"UTILITY OF RED BLOOD CELL HISTOGRAMS IN VARIOUS TYPES OF ANEMIAS IN PEDIATRIC AGE GROUP (1-18 YEARS)"

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ABSTRACT

Title: UTILITY OF RED BLOOD CELL HISTOGRAMS IN VARIOUS TYPES OF ANEMIAS IN PEDIATRIC AGE GROUP (1-18 YEARS)

Background:

Over the past few years, an automated haematology analyzer's complete blood count and a microscopic examination of a peripheral smear have complimented each other to produce a thorough report on patients' blood samples. When a simultaneous peripheral smear is recorded, it is frequently seen that histogram patterns exhibit a variety of properties. Additionally, it is evident that manual reporting of peripheral smears has a number of drawbacks, such as being subjective, time-consuming, and statistically erroneous. Microscopical peripheral smear examination does, nevertheless, also have its benefits.

Aims and Objectives:

1.Utility of red cell histograms in various types of anaemia in pediatric age group (1-18years)

2.To correlate peripheral smear findings with RBC histogram patterns

Materials and Methods:

The present study was undertaken inJNMC and KLE'S DR. PRABHAKAR KORE HOSPITAL and MRC. Hb levels were determined in EDTA sample by the 5-part differential autoanalyzer Sysmex XN 350. Peripheral blood smear of patients having lower limit of the normal range for the particular age and sex were made by push or wedge technique and Leishman staining is done(Procedure-Annexure). Then the slide is studied under microscope and provisional impression is made without referring to the histogram. The RBC histogram generated by the impedance principle and the RBC indices – MCV, MCHC, RDW-CV/RDW-SD were analysed. The impression derived by peripheral smear examination were then correlated with histogram patterns and RBC indices.

Results:

In the present study normocytic normochromic anemia was the most common anemia followed by microcytic hypochromic anemia both according to RBC indices and histogram analysis and on peripheral smear. The histogram and indices analysis correlated with peripheral smear findings in majority of microcytic hypochromic anemia, normocytic normochromic anemia and macrocytic anemia. Histogram pattern showed within normal limits in 46.43% of cases, 39.28%cases of left shift, right shift in 9.28% cases and bimodal in 5% of cases.

Conclusion:

Peripheral smear examination is a sensitive and gold standard method in diagnosing red blood cell disorders. Red blood cell histogram patterns and the red cell indices obtained from the hematology automated analysers act as an adjunct to the visual examination of peripheral smears.

Key words:

Hematology analyser, Peripheral smear, Red cell indices, Red cell histograms

LIST OF ABBREVIATIONS USED

WHO : World Health Organization

HCT : Hematocrit

RNA : Ribonucleic Acid

Hb : Hemoglobin

CV : Coefficient of Variation

SD : Standard Deviation

RBC: Red Blood Cell

PCV: Packed Cell Volume

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

RDW : Red cell distribution width

RDW-CV :Red cell distribution width- coefficient of variation

RDW-SD:Red cell distribution width -standard deviation .

fl: Femtoliters

pg: Picograms

DNA: Deoxyribonucleic acid

WBC: White Blood Cell

PLT: Platelet

IDA: Iron deficiency anemia

NRBC: Nucleated Red Blood Cell

CBC:Complete bloodcount

MPV: Mean plateletvolume

hpf: High power field

lpf:Low power field

EDTA: Ethylene Di amine Tetra Acetic Acid

2,3 DPG: 2, 3 - Diphosphoglyceric acid

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INTRODUCTION

One of the most common nutritional problems affecting mothers and children in India is childhood anaemia. In terms of anaemia rates in earlier demographic and health surveys, private and state humanitarian measures to boost child health have made little progress.[1]

The RBC histogram, in conjunction with RBC indices, Mean corpuscular volume, and RBC distribution width, is a crucial component of automated analysis and offers valuable insights in the diagnosis and treatment of red cell disorders.[2]

Additionally, it provides crucial details that are not immediately obvious from the numerical data, such as in the case of megaloblastic anaemia with progressive iron shortage. The small population of microcytic hypochromic cells is recognised as doublepeak by the histogram.[2]

A dual cell population is only indicated by a double peak histogram curve, which needs to be further connected with peripheral smear results.

