed – to- be- University)
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JNMC INSTITUTIONAL ETHICS COMMITTEE
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref No.MDC/JNMCIEC/ 133 **Date: 27/09/2022**

To.
REG NO: BN0121005
PG Student in Pathology,
J. N. Medical College,
BELAGAVI.

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Member Secretary
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,
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LIST OF ABBREVIATIONS

Hb	:	Hemoglobin
Hct	:	Hematocrit
MCV	:	Mean Corpuscular Volume
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
RDW	:	Red cell distribution width
CBC	:	Complete blood count
RBC	:	Red Blood Cell
HSCs	:	Hematopoietic stem cells
NK	:	Natural killer
GM-CSF	:	Granulocyte-monocyte colony-stimulating factor
M-CSF	:	Monocyte-macrophage colony-stimulating factor
G-CSF	:	Granulocyte colony-stimulating factor
BFU-E	:	Blast Forming Unit-Erythroid
EPO	:	Erythropoietin
CFU-E	:	Colony Forming Unit- Erythroid
GMP	:	Granulocyte-macrophage progenitor
TPO	:	Thrombopoietin
N:C ratio	:	Nuclear-to-cytoplasmic ratio
RNA	:	Ribonucleic Acid
fL	:	Femtoliters
pg	:	Picograms

WBC	:	White Blood Cell
PLT	:	Platelet
RDW-CV	:	Red cell distribution width- coefficient of variation
RDW-SD	:	Red cell distribution width -standard deviation
WHO	:	World Health Organization
PBS	:	Peripheral blood smear
PBF	:	Peripheral blood film
SD	:	Standard Deviation
NA	:	Normocytic anemia
MHA	:	Microcytic hypochromic anemia
MA	:	Macrocytic anemia
DA	:	Dimorphic anemia
EDTA	:	Ethylene Di amine Tetra Acetic Acid

ABSTRACT

Introduction- The most common and the most important hematological disorder in the world is Anemia. Since decades, the gold standard technique for the evaluation of RBC morphology is peripheral smear. With rising effectiveness and falling costs, cell counters have become a commonplace part of medical laboratory services all over the world. As RBC histogram and RBC indices do not give much information about the poikilocytosis of the red cells, there is a definite need of peripheral smear to look for RBC morphology like fragmented cells, dimorphic picture, agglutination, presence of polychromatophils etc. Modern devices may be sophisticated, but manual procedures are still needed for initial calibration. Despite the drive to automate everything, this highlights the importance of manual technical abilities.

Method- EDTA blood sample was run in the Sysmex XN-1500, 5-part differential autoanalyzer, analyzed by the machine and the data is generated which includes Hemoglobin, RBC count, RBC indices, Total Count, Differential Count, Platelet count, RDW-CV, RDW-SD etc. and all the 3 histograms. A peripheral blood smear was prepared from every sample with all the precautions and stained with Leishman stain using the standard procedure. Following that, histogram patterns and RBC indices were correlated with the impression obtained from the peripheral smear examination.

Results- When peripheral smear analysis was compared with RBC histograms and indices, a significant difference was observed between the two. The Pearson chi-squared test revealed a statistically significant p-value with $p<0.001$. We found that dimorphic anemia showed the maximum discordance (89.32%) between peripheral smear analysis and RBC indices and histograms.

Conclusion- Our study found a significant correlation between the RBC histogram and peripheral smear examination for diagnosing microcytic hypochromic anemia, normocytic anemia, and macrocytic anemia. when it came to diagnosing dimorphic anemia, the relationship between the histogram patterns, red cell indices, and peripheral smear examination raised questions about the reliability and validity of the RBC histogram. Thus, a detailed assessment of red blood cell morphology through a peripheral smear remains the gold standard for accurate classification and diagnosis of anemia types.

Keywords- Anemia, peripheral smear, RBC indices, Histograms, Hb, MCV, MCH, MCHC, RDW

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INTRODUCTION

One of the most frequent clinical conditions and laboratory results in haematology is anaemia.[1]

According to the National Family Health Survey 5 (2019–21), the prevalence of anemia is 57.0% in women aged 15–49 years, 25.0% in men aged 15–49 years, 59.1% in adolescent girls and 31.1% in teenage boys between 15–19 years, 67.1% in children of 6–59 months and 52.2% in pregnant women between 15–49 years.[2]

Functionally, anemia is described as decrease in the blood's ability to deliver oxygen to the tissues, consequently leading hypoxia of the tissues. Clinically, the term refers to reduction in the normal concentration of haemoglobin and/or erythrocytes.[3]

Haemoglobin (Hb), Haematocrit (Hct), Mean Corpuscular Haemoglobin (MCH), Red Cell Distribution Width (RDW), Mean Cell Volume (MCV), and Mean Corpuscular Haemoglobin Concentration (MCHC), are just a few of the factors used to classify anaemia.[4]

Since decades, peripheral blood smear examination has played a significant role in the investigation of many haematological disorders. It is also a key diagnostic tool, particularly for the etiopathological workup of many haematological disorders.[5]

With rising effectiveness and falling costs, cell counters have become a commonplace part of medical laboratory services all over the world. Complete blood count (CBC) generated from automated haematology analysers and peripheral smear examination under a microscope have complimented one another in recent years to produce a thorough report on a patient's blood sample.[6,7]

Along with Red Blood Cell (RBC) indices (MCV and RDW), the RBC histograms are an essential part of automated analysis and provides helpful information for the diagnosis and management of red cell disorders.

In the situation of megaloblastic anaemia with progressive iron deficiency, histogram reveals important features that are not immediately apparent from the numerical data. The histogram detects a twofold peak in the limited population of microcytic hypochromic cells.[8,9] Also, conditions like red cell inclusions and membrane abnormalities cannot be detected using red cell indices or the RBC histogram. The heterogeneity of the RBC population, which can be determined by peripheral smear examination, is not reflected by red cell indices like MCV, which is a mean value.[5] A dimorphic population curve cannot be utilized to determine MCV since it represents an average value and does not account for the variability within the RBC population. Additionally, for an accurate diagnosis, it is necessary to check for certain morphological features in peripheral smears. This requires knowledge of the aberrant curves of the RBC histogram and the flags.[10]

Modern devices may be sophisticated, but manual procedures are still needed for initial calibration. Despite the drive to automate everything, this highlights the importance of manual technical abilities.[7]

Comparing peripheral smear findings to RBC indices and histograms, there haven't been as many studies done. The current investigation is being carried out in the Department of Pathology, JNMC Belagavi, in an effort to correlate these in order to enhance the approach of diagnosing anaemia.

AIMS AND OBJECTIVES

- To study RBC morphology on peripheral smear in different types of anemia in adults.
- To study RBC histogram in different forms of anemia in adults.
- To study RBC indices in anemia in adults.
- To compare RBC morphology on peripheral smear with RBC histogram in various types of anemia in adults.

REVIEW OF LITERATURE

The examination of the blood's composition was made possible by Hans and Zacharias Jannsen's invention of the compound microscope in Holland around 1590.[11]

For the first time, red blood cells under a microscope were seen in 1658 by Dutch naturalist Jan Swammerdam. Antoni van Leeuwenhoek (1632–1723), a fellow Dutch microscopist and friend of Swammerdam, described the size and shape of "red corpuscles" and created the first depiction of them in 1695.[11,12]

Figure 1: Red corpuscles illustrated by A von Leeuwenhoek.[11]

William Hewson (1739–1744) gained the title "The founder of haematology" after publishing an essay on the significance of red blood cells.[13]

The first explanation of how to count blood cells was published in 1852 by Karl Vierordt, who recommended distributing a known volume of blood onto a microscope surface. Louis-Charles Malassez invented the hemocytometer in 1874, which made it straightforward to analyse blood cells under a microscope. In the late 19th century, Paul Ehrlich and Dmitri Leonidovich Romanowsky developed the white and red blood cell staining methods that are being used to check patients today.

James Homer Wright's invention of the Wright stain in 1902 paved the way for the visual examination of blood films under a microscope.[14,15]

Romanowsky stains are compound neutral dyes using a partly polychromed methylene blue in combination with Eosin Y or Eosin B.[16]

These include the Jenner, Wright, Wright-Giemsa, Leishman, and May-Grunwald stains.[17] Acid bichromate is used in known amounts by Giemsa stains to create the transformed azure chemicals. Methylene blue is changed into methylene azure by sodium bicarbonate in the Wright stain formula, which then stains the cell. Romanowsky stains of all kinds are insoluble in water but dissolvable in methyl alcohol.[18]

Hematopoiesis

All functional blood cells that are discharged from the bone marrow into the circulation are formed, developed, specialised, and replaced through a process known as hematopoiesis.[19-21]

Hematopoietic stem cells (HSCs), pool of undifferentiated cells, which are mostly found in the bone marrow, the primary site of adult hematopoiesis, are the source of all different types of blood cells.[20,22]

Hematopoiesis starts early during gestation and goes through a lot of changes during foetal and neonatal development. After blastocyst implantation, yolk sac is the first place of hematopoiesis. During the third week of embryonic development, day 18 of gestation, primitive erythroid cells can be seen in the yolk sac's blood islands. At days 21–22 of gestation, cardiac contractions begin to appear and along with that, primitive erythroblasts start to reach the embryo proper and continue to circulate for about 12 weeks. A few weeks later, the mesoderm of the intraembryonic aorta/gonad/mesonephros area begins to produce definitive hematopoietic stem cells.[23-26]

60% of liver cells between 7 and 15 weeks of gestation are hematopoietic.[24,27]

From the 9th week of gestation until the 24th, the liver is the main source of red blood cells.[24] Despite the fact that erythroid lineage cells predominate in the foetal liver, mature megakaryocytes, monocytes, macrophages, T cells, natural killer (NK) cells and B cells are also found. As the foetal liver develops throughout pregnancy, myeloid and lymphoid cells make up an increasing percentage of blood cells.[24,28] By 6 weeks of gestation, the liver has megakaryocytes, and by 8 to 9 weeks, the circulation begins to show signs of platelets.[24,27] As early as week seven of pregnancy, granulopoiesis can be seen in the liver parenchyma, and by week eleven of pregnancy, there are a few circulating leukocytes.[24] Hematopoietic cells first appear in the bone marrow of 10 to 11 week old embryos, and until 15 weeks of gestation, they are present exclusively in the diaphyseal areas of long bones.[24,26,29] In the beginning, the foetal marrow has roughly equal amounts of myeloid and erythroid cells. However, myeloid cells start to take over by 12 weeks of gestation, and by 21 weeks of gestation, the myeloid-to-erythroid ratio is approaching the adult level of 3:1.[24,27] After the 24th week of pregnancy, the marrow becomes the principal site of hematopoiesis and remains so for the remainder of the foetal life.[24] Hepatic hematopoiesis only persists in sporadic foci that quickly go dormant after delivery, and by the time of birth, the marrow throughout the skeleton is hematopoietically active. Hematopoiesis is limited to the axial skeleton after puberty and stops occurring in distal bones.

As a result, only about half of the marrow space in healthy adults is hematopoietically active.[23]

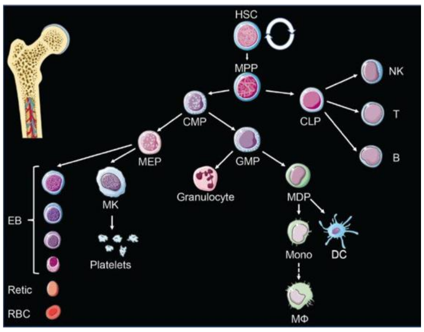


Figure 2: Hematopoiesis with the bone marrow precursors: HSC- hematopoietic stem cell, MPP- multipotent progenitor, CMP- *common myeloid progenitor, CLP- common lymphoid progenitor, NK- natural killer, MEP- megakaryocyte erythrocyte progenitor, GMP- granulocyte-macrophage progenitor, MDP- macrophage dendritic cell progenitor, Mono- monocyte, DC- dendritic cell, Mφ, macrophage, MK, megakaryocyte, EB- erythroblast, Retic, reticulocyte, RBC, red blood cell.*[30]

Regulation of Hematopoiesis

Normal hematopoiesis is a tightly controlled process whereby a primitive multipotent stem cell undergoes an organised sequence of maturation and proliferation to produce mature blood components. Regulating factors include erythropoietin, interleukin3, granulocyte-monocyte colony-stimulating factor (GM-CSF), monocyte-macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin5, interleukin4, and other less well-defined factors, such as the megakaryocyte growth factors.[31]

Regulation of Erythropoiesis

The earliest progenitor cells dedicated only to erythroid development, known as Blast Forming Unit-Erythroid (BFU-Es), are not Erythropoietin (EPO)-dependent, but Colony Forming Unit- Erythroid (CFU-Es), and their immediate offspring, proerythroblasts, rely on EPO to inhibit apoptosis.[32,33] In all stages of development, from proerythroblast to orthochromatic erythroblast, erythroid differentiation takes place within the erythroblastic islands, which are made up of a core stromal macrophage surrounded by adhering erythroid cells.[32,34,35] The main regulator of erythropoiesis, EPO, strictly limits production rates and is produced by the kidneys in response to low oxygen levels. Hypoxia is recognised by tissue oxygen supply, which in turn regulates the amount of circulating red blood cells.[32,36] Specialised sensor organs, kidney being the most important, experience decreased oxygen delivery as a result of a decrease in arterial blood's oxygen-carrying capacity or partial pressure of oxygen. In response, these organs produce more erythropoietin. When tissue oxygen delivery surpasses a predetermined level, erythropoietin production declines. By coordinating the production and removal of red blood cells, this feedback system, under normal conditions, keeps the blood's haemoglobin concentration relatively constant.[37]

Regulation of Granulopoiesis

Originating from stem cells and myeloid progenitor cells and concentrating in the subcortical sections of the hematopoietic cords, granulocytes are mature myeloid cells made up of neutrophils, eosinophils, and basophils.[32,38] By expressing a variety of transcription factors, granulocytes originate as the final differentiated form from the common granulocyte-macrophage progenitor (GMP) cells that develop from multipotent progenitors.[32,39] Hematopoietic growth factors that promote the viability and proliferation of granulocytic progenitor and precursor cells include KIT ligand/SCF, GM-CSF, G-CSF, M-CSF, IL-3, IL-6, and IL-5. Although some of them, like SCF and M-CSF, are commonly expressed by the marrow stroma and are produced in inflamed regions of peripheral organs. IL-5 for eosinophil progenitors and G-CSF for neutrophil progenitors are two hematopoietic growth factors with unique lineage targets for late-stage granulocytic cells.[32] A mechanism has been put forth to explain how neutrophil granulopoiesis is triggered. It states that a variety of cells, including the monocyte, release substances upon coming into contact with invading bacteria. These substances are then transported by the blood to the bone marrow, where they activate the neutrophil granulocyte series' proliferative activity. Significantly more eosinophils are produced during an allergic reaction, and the interaction between lymphocytes and eosinophil granulocyte precursors accounts for the connection between the identification of the allergen and the rise in eosinophil production.[37]

Regulation of Thrombopoiesis

Megakaryocytic progenitors produced by HSCs multiply and differentiate into proplatelets as they develop cytoplasmic demarcation membranes, branching extensions, and polyploidy acquired through endomitosis. Thrombopoietin, TPO, a key regulator of megakaryocyte formation, collaborates with a number of synergistic cytokines, such as IL-11, KIT ligand, IL-6, and leukaemia inhibitory factor, to provide the desired effects.[32,40,41]

TPO is predominantly produced by the liver and secondarily by other tissues, such as bone marrow, and is needed by HSC and the megakaryocytic lineage cells.[32,42] A straightforward feedback loop between relatively constant TPO production and intracellular catabolism causes TPO levels to be negatively correlated with megakaryocyte and platelet counts.[32,41] Megakaryocyte precursors spread throughout the marrow during steady state thrombopoiesis and move slowly in the direction of sinus vessels until mature megakaryocytes are close to the walls of the sinus vascular walls.[32,43,44] The generation of platelets is the primary role of megakaryocytes. A microtubule sliding mechanism in the mature megakaryocytes' proplatelets enables their extension and aids in the redistribution of cytoplasmic platelet granules to bulbous forms at their distal ends.[32,40] Megakaryocytes extend numerous proplatelets through the adjacent sinus endothelial cells. The sheer force of blood flow then assists in separating individual platelets and some proplatelets, which eventually disintegrate in the circulation.[32,45] As an alternative, megakaryocytes can enter sinusoidal blood under conditions of normal stress, move to the lungs where they dwell, create proplatelets, and generate approximately half of the circulating platelets.[32,46]

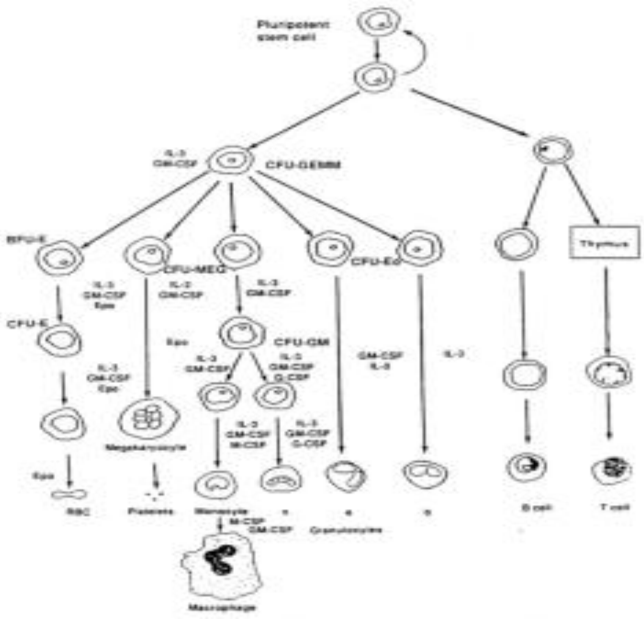


Figure 3: Hematopoietic differentiation and effect on progenitor cells of hematopoietic growth factors.[31]

Erythropoiesis

The process by which red blood cells are made in the bone marrow is called erythropoiesis. This can be broken down into several steps, including pluripotent stem cell progeny's commitment to erythroid differentiation, the early phase of erythropoiesis that is EPO-independent, and the late phase of erythropoiesis that is EPO-dependent.[47] It has been determined that the primary factor controlling the generation of red blood cells is the hormone EPO.[47,48] Colonies of fully hemoglobinized erythroblasts are produced by two erythroid progenitors called CFU-E and BFU-E, in response to EPO.[47]

The CFU-E progenitor cell develops from the maturation of the BFU-E. Within 7 days of culture, a single CFU-E, which experiences a limited number of cell divisions, produces a discrete colony of 8–64 distinguishable cells.[49]

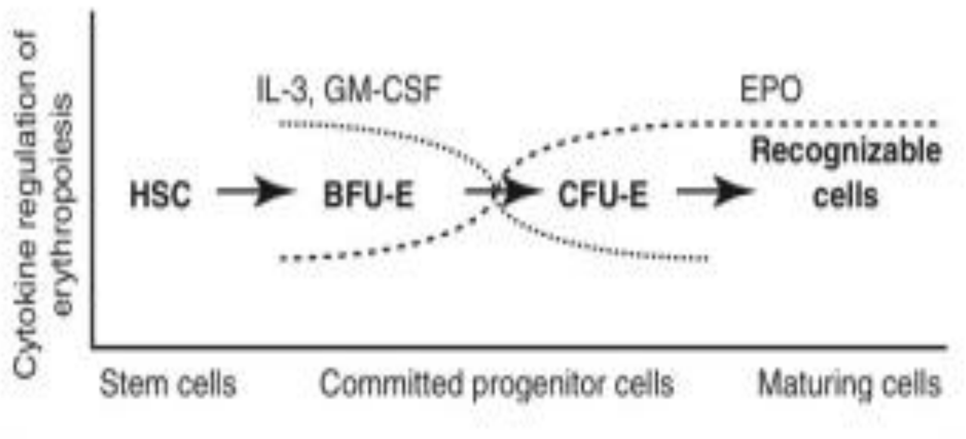


Figure 4: Maturation of erythroid cells.[49]

The pronormoblast, which is the earliest erythroid precursor that can be identified morphologically, is directly preceded by the CFU-E.[49]

Proerythroblast

Having a high nuclear-to-cytoplasmic (N:C) ratio, the proerythroblast is a round or oval cell that ranges in size from moderate to large (14–19 μM in diameter). With a rim of deep basophilic granular cytoplasm, the nucleus takes up 80% or more of the cell. Near the nucleus, a small, pale region that resembles the Golgi apparatus may be seen in the cytoplasm. The chromatin is a thin, linear network that is frequently called "lacy," and it appears coarser and stains darker than the chromatin of a white cell blast. One to three nucleoli may be prominent. Giemsa stain is unable to identify the little levels of Hb present.[47,49,50]

Basophilic erythroblast

This cell resembles the pronormoblast, although it is typically smaller (16–18 µM in diameter) and has a somewhat lower N:C ratio. About three-fourths of the cell's volume is taken up by the nucleus. Due to the increasing amount of ribosomes, the cytoplasm is still extremely basophilic, frequently much more so than it was in the preceding stage. Beginning with the condensation of chromatin (formation of heterochromatin), the chromatin may appear granular and coarse. The nucleoli are no longer apparent.[47,49]

Polychromatophilic erythroblast

The nuclear chromatin continues to condense, resulting in a lowered N:C ratio and a cell with a diameter of 12–15 µM. The appearance of extensive gray-blue cytoplasm, brought on by the synthesis of significant amounts of haemoglobin and a decline in the number of ribosomes (shown by one or more pink patches surrounding the nucleus in dry fixed preparations), is the most distinguishing feature of the cell at this stage. The nuclear chromatin is clumped and irregularly distributed. There are no discernible nucleoli.[47,49]

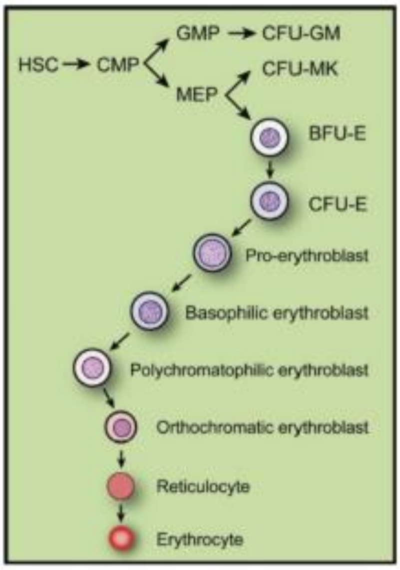


Figure 5: Schematic representation of multipotent HSC differentiating into erythroid cells.[47]

Orthochromatic erythroblast

Its low N:C ratio and modest size (8–12 µM) make it the smallest of the nucleated erythrocyte progenitors. Although the cytoplasm is mostly pink or salmon in colour due to increasing Hb concentration, there is still a faint blue tint present from the ribosomes that are still there. Pyknotic degeneration occurs in the nucleus, chromatin is highly condensed, the nucleus shrinks, and is frequently eccentric or even partially extruded.[47,49]

Reticulocyte

A cell is referred to as a reticulocyte once the nucleus has been ejected. These cells have a volume that is perhaps 20% bigger than mature erythrocytes with a diameter of 7-10 µM.[47,49,50]

Some cytoplasmic organelles, such as mitochondria, the Golgi complex, residual ribosomes and ribonucleic acid (RNA) are retained in the reticulocyte and have unique staining properties giving the developing cell a bluish tint when stained with Romanowsky stains. By reacting with supravital stains, new methylene blue N, or brilliant cresyl blue, which result in the precipitation of the RNA and mitochondria, one can identify these cells in vitro. [47,49]

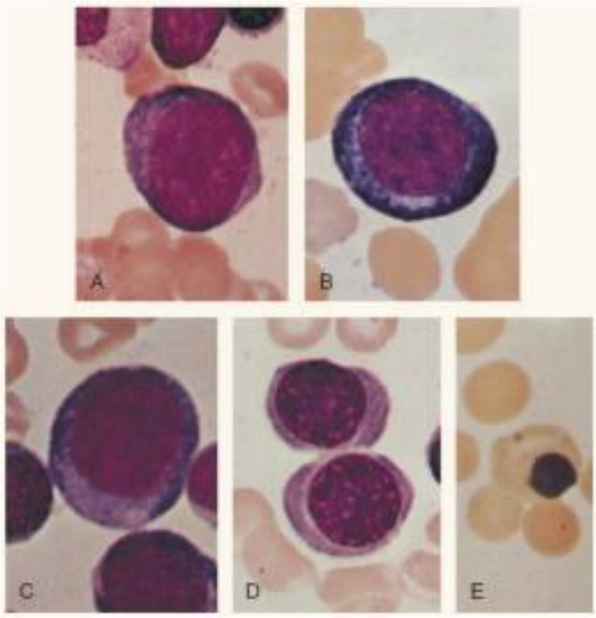


Figure 6: Erythroblasts. A- proerythroblast, B- basophilic erythroblast, C & D- early and late polychromatophilic erythroblast, E- orthochromatic erythroblast.[47]

Erythrocyte

The typical human erythrocyte has a resting diameter of 7-8 μM and a volume of 80–100fL, resembling a flattened, bilaterally indented sphere; this form is also known as a biconcave disc.[47,49]

The cell's membrane is made up of lipids and proteins, and its interior houses metabolic equipment that enables the cell to survive during its 120-day lifespan and preserve the integrity of haemoglobin function.[51]

Haemoglobin is a highly specialised internal erythrocyte protein that helps carbon dioxide go from tissue to the lungs and transports oxygen from the lungs to tissue for oxidative metabolism. 1.34 mL of oxygen may be carried by each gramme of

haemoglobin. Additionally, it is a nitric oxide (NO) transporter, which modifies vascular tone.[52]

Among the cells that make up human tissues, mature erythrocytes are distinct because they naturally lack nuclei and cytoplasmic elements including lysosomes, endoplasmic reticulum, and mitochondria.[53]

Since the red cell serves as the primary means of oxygen transport in the blood, its biconcave structure gives it a high surface area that makes it easier for oxygen to enter and depart the red cell.[53]

Age, sex, and geography all affect the normal erythrocyte concentration.[49]

The erythrocyte appears as a disc on a Romanowsky-stained blood smear, with a rim of pink-staining haemoglobin surrounding a core area of pallor (the centre staining lighter in colour than the rim). One third of the cell's diameter is typically taken up by the pallor in normal circumstances.[54] The size of a typical red blood cell is same as the size of the small lymphocyte nucleus on dried film.