Since MCV is a mean value and doesn't reflect the heterogeneity of the RBC population, further determination of MCV in a dimorphic population curve is useless.[3] In addition, knowledge of the abnormal curves of the RBC histogram and the flags is required to look for specific morphological features in peripheral smear for a correct diagnosis.[3]

Despite the sophistication of modern instruments, initial calibration still requires the use of manual processes. This emphasises the value of manual technical skills despite the desire to leave everything up to automation.

There haven't been many research on the use of peripheral smear findings compared to RBC indices and histograms. In order to correlate these in order to improve the method of diagnosing anaemia, the current study is being conducted in the Department of Pathology, JNMC Belagavi.

OBJECTIVES

- Utility of red cell histograms in various types of anaemia in pediatric age group (1-18years)
- To correlate the peripheral smear impression with RBC histogrampatterns

REVIEW OF LITERATURE

History and Nomenclature

Anemia is derived from two Greek words that collectively indicate "without blood." Any condition known as anemia is defined by a substantial decrease in the body's overall erythrocyte mass. After the invention of the microscope, the scientific study of blood could be conducted. Antonie Van Leeuwenhoek provided the first thorough description (1632-1723). With the publication of an essay on the significance of red blood cells, William Hewson (1739–1744) earned the moniker "The founder of haematology."[4]

Two stains are employed in standard blood smear examination for the diagnosis and classification of anemia: the Wright stain and the May Grunwald-Giemsa stain. D.L. Romanowsky, a native of St. Petersburg, Russia, created the eosin methylene blue stain in 1891.[5]

Modern haematology has improved methods for identifying and classifying anemia, and it has begun to examine the condition at the molecular level, which is what is required right now.[6]

Thanks to the research of George Richards Minot (1885–1955) and his assistant, William Parry Murph, pernicious anemia was successfully treated by consuming significant amounts of raw liver in their diet (1892-1987).

In 1852, Karl Vierordt published the first description of how to count blood cells, which entailed spreading a known volume of blood onto a microscope surface. The hemocytometer, created by Louis-Charles Malassez in 1874, made it simple to examine blood cells under a microscope, and in the late 19th century, Paul Ehrlich and Dmitri Leonidovich Romanowsky created staining techniques for white and red blood cells that are still used to examine patients today.

Automated methods for quantification of hemoglobin were developed in the 1920s, and Maxwell Wintrobe introduced the Wintrobe hematocrit method in 1929, which eventually allowed him to define the red blood cell indices.[6] A historic landmark in automation of blood cell counts was the Coulter principle, which was invented by Wallace H. Coulter in 1953. The Coulter principle uses the principle of electrical impedance to count blood cells and ascertain their sizes. It is a technology used in many automated analyzers. Further research in the 1970s included the use of optical measurements to count and identify cells, which helped the automation of the white blood cell differential.[7]

Definition and grading

WHO defines anemia as a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status of the individual. Anemia is the most common hematologic disorder, diagnosed in males when hemoglobin is <13g/dl and HCT <0.39. In females, when hemoglobin <12g/dl andHCT<0.36.[8,9]

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

Table 1: Grading of anemia according to WHO[10]

CLASSIFICATION OF ANEMIAS

Anemias can be categorised using morphological parameters, such as cell size and hemoglobin-content parameters, like MCV and MCHC, or erythrokinetic schemes, which consider the rates of RBC production and destruction, and biochemical/molecular schemes, where the aetiology of the anemia at a molecular level is taken into account.

Morphological classification[11]

It is the most practical way since morphological characteristics are easier and less expensive to evaluate than erythrokinetic and biochemical ones. Wintrobe originally identified anemia based on variations in the average volume and haemoglobin content of RBC.

Iron deficiency anemia is the most prevalent type of anemia, while chronic disease-related anemia is the second most common. Microcytic anemia is characterised by disordered haemoglobin synthesis from iron deficiency, incorrect globin production, or impairment in heme and porphyrin synthesis.

Deficiencies in folic acid and vitamin B12, which result in unusually large RBC precursors in the bone marrow, are the most prevalent causes of macrocytosis; however, inherited conditions and drugs that interfere with DNA synthesis can also result in macrocytosis

Normocytic anemia is seen in anemia of chronic disease, hemolytic anemia, acute hemorrhage and in aplastic anemia.

Etiological Classification

- Impaired RBC production

1. Abnormal bone marrow- aplastic anemia, myelofibrosis, leukemia, cancer metastasis

2. Essential factor deficiency- Iron, vitamin B12, folic acid etc
3. Stimulation factor deficiency- Anemia in chronic disease, anemia in hypopituitarism and anemia in hypothyroidism

- Excessive destruction of RBCHemolytic anemia- Intracorpuscular and extra corpuscular defect
- Blood Loss

1. Acute blood loss –Accident, GI bleeding
2. Chronic blood loss- Hypermenorrhoea, Parasite infestation

Erythrokinetic classification

The reticulocyte count, which should range from 0.5 to 1.5% normally, aids in the distinction between peripheral bleeding or hemolysis and decreased marrow production. This classification is based on RBC turnover. If RBC turnover is high, a non-regenerative anemia exists, as seen in hemolysis or haemorrhage. In these cases, marrow responds and produces an increased number of reticulocytes

Biochemical classification

Different biochemical tests assist in diagnosing the aetiology of anemia, such as a depleted cofactor necessary for proper hematopoiesis (iron, ferritin, folate, or B12), incorrectly functioning enzymes (glucose -6-phosphate dehydrogenase, pyruvate kinase), or abnormal immune system function (The direct antiglobulin test).

Based on MCV and RDW, Bessman et al characterised anemia. They divided the RBC population into homogeneous (with a normal RDW) and heterogeneous (with abnormal RDW) (with increased RDW).[12]

Epidemiology[13]

1.62 billion individuals (95% CI: 1.50-1.74 billion) or 24.8% of the world's population (95% CI: 22.9-26.7%) suffer from anemia, according to WHO data; nonetheless, reticulocytes percentage %) x Ht (%) Ht (%) Normal * Due to the fact that anemia has varying frequency and root causes in various contexts, worldwide data cannot accurately depict the true scope of the issue. The highest anemia prevalence is found in children under the age of four. Kassebaum et al. found that over a 20-year period, the prevalence of anemia fell from 40.2% to 32%. Due to unclear reasons, the decline in prevalence was more pronounced in males, expanding the gender difference. As a result, females continue to outnumber males in prevalence throughout all regions, but particularly in sub-Saharan Africa starting at age 15 and continuing into adulthood. Poor dietary iron intake and reproductive factors like pregnancy and lactation continue to be the most common causes of anemia in women. It is obvious that the scenario differs depending on the geographic location and, as a result, the economic standing of various nations. Notably, prevalence is higher in those who are undernourished, have low socioeconomic position, and are new mothers. The leading causes of anemia in lower-income nations continue to be diseases including malaria, hookworms, schistosomiasis, and iron-related etiologies. Among contrast, cancer, gastrointestinal bleeding, and chronic kidney disease (CKD), which is quite common in senior persons, are the most common causes in high-income areas. Hereditary anemias like thalassemia and sickle cell disease are endemic in some nations (for example, in Africa, India, the Middle East, and the Mediterranean region) and are a significant socioeconomic burden. However, migration is changing the epidemiology of these anemias, which is challenging for doctors and healthcare systems worldwide.