The average amount of erythrocytes in one microliter of human blood is $\sim 5 \times 10^6$ with normal ranges for males and females being $5.0 \pm 0.5 \times 10^{12}$/l and $4.3 \pm 0.5 \times 10^{12}$/l respectively.[55,56]

A high erythrocyte count and haemoglobin concentration after birth are followed by a steady decline that lasts until around the second or third month of extrauterine life, the outcome of a brief halt in bone marrow erythropoiesis following delivery due to a low level of EPO. Because of the strong oxygen affinity of foetus for haemoglobin F (foetal haemoglobin) and the relatively hypoxic environment in gestation, EPO levels are high in foetus.[49]

Because testosterone is present after puberty, males have a higher erythrocyte content than females. Males and females have similar erythrocyte levels prior to puberty and following "male menopause."[49,57,58]

In addition to directly enhancing the differentiation of marrow stem and progenitor cells, testosterone boosts the generation of extrarenal and renal EPO.[49,59]

The mean erythrocyte concentration is higher in people who live at high altitudes than it is in people who reside at sea level.[49]

The haemoglobin concentration and size of the erythrocytes are classified using the erythrocyte indices, MCV, MCH, and MCHC. These indices provide substantial diagnostic information (most frequently for the diagnosis of anaemias) and predict how the RBCs will look under a microscope·[54]

Automated instrumentation is used to calculate the haemoglobin, hematocrit, platelet count (PLT count), erythrocyte count (RBC count), and leukocyte count (WBC count).[54]

The hematocrit, which is expressed in SI units as a percentage (%) or as the volume of RBCs in litres per volume of whole blood in litres (L/L), measures the volume that RBCs occupy within whole blood. After the haemoglobin has been liberated from the lysed erythrocytes, it is quantified spectrophotometrically. In either grammes per decilitre or grammes per litre, it is measured.[54]

The rule of three is a fast mathematical check that the laboratory professional uses to determine whether the RBC count, hematocrit, and haemoglobin readings are accurate. RBC count * 3 = haemoglobin * 3 = hematocrit (%) is a simple formula.[54]

The MCV is measured in femtoliters and represents the average volume of a single erythrocyte. The Hct and RBC count can be used to compute it, and it is measured via automated instrumentation.[54]

The MCV is used to categorise cells as normocytic, microcytic, or macrocytic and typically correlates with how the cells appear on stained blood smears (cells with a higher MCV appear larger [macrocytic], whereas cells with a decreased MCV appear smaller [microcytic]). 80 to 100fL is the reference range for MCV.[54]

Terminology	Description
Normocytic	80.0–100.0 fL
Microcytic	Red cells with a reduced volume (less than 80 fL)
The Hb (Macrocytic)	Red cells with an increased volume (greater than 100 fL)
Anisocytosis	Increased variation in the range of red cell sizes

Figure 7: MCV based erythrocyte classification.[54]

The MCH measures the average haemoglobin weight in individual erythrocytes, which is measured in picograms. Using the haemoglobin and erythrocyte counts, the MCH is determined.[54]

The MCV should always be taken into account when interpreting the MCH because the MCH varies in a direct linear relationship with the MCV. The MCH does not account for the size of a cell. Haemoglobin concentrations vary depending on cell volume, with smaller cells normally containing less haemoglobin and larger cells typically containing more.[54]

The suffix -chromia, which denotes colour, is used to define the MCHC, which represents the concentration of haemoglobin in the overall cell population. If the central pallor area covers more than one-third of the cell's surface area, the cell can be morphologically defined as hypochromic. Hyperchromic should only ever be utilised in very specific circumstances. Spherocytes are the only erythrocytes that are hyperchromic and have MCHC values greater than 36.0 g/dL. The mean haemoglobin concentration in a decilitre of erythrocytes, or MCHC, is measured in grammes per decilitre (g/dL), which is the ratio of haemoglobin mass to the volume in which it is contained. The Hb and Hct are used to compute the MCHC.[54]

Normochromic	32.0–36.0 g/dL
Hypochromic	Less than 32.0 g/dL
Hyperchromic	More than 36.0 g/dL

Figure 8: MCHC based erythrocyte classification.[54]

When there is significant fluctuation in erythrocyte volume/size (anisocytosis), the MCV is less accurate in defining the erythrocyte population since it represents an average of erythrocyte volume. The RDW, which is also known as the RDW-coefficient of variation (RDW-CV), is the MCV's coefficient of variation. The RDW-CV, a calculated index from haematology tools that aids in the detection of anisocytosis, has the following formula:[54]

$$\text{RDW} - \text{CV} = \frac{\text{1 standard deviation (SD) of MCV}}{\text{MCV}} \times 100$$

RDW values that are elevated (greater than 14.5%) signify an increase in the variability of erythrocyte size. A reduced RDW does not signify any recognised anomalies.[54]

Reticulocytes are immature, anuclear erythrocytes with organelles and lingering ribosomes for haemoglobin production. Before their RNA is broken down and they mature into erythrocytes, these cells typically spend 2-3 days in the bone marrow and another day in peripheral blood. One of the most beneficial and economical laboratory tests for assessing anaemia pathophysiology and treatment response is the peripheral blood reticulocyte count, which reveals the level of effective bone marrow activity. Compared to the relative reticulocyte count, the absolute reticulocyte count provides a more accurate indicator of erythropoietic activity. The absolute count for reticulocytes can be provided by automated analysers, or it can be computed manually:[54]

$$\text{Absolute reticulocyte } (\times 10^3/\text{mcL}) = \text{RBC count } (\times 10^6/\text{mcL}) \times \text{Reticulocyte } (\%)$$

Anemia

Definition

According to the World Health Organization (WHO), anemia is a condition in which a person's red blood cell count or oxygen-carrying capacity is insufficient to meet their physiologic needs, which differ depending on the person's age, sex, altitude, smoking status, and stage of pregnancy. When Hb is below 13 g/dl in males and below 12 g/dl in females, anemia is diagnosed.[60,61]

Population	Non-Anemia*	Mild Anemia*	Moderate Anemia*	Severe Anemia*
6-59 months of age	≥ 11	10-10.9	7-9.9	<7
5-11 years of age	≥ 11.5	11-11.4	8-10.9	<8
12-14 years of age	≥ 12	11-11.9	8-10.9	<8
Non-pregnant women (≥ 15 years)	≥ 12	11-11.9	8-10.9	<8
Pregnant women	≥11	10-10.9	7-9.9	<7
Men (≥ 15 years)	≥13	11-12.9	8-10.9	<8

Figure 9: Grading of anemia according to WHO.[62]

Classification

Anemia can be categorized as (1) relative or (2) absolute depending on the red cell mass measurement. A normal total red cell mass in an elevated plasma volume is indicative of relative anemia, which causes dilution anemia, or a disruption in the regulation of plasma volume.

It may be difficult to classify absolute anemias with reduced red cell mass since kinetic, morphologic, and pathophysiologic interaction factors must be taken into account. The first step in classifying all anemias is to separate those that result from decreased red cell production from those that result from increased red cell death. The reticulocyte count plays a major role in the differentiation process. Pathophysiologic or morphologic criteria may be used for the subsequent diagnostic breakdown.[63]

Pathophysiologic (etiologic) Classification[63] **(Annexure I)**

The best method for connecting possible treatments to disease processes is pathophysiologic categorization.

Morphologic Classification

The erythrocyte indices can be used to initially classify anemias morphologically according to the average red cell size and hemoglobin concentration. Anemia is classified based on its morphology into three categories: macrocytic anemia, normocytic anemia, and microcytic hypochromic anemia.[63,64]

NORMOCYTIC NORMOCYTIC ANEMIA

The term "normocytic normochromic anemia" refers to anemia in which the RBCs in circulation have a normal red color and are of the same size (normocytic). When viewed under a microscope, red blood cells often resemble normal cells.

When a person has either hyperproliferative (corrected reticulocyte count >2%) or hypoproliferative (corrected reticulocyte count <2%) anemia, the etiology of their normocytic normochromic anemia alters.[65-67]

Reticulocyte Count Normal/Reduced

Normal Bone Marrow

Anemia of chronic disorders (neoplastic, infections)

Anemia of renal failure

Endocrinopathy (myxedema, Addison disease, hypothyroidism, panhypopituitarism)

Anemia of liver disease

Anemia of early iron deficiency

Abnormal Bone Marrow

Anemia due to Marrow infiltration (leukemia, myelofibrosis, metastasis)

Hypoplastic anemia

Aplastic anemia

Medication side effect

Reticulocyte Count Increased

Hemolysis

1. Intrinsic- Inherited defects of hemoglobin, RBC membrane, or enzyme; paroxysmal nocturnal hemoglobinuria
2. Extrinsic- Autoimmune hemolytic anemia, microangiopathic hemolytic anemia, disseminated intravascular coagulation (DIC).

Hemorrhage

MICROCYTIC HYPOCHROMIC ANEMIA

As the name implies, microcytic hypochromic anemia is a kind of anemia characterized by smaller than average (microcytic) circulating red blood cells and have a diminished red color (hypochromic). Reduced iron reserves in the body, which can result from various underlying factors, are the most frequent cause of this kind of anemia. This could be the result of low iron levels in the diet, inadequate iron absorption from the intestines, both acute and chronic blood loss, and an increased need for iron during pregnancy and the healing process following major surgery or trauma.[68]

Defective hemoglobin synthesis is linked to microcytic, hypochromic anemias. Thalassemia, iron deficiency, anemia of chronic disease, lead poisoning, and congenital sideroblastic anemia are the five primary causes of microcytic anemia. To distinguish between the various causes of these anemias, serum iron tests and occasionally hemoglobin electrophoresis are typically sufficient.[64]

MACROCYTIC ANEMIA

When anemia is present together with macrocytosis (MCV larger than 100fL), the condition is known as macrocytic anemia.

The most common causes of macrocytosis are deficiency in folic acid and vitamin B12, because of decreased intake (malnourishment or alcohol misuse), increased consumption (hemolysis or pregnancy), or malabsorption (gastric bypass), which create abnormally large RBC precursors in the bone marrow. However, medicines that disrupt DNA synthesis, hereditary disorders, hypothyroidism and chronic liver diseases can also cause macrocytosis.[64,69]

DIMORPHIC ANEMIA

Dimorphic anemia refers to a condition characterized by the presence of RBCs of two distinct population and different sizes. An elevated RDW in the presence of a normal MCV and a dimorphic blood picture with two RBC populations may be indicative of this disease. The pathophysiology of dimorphic anemia is complicated and involves multiple deficient states. The underlying cause of dimorphic anemia is typically a combination of iron and vitamin B12 or folic acid insufficiency.[70,71]

Several other conditions can also result in the appearance of a dimorphic blood film. It can happen when a patient receiving treatment for megaloblastic anemia develops iron deficiency, when an iron deficiency anemia responds to iron therapy, or when normal blood transfusion is administered to a patient with hypochromic anemia.[72]

Haemoglobin could be measured automatically in the 1920s, and Maxwell Wintrobe devised the Wintrobe hematocrit method in 1929. This approach finally allowed him to define the red blood cell indices.[64]

Wallace H. Coulter created the Coulter principle in 1953, which became a historical milestone in the automation of blood cell counts and were based on Coulter's electrical impedance theory.[63,64]

In order to count blood cells, WH Coulter employed a patent-protected method for measuring metal ions in suspension.

Several concepts related to differential analysis and cell counting have been applied in the past. Impedance and optical light scattering are the two main concepts of blood cell counts that are now employed by haematology equipment.[73-75]

The increased resistance that results from a blood cell with low conductivity passing through an electrical field is the foundation of the impedance concept of blood cell counts. The quantity of pulses signifies the number of blood cells present, and the height, or amplitude, of each pulse, is directly proportional to the cell volume.[73,76] Beckman Coulter, Inc., Sysmex Corporation, and Abbott Diagnostics are some of the examples of instruments that utilise this approach.[73]

The light-scattering measurements made when a single blood cell travels through an optical or laser light beam form the basis of the optical light-scattering principle of blood cell counting. Using photodetectors, blood cells produce side and forward scatter. Cell size can be determined by measuring the degree of forward scatter, and cell complexity or granularity can be determined by measuring the degree of side scatter.[73,77] This approach is used for all blood cell counts in Siemens Healthcare devices. It is also used for leukocyte enumeration and differentials in newer versions made by Beckman Coulter, Inc., Sysmex Corporation, and Abbott Diagnostics.[73]

Analysers with the capacity to do accurate WBC differential counts are essential, particularly those that are able to conduct a five-part differential (counting neutrophils, lymphocytes, monocytes, and eosinophils). Most automated haematology analysers do leukocyte differentials, haemoglobin concentration, and red cell size in addition to counting cells.[78-80]

To count blood cells, two techniques with similar structural characteristics are used: the direct current impedance approach and the capacitance method. Both techniques magnify and show on an oscillograph the change in capacitance or electric impedance of particles in suspension going through an aperture; each particle then produces a signal, or pulse, which is counted after it has been generated.[81,82]

The electrical impedance differential of a blood cell is directly proportional to its volume as it flows through the aperture, so the volume of the cell may be calculated by multiplying the impedance differential by a fixed value. The behaviours of various blood cells vary. The normal RBC is very pliable, assuming a fusiform morphology and exhibiting prodding. In order to prevent changes in cell sizes, effectively mixed blood is significantly diluted or suspended in an isotonic electrolyte solution. In contrast to blood cells, which are non-conductive, this isotonic electrolyte solution exhibits excellent electrical conductivity. Haematology counters are based on this procedure.

A high pulse that indicates a very large cell is produced by the coincidence error, which happens when two or more cells reach the aperture at the same time and can be recorded and counted as one cell (as one high pulse). However, the vast dilution, the reduction in aperture dimensions, and the statistical correction for this error made by the computer considerably diminish this error.[81,82]

An aperture, which is a tiny opening with predefined dimensions, two electrodes with direct current running through them, a beaker, and suspended cells or particles are the components that make up a counting chamber. The suspended cells or particles will displace their own volume of the isotonic electrolyte solution as they pass through the aperture, increasing the electrical resistance (impedance) since they are non-conductive between the two electrodes that are positioned on either side of the aperture.[81,82]

Instead of identifying the cells that are referred to as particles in this context, the impedance haematology counters, which operate on the impedance principle, count and size them.[83]

The RBC aperture is smaller than the WBC aperture due to the bigger size of WBCs compared to RBCs and platelets.

Along with the RBC, platelet, and WBC counts, the counter provides us three-part differential that comprises haemoglobin, neutrophil, and lymphocyte count in addition to a comprehensive haematology menu.

Once the previously described data is received, the counter computer uses it to do calculations that yield valuable data.[83]

Automated blood counters have developed in the contemporary world, and numerous additional state-of-the-art devices are in use.

Examples

- Mindray: It makes use of fluorescent flow cytometry and low frequency direct current in conjunction with the notion of impedance.
- Sysmex SE: This device employs the idea of impedance in fluorescence flow cytometry as well as low frequency direct current and radiofrequency current applications.
- The Siemens Advia series makes use of the principles of absorbance and light scattering after the peroxidase reaction.

A basic, highly informative and routinely conducted test in the hematopathology laboratory is the inspection of a peripheral blood smear (PBS) that has been well-stained. Comprehensive analysis of a peripheral blood smear can be utilized for three purposes: (1) determining the final diagnosis of specific hematologic and nonhematologic disorders; (2)

monitoring the patient's response to therapy; and (3) serving as a screening tool to detect illness.[17,84,85]

PBFs have significant diagnostic utility. Since the peripheral blood film (PBF) reveals the morphology of peripheral blood cells, it can be used to diagnose a variety of primary, secondary, and blood-related disorders based on their morphology.[86]

Preparation of peripheral smear

Blood smears are frequently made from anticoagulated blood samples that are left over from automated hematologic analysis. Anticoagulants, however, may cause artefacts in the appearance and staining of cells. To prepare blood films, glass slides are used.

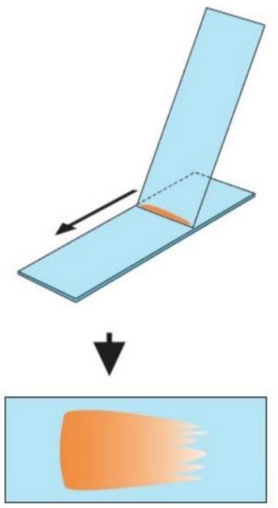


Figure 10: Peripheral smear preparation.[87]

Blood smears are prepared on clean glass slides by the wedge method. One end of the slide should have a drop of blood about one to two centimeters from the center in order to make a slide blood smear. Positioned at an angle ranging from 30° to 45°, a second spreader slide is advanced rearward to establish contact with the blood drop. After the drop of blood has spread down the edge of the slide, the spreader slide is quickly advanced. Utilizing this method yields a 3–4 cm long blood film.[17,87]

Uneven blood distribution and mechanical dragging of the cells across the slide's glass or coverslip can also cause cell distortion; although, these artefacts can be reduced with appropriate technique.

Features of ideal Peripheral Smear:

1. 1.The dimensions and thickness of the slides should be 75*25 mm.
2. For easier labelling, they should ideally have frosted ends.
3. The film should end at least one centimeter (cm) before the slide's end and be roughly 3 cm long.
4. The optimal thickness is one that, when viewed under a microscope, permits some red cell overlap along the majority of the film's length.

Parts of Peripheral Smear:

Head

Body

Tail

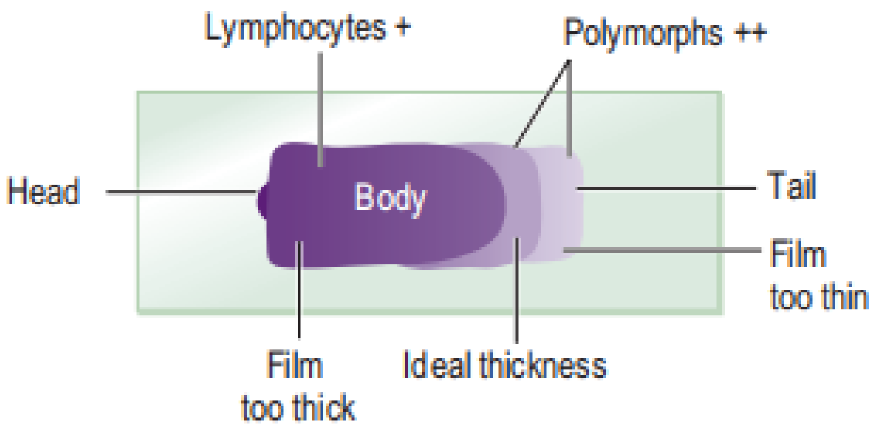


Figure 11: Examination of peripheral smear.[88]

Routine staining

Wright or Leishman stains are typically used to color blood smears. Both stains are adaptations of the Romanowsky method. The different Romanowsky stains include:

- May-Grunwald stain
- Jenner stain
- Giemsa stain
- Azure B-EosinY stock solution
- Leishman stain
- Wright stain

Examination of peripheral smear

Blood films should be inspected methodically, moving from low-power to high-power microscopic analysis after beginning with macroscopic inspection of the stained film. A macroscopical examination of the film is necessary to evaluate the quality of the spreading technique and the staining features.[89]

To check for adequate staining and cellular distribution, the blood smear should first be viewed with an intermediate power (10 to 20 objective). It is crucial to thoroughly examine the blood smear to make sure that any aberrant populations—which could be concentrated around the smear's edges—are not overlooked. To inspect and characterize every type of cell in the blood smear, a methodical assessment is necessary. It is important to assess any anomalies that are both quantitative and qualitative for each type of cell.[87,90,91]

Quantitatively assessing red cell abnormalities on a blood smear is challenging; still, the RBCs should be assessed for differences in size, shape, distribution and amount of hemoglobin, and the existence of cellular inclusions. Normally, the blood film's red cell distribution is not uniform. The region of the smear where the red cells are near to one another but do not overlap exhibits optimal red cell morphology. Artefacts are more prevalent in areas where red blood cells are dispersed either too thinly or too thickly. Rouleaux, or seemingly stacking red blood cells, are the result of red blood cells adhering to one another in some blood smears. Red blood cells ought to have a consistent size and shape, with an average diameter ranging from 7.2 to 7.9 μm. This can be measured by comparing its diameter to that of a small lymphocyte nucleus, which is either slightly smaller or around the same size.

Typical red cells are round and have smooth contours. Due to the normal biconcavity of the red cells, the eosin component of Romanowsky dyes stains the red cells fairly deeply, especially around the edges of the cells. A pale center (central pallor) surrounded by a reddish-orange Hb ring characterizes a red cell.[87,89-91]

The term anisocytosis refers to variations in the size of the red cells. This can be caused by the existence of cells that are either larger than normal (macrocytosis) or smaller than usual (microcytosis); in many cases, both types of cells are present. Macrocytes are defined as well-hemoglobinized cells larger than 9 μm. Because of the greater cell thickness, macrocytes do not have a central pallor.

A microcyte is a cell that has a diameter less than 6 μm. [87,89-91]

The term poikilocytosis refers to variations in the morphology of red cells. Among the poikilocytes that may be seen in dyserythropoiesis are ovalocytes and elliptocytes; these cells are frequently seen in iron deficiency anemia (also known as "pencil cells") and megaloblastic anemia (also known as macro-ovalocytes).[87,89]

When red cells stain abnormally pale, it is referred to as hypochromia (before often called hy- pochromasia). This manifests as a very narrow ring of Hb or an enlarged area of central pallor, which is indicative of inadequate hemoglobinization.[87,89]

It is important to distinguish between a dimorphic image, which shows two separate populations, and anisochromasia, which exhibits aberrant diversity in red cell staining.

Anisochromasia, a condition where some red blood cells stain palely but not all of them do, is indicative of a situation when chronic disease-related anemia or iron deficient anemia develops or resolves. Several conditions can result in the appearance of a dimorphic blood film. It may happen when a patient receiving treatment for megaloblastic anemia develops iron deficit, when an iron deficiency anemia reacts to iron therapy, or following the transfusion of normal blood to a patient suffering from hypochromic anemia.[89,92]

A crucial component of automated analysis is the red blood cell histogram, which when combined with hematocrit, red blood cell distribution width, and red blood cell indices offers important hints for the diagnosis and treatment of red cell disorders.[9,93]

The foundation for creating a histogram is the well-known Coulter principle, which counts and sizes the cells.[9,94,95]

It is a graphic representation of cell frequencies vs size.[93,96]

The X axis shows the cell's size, and the Y axis shows the total number of cells.

Major hints for the diagnosis and treatment of serious red cell diseases can be found in the histograms. All automated cell counters now regularly display the RBC histogram, which is a crucial component of automated haematology analysis.[97]

Numerous automatic haematology analysers have been created, the majority of which measure MCV and RBC count directly.[93,98]

In addition to the histogram, complete blood count metrics such as MCV and RDW are helpful in interpreting aberrant red blood cell morphology.[93,99]

Unless it is compared to a reference normal curve and verified microscopically, the general histogram pattern in the red cell distribution curve has no significance on its own.

Within the usual range of 80-100fL for MCV, lies the normal curve.[100]

The typical red cell histogram has a Gaussian, or "bell-shaped," curve, is symmetric, and has only one peak. The distribution, with majority of each cell falls between 55fL and

125fL, should always begin and terminate on the base line and be situated between the two discriminators.[94,101]

A homogeneous cell population is shown by a small red cell distribution curve, whereas a heterogeneous red cell population is indicated by a larger red cell distribution curve.[93]

The RDW is calculated from the width of the histogram at 1 SD from the mean divided by MCV. 11.5% to 14.5% is the normal range for RDW-CV.