THE NORMAL AND PATHOLOGIC RED BLOOD CELL

The bone marrow contains pluripotent stem cells that have the capacity for selfrenewal and differentiation into mature cells. Erythropoietin stimulates primitive stem cells to undergo divisions. During the final step of differentiation the nucleusisextruded from the red blood cell precursor of reticulocyte that still retainscytoplasmic RNA.A mature red blood cell is formed up on the loss of RNA.A redbloodcell remains in the peripheral circulation for 120 days before being removedby the spleen and reticuloendothelial system. Usually the red blood cell numberremains relatively constant with removal from the peripheral circulation balanced byproliferation and differentiation within the marrow.Many disease states disruptedblood cell homeostasis,the most common is the decrease in overall red blood cellnumbers.

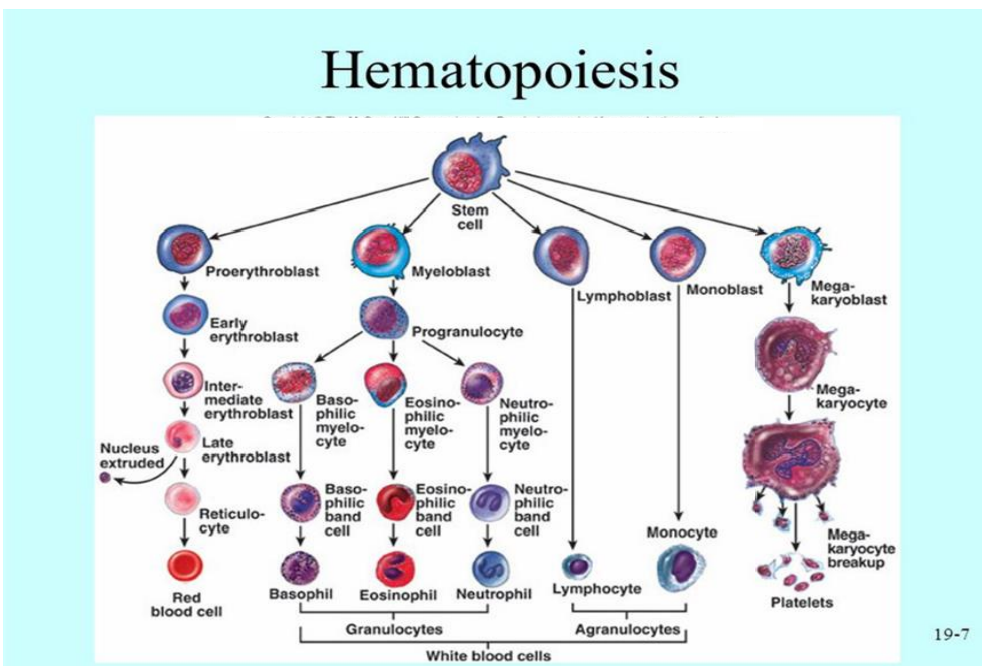


Fig 1: Hematopoiesis[14]

A mature red cell has a diameter that falls within the specific range of 6.0-8.5um, is round, biconcave, and has smooth outlines. The nucleus of a tiny lymphocyte on dried film is the same size as a typical red blood cell. In healthy people, there is relatively little variation in the size and form of the red blood cells. In well-made films, a small but variable percentage of the cells are oval rather than spherical (often less than 10%), and a very small percentage may be constricted, have an uneven shape, or appear to have lost some of their substance due to fragmentation (Schistocytes).

HEMOGLOBIN CONCENTRATION

Because of its vibrant hue, haemoglobin (Hb) can be measured using spectrophotometric methods. Oxyhemoglobin, carboxyhemoglobin, methemoglobin, and other minor components are only a few of the several types of haemoglobin that can be found in the blood. Mixing blood with Drabkins solution will result in the creation of cyanmethemoglobin, a single stable molecule (containing potassium ferricyanide and potassium cyanide).[14,15]Although it is rarely found in large levels, sulfhemoglobin is not transformed. Hb is determined by measuring the cyanmethhemoglobin's absorption at 540 nm in a spectrophotometer. This method is applied to both manual and automatic haematological analyzers. Hb is measured in whole blood grammes per deciliter (g/dl). Leukocytosis, inappropriate red cell lysis, dilution mistakes, or elevated levels of fat or protein in the plasma are the main causes of measurement errors.[16,17,18]The accuracy for measuring haemoglobin using automated techniques is 1%. (CV).[19]