The distribution curve's arithmetic width, as measured at the 20% frequency curve, is known as the RDW-SD. Normal range for RDW-SD is 39-47fL.[9,95]

Raised RDW has a somewhat broad base histogram.[94,102]

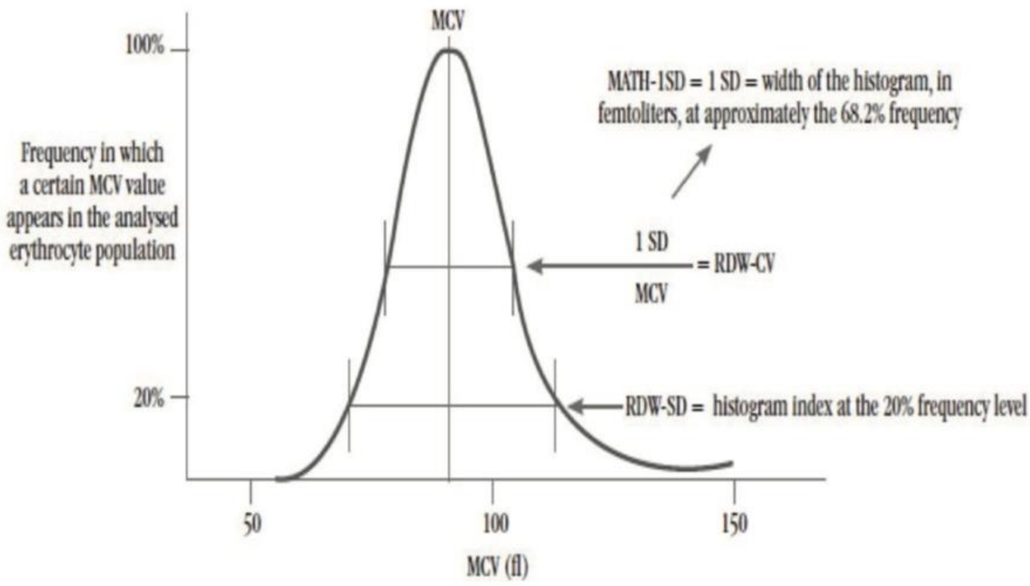


Figure 12: Typical Gaussian curve of histogram.[94]

A shift in either direction can be crucial for diagnosis, and it has been found to be aberrant in a number of haematological diseases when paired with other CBC characteristics such as RDW and RBC indices (MCV, MCH, and MCHC).[100]

The size of the RBC determines how the histogram shifts:

if the cell is larger than normal, >100fL (macrocytic RBC), the shift is towards the right, and if it is smaller than normal, <100fL (microcytic RBC), the shift is towards the left.[93,103] When a population of red blood cells exhibits two distinct morphologies, the corresponding peaks on the graph will be identified as dimorphic red blood cells.[104]

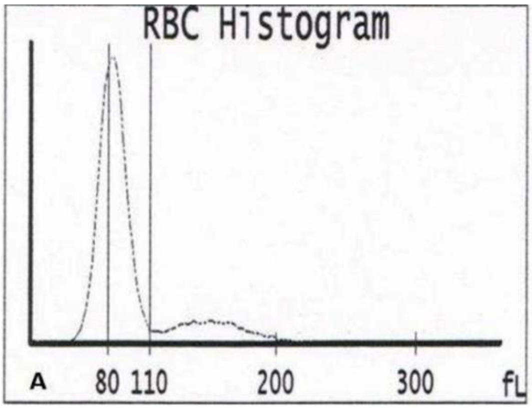


Figure 13: Normal histogram[8]

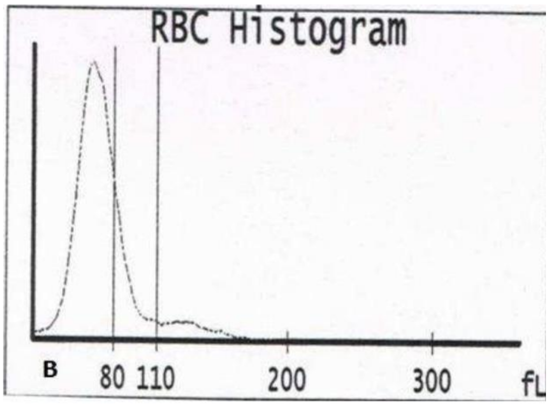


Figure 14: Left shift of histogram[8]

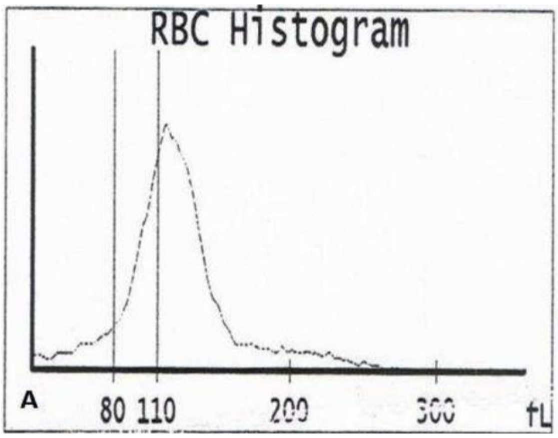


Figure 15: Right shift of histogram[8]

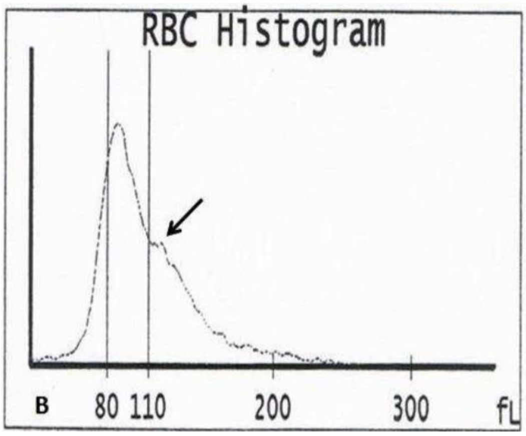


Figure 16: Double peak of histogram[8]

Since decades, peripheral blood smear examination has been a crucial component of the investigation of a wide range of haematological disorders. It is also a vital diagnostic tool, particularly for the etiopathological work up of various haematological disorders.

Cell counters are becoming more and more common in medical laboratory services, and their cost has decreased while their effectiveness has increased globally. In recent years, microscopic analysis of peripheral smears and CBC by automated haematology analysers have complimented one another to offer a thorough report on the patient's blood sample.

Peripheral smears are necessary for the diagnosis of certain conditions, such as dimorphic anaemia and several hemoglobinopathies, which go undetected due to automation. Numerous studies have confirmed the usage of peripheral smears in cases of such discrepancies.

One such study by Singla S et al[105] underscores the necessity of examining the peripheral blood smear to evaluate all cell populations. This is important because a dimorphic blood picture can manifest as a combination of microcytic and normocytic, or normocytic and macrocytic red cells, or as a mix of small, normal, and large cells of different sizes and shapes, potentially with normal red blood cell indices. Sole reliance on automated values can lead to incorrect diagnoses. Therefore, it is crucial to correlate morphological findings with graphical and numerical data for a clearer understanding of the results, as dimorphic patterns are often associated with abnormal red cell populations. The study concludes that even with modern advancements in automation and molecular analysis, the peripheral smear test, along with the patient's medical history, remains an essential diagnostic tool for treating hematological disorders. Visual evaluation of peripheral smears is enhanced by red cell histograms and numerical parameters such as MCV, MCH, MCHC, and RDW.

Studies by Bhargava OP et al[106], Garg M et al[107], Phukan JP et al[108], Bhadran R et al[109], Rao BSS et al[104], also concluded that Histograms can provide diagnostic insights for microcytic hypochromic anemia and normocytic normochromic anemia. However, for dimorphic and hemolytic anemias, where RBC indices and histograms can vary, a peripheral smear analysis is essential to make an accurate diagnosis.

Study done by Goyal SC et al[5], concludes that even though automated analysers use advances in graphical representation to reduce overall workload, microscopy should be used to confirm this. Numerous elements, such as leukocyte inclusion, red cell agglutination, alterations in red cell morphology, inclusion of parasite infection in red blood cells, and inclusion of Hb H, might impact the histogram. These elements all affect the appearance of the histogram in some way, which causes the histogram to be inaccurate. The gold standard for diagnosing a variety of haematological disorders is still peripheral blood film microscopy.

The study by Choudhary S et al[110] concludes that anemia can be misdiagnosed due to differences between manual and automated blood scans for hemoglobin and red blood cell counts as Peripheral smear analysis, which can identify abnormalities like large platelets, platelet satellitism, platelet clusters, and various kinds of hemoglobinopathies, is superior to automated methods. While automated analysers are useful for screening, manual peripheral smear analysis is preferred for accurately diagnosing and differentiating various types of anemia and blood diseases.

MATERIALS AND METHODS

In the Pathology department of, KLE's Dr Prabhakar Kore Hospital and MRC, Belagavi, this study was conducted. The patient's identity has been kept confidential and approval from the University Research Ethical Committee was obtained before conducting the study.

Study design: Observational study

Study period: 1st January 2023 to 31st December 2023

1year prospective study

Source of data: With due consent, the current study included, four hundred seventy-eight adult patients with anaemia in the age range of 19 years to 60 years, as identified by decreased haemoglobin levels of less than the lower limit of the normal range for the specific age and sex, at the diagnostic laboratory of KLE's Dr Prabhakar Kore Hospital and MRC, Belagavi.

Study population: Adult patients aged 19-60 years who have a clinical diagnosis of anemia from January 1, 2023, to December 31, 2023, at KLE's Dr Prabhakar Kore Hospital and MRC.

Sample size: 478

Inclusion Criteria :

Adult females (19-60 years) with Hb<12gm/dl

Adult males (19-60 years) with Hb<13gm/dl

Exclusion Criteria :

Adult females with Hb 12 gm/dl or above

Adult males with Hb 13gm/dl or above

All pregnant females

Adults above 60 years

Data collection

In this study, patients presenting to KLE's Dr Prabhakar Kore Hospital and MRC, having hemoglobin levels below the lower limit of the normal range for their specific age and sex were the subjects.

The study was explained to the participants and informed consent was obtained from the prospective cases after the ethical clearance permission.

Method of collection

EDTA blood sample was run in the Sysmex XN-1500, 5-part differential autoanalyzer, analyzed by the machine and the data is generated which includes Hemoglobin, RBC count, RBC indices, Total Count, Differential Count, Platelet count, RDW-CV, RDW-SD etc. and all the 3 histograms. A peripheral blood smear was prepared from every sample with all the precautions and stained with Leishman stain using the standard procedure. Following that, histogram patterns and RBC indices were correlated with the impression obtained from the peripheral smear examination.

Statistical analysis: The data was analysed using Stata 17 software and The Pearson chi-squared test.

The p-value <0.05 was considered statistically significant.

RESULTS

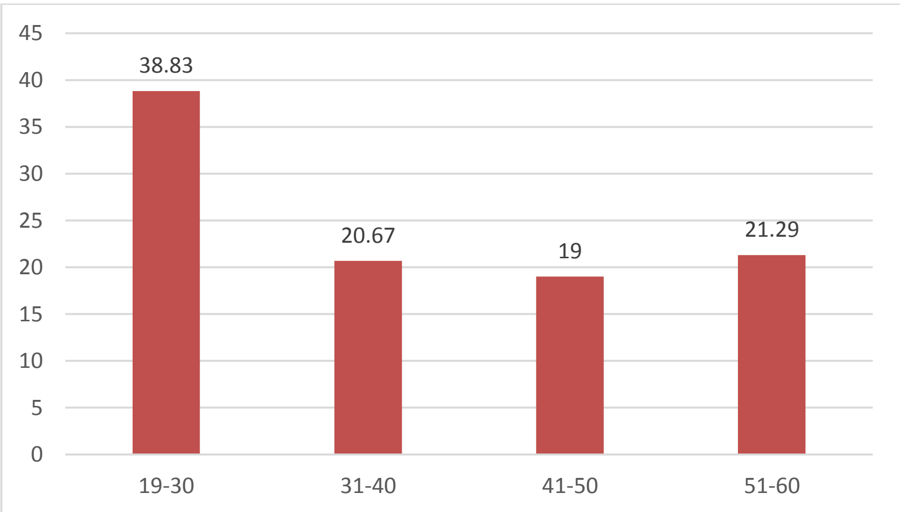
In this study, the peripheral smears were compared to RBC indices and histograms, generated by the automated haematology analyser, in 478 cases of anaemic adults from 19-60 years of age, gender irrespective, within the time period of 1st January'23 to 31st December'23.

The majority of cases fell within the age group of 19-30 years, encompassing 186 participants (38.83%) of the total study population, followed by 51-60 years,102 cases (21.29%), 31-40 years, 99 cases (20.67%) and 41-50 years, 91 cases (19.0%). Table 1 and graph 1.

Table 1: Age distribution of participants (n=478)

Age group(years)	No of cases	Percentage (%)
19-30	186	38.83
31-40	99	20.67
41-50	91	19.00
51-60	102	21.29
Total	**478**	**100.00**

Graph 1: Age distribution of participants

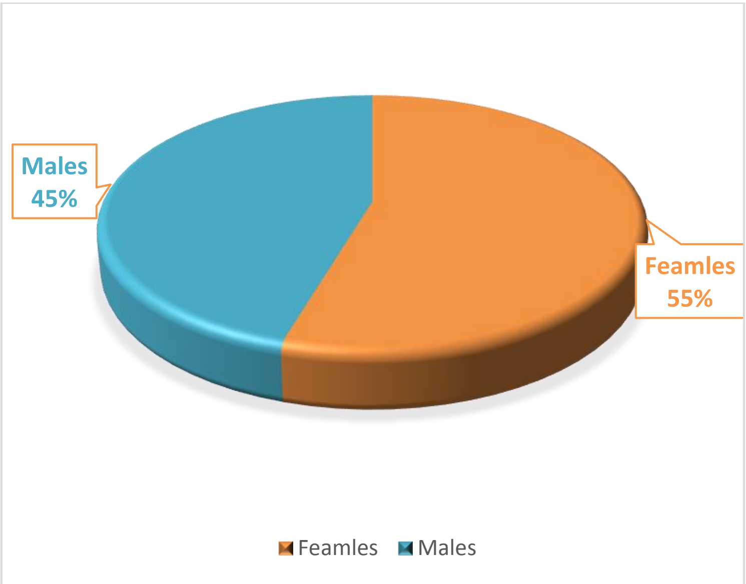


Females constituted the majority, with 261 participants representing 54.60% of the total population, while male participants numbered 217, accounting for 45.40%. Table 2 and graph 2.

Table 2: Gender distribution of Participants (n=478)

Gender	No of cases	Percentage (%)
Female	261	54.60
Male	217	45.40
Total	**478**	**100.00**

Graph 2: Gender distribution of participants

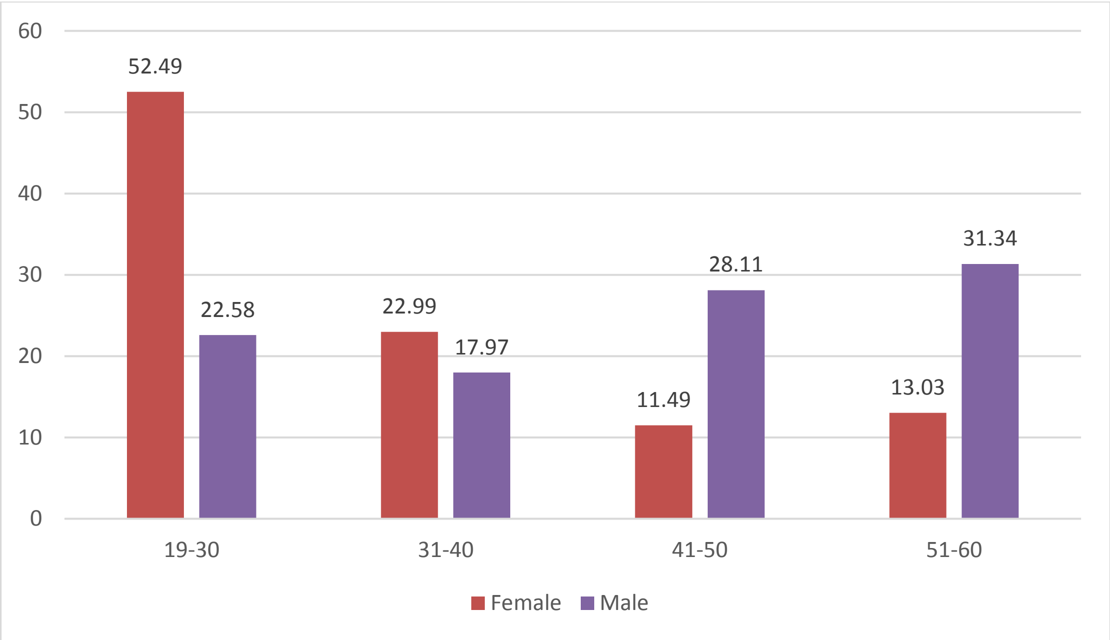


It was observed that females were predominant in the age group of 19-40 years, whereas males formed the majority in 41-60 years of age. Table 3 and graph 3.

Table 3: Age wise gender distribution of participants (n=478)

Age group (years)	Female	Male	Total
	No of cases (%)	No of cases (%)	No of cases (%)
19-30	137 (**52.49**)	49 (**22.58**)	186 (**38.91**)
31-40	60 (**22.99**)	39 (**17.97**)	99 (**20.71**)
41-50	30 (**11.49**)	61 (**28.11**)	91 (**19.04**)
51-60	34 (**13.03**)	68 (**31.34**)	102 (**21.34**)
Total	**261**	**217**	**478**

Graph 3: Age wise gender distribution of participants

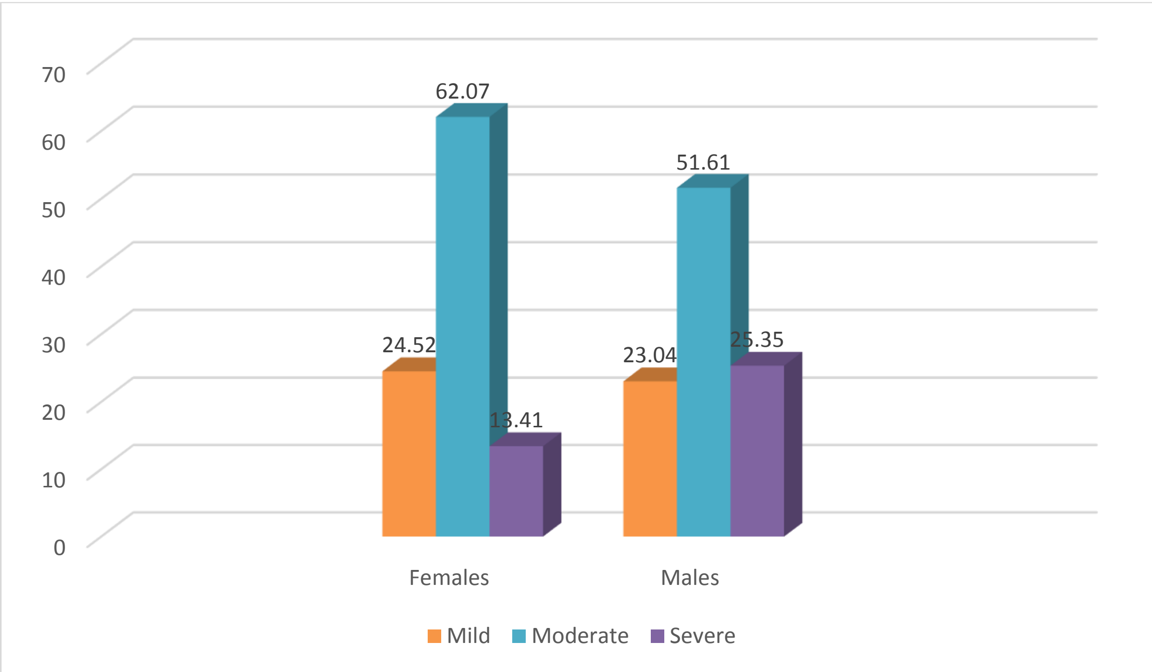


Majority of the females and males were moderately anemic, constituting 62.07% (162 cases) and 51.61% (112 cases) respectively. 24.52% of females were mildly anemic and 13.41% severely anemic. In males 25.35% were found to have severe anemia while 23.04% had mild anemia. Table 4 and graph 4.

Table 4: Gender distribution according to Hb

Hemoglobin (gm%)	**Females (%)**	**Males (%)**	**Total (%)**
Mild	64 (24.52)	50 (23.04)	114 (23.85)
Moderate	162 (62.07)	112 (51.61)	274 (57.32)
Severe	35 (13.41)	55 (25.35)	90 (18.83)
Total	**261 (100%)**	**217 (100%)**	**478 (100%)**

Graph 4: Gender distribution according to Hb

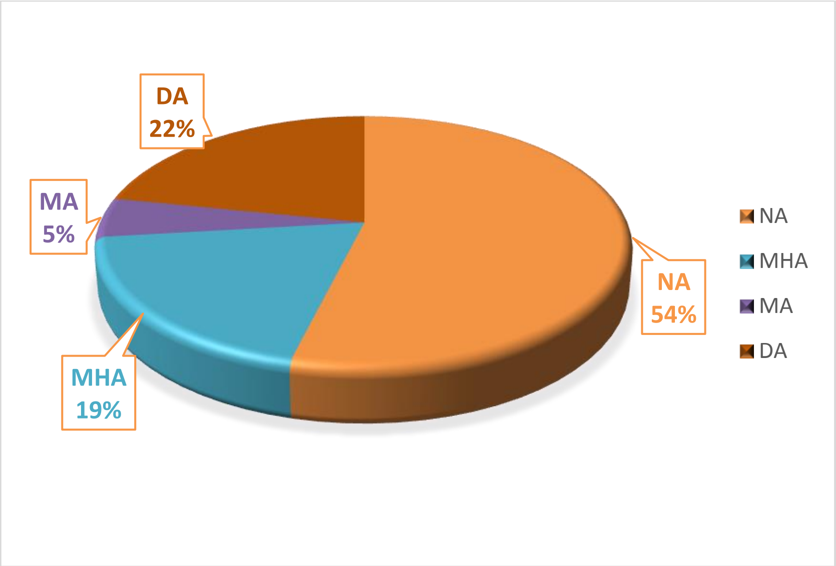


On peripheral smear, Normocytic Anemia was the most frequently observed diagnosis, with 260 cases or 54.39% of the study population, followed by Dimorphic Anemia with 103 participants, (21.55%), Microcytic Hypochromic Anemia, with 91 cases, (19.04%) and Macrocytic Anemia, with 24 subjects or 5.02%. Table 5 and graph 5.

Table 5: Various types of anemia on Peripheral Smear

Types of anemia on peripheral smear	No of cases	Percentage (%)
Normocytic anemia (NA)	260	54.39
Microcytic hypochromic anemia (MHA)	91	19.04
Macrocytic anemia (MA)	24	5.02
Dimorphic anemia (DA)	103	21.55
Total	**478**	**100.00**

Graph 5: Types of anemia reported on Peripheral Smear

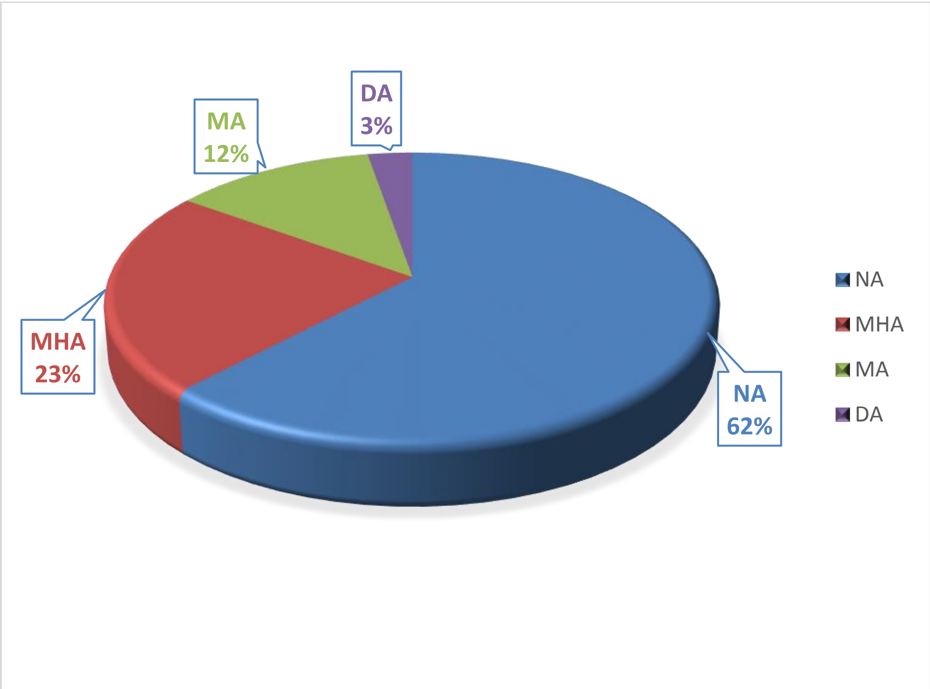


On Histogram and RBC Indices, it was observed that, Normocytic Anemia was the most prevalent type, diagnosed in 297 participants, accounting for 62.13% of the total population, followed by Microcytic Hypochromic Anemia with 109 cases (22.08%), Macrocytic Anemia in 59 cases (12.34%) and Dimorphic Anemia in 13 cases which accounts for 2.72%. Table 6 and graph 6.

Table 6: Anemia types based on RBC Indices and Histograms

Types of anemia	No of cases	Percentage (%)
NA	297	62.13
MHA	109	22.80
MA	59	12.34
DA	13	2.72
Total	**478**	**100.00**

Graph 6: Various anemias on RBC Histogram and Indices



When peripheral smears were compared with histogram patterns, it was observed that, out of 260 normocytic anemia reported on peripheral smear, 249 cases showed normal position, 1 case with left shift, 10 with right shift. All the cases had bell shape curve and 148 had broad base. In microcytic hypochromic anemia, amongst the 91 cases, 88 had left shift, 3 showed normal position and no right shift was observed. None of the cases showed bimodal curve. 79 cases had a broad base. All the 24 cases of macrocytic anemia had right shift with bell shape curve and broad base was seen in 14 cases. Out of 103 cases of dimorphic anemia, 57 were positioned normal, 21 had a left shift and 25 showed right shift of the curve. 79 cases were bell shaped, 24 bimodal and broad base of the curve was found in 101 cases.

Table 7, 8 and graph, 7, 8.

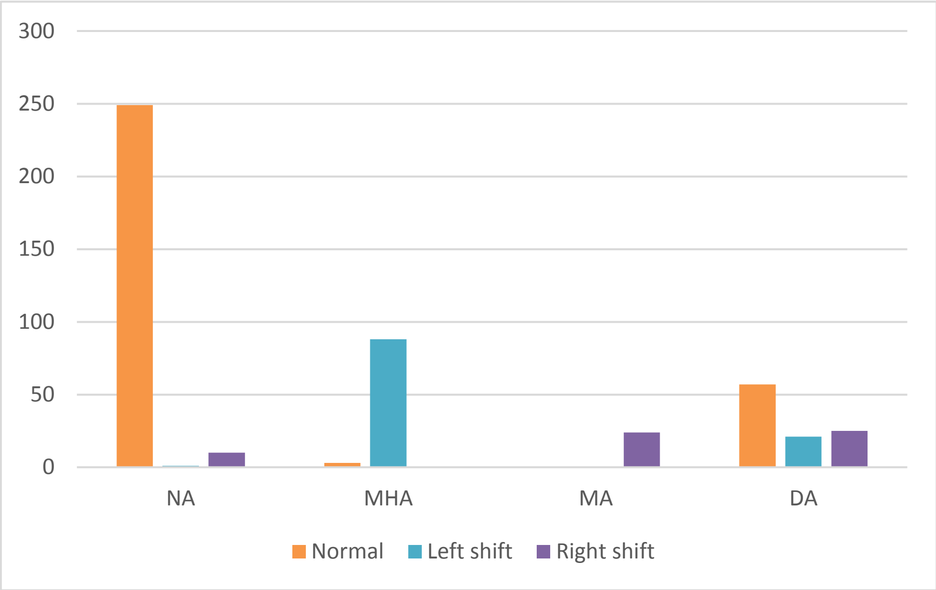
Table 7: Position of Histogram in different types of Anemia

Peripheral smear	Normal position	Left shift	Right shift	Total cases
NA	249	1	10	**260**
MHA	3	88	0	**91**
MA	0	0	24	**24**
DA	57	21	25	**103**

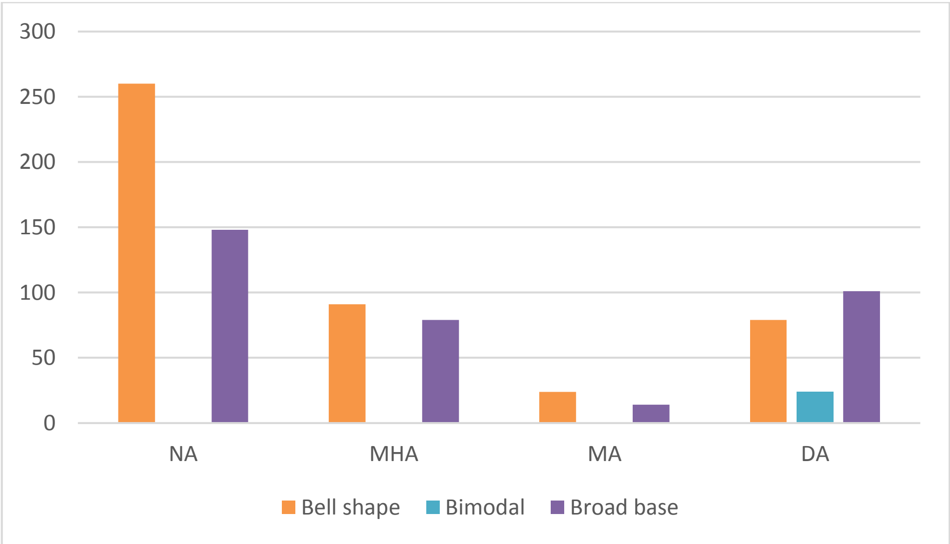
Table 8: Characteristics of Histogram curve in different types of Anemia

Peripheral smear	Bell shape	Bimodal curve	Broad base	Total cases
NA	260	0	148	**260**
MHA	91	0	79	**91**
MA	24	0	14	**24**
DA	79	24	101	**103**

Graph 7: Histogram position in different anemia



Graph 8: Histogram curve in different anemia

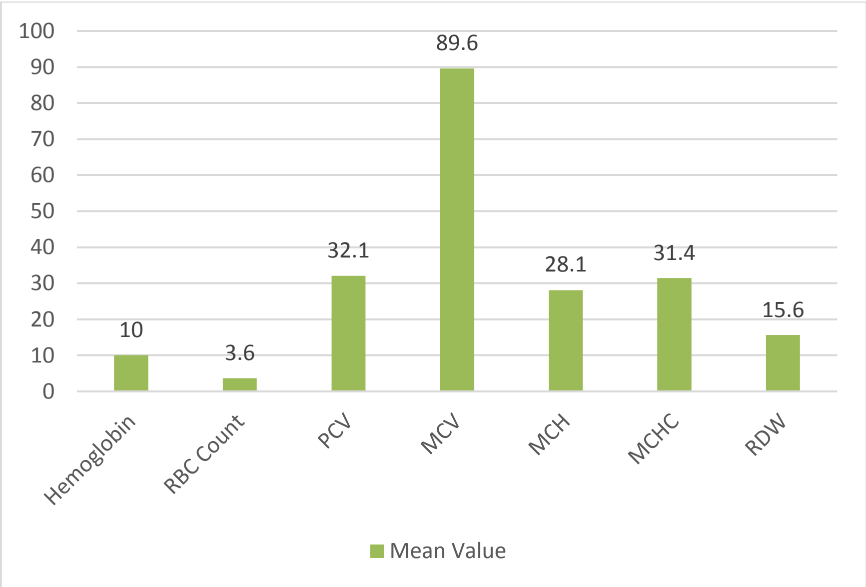


It was observed that, the RBC indices were within normal limits in cases of normocytic anemia with mean MCV, MCH, MCHC, RDW being 89.6, 28.1, 31.4, 15.6 respectively and the mean Hb with 10. Table 9 and graph 9.

Table 9: Mean RBC parameters in Normocytic Anemia

Parameters	**NA**	
	Mean	SD
Hemoglobin	10	1.59
RBC Count	3.6	0.62
PCV	32.1	5.08
MCV	89.6	5.98
MCH	28.1	2.79
MCHC	31.4	3.54
RDW	15.6	2.19

Graph 9: Mean RBC parameters in Normocytic Anemia



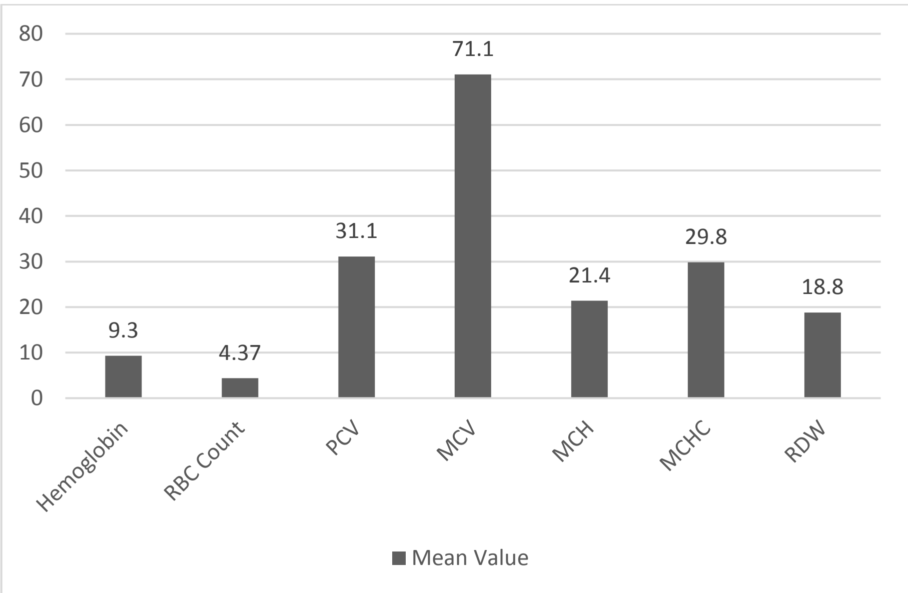
In microcytic hypochromic anemia, the mean Hb was 9.3 and the mean MCV and MCH were decreased with values of 71.1 and 21.4, respectively, mean MCHC was 29.8 and RDW, 18.8.

Table 10 and graph 10.

Table 10: Mean RBC parameters in Microcytic Hypochromic Anemia

Parameters	MHA	
	Mean	SD
Hemoglobin	9.3	1.97
RBC Count	4.37	0.72
PCV	31.1	5.64
MCV	71.1	6.97
MCH	21.4	3.43
MCHC	29.8	2.41
RDW	18.8	4.08

Graph 10: Mean RBC parameters in Microcytic Hypochromic Anemia

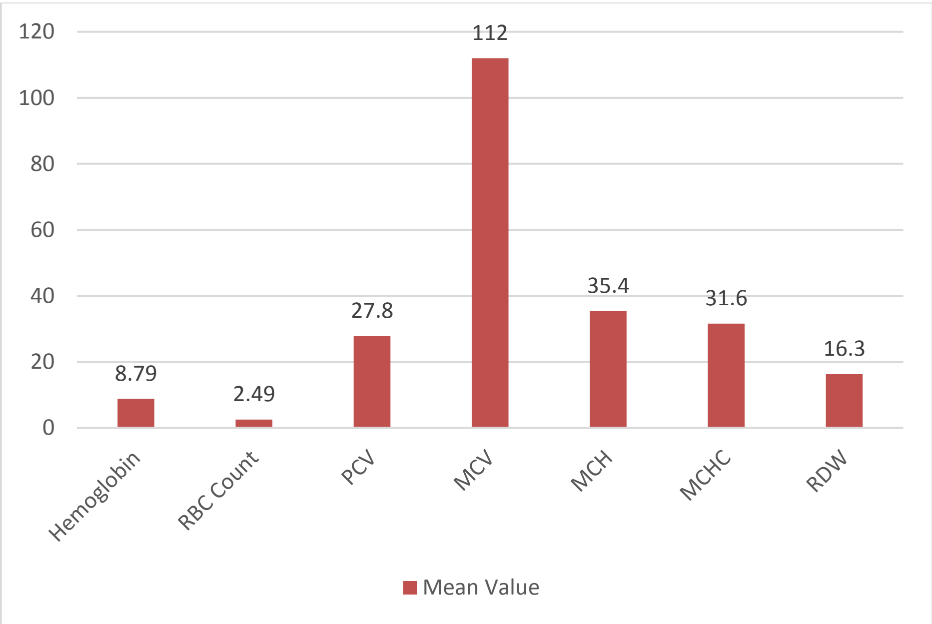


The mean Hb was 8.7, with 112, 35.4, 31.6 and 16.3 being mean MCV, MCH, MCHC and RDW respectively, in cases of macrocytic anemia. Table 11 and graph 11.

Table 11: Mean RBC parameters in Macrocytic Anemia

Parameters	**MA**	
	Mean	SD
Hemoglobin	8.79	2.26
RBC Count	2.49	0.60
PCV	27.8	6.52
MCV	112	6.32
MCH	35.4	3.14
MCHC	31.6	1.88
RDW	16.3	2.83

Graph 11: Mean RBC parameters in Macrocytic Anemia

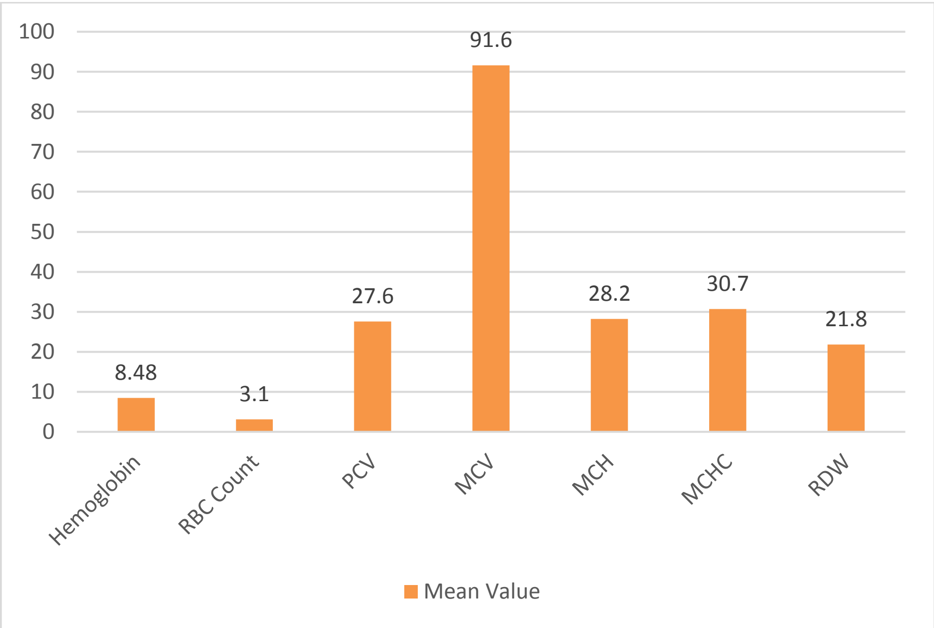


In dimorphic anemia, the mean values of MCV, MCH, MCHC and RDW were 91.6, 28.2, 30.7, 21.8 respectively, with mean Hb of 8.48. Table 12 and graph 12.

Table 12: Mean RBC parameters in Dimorphic Anemia

Variable	DA	
	Mean	SD
Hemoglobin	8.48	1.89
RBC Count	3.1	0.87
PCV	27.6	6
MCV	91.6	13.4
MCH	28.2	4.9
MCHC	30.7	2.15
RDW	21.8	3.98

Graph 12: Mean RBC parameters in Dimorphic Anemia

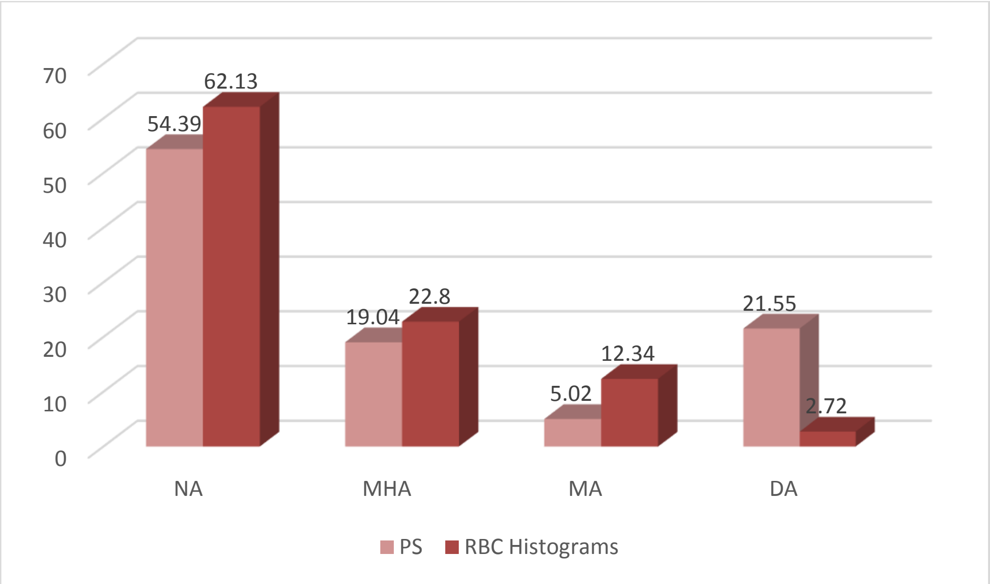


When peripheral smear analysis was compared with RBC histograms and indices, a significant difference was observed between the two. The Pearson chi-squared test revealed a statistically significant p-value with $p<0.001$ and the observations can be seen in the below Table 13 and graph 13.

Table 13: Comparison of Peripheral Smear analysis with Histogram and RBC Indices

Type of anemia	Peripheral smear findings		RBC Indices and Histograms	
	No of cases	%	No of cases	%
NA	260	54.39	297	62.13
MHA	91	19.04	109	22.80
MA	24	5.02	59	12.34
DA	103	21.55	13	2.72
Total	**478**	**100**	**478**	**100**
p<0.001				

Graph 13: Comparison of Peripheral Smear analysis with Histogram and RBC Indices



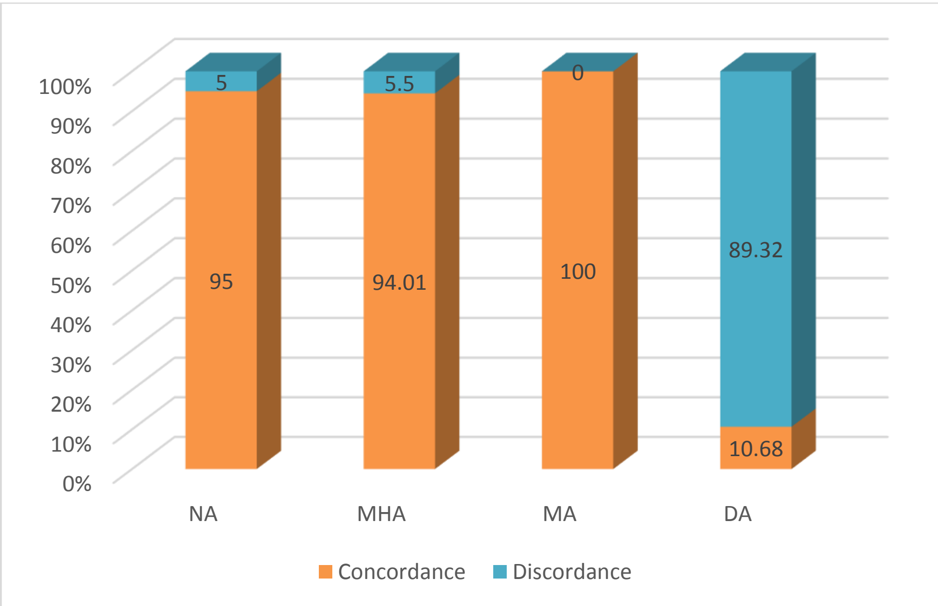
We found that dimorphic anemia showed the maximum discordance (89.32%) between peripheral smear analysis and RBC indices and histograms, followed by microcytic hypochromic anemia (5.50%), normocytic anemia (5.0%).

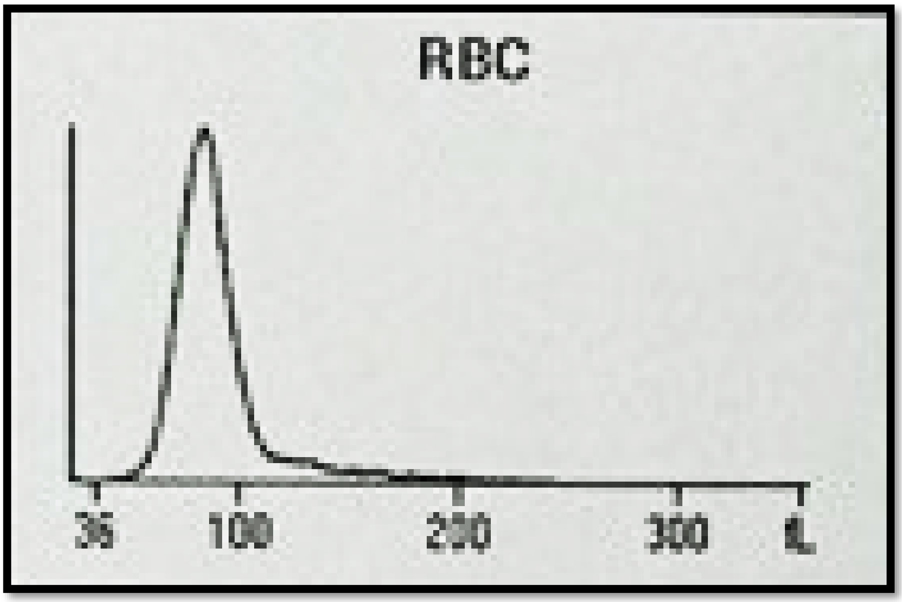
All the cases of macrocytic anemia were consistent when compared between peripheral smear and RBC indices and histograms. Table 14 and graph 14.

Table 14: Concordance and discordance between Peripheral Smear analysis with Histogram and RBC Indices

	NA	MHA	MA	DA
Concordance	95%	94.01%	100.0%	10.68%
Discordance	5.0%	5.50%	0.0	89.32%

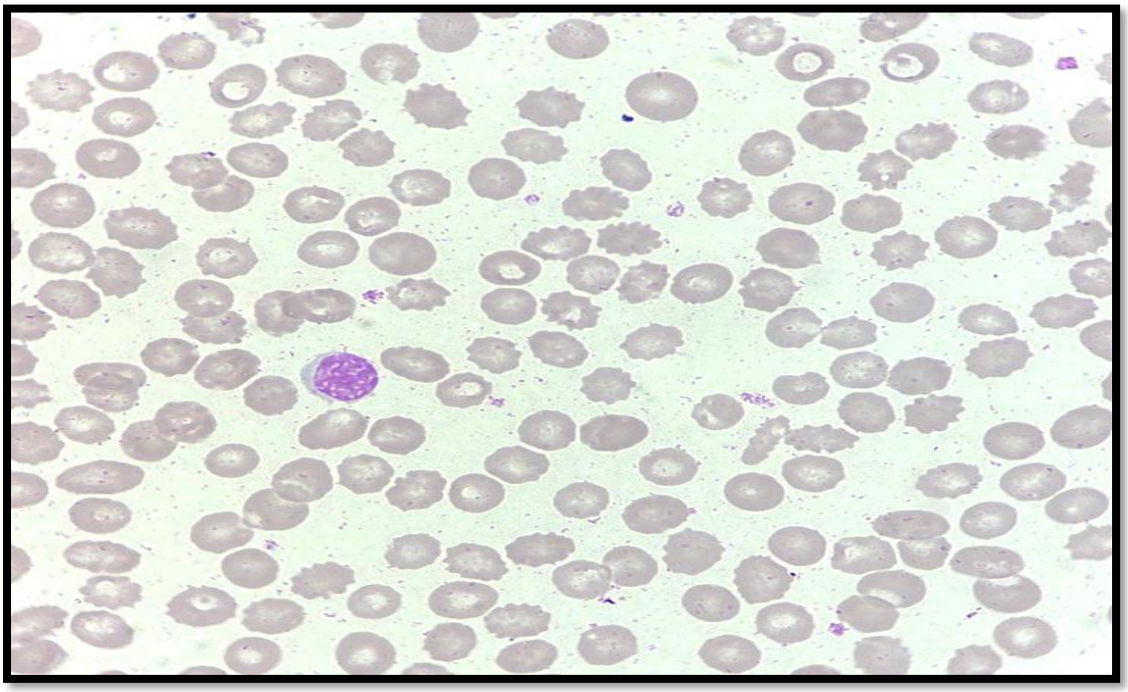
Graph 14: Concordance and discordance between Peripheral Smear analysis with Histogram and RBC Indices



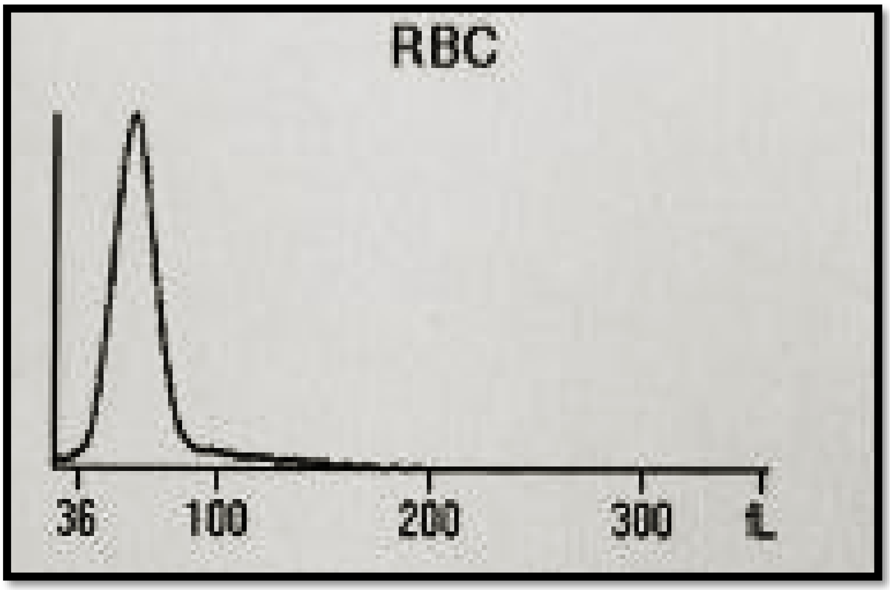


Hb- 11.2gm/dL
MCV- 83.6fL
MCH- 27.1pg
MCHC- 32.4gm/dL
RDW- 14.0%

Picture 1: Normocytic anemia with Normal RBC Indices and Histogram curve

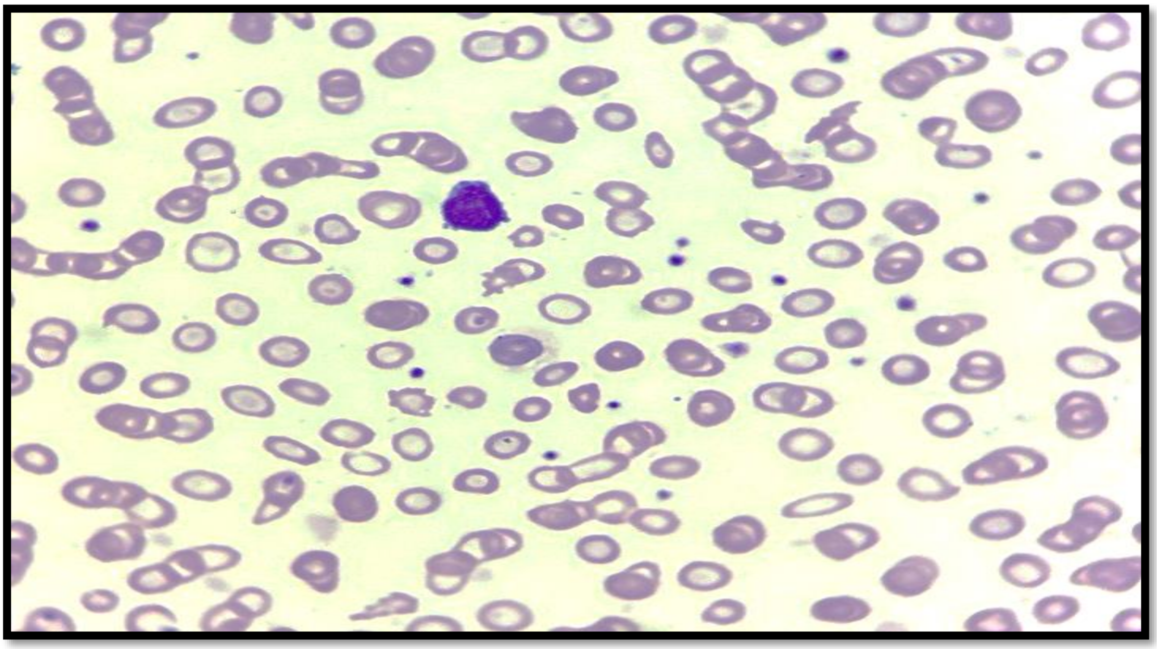


Picture 2: Peripheral smear of Normocytic anemia showing predominantly normocytic normochromic and RBCs.

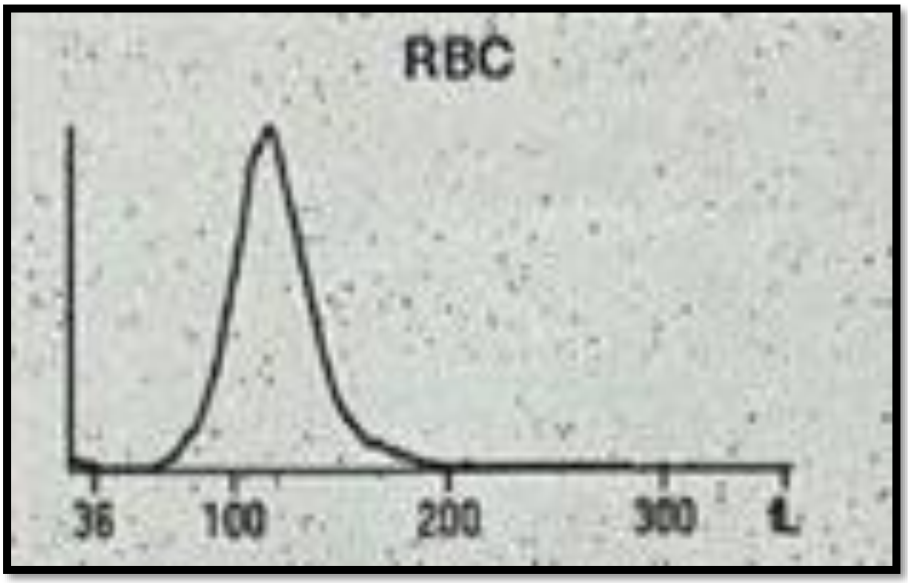


Hb– 9.0gm/dL
MCV- 60.1fL
MCH- 18.6pg
MCHC-31.0gm/dL
RDW-16.6%

Picture 3: Microcytic Hypochromic anemia with decreased RBC Indices and Left shift of the Histogram curve

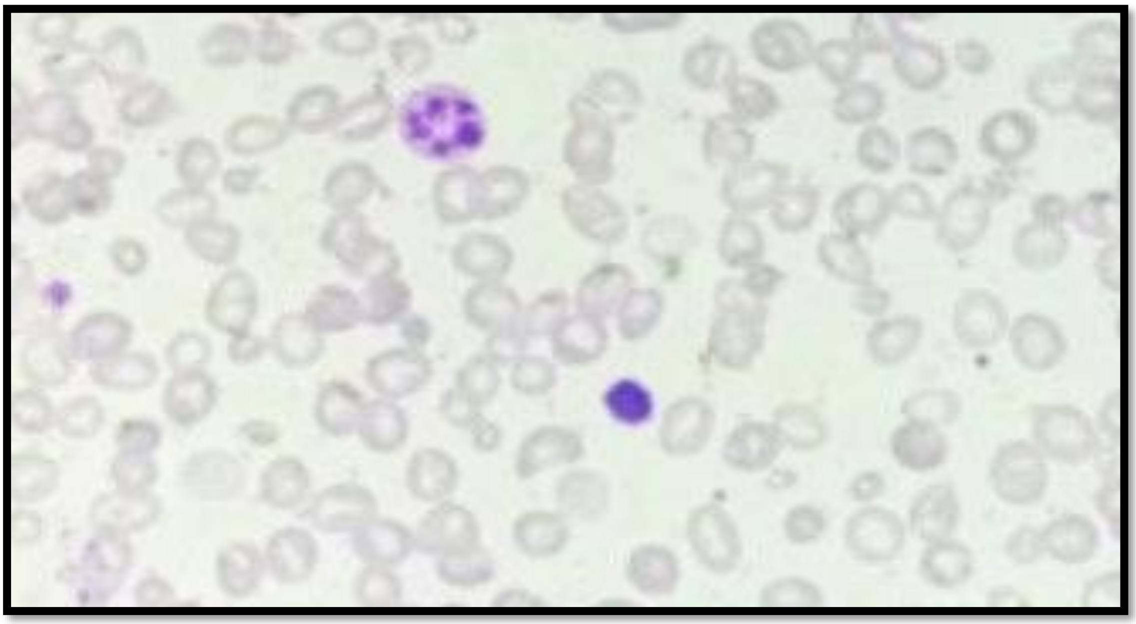


Picture 4: Peripheral smear of Microcytic Hypochromic anemia showing predominantly microcytic hypochromic RBCs with few pencil cells

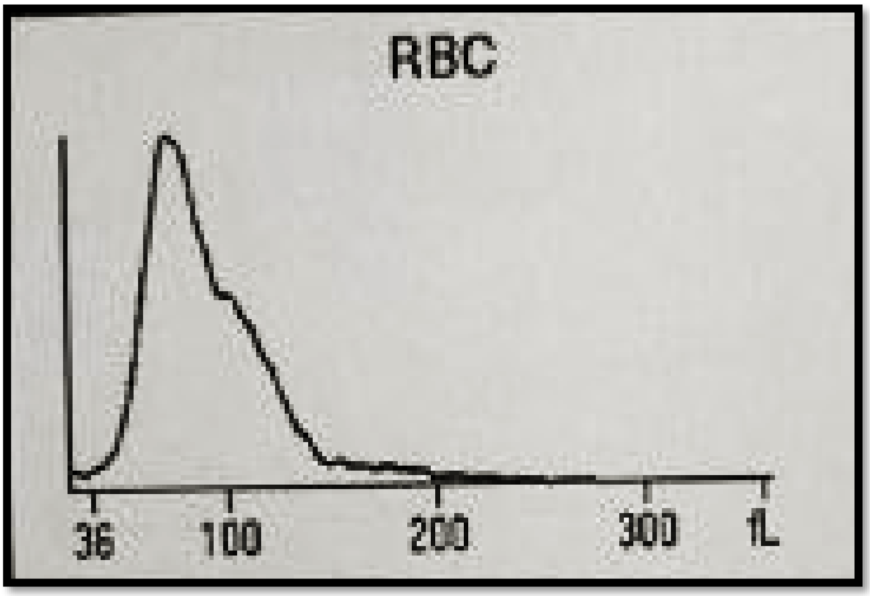


Hb- 7.3gm/dL
MCV- 114.9fL
MCH-35.3pg
MCHC-30.7gm/dL
RDW- 17.2%

Picture 5: Macrocytic Anemia with increased MCV and Right shift of the Histogram curve

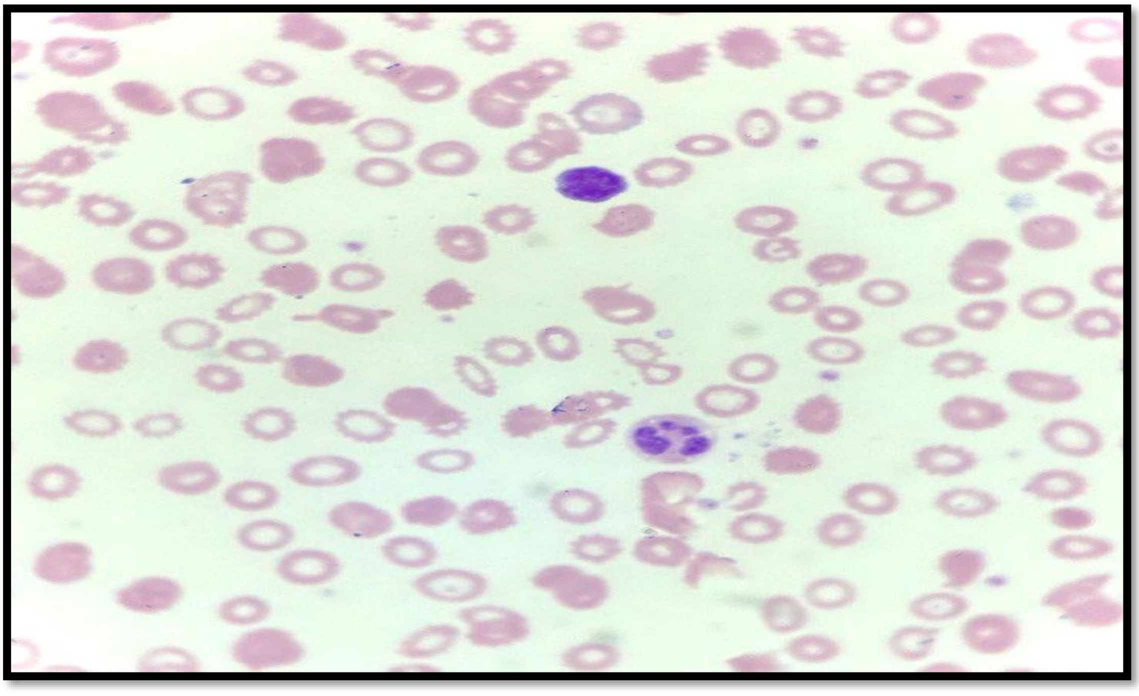


Picture 6: Peripheral smear of Macrocytic Anemia showing macrocytic RBC predominantly and Hypersegmented Neutrophil



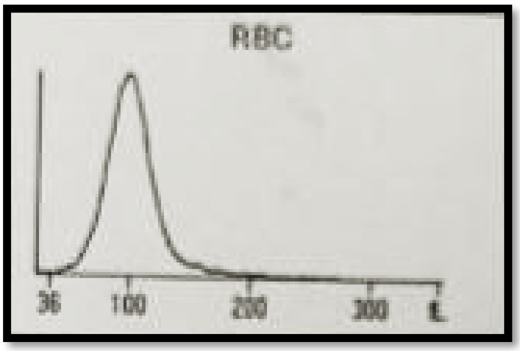
Hb- 11.8gm/dL
MCV- 84.9fL
MCH- 28.3pg
MCHC- 33.3gm/dL
RDW- 28.6%

Picture 7: Dimorphic anemia with normal MCV and increased RDW showing Bimodal peak of the Histogram with a broad base.

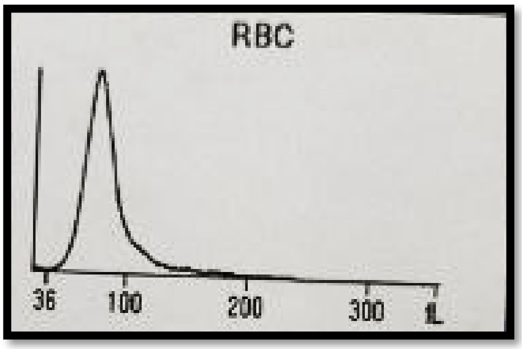


Picture 8: Peripheral smear of Dimorphic anemia with anisopoikilocytosis showing Microcytic and Macrocytic RBCs, pencil cells and Hypersegmented neutrophil

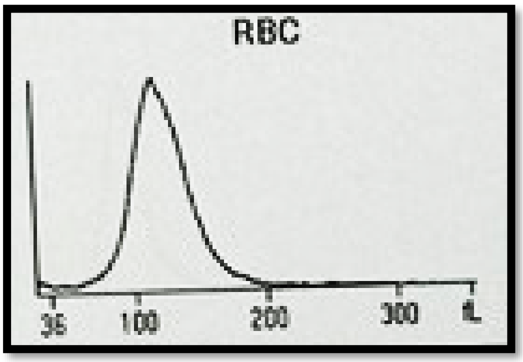
RBC Histograms: Visualizing Variations from Normal Patterns



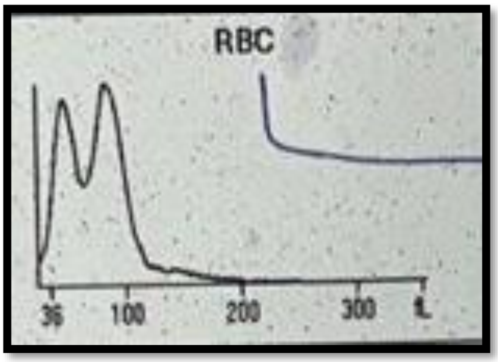
Picture 9: Normal position of the curve with broad base



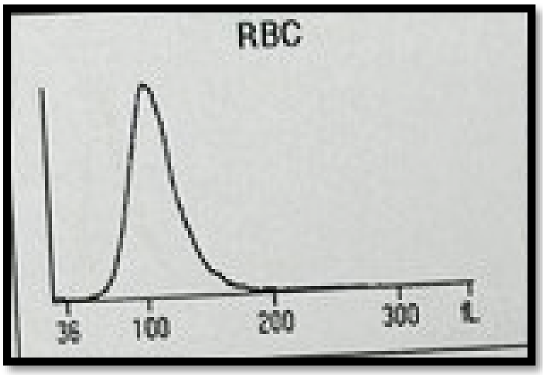
Picture 10: Left shift of the curve with broad base



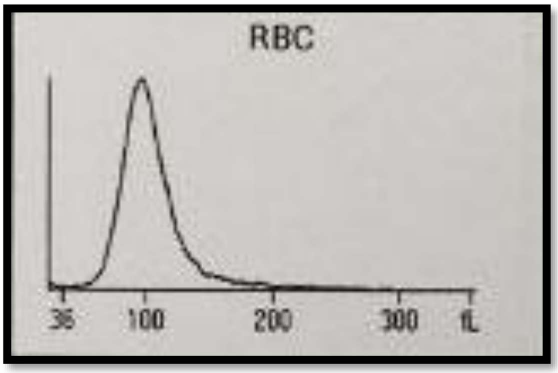
Picture 11: Right shift of the curve with broad base



Picture 12: Bimodal peak with broad base of the curve



Picture 13: The histogram shows right shift with broad base but on PS showed normocytic anemia



Picture 14: In this histogram, bell shape of the curve with broad base is seen, but showed dimorphic anemia on PS

DISCUSSION

Anemia represents a significant clinical and diagnostic category of hematological disorders found globally. Misclassification for the diagnosis of anaemia may arise from differences in peripheral blood scanning performed manually versus automatically in order to detect haemoglobin and red blood cell count.[111]

An essential tool for assessing haematological diseases is the PBS examination.[112]

The present study was done to evaluate the importance of peripheral smear over the advanced automated hematology analysers in today's era.

In this study, 478 anemic patients' peripheral blood smears were assessed and their results were correlated with red cell indices produced by an automatic cell counter. The anemic individuals in the study were predominantly within the age group of 19-30 years. This was in concordance with study done by Phukan JP et al[108], (21 to 30 years majority of cases), Preethi M et al[101], (18-25 years maximum cases). In the study done by Singla et al[105], majority cases involve adults between the ages of 21 and 49 and similar age range of 21-50 years was predominant in study by Aslam H et al[113] and Garg M et al[107].

Amongst the total 478 cases, 217 (45.4%) were males and 261 (54.6%) were females and among the females majority fall in the reproductive age group. Similar findings were reported in studies by Sinha R et al[100], Aslam H et al[113], and Singla S et al[105].

In our study it was observed that majority cases were moderately anemic (57.32%), followed by mild anemia (23.85%) and severe anemia (18.83%). Similar findings were observed in study done by Maqsood S et al[94]. When compared to studies done by Korgaonker KA et al[97] and Bhadran R et al[109], it was observed that majority had moderate anemia followed by severe anemia and mild anemia.

Table 15: Comparison of anemia grading with different studies

Anemia grading	Maqsood S et al[94]	Korgaonker KA et al[97]	Bhadran Ret al[109]	Present study
Mild	21%	12.4%	10.62%	23.85%
Moderate	60%	50.3%	64.58%	57.32%
Severe	19%	32.15%	24.8%	18.83%

Morphologically, the most prevalent type of anemia in our study was Normocytic Anemia, which accounts for 54.39% of the total, and is similar with study findings of Thomas RM et al[114], Preethi M et al[101], Varghese AM et al[111].

This was followed by DA with 103 cases, 21.55%, then MHA with 91 cases, 19.04% and Macrocytic anemia with 24 cases, 5.02%. Our findings are in contrast with Garg M et al[107], Sinha R et al[100] and Hussain S et al[115], in which Microcytic Hypochromic Anemia the most common followed by normocytic anemia. The difference between our findings as compared to other authors could be possibly due to our hospital being tertiary care centre with advanced specialty services, majority cases in our hospital are of anemia of chronic disease.

Table 16: Comparison of peripheral smear findings with other studies

PS examination	Thomas RM et al[114]	Hussain S et al[115]	Garg M et al[107]	Present study
Normocytic anemia	53.7%	15.4%	25.14%	54.39%
Microcytic Hypochromic anemia	28.9%	72.8%	50.86%	19.04%
Macrocytic anemia	4.8%	4.2%	2.29%	5.02%
Dimorphic anemia	6.2%	7.6%	15.43%	21.55%

In our study out of 478 RBC Histograms, Normal position was found in 64.64%, Left shift in 23.01%, Right shift in 12.34%, Bimodal Peak in 5.02% and 71.40% showed broad base. The results were compared with other studies and our findings were coinciding with the study by Preethi M et al[101], whereas, contradicting findings were seen in studies of Chavda et al[116] and Patel A et al[117].

Table 17: Comparison of histogram findings with different studies

Histogram	Preethi M et al[101]	Chavda J et al[116]	Patel A et al[117]	Present study
Normal	34.54%	19%	19.4%	64.64%
Left shift	30.90%	27%	30.60%	23.01%
Right shift	3.63%	7%	10.80%	12.34%
Bimodal peak	3.63%	3%	3.60%	5.02%
Broad base	23.63%	38%	35.60%	71.40%

The normal limits of mean MCV, MCH, and MCHC were demonstrated in normocytic anaemia patients, with slightly elevated RDW. Parallel results were seen in study of Thomas RM et al[114], Srivastava A et al[103].

In microcytic hypochromic anemia, the average values of MCV, MCH, and MCHC were observed to be reduced, with mean RDW slightly elevated. Our study shows similar findings with Thomas RM et al[114], Garg M et al[107].

Macrocytic anemia shows increased mean MCV and MCH with normal MCHC with slightly raised mean RDW. Corresponding outcomes were seen in study of Garg M et al[107].

Dimorphic anaemia was characterised by normal ranges for MCV, MCH, and MCHC, but elevated RDW as a result of high levels of poikilocytosis and anisocytosis in the PBS. Comparable findings were reported in studies of Garg M et al[107], Srivastava A et al[103].

Table 18: Comparison of concordant and discordant cases in different studies

Studies	Total no of cases	Concordant	Discordant
Farah E et al[118]	350	78.3%	21.7%
Preethi M et al[101]	55	76.36%	23.63%
Radadiya P et al[102]	100	72%	28%
Chaudhary S et al[110]	600	67%	33%
Present study	478	76.9%	23.1%

According to Pierre[119] and Novis et al[120], manual counting by peripheral blood film was less accurate than automated haematology analysers in identifying morphological abnormalities.

This contrasted with our findings. Since the MCV represented the average of the distribution curve and did not account for the presence of very small number of macrocytes, Florence Aslina et al[121] found that peripheral blood smears were more sensitive than RBC indices for detecting early microcytic alterations. The results are in agreement with the current investigation.

Similarly, findings of Chaudhary S et al[110], Lantis et al[122], Preethi M et al[101] supported our study.

Based on this research, we conclude that although automated analyzers decrease total effort through their advancements in graphical depiction, microscopy should be used to verify it.[102]

CONCLUSION

Our study revealed an important correlation between RBC parameters, histogram and peripheral smear diagnosis. Our study found a significant relationship between the RBC histogram and peripheral smear examination for diagnosing microcytic hypochromic, normocytic and macrocytic anemia. This suggests that RBC histograms can be a reliable tool in identifying these types of anemia. However, when it came to diagnosing dimorphic anemia, the correlation between the histogram patterns, red cell indices, and peripheral smear examination raised questions about the reliability and validity of the RBC histogram. This indicates that while RBC histograms are useful for certain anemia types, they may not be as dependable for diagnosing conditions like dimorphic anemia. Automated hematology analysers are dependable tools for diagnosing anemia and are effective for initial screening. However, for precise diagnosis of anemia and differentiating amongst its various types, a manual examination using a peripheral blood smear is essential. While automated analysers provide valuable preliminary information, the detailed assessment of red blood cell morphology through a peripheral smear remains the gold standard for accurate classification and diagnosis of anemia types. Thus, integrating both automated analysis and manual peripheral smear examination ensures a comprehensive and accurate hematological evaluation.

SUMMARY

The present study was an observational prospective study done in a tertiary care hospital which included 478 anemic adults for a time period of 1st January'23 to 31st December'23 to evaluate the need of peripheral smear to study the morphology of different types of anemia in this automated era of hematology.

EDTA blood samples of the patients were analysed by the automated hematology analysers and for each sample a peripheral smear was prepared which was examined under a microscope.

The RBC indices and histograms generated by the automated analysers were compared with the peripheral smears in different types of anemia reported.

The maximum number of cases in our study were in the age group of 19-30 years.

Females accounted a slightly higher percent (54.6%) of the total study population and were predominant in the age group 19-40 years, while males constituted predominant population in 41-60 years.

Maximum study population was moderately anemic (57.32%).

We found in our study that the most frequently reported anemia on peripheral smear examination was normocytic anemia (54.39%). On RBC indices and histograms also, normocytic anemia was the most prevalent reported (62.13%).

The RBC indices were studied in different types of anemias and the mean values were calculated for each type of anemia.

When findings of peripheral smears were compared to RBC indices and histograms, it was observed that Dimorphic anemia showed the maximum discrepancy (89.32%) between the two which was statistically significant ($p<0.001$).

This highlights that peripheral smear examination is still the gold standard technique to study morphology of different types anemia.

LIMITATIONS

A notable limitation of our study is the exclusion of conditions like hemolysis, RBC agglutination, Rouleux formation and hemoglobinopathies which can significantly impact the RBC morphology leading to disparity between the RBC Histogram and Peripheral smear correlation.

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ANNEXURE I
Classification of Anemia

I. Absolute anemia (decreased red cell volume)

A. Reduced red cell production

1. Acquired

a. Pluripotential hematopoietic stem cell failure

(1) Autoimmune (aplastic anemia)

(a) Radiation induced

(b) Drugs and chemicals (chloramphenicol, benzene, etc.)

(c) Viruses (Non-A-G, H hepatitis, Epstein-Barr virus, etc.)

(d) Idiopathic

(2) Anemia of leukemia and of myelodysplastic syndromes

(3) Anemia associated to bone marrow infiltration

(4) Post chemotherapy

b. Erythroid progenitor cell failure

(1) Pure red cell aplasia (parvovirus B19 infection, drugs, associated with thymoma, autoantibodies, etc.)

(2) Endocrine disorders

(3) Acquired sideroblastic anemia (drugs, copper deficiency, etc.)

c. Functional impairment of erythroid and other progenitors from nutritional and other causes

(1) Megaloblastic anemias

(a) Vitamin B12 deficiency

(b) Folic acid (Folate) deficiency

(c) Acute megaloblastic anemia because of nitrous oxide (N2O)

(d) Drug induced megaloblastic anemia (pemetrexed, methotrexate, phenytoin toxicity, etc.)

(2) Iron deficiency anemia

(3) Anemia due to other nutritional deficiencies

(4) Anemia of chronic disease and inflammation

(5) Anemia of renal failure

(6) Anemia caused by chemical agents (lead toxicity)

(7) Acquired thalassemias (in some clonal hematopoietic disorders)

(8) Erythropoietin antibodies

2. Hereditary

a. Pluripotential hematopoietic stem cell failure

(1) Fanconi anemia

(2) Shwachman syndrome

(3) Dyskeratosis congenita

b. Erythroid progenitor cell failure

(1) Diamond Blackfan syndrome

(2) Congenital syndromes of dyserythropoiesis

c. Functional impairment of erythroid and other progenitors from nutritional and other causes

(1) Megaloblastic anemias

(a) Selective malabsorption of vitamin B12 (Imerslund Gräsbeck disease)

(b) Congenital deficiency of intrinsic factor

(c) Deficiency of Transcobalamin II

(d) Inborn errors of cobalamin metabolism (methylmalonic aciduria, homocystinuria, etc.)

(e) Inborn errors of folate metabolism (congenital folate malabsorption, dihydrofolate deficiency, methyltransferase deficiency, etc.)

(2) Inborn purine and pyrimidine metabolism defects (Lesch-Nyhan syndrome, hereditary orotic aciduria, etc.)

(3) Disorders of iron metabolism

(a) Hereditary atransferrinemia

(b) Hypochromic anemia due to divalent metal transporter (DMT)-1 mutation

(4) Hereditary sideroblastic anemia

(5) Thalassemias

B. Increased red cell destruction

1. Acquired
    a. Mechanical
        (1) Macroangiopathic (march hemoglobinuria, artificial heart valves)
        (2) Microangiopathic (disseminated intravascular coagulation [DIC]; thrombotic thrombocytopenic purpura [TTP]; vasculitis)
        (3) Parasites and microorganisms (malaria, bartonellosis, babesiosis, Clostridium perfringens, etc.)
    b. Antibody and complement mediated
        (1) Warm-type autoimmune hemolytic anemia
        (2) Cryopathic syndromes (cold agglutinin disease, paroxysmal cold hemoglobinuria, cryoglobulinemia)
        (3) Transfusion reactions (immediate and delayed)
        (4) Paroxysmal nocturnal hemoglobinuria
    c. Hypersplenism
    d. Disorders of Red cell membrane
        (1) Spur cell hemolysis
        (2) Acquired acanthocytosis and acquired stomatocytosis, etc.
    e. Chemical injury and complex chemicals (arsenic; copper; chlorate; spider, scorpion, and snake venoms, etc.)
    f. Physical injury (heat, oxygen, radiation)
2. Hereditary
    a. Hemoglobinopathies
        (1) Sickle cell disease
        (2) Unstable hemoglobins
    b. Red cell membrane disorders
        (1) Cytoskeletal membrane disorders (hereditary spherocytosis, elliptocytosis, pyropoikilocytosis)

(2) Lipid membrane disorders (hereditary abetalipoproteinemia, hereditary stomatocytosis, etc.)

(3) Membrane disorders related to abnormalities of erythrocyte antigens (McLeod syndrome, Rh deficiency syndromes, etc.)

(4) Membrane disorders associated with abnormal transport (hereditary xerocytosis)

c. Red cell enzyme defects (pyruvate kinase, 5′ nucleotidase, glucose-6-phosphate dehydrogenase deficiencies, other red cell enzyme disorders)

d. Porphyrias (congenital erythropoietic and hepatoerythropoietic porphyrias, rarely congenital erythropoietic protoporphyria)

C. Blood loss and blood redistribution

1. Acute blood loss

2. Splenic sequestration crisis

II. Relative (increased plasma volume)

A. Macroglobulinemia

B. Pregnancy

C. Athletes

D. Postflight astronauts

E. Rapid descent from high to low altitude, i.e., neocytolysis

ANNEXURE II

Preparation of stain and staining procedure

Preparation of stain

- Dissolve 100 ml of 100% methanol with 0.15 gramme of Leishman stain powder.
- Next, the stain is filtered and placed in a stock bottle.
- In a water bath, place at 50deg C for 15 minutes.
- Once more, pour the filtrate into a clean brown borosilicate glass bottle and keep it at room temperature in the dark.

Procedure

- By counting the droplets of Leishman stain, the thin blood smear that has been well-dried is covered with the undiluted stain solution.
- It is intended to stand for two minutes. The stain's methanol helps adhere the smear to the glass slide.
- Two minutes later, twice as much distilled water or phosphate buffer solution is added. The mixture is then gently stirred by blowing or spinning, and it is left to stand for five to seven minutes.
- To make it easy to remove water from the slides, they are slanted and air dried.
- After the slides have air dried, they are examined using a microscope.

ANNEXURE III

Informed Consent

"COMPARATIVE STUDY OF PERIPHERAL SMEAR WITH RBC INDICES AND RBC HISTOGRAMS IN CASES OF ANEMIA IN ADULTS"

Name of Student/Principal Investigator: REG NO.: BN0121005

Name of Guide/Co Investigators: DR ______________

Objective: The purpose of this study is to compare peripheral smear with RBC indices and RBC histogram in adult anemic patients.

Explanation of procedure: During this study, your CBC reports will be used and a peripheral smear prepared from your blood sample will be compared with the CBC report for the diagnosis of anemia. The principal investigator of the study is REG NO.: BN0121005 under the guidance of DR ___________.

If you agree to enroll yourself in this study, your CBC report and peripheral smear prepared from your blood sample for anemia diagnosis will be used for research purpose.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator

Possible benefits from participating in the study: The benefit would be to know a better way to predict anemia with the help of CBC reports and peripheral smear of the anemic patients and the importance of both peripheral smear and RBC histograms and RBC indices.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

1. Reg No.: BN0121005, Department of Pathology, J.N. Medical College,
2. DR _______________, Department of Pathology, J.N. Medical College.

If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "**COMPARATIVE STUDY OF PERIPHERAL SMEAR WITH RBC INDICES AND RBC HISTOGRAMS IN CASES OF ANEMIA IN ADULTS**". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE IV

Proforma

PATIENT HISTORY

Name: Sample Id:

Age: IP no.:

Sex: Date:

Department:

Brief clinical history:

INVESTIGATIONS: -

1. Hemoglobin
2. RBC Count
3. Hct
4. MCV
5. MCH
6. MCHC
7. RDW
8. RBC Histogram
9. Peripheral Blood Smear

ANNEXURE V

Key to Masterchart

THE COLUMNS

Hb:	Hemoglobin
PCV:	Packed Cell Volume
MCV:	Mean Corpuscular Volume
MCH:	Mean Corpuscular Hemoglobin
MCHC:	Mean Corpuscular Hemoglobin Concentration
RDW-CV:	Red cell distribution width- coefficient of variation

RBC HISTOGRAM-POSITION AND SHAPE

Y:	Yes
N:	No

IMPRESSION ON RBC HISTOGRAM AND INDICES

1-	Normocytic Anemia
2-	Microcytic Hypochromic Anemia
3-	Macrocytic Anemia
4-	Dimorphic Anemia

IMPRESSION ON PERIPHERAL SMEAR

NA:	Normocytic Anemia
MHA:	Microcytic Hypochromic Anemia
MA:	Macrocytic Anemia
DA:	Dimorphic Anemia

NAME	IP NO.	AGE	SEX	Hb(g/dL)	RBC COUNT(10^6/uL)	PCV(%)	MCV(fL)	MCH(pg)	MCHC(g/L)	RDW-CV(%)	RBC HISTOGRAM POSITION			RBC HISTOGRAM SHAPE			IMPRESSION ON RBC HISTOGRAM AND INDICES	IMPRESSION ON PERIPHERAL SMEAR
											NORMAL	LEFT SHIFT	RIGHT SHIFT	BELL	BIMODAL	BROAD BASE		
X	23361198	24	F	8.1	3.22	27.1	84.2	27.1	29.9	16.8	Y	N	N	Y	N	Y	1	NA
X	23107419	55	F	8.6	3.34	28.1	84.1	26.9	30.5	14.3	Y	N	N	Y	N	N	1	NA
X	23362596	31	F	9.2	3.50	30.2	86.3	27.5	30.5	20.6	Y	N	N	Y	N	Y	1	NA
X	23358626	18	F	8.9	3.40	30.2	88.8	28.3	29.5	16.6	Y	N	N	Y	N	Y	1	NA
X	23354188	25	M	12.7	4.86	41.4	85.2	27.3	30.7	13.8	Y	N	N	Y	N	N	1	NA
X	23354186	48	F	8.5	3.30	27.0	81.8	26.8	31.5	13.2	Y	N	N	Y	N	N	1	NA
X	23352814	44	M	6.2	3.12	26.7	85.4	27.4	23.3	15.6	Y	N	N	Y	N	Y	1	NA
X	23352776	59	M	5.8	2.51	24.1	96.2	29.8	23.9	18.3	Y	N	N	Y	N	Y	1	NA
X	23127697	51	M	8.7	3.28	29.1	88.6	28.2	29.9	19.0	Y	N	N	Y	N	Y	1	NA
X	23125761	59	F	9.4	3.56	29.1	81.7	27.1	32.2	13.7	Y	N	N	Y	N	N	1	NA
X	23120552	42	M	12.2	4.74	38.0	80.3	26.9	32.2	16.7	Y	N	N	Y	N	Y	1	NA
X	23119978	51	M	8.1	3.12	26.7	85.5	29.4	30.2	18.5	Y	N	N	Y	N	Y	1	NA
X	23113887	55	M	8.0	3.12	25.1	80.5	27.3	32.1	15.4	Y	N	N	Y	N	Y	1	NA
X	23108594	40	F	9.9	3.84	32.2	83.9	27.1	30.8	15.5	Y	N	N	Y	N	Y	1	NA
X	23107419	55	F	8.6	3.34	28.1	84.1	28.5	30.5	14.3	Y	N	N	Y	N	N	1	NA
X	23125330	29	F	9.8	3.65	32.1	87.9	28.6	30.5	15.2	Y	N	N	Y	N	Y	1	NA
X	23121794	24	M	11.9	4.39	37.1	84.4	28.1	32.1	13.3	Y	N	N	Y	N	N	1	NA
X	23120586	22	F	10.0	3.27	32.0	97.8	30.7	31.4	13.5	Y	N	N	Y	N	N	1	NA
X	23119966	36	M	8.0	2.91	24.0	82.3	27.3	33.2	14.1	Y	N	N	Y	N	N	1	NA
X	23119899	59	F	10.8	3.93	34.4	87.6	27.6	31.5	13.2	Y	N	N	Y	N	N	1	NA
X	23362084	52	F	9.2	3.00	29.0	96.7	30.7	32.2	17.1	Y	N	N	Y	N	Y	1	NA
X	23362915	55	F	9.8	4.00	33.0	82.5	27.2	29.7	16.2	Y	N	N	Y	N	Y	1	NA
X	23358259	44	M	9.5	2.85	27.9	97.9	33.3	34.1	15.8	Y	N	N	Y	N	Y	1	NA
X	23352659	56	F	10.6	3.32	34.4	103.6	31.9	30.8	16.4	N	N	Y	Y	N	Y	3	NA
X	23353140	56	M	9.7	3.19	30.2	94.7	30.4	32.1	15.7	Y	N	N	Y	N	Y	1	NA
X	23353139	52	M	11.3	4.13	35.8	86.7	27.4	31.6	16.3	Y	N	N	Y	N	Y	1	NA
X	23352342	54	F	10.9	3.84	34.2	89.1	28.4	32.0	19.2	Y	N	N	Y	N	Y	1	NA
X	23351680	25	M	12.8	4.40	41.2	93.6	29.1	31.1	12.7	Y	N	N	Y	N	N	1	NA
X	23136258	51	M	10.4	3.40	30.5	89.8	30.7	34.2	15.1	Y	N	N	Y	N	Y	1	NA
X	23361393	60	F	10.8	4.79	35.8	74.7	22.5	30.2	15.7	N	Y	N	Y	N	Y	2	MHA
X	23361713	27	F	5.4	3.84	21.5	56.0	14.1	25.1	23.1	N	Y	N	Y	N	Y	2	MHA
X	23362106	18	F	7.7	3.59	25.7	71.6	21.4	30.0	20.0	N	Y	N	Y	N	Y	2	MHA
X	23362151	51	M	9.0	4.61	32.5	70.5	19.5	27.7	20.1	N	Y	N	Y	N	Y	2	MHA
X	23358157	19	M	7.5	3.31	23.4	70.7	22.7	32.1	22.5	N	Y	N	Y	N	Y	2	MHA
X	23358654	18	F	7.8	3.70	26.8	72.4	21.1	29.1	20.0	N	Y	N	Y	N	Y	2	MHA
X	23358794	51	M	7.7	4.06	28.0	69.0	19.0	27.5	19.5	N	Y	N	Y	N	Y	2	MHA
X	23359051	52	F	9.8	4.05	31.2	77.0	24.2	31.4	18.0	N	Y	N	Y	N	Y	2	MHA
X	23354675	48	M	6.5	2.63	20.7	78.7	24.7	31.4	16.5	N	Y	N	Y	N	Y	2	MHA
X	23352560	21	M	9.4	5.13	32.5	63.4	18.3	28.9	17.5	N	Y	N	Y	N	Y	2	MHA
X	23353528	42	F	10.7	5.04	35.3	70.0	21.2	30.3	15.9	N	Y	N	Y	N	Y	2	MHA
X	23351639	40	M	10.5	5.01	36.2	69.8	20.8	31.5	14.8	Y	N	N	N	Y	Y	4	MHA
X	23351561	22	F	8.4	4.75	30.3	63.8	17.7	27.7	19.9	N	Y	N	Y	N	Y	2	MHA
X	23350404	52	M	9.1	4.98	35.2	70.7	18.3	25.9	20.4	N	Y	N	Y	N	Y	2	MHA
X	23349165	54	M	8.9	4.18	28.8	68.9	21.4	31.0	23.9	N	Y	N	Y	N	Y	2	MHA
X	23232142	52	F	8.2	3.88	26.2	67.5	21.1	31.3	16.4	N	Y	N	Y	N	Y	2	MHA
X	23230786	41	F	4.6	3.26	17.4	53.3	14.1	26.4	21.0	N	Y	N	Y	N	Y	2	MHA
X	23140069	18	M	10.7	4.2	35.6	84.8	25.5	30.1	14.7	Y	N	N	Y	N	N	1	MHA
X	23140117	18	M	10.1	4.03	31.5	78.1	25.0	32.0	14.6	N	Y	N	Y	N	N	2	MHA
X	23137234	41	F	11.4	4.62	35.7	77.1	24.6	31.9	16.3	N	Y	N	Y	N	Y	2	MHA
X	23136015	42	F	9.0	4.84	29.1	60.1	18.6	31.0	16.6	N	Y	N	Y	N	Y	2	MHA
X	23108148	19	F	10.7	4.41	33.8	76.8	24.2	31.5	17.0	N	Y	N	Y	N	Y	2	MHA
X	23119943	18	F	9.6	4.28	31.3	73.0	22.4	30.7	15.2	N	Y	N	Y	N	Y	2	MHA
X	23362793	53	F	7.1	2.70	24.9	92.2	26.3	28.5	23.1	Y	N	N	Y	N	Y	1	DA
X	23362931	31	M	6.8	1.94	20.7	106.7	35.1	32.9	23.3	N	N	Y	Y	N	Y	3	DA
X	23357586	50	M	5.3	1.71	17.9	104.7	31.0	29.6	23.6	N	N	Y	Y	N	Y	3	DA
X	23357574	22	M	8.0	3.31	25.2	76.1	24.2	31.7	26.9	N	Y	N	Y	N	Y	2	DA
X	23357932	28	M	7.6	2.07	23.2	112.2	36.7	32.8	22.4	N	N	Y	Y	N	Y	3	DA
X	23358501	53	F	6.1	2.27	21.4	94.3	26.9	28.5	25.1	Y	N	N	N	Y	Y	4	DA
X	23358927	40	M	9.9	3.38	32.6	96.4	29.3	30.4	20.4	Y	N	N	N	Y	Y	4	DA
X	23359326	38	M	7.4	2.48	24.1	97.2	29.8	30.7	20.6	Y	N	N	Y	N	Y	1	DA
X	23352797	32	F	6.2	2.90	28.4	98.1	21.4	21.8	17.4	Y	N	N	Y	N	Y	1	DA
X	23353367	28	M	9.5	3.17	32.4	102.2	30.0	29.3	17.6	N	N	Y	Y	N	Y	3	DA
X	23260948	32	M	6.0	1.61	18.1	112.5	37.5	33.3	16.5	N	N	Y	Y	N	Y	3	DA
X	23251068	46	M	7.4	2.97	23.5	79.2	25.1	31.6	22.2	N	Y	N	Y	N	Y	2	DA
X	23237351	22	F	8.9	3.73	29.7	79.6	23.9	30.0	22.1	N	Y	N	Y	N	Y	2	DA
X	23237958	40	F	9.7	4.60	31.4	68.2	21.1	31.0	26.7	N	Y	N	N	Y	Y	2	DA
X	23238843	28	F	8.0	2.76	24.7	89.7	29.0	32.4	25.2	Y	N	N	Y	N	Y	1	DA
X	23232334	51	M	12.3	3.28	35.3	107.7	37.3	34.7	23.3	N	N	Y	Y	N	Y	3	DA
X	23231975	60	M	8.2	3.68	29.7	80.7	22.3	27.6	22.5	Y	N	N	Y	N	Y	1	DA
X	23233117	23	M	10.0	3.76	29.1	77.3	26.6	34.3	23.1	N	Y	N	Y	N	Y	2	DA
X	23137266	40	F	8.5	4.10	27.3	66.6	20.9	31.3	25.0	N	Y	N	Y	N	Y	2	DA
X	23137249	27	F	11.8	4.53	37.0	81.8	26.1	32.1	20.1	Y	N	N	Y	N	Y	1	DA
X	23137279	26	F	11.8	4.17	35.4	84.9	28.3	33.3	28.6	Y	N	N	N	Y	Y	4	DA
X	23126427	25	M	6.8	2.49	22.1	88.8	27.4	30.9	19.0	Y	N	N	Y	N	Y	1	DA
X	23107351	28	M	8.4	2.81	25.8	91.9	29.7	32.4	17.6	Y	N	N	Y	N	Y	1	DA
X	23514414	32	F	11.3	4.28	39.7	92.8	30.4	28.5	19.8	Y	N	N	Y	N	Y	1	NA
X	23515160	25	F	8.1	2.55	26.3	103.1	31.8	30.8	21.2	N	N	Y	Y	N	Y	3	DA
X	23515273	49	M	10.0	3.99	32.3	81.0	28.7	31.0	15.9	Y	N	N	Y	N	Y	1	NA
X	23514459	39	F	7.9	4.38	29.5	67.4	18.0	26.8	18.9	N	Y	N	Y	N	Y	2	MHA
X	23514417	43	F	11.3	4.58	37.5	81.9	27.1	30.1	16.5	Y	N	N	Y	N	Y	1	NA
X	23514255	23	F	11.0	3.58	35.0	97.8	30.7	31.4	12.9	Y	N	N	Y	N	N	1	NA
X	23514432	32	F	9.8	2.16	27.2	125.9	45.4	36.0	27.9	N	N	Y	Y	N	Y	3	DA
X	23514828	26	F	11.1	4.13	35.8	86.7	28.1	31.0	23.2	Y	N	N	Y	N	Y	1	NA
X	23514500	23	F	10.7	3.49	32.9	94.3	30.7	32.5	13.9	Y	N	N	Y	N	N	1	NA
X	23514224	45	M	9.5	4.31	33.8	78.4	22.0	28.1	16.4	N	Y	N	Y	N	Y	2	MHA
X	23514765	34	F	9.2	3.02	29.9	99.0	30.5	30.8	21.1	Y	N	N	Y	N	Y	1	DA
X	23514427	55	F	9.4	4.36	33.6	77.1	21.6	28.0	21.5	N	Y	N	Y	N	Y	2	MHA
X	23514819	23	F	10.1	3.57	32.9	92.2	28.3	30.7	13.1	Y	N	N	Y	N	N	1	NA
X	23514818	28	F	11.2	3.56	34.9	98.0	31.5	32.1	13.1	Y	N	N	Y	N	N	1	NA

X	23514801	27	M	12.8	4.59	40.7	88.7	27.9	31.4	13.2	Y	N	N	Y	N	N	1	NA
X	23514689	28	M	7.8	2.18	24.3	111.5	35.8	32.1	22.7	N	N	Y	N	Y	Y	3	DA
X	23514179	29	F	10.3	3.30	31.4	95.1	31.2	32.8	14.0	Y	N	N	Y	N	N	1	NA
X	23513803	55	M	2.9	1.57	10.4	66.2	18.7	28.2	23.2	N	Y	N	Y	N	Y	2	MHA
X	23514006	32	F	11.2	4.13	34.5	83.6	27.1	32.4	14.0	Y	N	N	Y	N	N	1	NA
X	23504115	27	F	8.2	3.41	27.6	80.9	28.2	29.6	22.6	Y	N	N	Y	N	Y	1	NA
X	23504106	21	F	10.3	4.03	34.2	84.9	29.8	30.0	17.3	Y	N	N	Y	N	Y	1	NA
X	23504412	28	F	8.9	3.09	26.6	86.1	28.7	33.3	15.6	Y	N	N	Y	N	Y	1	NA
X	23504290	47	F	11.9	3.94	33.3	84.6	30.2	35.7	12.8	Y	N	N	Y	N	N	1	NA
X	23504494	27	F	11.6	4.28	34.9	81.6	27.0	33.1	13.9	Y	N	N	Y	N	N	1	NA
X	23504487	42	F	11.5	4.61	36.0	78.2	25.0	32.0	16.5	N	Y	N	Y	N	Y	2	MHA
X	23504480	24	M	9.3	3.15	29.0	92.1	29.6	32.2	19.1	Y	N	N	Y	N	Y	1	DA
X	23503530	35	M	8.9	2.98	28.6	96.0	29.9	31.1	16.8	Y	N	N	Y	N	Y	1	DA
X	23503513	23	F	10.3	3.71	36.1	97.3	27.8	28.5	14.2	Y	N	N	Y	N	N	1	DA
X	23503899	33	F	10.7	3.58	32.5	90.9	30.0	33.0	14.7	Y	N	N	Y	N	N	1	NA
X	23503627	56	M	9.9	3.69	33.1	89.7	29.4	29.9	17.2	Y	N	N	Y	N	Y	1	NA
X	23502532	23	F	10.8	4.29	34.2	79.7	25.2	31.6	21.1	N	Y	N	Y	N	Y	2	MHA
X	23493932	42	F	9.3	3.25	28.9	89.2	28.7	32.2	16.2	Y	N	N	Y	N	Y	1	NA
X	23493873	28	F	10.6	3.64	33.0	90.8	29.1	32.0	14.3	Y	N	N	Y	N	N	1	NA
X	23493860	45	M	11.1	3.89	34.2	88.0	28.5	32.4	15.1	Y	N	N	Y	N	Y	1	NA
X	23493899	37	F	8.9	4.47	29.5	66.0	19.9	30.1	21.7	N	Y	N	Y	N	Y	2	MHA
X	23494474	40	F	4.5	3.45	17.6	51.0	13.1	25.7	23.8	N	Y	N	Y	N	Y	2	MHA
X	23494551	36	F	8.2	3.73	26.7	71.5	21.9	30.6	17.1	N	Y	N	Y	N	Y	2	MHA
X	23494499	58	M	12.7	4.55	38.7	85.1	27.9	32.7	16.7	Y	N	N	Y	N	Y	1	NA
X	23494514	39	F	10.0	3.62	30.6	84.5	27.5	32.6	17.3	Y	N	N	Y	N	Y	1	NA
X	23494516	55	F	11.5	4.18	35.7	85.3	28.4	33.3	14.2	Y	N	N	Y	N	N	1	NA
X	23494523	29	F	11.5	3.86	34.5	89.4	29.7	33.3	14.3	Y	N	N	Y	N	N	1	NA
X	23494550	27	F	10.7	3.73	33.5	90.0	28.8	32.0	13.7	Y	N	N	Y	N	N	1	NA
X	23494647	45	M	8.9	3.25	27.5	84.7	27.4	32.3	15.1	Y	N	N	Y	N	Y	1	NA
X	23494659	43	M	12.1	5.02	37.9	75.5	24.2	32.1	14.4	N	Y	N	Y	N	N	2	MHA
X	23494851	26	F	10.0	4.10	33.8	82.4	28.6	29.6	17.1	Y	N	N	Y	N	Y	2	NA
X	23494745	23	F	11.6	4.07	35.9	88.3	29.1	33.0	12.7	Y	N	N	Y	N	N	1	NA
X	23495093	34	F	7.9	2.75	25.6	93.1	28.7	30.9	16.6	Y	N	N	Y	N	Y	1	NA
X	23494340	33	F	10.2	3.48	31.0	89.1	29.4	32.9	15.0	Y	N	N	Y	N	Y	1	NA
X	23494192	48	M	11.6	4.61	35.6	77.3	25.1	32.5	13.1	N	Y	N	Y	N	N	1	MHA
X	23494249	50	F	11.2	4.21	36.4	86.5	27.9	32.2	13.7	Y	N	N	Y	N	N	1	NA
X	23494240	26	F	11.5	4.15	35.9	86.5	28.3	32.8	17.0	Y	N	N	Y	N	Y	1	NA
X	23494236	48	M	11.5	3.95	35.7	90.5	29.1	32.1	15.9	Y	N	N	Y	N	Y	1	NA
X	23494227	35	M	9.8	2.74	28.8	105.2	35.9	34.1	21.7	N	N	Y	N	Y	Y	3	MA
X	23494218	40	M	12.6	4.48	39.8	88.9	28.2	31.7	16.8	Y	N	N	Y	N	Y	1	NA
X	23494134	40	M	7.3	2.65	21.6	81.6	27.4	33.6	15.9	Y	N	N	Y	N	Y	1	NA
X	23494095	30	F	10.0	4.20	31.7	75.6	23.8	31.5	18.4	N	Y	N	Y	N	Y	2	MHA
X	23495226	55	M	12.5	5.46	38.9	71.3	22.9	32.1	18.1	N	Y	N	Y	N	Y	2	MHA
X	23495264	55	M	8.8	3.63	29.1	80.3	27.1	30.4	18.3	Y	N	N	Y	N	Y	1	NA
X	23494924	40	M	11.5	3.88	35.4	91.2	29.6	32.5	12.0	Y	N	N	Y	N	N	1	NA
X	23495044	35	M	9.2	3.09	30.9	100.0	29.8	29.8	17.9	Y	N	N	Y	N	Y	1	DA
X	23495063	27	F	9.1	4.18	30.0	71.8	21.8	30.3	19.5	N	Y	N	Y	N	Y	2	MHA
X	23495440	43	M	8.2	3.15	25.9	82.1	27.7	32.1	20.3	Y	N	N	Y	N	Y	1	NA
X	23494802	41	M	8.1	2.37	25.3	106.6	34.1	32.0	18.1	N	N	Y	Y	N	Y	3	MA
X	23512090	40	F	11.4	4.85	37.9	78.1	32.5	30.1	23.9	N	Y	N	N	Y	Y	2	MHA
X	23512075	27	F	11.6	3.68	35.9	97.6	31.5	32.3	13.2	Y	N	N	Y	N	N	1	NA
X	23512618	42	F	11.3	4.34	38.9	89.6	27.0	30.1	16.4	Y	N	N	Y	N	Y	1	NA
X	23512975	36	F	6.5	3.78	26.7	70.6	17.2	24.3	19.8	N	Y	N	Y	N	Y	2	MHA
X	23513078	50	M	10.0	3.72	33.2	89.2	26.9	30.1	18.4	Y	N	N	Y	N	Y	1	NA
X	23513207	50	F	10.7	4.31	35.1	81.4	28.1	30.5	15.7	Y	N	N	Y	N	Y	1	NA
X	23513219	57	M	8.6	3.20	27.8	86.9	26.9	30.9	17.3	Y	N	N	Y	N	Y	1	NA
X	23513215	50	F	5.8	1.77	19.6	110.7	32.8	29.6	15.9	N	N	Y	Y	N	Y	3	MA
X	23517501	23	F	10.9	3.73	36.1	96.8	29.2	30.2	17.1	Y	N	N	Y	N	Y	1	NA
X	23518033	24	M	9.9	3.29	31.8	96.7	30.1	31.1	17.2	Y	N	N	Y	N	Y	1	NA
X	23518104	54	M	9.8	3.59	33.4	93.0	27.3	29.3	14.5	Y	N	N	Y	N	N	1	NA
X	23518132	35	F	11.9	4.46	38.1	85.4	29.2	31.2	12.9	Y	N	N	Y	N	N	1	NA
X	23518135	39	F	10.9	3.78	36.0	95.2	28.8	30.3	13.5	Y	N	N	Y	N	N	1	NA
X	23518137	30	F	10.2	3.83	34.5	90.1	29.8	29.6	15.3	Y	N	N	Y	N	Y	1	NA
X	23518147	36	M	10.8	3.49	26.7	76.5	30.9	40.4	10.4	N	Y	N	Y	N	N	2	MHA
X	23518466	21	F	10.6	3.25	33.1	101.8	32.6	32.0	14.5	N	N	Y	Y	N	N	3	NA
X	23518467	23	F	9.8	3.44	31.1	90.4	28.5	31.5	13.6	Y	N	N	Y	N	N	1	NA
X	23518468	22	F	9.1	3.24	29.9	92.3	28.1	30.4	13.4	Y	N	N	Y	N	N	1	NA
X	23518523	51	F	8.3	2.69	25.7	95.5	30.9	32.3	19.0	Y	N	N	Y	N	Y	1	NA
X	23518532	40	F	10.4	4.04	33.0	81.7	27.9	31.5	12.9	Y	N	N	Y	N	N	1	NA
X	23518013	21	F	11.4	4.93	40.4	81.9	28.4	82.2	16.2	Y	N	N	Y	N	Y	1	NA
X	23517893	28	F	7.7	2.25	25.2	112.0	34.2	30.6	18.9	N	N	Y	Y	N	Y	3	MA
X	23517895	23	F	10.5	4.39	35.3	80.4	27.2	29.7	14.9	Y	N	N	Y	N	N	1	NA
X	23517992	20	F	11.8	4.43	39.1	88.3	29.2	30.2	13.3	Y	N	N	Y	N	N	1	NA
X	23517213	52	M	10.1	3.11	32.6	104.8	32.5	31.0	14.5	N	N	Y	Y	N	N	3	MA
X	23517317	22	M	8.5	2.88	28.4	98.6	29.5	29.9	13.9	Y	N	N	Y	N	N	1	NA
X	23517315	50	M	9.2	3.02	29.4	97.4	30.5	31.3	13.7	Y	N	N	Y	N	N	1	NA
X	23517495	29	F	8.2	3.01	26.8	89.0	27.2	30.6	16.3	Y	N	N	Y	N	Y	1	NA
X	23516594	50	F	12.6	4.80	42.5	88.5	29.4	29.6	12.9	Y	N	N	Y	N	N	1	NA
X	23516662	38	M	8.2	2.95	25.9	87.8	27.8	32.1	15.9	Y	N	N	Y	N	Y	1	NA
X	23516665	42	M	9.3	3.24	30.1	92.9	28.7	30.9	16.9	Y	N	N	Y	N	Y	1	NA
X	23516636	29	F	11.8	4.26	38.1	89.4	27.7	31.0	13.7	Y	N	N	Y	N	N	1	NA
X	23516722	43	M	6.8	2.65	24.4	92.1	30.2	27.9	22.3	Y	N	N	Y	N	N	1	NA
X	23516595	20	F	9.5	3.22	29.0	90.1	29.5	32.8	13.7	Y	N	N	Y	N	N	1	NA
X	23516587	21	F	9.8	3.49	31.0	88.8	28.1	31.6	15.7	Y	N	N	Y	N	Y	1	NA
X	23516576	32	F	10.8	4.12	35.3	85.7	29.7	30.6	16.1	Y	N	N	Y	N	Y	1	NA
X	23516578	29	F	7.8	3.58	27.6	77.1	21.8	28.3	15.4	N	Y	N	Y	N	Y	2	MHA
X	23516719	26	F	11.0	3.90	32.0	82.1	28.2	34.4	15.3	Y	N	N	Y	N	Y	1	NA
X	23517033	40	F	11.2	4.62	36.6	79.2	24.2	30.6	22.4	N	Y	N	N	Y	Y	2	DA
X	23516869	55	M	6.4	2.70	21.7	80.4	23.7	29.5	22.5	Y	N	N	Y	N	Y	1	DA
X	23528405	43	M	12.6	2.78	36.5	131.3	45.3	34.5	15.8	N	N	Y	Y	N	Y	3	MA
X	23528266	27	F	9.6	3.86	31.1	80.6	27	30.9	15.7	Y	N	N	Y	N	Y	1	NA
X	23528438	28	M	7.2	2.71	22.3	82.3	26.6	32.3	19.9	Y	N	N	Y	N	Y	1	DA
X	23528068	59	M	10.1	3.45	34.0	98.6	29.3	29.7	16.1	Y	N	N	Y	N	Y	1	NA

X	23528099	45	F	8.3	2.83	27.2	96.1	29.3	30.5	14.3	Y	N	N	Y	N	N		1	NA
X	23528436	23	M	8.4	3.19	26.3	82.4	26.3	32.1	23.8	Y	N	N	Y	N	Y		1	DA
X	23528442	20	M	9.0	3.25	27.9	85.8	27.7	32.3	18.2	Y	N	N	Y	N	Y		1	DA
X	23519392	22	F	9.0	3.49	31.2	89.4	29.6	28.8	12.9	Y	N	N	Y	N	N		1	NA
X	23527975	56	M	9.5	3.16	28.5	90.2	30.1	33.3	14.0	Y	N	N	Y	N	N		1	NA
X	23527531	40	F	9.5	3.85	32.4	84.2	28.7	29.3	17.3	Y	N	N	Y	N	Y		1	NA
X	23527065	59	F	10.6	3.82	35.5	93.1	27.8	29.8	17.9	Y	N	N	Y	N	Y		1	NA
X	23526400	52	F	9.7	3.78	30.9	81.9	27.9	31.4	17.9	Y	N	N	Y	N	Y		1	NA
X	23526777	45	M	10.4	3.43	33.1	96.4	30.4	31.5	16.9	Y	N	N	Y	N	Y		1	NA
X	23526409	49	F	11.4	4.49	35.2	78.5	25.3	32.3	14.8	N	Y	N	Y	N	N		2	MHA
X	23526376	48	M	9.2	3.25	28.3	87.1	28.3	32.5	16.6	Y	N	N	Y	N	Y		1	NA
X	23526639	32	F	11.7	4.77	36.4	76.2	24.6	32.2	15.0	N	Y	N	Y	N	Y		2	MHA
X	23526696	33	M	9.1	4.12	29.2	71.0	22.1	31.2	21.3	N	Y	N	Y	N	Y		2	MHA
X	23526768	31	F	11.1	4.38	34.8	79.4	25.3	31.8	14.6	N	Y	N	Y	N	N		2	MHA
X	23527367	49	M	11.5	3.74	33.9	90.6	30.7	33.9	16.6	Y	N	N	Y	N	Y		1	DA
X	23527385	44	F	10.9	4.45	37.1	83.4	24.5	29.4	20.9	Y	N	N	N	Y	Y		4	DA
X	23527373	23	F	11.9	4.29	38.1	88.8	27.7	31.2	17.1	Y	N	N	Y	N	Y		1	DA
X	23527166	56	F	10.8	3.95	35.4	89.6	27.3	30.5	14.6	Y	N	N	Y	N	N		1	NA
X	23527157	28	M	10.1	3.60	35.8	99.4	28.1	28.2	16.3	Y	N	N	Y	N	Y		1	DA
X	23527113	50	M	7.7	2.47	23.6	95.4	31.1	32.6	20.0	Y	N	N	Y	N	Y		1	DA
X	23527120	41	F	11.4	4.77	36.5	76.7	24.0	31.3	19.8	N	Y	N	Y	N	Y		2	MHA
X	23527088	38	M	6.3	2.17	20.3	93.2	29.2	31.3	17.6	Y	N	N	Y	N	Y		1	DA
X	23526426	20	M	10.0	4.19	32.6	77.9	23.9	30.7	26.5	N	Y	N	Y	N	Y		2	MHA
X	23526429	29	F	10.2	3.71	31.7	85.5	27.4	32.1	15.1	Y	N	N	Y	N	Y		1	NA
X	23526428	23	F	8.6	3.49	26.6	76.3	24.7	32.4	24.0	N	Y	N	Y	N	Y		2	DA
X	23526205	25	F	11.8	4.26	37.5	88.0	27.7	31.5	15.1	Y	N	N	Y	N	Y		1	NA
X	23526306	43	F	9.2	4.63	30.0	64.8	19.8	30.5	21.1	N	Y	N	Y	N	Y		2	DA
X	23526421	28	M	8.3	2.96	25.3	85.7	28.1	32.8	15.0	Y	N	N	Y	N	Y		1	NA
X	23530583	49	M	8.8	3.08	26.1	84.8	28.5	33.6	16.0	Y	N	N	Y	N	Y		1	NA
X	23529686	23	F	10.9	3.86	35.7	92.5	28.2	30.5	13.1	Y	N	N	Y	N	N		1	NA
X	23529675	57	M	9.1	3.41	29.9	87.7	29.8	30.4	18.1	Y	N	N	Y	N	Y		1	NA
X	23529680	30	F	11.5	3.73	34.7	93.0	30.8	33.1	14.0	Y	N	N	Y	N	N		1	NA
X	23529344	40	F	9.6	4.63	32.6	70.5	20.9	29.6	17.5	N	Y	N	Y	N	Y		2	MHA
X	23528728	38	F	10.2	3.69	30.2	81.7	27.5	33.7	19.2	Y	N	N	Y	N	Y		1	NA
X	23528715	24	M	9.6	3.25	30.3	93.3	29.4	31.5	18.4	Y	N	N	Y	N	Y		1	NA
X	23528674	41	M	7.2	2.01	21.7	108.1	36.0	33.3	15.8	N	N	Y	Y	N	Y		3	NA
X	23528633	40	F	9.2	4.86	30.7	63.2	19.0	30.0	16.4	N	Y	N	Y	N	Y		2	MHA
X	23528513	51	F	10.0	3.48	32.0	92.0	28.7	31.3	12.9	Y	N	N	Y	N	N		1	NA
X	23527719	54	M	7.5	2.90	25.9	89.3	25.9	29.0	20.2	Y	N	N	Y	N	Y		1	DA
X	23527797	52	M	9.4	2.92	30.8	105.5	32.2	30.5	14.4	N	N	Y	Y	N	N		3	MA
X	23527168	28	F	10.8	4.51	33.8	74.9	23.9	32.0	28.3	N	Y	N	N	Y	Y		2	MHA
X	23527169	22	F	10.5	4.00	35.5	88.8	28.6	29.6	14.3	Y	N	N	Y	N	N		1	NA
X	23527177	24	F	10.4	4.67	35.4	75.8	22.3	29.4	17.2	N	Y	N	Y	N	Y		2	MHA
X	23527621	25	M	11.4	4.13	37.0	89.6	27.6	30.8	16.0	Y	N	N	Y	N	Y		1	NA
X	23527630	45	F	9.8	3.46	31.7	91.6	28.3	30.9	12.4	Y	N	N	Y	N	N		1	NA
X	23527792	35	M	10.3	3.81	32.7	85.8	27.0	31.5	15.5	Y	N	N	Y	N	Y		1	NA
X	23522850	50	M	12.2	3.90	37.3	95.6	31.3	32.7	17.6	Y	N	N	Y	N	Y		1	NA
X	23522817	53	M	9.7	2.96	30.3	102.4	32.8	32.0	18.6	N	N	Y	Y	N	Y		3	NA
X	23522792	45	M	8.1	2.75	25.9	94.2	29.5	31.3	14.3	Y	N	N	Y	N	N		1	NA
X	23522752	29	F	11.2	3.79	37.2	98.2	29.6	30.1	14.9	Y	N	N	Y	N	N		1	NA
X	23522701	31	F	10.9	4.32	36.6	84.7	25.2	29.8	24.3	Y	N	N	N	Y	Y		4	DA
X	23522638	31	F	9.1	2.96	29.4	99.3	30.7	31.0	23.3	Y	N	N	N	Y	Y		4	DA
X	23522629	52	M	9.8	3.00	34.2	114.0	32.7	28.7	14.7	N	N	Y	Y	N	N		3	MA
X	23522422	54	M	12.5	4.71	40.4	85.8	27.8	30.9	13.4	Y	N	N	Y	N	N		1	NA
X	23522424	50	F	11.5	4.49	37.0	82.4	28.3	31.1	13.7	Y	N	N	Y	N	N		1	NA
X	23522390	35	M	11.6	4.27	39.3	92.0	27.2	29.5	15.3	Y	N	N	Y	N	Y		1	NA
X	23522343	57	M	9.3	3.52	31.3	88.9	29.2	29.7	17.2	Y	N	N	Y	N	Y		1	NA
X	23522349	43	M	7.2	2.87	24.1	84.0	25.1	29.9	22.5	Y	N	N	Y	N	Y		1	DA
X	23522392	48	M	10.0	2.93	29.2	99.7	30.8	34.2	14.9	Y	N	N	Y	N	N		1	NA
X	23530583	49	M	8.8	3.08	26.1	84.8	28.5	33.6	16.0	Y	N	N	Y	N	Y		1	NA
X	23529686	23	F	10.9	3.86	35.7	92.5	28.2	30.5	13.1	Y	N	N	Y	N	N		1	NA
X	23529675	57	M	9.1	3.41	29.9	87.7	29.1	30.4	18.1	Y	N	N	Y	N	Y		1	NA
X	23529680	30	F	11.5	3.73	34.7	93.0	30.8	33.1	14.0	Y	N	N	Y	N	N		1	NA
X	23529344	40	F	9.6	4.63	32.6	70.5	20.9	29.6	17.5	N	Y	N	Y	N	Y		2	MHA
X	23528728	38	F	10.2	3.69	30.2	81.7	27.5	33.7	19.2	Y	N	N	Y	N	Y		1	NA
X	23528715	24	M	9.6	3.25	30.3	93.3	29.4	31.5	18.4	Y	N	N	Y	N	Y		1	NA
X	23528623	20	F	10.5	4.06	32.6	80.5	26.8	32.1	17.3	Y	N	N	Y	N	Y		1	NA
X	23528674	41	M	7.2	2.01	21.7	108.1	36.0	33.3	15.8	N	N	Y	Y	N	Y		3	NA
X	23528633	40	F	9.2	4.86	30.7	63.2	19.0	30.0	16.4	N	Y	N	Y	N	Y		2	MHA
X	23531817	23	F	10.4	3.74	32.7	87.4	27.9	32.0	13.1	Y	N	N	Y	N	N		1	NA
X	23531777	35	M	5.7	1.66	17.4	105.0	34.1	32.5	17.3	N	N	Y	Y	N	Y		3	NA
X	23531923	38	F	8.9	3.20	26.4	82.4	28.0	33.9	19.0	Y	N	N	Y	N	Y		1	NA
X	23531717	37	M	10.0	3.29	30.4	92.6	30.3	32.7	16.8	Y	N	N	Y	N	Y		1	NA
X	23531685	21	F	11.4	4.44	35.4	79.6	25.6	32.2	15.6	N	Y	N	Y	N	Y		2	MHA
X	23531654	50	M	13.9	7.34	45.7	62.3	18.9	30.4	16.7	N	Y	N	Y	N	Y		2	MHA
X	23531619	38	M	7.8	2.69	23.5	87.1	28.9	33.1	17.2	Y	N	N	Y	N	Y		1	NA
X	23531577	27	F	11.3	4.40	35.3	80.2	26.9	32.1	16.1	Y	N	N	Y	N	Y		1	NA
X	23531540	22	F	11.9	4.08	39.1	95.8	29.1	30.4	16.2	Y	N	N	Y	N	Y		1	NA
X	23532654	51	M	8.0	4.10	26.8	65.5	19.4	29.6	22.3	N	Y	N	Y	N	Y		2	MHA
X	23531813	22	F	10.9	3.99	33.4	83.6	27.3	32.7	17.2	Y	N	N	Y	N	Y		1	NA
X	23533230	27	F	11.7	4.68	36.6	78.2	25.0	31.9	14.4	N	Y	N	Y	N	N		2	MHA
X	23533187	35	M	9.4	3.61	30.0	83.2	28.2	31.3	16.7	Y	N	N	Y	N	Y		1	NA
X	23533632	38	F	9.9	4.51	31.5	69.9	22.0	31.5	15.4	N	Y	N	Y	N	Y		2	MHA
X	23533406	32	F	9.1	3.57	28.8	80.4	27.3	32.2	16.3	Y	N	N	Y	N	Y		1	NA
X	23533487	53	F	10.4	4.68	32.9	70.3	22.3	31.8	19.0	N	Y	N	Y	N	Y		2	MHA
X	23533432	38	F	8.8	3.17	25.9	81.9	27.7	33.8	18.6	Y	N	N	Y	N	Y		1	NA
X	23533351	26	F	11.7	4.42	35.1	79.4	26.5	33.4	14.3	N	Y	N	Y	N	N		2	MHA
X	23533346	23	F	11.2	4.23	35.3	83.4	27.8	32.3	14.8	Y	N	N	Y	N	N		1	NA
X	23533240	23	F	10.3	3.74	32.6	87.1	27.4	31.5	12.7	Y	N	N	Y	N	N		1	NA
X	23533238	22	F	10.6	3.96	33.2	83.9	26.9	32.1	17.3	Y	N	N	Y	N	Y		1	NA
X	23533299	38	M	7.9	2.75	25.4	92.2	28.6	31.0	18.6	Y	N	N	Y	N	Y		1	NA
X	23533305	41	M	7.3	2.07	23.8	114.9	35.3	30.7	17.2	N	N	Y	Y	N	Y		3	MA
X	23533310	28	M	9.9	3.45	30.8	89.3	28.6	32.0	15.5	Y	N	N	Y	N	Y		1	NA

X	23533122	52	M	9.2	3.13	28.8	92.0	29.5	32.1	15.7	Y	N	N	Y	N	Y	1	NA
X	23533030	54	F	8.6	2.82	26.8	94.8	30.3	32.0	17.9	Y	N	N	Y	N	Y	1	DA
X	23533040	25	F	10.5	3.68	33.3	90.5	28.4	31.4	14.6	Y	N	N	Y	N	N	1	NA
X	23533050	47	F	11.6	4.36	39.7	91.1	29.7	29.1	18.4	Y	N	N	Y	N	Y	1	NA
X	23533014	40	F	7.7	4.47	26.4	59.1	17.3	29.3	22.2	N	Y	N	Y	N	Y	2	MHA
X	23532936	57	M	9.4	3.54	30.6	86.3	26.6	30.8	20.2	Y	N	N	Y	N	Y	1	DA
X	23533319	53	M	10.5	4.58	34.9	76.2	22.9	30.1	18.5	N	Y	N	Y	N	Y	2	MHA
X	23532843	19	M	5.5	2.29	18.4	80.3	23.9	29.8	25.0	Y	N	N	Y	N	Y	1	DA
X	23535142	53	F	10.9	4.88	37.3	76.4	22.3	29.2	18.8	N	Y	N	Y	N	Y	2	DA
X	23535107	25	F	10.6	4.39	35.9	81.8	26.9	29.5	14.6	Y	N	N	Y	N	N	1	NA
X	23535370	25	F	11.5	4.27	36.0	84.3	28.9	32.2	13.3	Y	N	N	Y	N	N	1	NA
X	23534582	23	F	9.5	3.61	32.5	90.0	26.3	29.2	20.9	Y	N	N	Y	N	Y	1	DA
X	23535000	31	M	10.0	3.19	30.7	96.2	31.3	32.6	13.0	Y	N	N	Y	N	N	1	NA
X	23535041	43	M	12.7	3.30	38.5	116.7	38.5	33.0	13.8	N	N	Y	Y	N	N	3	MA
X	23534926	35	M	8.1	3.07	26.3	85.7	28.7	30.8	15.2	Y	N	N	Y	N	Y	1	NA
X	23533694	24	F	7.1	2.96	23.2	78.3	23.8	30.4	17.2	N	Y	N	Y	N	Y	2	MHA
X	23533693	53	F	10.2	4.64	33.4	72.0	21.9	30.5	19.1	N	Y	N	Y	N	Y	2	MHA
X	23533698	21	F	11.6	4.36	39.1	89.6	29.8	29.7	15.9	Y	N	N	Y	N	Y	1	NA
X	23533729	42	F	10.9	3.53	32.2	91.4	30.8	33.7	13.2	Y	N	N	Y	N	N	1	NA
X	23533759	52	M	9.6	3.02	30.0	99.4	31.9	32.1	16.0	Y	N	N	Y	N	Y	1	NA
X	23533794	28	M	11.1	3.99	36.4	91.3	28.0	30.6	18.3	Y	N	N	Y	N	Y	1	DA
X	23533792	48	M	8.0	3.24	24.7	76.3	24.6	32.3	13.2	N	Y	N	Y	N	N	2	MHA
X	23540289	50	M	11.7	4.07	37.0	91.0	28.7	31.6	15.9	Y	N	N	Y	N	Y	1	NA
X	23546262	45	F	6.4	4.59	26.9	58.6	13.9	23.8	25.6	N	Y	N	Y	N	Y	2	MHA
X	23546268	27	F	8.7	3.25	28.9	88.9	29.5	30.1	15.4	Y	N	N	Y	N	N	1	NA
X	23546271	32	F	8.7	4.60	30.7	66.7	18.9	28.3	27.8	N	Y	N	N	Y	Y	2	MHA
X	23546318	38	F	9.1	3.03	28.3	93.4	30.1	32.2	16.0	Y	N	N	Y	N	Y	1	NA
X	23546321	28	F	9.2	2.90	27.7	95.6	31.8	33.2	16.7	Y	N	N	Y	N	Y	1	NA
X	23546073	29	F	6.0	1.91	19.4	101.6	31.4	30.9	16.2	Y	N	N	Y	N	Y	1	NA
X	23546175	59	M	10.7	4.58	37.6	82.1	26.9	28.5	23.3	Y	N	N	Y	N	Y	1	NA
X	23546187	51	F	9.0	3.89	30.5	78.4	23.1	29.5	14.0	N	Y	N	Y	N	N	2	MHA
X	23546494	23	F	9.9	3.86	34.6	89.6	30.1	28.6	18.0	Y	N	N	Y	N	Y	1	NA
X	23546457	46	M	7.0	2.39	20.6	86.0	29.2	34.0	14.9	Y	N	N	Y	N	N	1	NA
X	23545724	29	F	11.2	3.90	34.9	89.5	28.7	32.1	15.6	Y	N	N	Y	N	Y	1	NA
X	23545688	36	F	11.9	3.82	35.7	93.5	31.2	33.3	12.7	Y	N	N	Y	N	N	1	NA
X	23545880	30	M	7.8	2.94	25.1	85.4	30.2	31.1	15.9	Y	N	N	Y	N	Y	1	NA
X	23545623	19	F	9.1	3.33	28.9	86.8	27.3	31.5	23.5	Y	N	N	Y	N	Y	1	DA
X	23545615	21	F	8.6	3.80	28.4	74.7	22.6	30.3	29.6	N	Y	N	N	Y	Y	2	DA
X	23545669	33	F	8.3	3.08	30.4	98.7	26.9	27.3	14.9	Y	N	N	Y	N	N	1	NA
X	23545810	30	F	8.7	3.05	28.7	94.1	28.5	30.3	14.9	Y	N	N	Y	N	N	1	NA
X	23545619	19	M	5.5	1.85	17.7	95.7	29.7	31.1	18.6	Y	N	N	Y	N	Y	1	DA
X	23545660	19	F	11.6	4.98	39.9	80.1	23.3	29.1	15.4	Y	N	N	Y	N	Y	1	NA
X	23545736	28	F	8.7	3.95	29.9	75.7	22.0	29.9	18.0	N	Y	N	Y	N	Y	2	MHA
X	23545694	55	M	7.9	2.75	25.1	91.3	28.7	31.5	14.2	Y	N	N	Y	N	N	1	NA
X	23545544	30	F	9.6	3.63	31.8	87.6	26.4	30.2	17.9	Y	N	N	Y	N	Y	1	NA
X	23545084	21	F	8.7	3.48	27.8	79.9	25.0	31.3	16.6	Y	N	N	Y	N	Y	1	MHA
X	23545489	36	F	9.1	4.92	31.6	64.2	18.5	28.8	20.7	N	Y	N	Y	N	Y	2	MHA
X	23545671	28	F	8.2	2.61	26.8	102.7	31.4	30.6	15.6	N	N	Y	Y	N	Y	3	MA
X	23545156	48	F	11.9	4.18	36.9	88.3	28.5	32.2	14.0	Y	N	N	Y	N	N	1	NA
X	23566299	43	M	9.7	2.69	28.6	106.3	36.1	33.9	13.5	N	N	Y	Y	N	N	3	MA
X	23566091	25	F	10.3	3.36	33.1	98.5	30.7	31.1	14.7	Y	N	N	Y	N	N	1	NA
X	23566179	26	F	7.5	2.36	24.1	102.1	31.8	31.1	17.1	N	N	Y	Y	N	Y	3	DA
X	23565929	26	F	9.6	3.07	30.6	99.7	31.3	31.4	19.7	Y	N	N	Y	N	Y	1	DA
X	23566044	35	F	6.8	3.83	26.6	69.6	17.8	25.6	17.3	N	Y	N	Y	N	Y	2	MHA
X	23565957	46	M	11.1	3.88	33.8	87.1	28.6	32.8	13.4	Y	N	N	Y	N	N	1	NA
X	23565177	38	M	5.9	1.82	20.9	114.8	32.4	28.2	22.0	N	N	Y	N	Y	Y	3	MA
X	23565247	53	M	12.1	4.05	37.1	91.6	29.9	32.6	12.8	Y	N	N	Y	N	N	1	NA
X	23565493	43	M	5.9	2.03	19.0	93.6	29.1	31.1	19.9	Y	N	N	Y	N	Y	1	DA
X	23565732	19	M	11.6	4.47	39.2	87.7	27.2	29.6	14.7	Y	N	N	Y	N	N	1	NA
X	23565693	31	F	11.4	4.24	37.8	89.2	27.1	30.2	13.6	Y	N	N	Y	N	N	1	NA
X	23565675	26	F	8.2	3.06	28.1	91.8	27.2	29.2	15.6	Y	N	N	Y	N	Y	1	NA
X	23565698	36	M	8.9	3.07	27.1	88.3	29.0	32.8	17.3	Y	N	N	Y	N	Y	1	NA
X	23565652	23	F	10.4	3.66	34.0	92.9	28.4	30.6	13.7	Y	N	N	Y	N	N	1	NA
X	23564631	46	M	8.6	2.21	25.3	114.5	38.9	34.0	17.9	N	N	Y	Y	N	Y	3	MA
X	23564926	39	M	12.7	4.00	38.4	96.0	31.8	33.1	12.7	Y	N	N	Y	N	N	1	NA
X	23564811	56	M	10.9	4.49	36.2	80.6	27.9	30.1	16.0	Y	N	N	Y	N	Y	1	NA
X	23564760	37	F	10.7	3.71	35.5	95.7	28.8	30.1	15.4	Y	N	N	Y	N	Y	1	NA
X	23565099	25	F	10.6	4.65	35.3	75.9	22.8	30.0	15.5	N	Y	N	Y	N	Y	2	MHA
X	23565168	31	M	10.1	3.52	32.3	91.8	28.7	31.3	14.1	Y	N	N	Y	N	N	1	NA
X	23564979	54	M	11.9	5.25	40.7	77.5	22.7	29.2	16.5	N	Y	N	Y	N	Y	2	MHA
X	23565005	53	M	10.9	3.52	36.2	102.8	31.0	30.1	18.9	N	N	Y	Y	N	Y	3	NA
X	23565125	56	M	8.0	2.83	25.7	90.8	28.3	31.1	14.4	Y	N	N	Y	N	N	1	NA
X	23565159	55	M	7.3	2.59	23.9	92.3	28.2	30.5	15.5	Y	N	N	Y	N	Y	1	NA
X	23565183	26	F	11.0	4.55	36.4	80.0	26.9	30.2	19.6	Y	N	N	Y	N	Y	1	NA
X	23566038	20	F	4.9	1.33	17.0	127.8	36.8	28.8	31.2	N	N	Y	N	Y	Y	3	DA
X	23564584	58	M	12.6	4.44	38.8	87.4	28.4	32.5	13.3	Y	N	N	Y	N	N	1	NA
X	23564725	19	M	8.5	3.17	26.6	83.9	26.8	32.0	30.2	Y	N	N	N	Y	Y	4	DA
X	23564726	19	M	7.8	2.89	24.5	84.8	27.0	31.8	24.4	Y	N	N	Y	N	Y	1	DA
X	23564424	58	M	11.9	3.35	36.1	107.8	35.5	33.0	18.5	N	N	Y	Y	N	Y	3	MA
X	23564272	56	F	9.7	4.01	32.7	81.5	27.1	29.7	13.8	Y	N	N	Y	N	N	1	NA
X	23564768	38	M	6.7	2.53	21.6	85.4	27	31.0	15.1	Y	N	N	Y	N	Y	1	NA
X	23564643	50	M	8.1	2.96	25.6	86.5	27.4	31.6	15.7	Y	N	N	Y	N	Y	1	NA
X	23564790	38	F	8.0	4.07	28.9	71.0	19.7	27.7	23.8	N	Y	N	Y	N	Y	2	MHA
X	23564549	54	M	11.1	3.80	32.8	86.3	29.2	33.8	12.7	Y	N	N	Y	N	N	1	NA
X	23564477	35	F	10.3	5.04	32.6	64.6	20.5	31.7	14.8	N	Y	N	Y	N	Y	2	MHA
X	23564194	41	F	8.8	4.56	30.2	66.2	19.3	29.1	19.0	N	Y	N	Y	N	Y	2	MHA
X	23563853	38	M	9.2	2.90	30.6	105.5	31.7	30.1	21.5	N	N	Y	Y	N	Y	3	DA
X	23563591	30	F	11.7	4.28	36.9	86.2	27.3	31.7	14.2	Y	N	N	Y	N	N	1	NA
X	23563590	30	F	10.4	3.59	32.6	90.8	29.0	31.9	13.6	Y	N	N	Y	N	N	1	NA
X	23563586	28	M	7.0	2.37	23.1	97.5	29.5	30.3	14.6	Y	N	N	Y	N	N	1	NA
X	23563191	28	F	10.0	3.65	33.1	90.7	27.4	30.2	12.8	Y	N	N	Y	N	N	1	NA
X	23563850	57	M	12.0	4.12	39.6	96.1	29.1	30.3	12.9	Y	N	N	Y	N	N	1	NA
X	23563711	49	M	5.8	2.33	20.1	86.3	28.7	28.9	16.0	Y	N	N	Y	N	Y	1	NA

X	23563735	32	F	9.2	3.86	31.9	82.6	29.9	28.8	15.1	Y	N	N	Y	N	Y	1	NA
X	23563712	50	M	11.8	3.04	31.6	103.9	38.8	37.3	18.2	N	N	Y	Y	N	Y	3	DA
X	23563623	54	M	10.7	4.75	38.1	80.2	28.5	28.1	16.7	Y	N	N	Y	N	Y	1	NA
X	23563616	31	F	8.9	3.05	30.1	98.7	29.2	29.6	19.1	Y	N	N	Y	N	Y	1	NA
X	23563632	43	M	10.6	3.59	35.1	97.8	29.5	30.2	19.8	Y	N	N	Y	N	Y	1	DA
X	23563626	48	M	12.2	4.26	37.1	87.1	28.6	32.9	13.6	Y	N	N	Y	N	N	1	NA
X	23563507	37	F	9.5	4.76	32.9	69.1	20.0	28.9	18.4	N	Y	N	Y	N	Y	1	MHA
X	23563023	46	M	8.4	3.23	26.8	83.0	28.1	31.3	18.7	Y	N	N	Y	N	Y	1	NA
X	23563557	23	F	9.8	3.22	32.7	101.6	30.4	30.0	12.7	Y	N	N	Y	N	N	1	NA
X	23563558	22	F	10.6	3.95	36.0	91.1	27.2	29.4	16.0	Y	N	N	Y	N	Y	1	NA
X	23564182	42	F	9.9	3.12	32.0	102.6	31.7	30.9	15.0	N	N	Y	Y	N	Y	3	NA
X	23563475	35	F	7.0	3.86	27.2	70.5	18.1	25.7	17.6	N	Y	N	Y	N	Y	2	DA
X	23563318	55	M	11.9	4.26	37.2	87.3	27.9	32.0	14.2	Y	N	N	Y	N	N	1	NA
X	23563373	58	M	9.1	4.61	31.0	67.2	19.7	29.4	28.8	N	Y	N	Y	N	Y	2	MHA
X	23563174	56	M	8.3	4.97	33.7	67.8	16.7	24.6	19.7	N	Y	N	Y	N	Y	2	MHA
X	23562797	38	F	11.4	3.55	36.5	102.8	32.1	31.2	18.2	N	N	Y	Y	N	Y	3	NA
X	23562823	21	F	5.7	3.29	21.1	64.1	17.3	27.0	37.7	N	Y	N	Y	N	Y	2	MHA
X	23563090	25	M	11.3	4.13	36.6	88.6	27.4	30.9	17.6	Y	N	N	Y	N	Y	1	NA
X	23572133	27	M	8.9	4.17	31.4	75.3	21.3	28.3	27.7	N	Y	N	Y	N	Y	2	DA
X	23572148	32	M	8.2	2.64	25.7	97.3	31.1	31.9	13.5	Y	N	N	Y	N	N	1	NA
X	23572155	44	M	10.8	2.79	30.5	109.3	38.7	35.5	19.9	N	N	Y	N	Y	Y	3	DA
X	23572266	55	M	11.3	5.27	38.2	72.5	21.4	29.6	19.0	N	Y	N	Y	N	Y	2	MHA
X	23572034	46	M	10.2	3.59	32.0	89.1	28.4	31.9	14.1	Y	N	N	Y	N	N	1	NA
X	23571887	23	F	11.5	3.72	34.7	93.3	30.9	33.1	13.6	Y	N	N	Y	N	N	1	NA
X	23569376	39	M	11.4	3.80	34.0	89.7	30.2	33.6	15.1	Y	N	N	Y	N	Y	1	NA
X	23569677	20	F	5.7	1.56	19.8	126.9	36.5	28.8	29.5	N	N	Y	N	Y	Y	3	DA
X	23569679	46	M	10.6	3.70	32.2	87.0	28.6	32.9	13.9	Y	N	N	Y	N	N	1	NA
X	23569591	55	F	11.8	4.08	39.6	97.1	28.9	29.8	13.1	Y	N	N	Y	N	N	1	NA
X	23569332	30	M	8.1	2.66	25.9	97.4	30.5	31.3	18.6	Y	N	N	Y	N	Y	1	DA
X	23569310	41	M	5.6	2.06	18.5	89.9	27.2	30.3	14.0	Y	N	N	Y	N	N	1	NA
X	23569311	24	M	9.1	3.13	29.6	94.6	29.1	30.7	16.8	Y	N	N	Y	N	Y	1	NA
X	23569302	35	M	11.4	4.41	39.5	89.6	27.2	28.9	14.0	Y	N	N	Y	N	N	1	NA
X	23569806	55	M	4.3	1.12	12.9	115.2	38.4	33.3	21.2	N	N	Y	Y	N	Y	3	MA
X	23569743	21	F	8.1	3.63	27.1	74.7	22.3	29.9	20.5	N	Y	N	N	Y	Y	2	DA
X	23569882	58	M	12.2	5.10	40.4	79.2	23.9	30.2	15.2	N	Y	N	Y	N	Y	2	NA
X	23569685	54	M	10.1	2.88	32.2	111.8	35.1	31.4	13.6	N	N	Y	Y	N	N	3	MA
X	23568929	38	M	7.7	2.39	26.3	110.0	32.2	29.3	21.2	N	N	Y	Y	N	Y	3	DA
X	23568330	43	M	9.1	2.41	28.5	118.3	37.8	31.9	19.2	N	N	Y	Y	N	Y	3	MA
X	23559635	41	M	6.3	1.96	20.5	104.3	32.3	31.0	19.8	N	N	Y	Y	N	Y	3	DA
X	23559585	39	F	6.9	3.53	23.3	66.0	19.5	29.5	20.8	N	Y	N	Y	N	Y	2	MHA
X	23559281	26	F	8.8	2.83	29.5	104.2	31.1	29.8	20.0	N	N	Y	Y	N	Y	3	DA
X	23559202	57	M	7.7	2.81	25.9	92.2	27.4	29.7	20.6	Y	N	N	Y	N	Y	1	DA
X	23559498	32	F	8.5	3.46	27.8	80.3	24.6	30.6	19.4	Y	N	N	Y	N	Y	1	DA
X	23559453	55	M	6.5	2.79	21.7	77.8	23.3	30.0	22.5	N	Y	N	Y	N	Y	2	DA
X	23558861	46	F	11.5	5.01	39.1	78.2	23.0	29.4	18.4	N	Y	N	Y	N	Y	2	MHA
X	23558699	20	M	7.1	2.75	23.3	84.7	30.2	30.5	23.6	Y	N	N	N	Y	Y	4	NA
X	23559105	59	F	8.1	3.17	28.2	89.0	29.2	28.7	18.5	Y	N	N	Y	N	Y	1	NA
X	23617697	59	M	6.2	2.53	24.4	96.4	24.5	25.4	22.3	Y	N	N	Y	N	Y	1	DA
X	23617541	48	F	9.0	3.44	27.7	80.5	27.1	32.5	19.9	Y	N	N	Y	N	Y	1	NA
X	23617544	24	F	9.3	3.73	31.6	84.8	27.2	29.5	19.7	Y	N	N	Y	N	Y	1	NA
X	23617537	30	F	9.4	3.69	29.5	80.1	25.4	31.7	22.0	Y	N	N	Y	N	Y	1	DA
X	23617359	23	F	9.7	5.00	34.9	69.8	19.3	27.7	33.0	N	Y	N	N	Y	Y	2	DA
X	23617305	48	M	10.4	4.93	33.5	67.8	21.1	31.1	19.2	N	Y	N	Y	N	Y	2	MHA
X	23617427	28	F	5.3	1.48	17.3	116.5	35.5	30.5	15.4	N	N	Y	Y	N	Y	3	MA
X	23617496	59	F	9.4	3.07	28.0	91.9	30.5	33.5	18.2	Y	N	N	Y	N	Y	1	DA
X	23617168	28	M	10.4	5.51	34.5	62.5	18.8	30.1	18.4	N	Y	N	Y	N	Y	2	MHA
X	23617308	58	M	10.7	4.23	34.4	81.1	25.4	31.3	23.2	Y	N	N	Y	N	Y	1	DA
X	23616792	50	M	12.6	3.85	41.1	106.8	32.7	30.7	17.6	N	N	Y	Y	N	Y	3	DA
X	23616563	24	F	10.5	3.38	35.7	105.6	31.1	29.4	13.2	N	N	Y	Y	N	N	3	MA
X	23616908	57	F	6.9	2.06	22.3	108.3	33.5	30.9	23.9	N	N	Y	N	Y	Y	3	DA
X	23616685	19	M	8.4	3.16	26.7	84.5	26.6	31.5	22.3	Y	N	N	Y	N	Y	1	DA
X	23616443	57	F	9.0	3.14	29.8	94.8	28.7	30.2	15.1	Y	N	N	Y	N	Y	1	NA
X	23616647	28	M	8.7	2.49	27.7	111.2	34.9	31.4	12.7	N	N	Y	Y	N	N	3	MA
X	23616654	48	M	9.1	3.45	32.1	93.0	26.4	28.3	21.8	Y	N	N	Y	N	Y	1	DA
X	23616849	20	F	10.4	4.94	35.9	72.7	21.1	29.0	16.4	N	Y	N	Y	N	Y	2	DA
X	23616646	55	F	7.4	3.79	28.4	74.9	19.5	26.1	23.7	N	Y	N	Y	N	Y	2	DA
X	23615785	20	F	7.0	2.49	22.5	90.4	28.1	31.1	23.5	Y	N	N	N	Y	Y	4	DA
X	23615550	28	F	6.1	1.73	20.9	120.8	35.3	29.2	14.3	N	N	Y	Y	N	N	3	MA
X	23616071	23	F	6.9	2.45	22.1	90.2	28.2	31.2	21.6	Y	N	N	N	Y	Y	4	DA
X	23615229	20	M	9.1	3.96	31.9	80.6	23.0	28.5	23.1	Y	N	N	Y	N	Y	1	DA
X	23615409	25	F	7.4	2.4	25.9	107.9	30.8	28.6	14.7	N	N	Y	Y	N	N	3	DA
X	23615294	28	M	8.0	2.26	25.6	113.3	35.4	31.3	12.9	N	N	Y	Y	N	N	3	MA
X	23614608	20	M	6.7	2.71	21.5	79.3	24.7	31.2	27.3	N	Y	N	Y	N	Y	2	DA
X	23618784	58	F	8.6	3.13	29.8	95.2	27.5	28.9	17.9	Y	N	N	Y	N	Y	1	NA
X	23614458	32	F	9.0	3.09	28.1	90.9	29.1	32.0	18.6	Y	N	N	Y	N	Y	1	NA
X	23619323	27	M	4.5	3.71	18.3	49.1	12.1	24.7	23.1	N	Y	N	Y	N	Y	2	MHA
X	23619759	40	M	9.0	3.9	27.8	71.3	23.1	32.4	37.4	N	Y	N	N	Y	Y	2	DA
X	23582103	23	F	10.7	4.61	35.8	77.7	23.2	29.9	14.5	N	Y	N	Y	N	N	2	MHA
X	23581446	27	M	11.7	3.75	35.4	94.4	31.2	33.1	13.2	Y	N	N	Y	N	N	1	NA
X	23581248	22	F	10.6	3.79	37.0	97.6	28.0	28.6	18.3	Y	N	N	Y	N	Y	1	NA
X	23581274	32	F	10.1	5.57	36.3	65.2	18.1	27.5	15.8	N	Y	N	Y	N	Y	2	MHA
X	23580006	52	M	12.5	4.56	38.3	84.1	27.4	32.6	12.8	Y	N	N	Y	N	N	1	NA
X	23579815	27	F	11.1	4.07	36.3	89.2	27.3	30.6	13.1	Y	N	N	Y	N	N	1	NA
X	23579964	53	M	11.3	2.91	32.8	112.7	38.8	34.5	15.1	N	N	Y	Y	N	Y	3	MA
X	23579982	48	M	12.6	4.39	43.5	99.1	28.7	29.1	12.9	Y	N	N	Y	N	N	1	NA
X	23579145	55	M	5.7	1.67	17.1	102.4	34.1	33.3	25.6	N	N	Y	N	Y	Y	3	DA
X	23579379	55	M	8.5	3.6	26.7	74.2	23.6	31.8	23.8	N	Y	N	N	Y	Y	2	DA
X	23579230	23	F	5.2	1.61	16.7	103.7	32.3	31.1	16.5	N	N	Y	Y	N	Y	3	DA
X	23579265	50	F	9.1	2.95	29.4	99.7	30.8	31.1	19.1	Y	N	N	Y	N	Y	1	DA
X	23579287	38	M	3.5	0.95	10.9	114.7	36.8	32.1	27.3	N	N	Y	Y	N	Y	3	DA
X	23579469	19	F	4.4	1.39	13.5	97.1	31.7	32.6	16.7	Y	N	N	Y	N	Y	1	NA
X	23579664	32	M	5.5	2.32	20.8	89.7	27.1	26.4	19.6	Y	N	N	Y	N	Y	1	NA
X	23579689	50	M	12.3	3.73	39.4	105.6	33.1	31.2	19.5	N	N	Y	Y	N	Y	3	DA

X	23579760	19	F	10.5	3.8	35.5	93.4	27.6	29.6	16.4	Y	N	N	Y	N	Y	1	NA
X	23579608	22	F	11.1	3.9	36.1	92.3	28.5	30.8	15.9	Y	N	N	Y	N	Y	1	NA
X	23578740	35	M	12.8	4.26	40.6	95.3	30.1	31.5	15.9	Y	N	N	Y	N	Y	1	NA
X	23578741	19	F	11.1	4.53	39.2	86.5	24.3	28.1	22.4	Y	N	N	N	Y	Y	4	DA
X	23578761	52	M	10.3	4.73	35.6	75.3	21.8	28.9	18.1	N	Y	N	Y	N	Y	2	MHA
X	23578608	52	F	7.4	4.01	28.6	71.3	18.5	25.9	19.9	N	Y	N	Y	N	Y	2	MHA
X	23578620	56	M	7.9	2.82	25.7	91.1	28.1	30.7	15.1	Y	N	N	Y	N	Y	1	NA
X	23578341	47	M	12.1	4.67	39.8	85.2	30.8	30.4	12.8	Y	N	N	Y	N	N	1	NA
X	23578384	24	F	11.2	4.17	35.6	85.4	28.6	31.5	12.7	Y	N	N	Y	N	N	1	NA
X	23578349	26	F	11.6	4.18	36.7	87.8	27.8	31.6	12.9	Y	N	N	Y	N	N	1	NA
X	23577961	43	M	8.3	2.43	25.3	104.1	34.2	32.8	17.1	N	N	Y	Y	N	Y	3	NA
X	23577879	28	F	11.1	3.37	34.3	99.7	32.6	32.1	13.1	Y	N	N	Y	N	N	1	NA
X	23577888	29	F	10.1	3.31	32.9	99.4	30.5	30.7	12.4	Y	N	N	Y	N	N	1	NA
X	23577437	55	M	5.9	1.71	17.7	99.9	29.1	32.1	23.8	Y	N	N	N	Y	Y	4	DA
X	23576628	38	F	11.1	4.07	34.5	84.8	27.1	31.9	11.8	Y	N	N	Y	N	N	1	NA
X	23577802	23	F	10.2	3.84	32.7	85.2	30.6	31.2	15.1	Y	N	N	Y	N	Y	1	NA
X	23577446	30	F	10.2	3.76	31.3	83.2	27.1	32.6	13.9	Y	N	N	Y	N	N	1	NA