ERYTHROCYTE COUNT

When performing an erythrocyte count, one counts the RBCs per litre of whole blood. The test's outcome is given as the number of cells per unit volume, or cells/uL. Automated counts offer a considerably more precise reflection of red cell populations than do manual approaches, which have been shown to be quite inaccurate12 Therefore, computerised counting has completely replaced manual hemocytometer usage. As a result of RBCs sticking together in the sample tube under some circumstances, two or more cells are counted as one, which is the main cause of inaccuracies in the RBC count when using an automated method. When employing automated haematology analyzers, the observed precision for RBC counts is 1% (CV), but manual approaches have a minimum estimated value of 11%.[20]

HEMATOCRIT

A measurement of the proportional volume occupied by RBCs in capillary or venous whole blood samples is known as packed cell volume (PCV), also.[21] The RCM of the body is divided by the blood volume to measure the HCT. The presence or absence of polycythemia and anemia is assessed using the HCT. Hematocrit is manually determined by centrifuging blood in a standardised glass tube with a uniform bore at a set speed and time, as stated in Wintrobe's original description.[22] By comparing the height of the red cell column after centrifugation to the volume of the entire blood sample, the heamatocrit is calculated

Larger laboratories employ automated procedures that measure the amount of each of the thousands of red cells in a measured volume of whole blood separately and tally them together. Electronic measurement of erythrocyte volume is possible.

Either their electrical impedance or the way they scatter light can be measured. The test's outcome is given as "volumes percent" or simply "percent."Patients with polycythemia [23]or aberrant plasma osmotic pressures are more likely to experience automated Hct calculation errors than other patients.[24]

In these circumstances, manual methods of Hct determination may be preferred. The majority of automated HCTs have a 1% precision (CV).[19]

The conditions where we can get deranged hematocrit are:

- Increased in dehydration or polycythemia
- Decreased in anemia, over hydration, kidney failure or chronic inflammatory conditions.

Principle of Threes:

The Rule of Three formula traditionally reads: HB (g/dL) X 3 = HCT (%) (+ or - 2%)The haemoglobin value is 1/3 (or 33%) of the hematocrit number, to put it another way. Since 33% is included in the MCHC calculation's reference interval (32–36%), this is effectively a rearranging of the MCHC calculation. MCHC = HB/HCT X 100% without the units. Rearranging the equation with 33% in place of MCHC yields: 33% = HB/HCT X 100%; multiply both sides by HCT; 33% x HCT X 100%; divide both sides by 33%. HCT = HB * 3. When the MCHC falls inside the reference range, the Rule of Three is valid.

The Rule of Three serves as a second check on the MCHC as a result. The Rule of Three will not apply in cases of hypochromia, spherocytosis, or misleading results, though, thus it is a quick way to check for those conditions.A second, less common application of the Rule of Three is HB = RBC (without powers of 10) X 3.

Or to put it another way, 1/3 of the HB value is the RBC (without powers of ten). The MCH equation has basically been rearranged in this case.

1) Hemoglobin, one of the three cardinal RBC measures mentioned above the erythrocyte indices—Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, and Mean Corpuscular haemoglobin Concentration—are derived mathematically from 2) Hematocrit and

3)RBC count.

MEAN CORPUSCULAR VOLUME

Is a way to measure cell volume. Compared to the RBC size data obtained from the blood film, it is more reproducible. The average red blood cell volume is a helpful metric for classifying anemias and may shed light on the pathophysiology of diseases of the red blood cells.[25,26,27] The MCV will be normal with a low HCT in settings of severe chronic disease or quick blood loss. Thalassemia and iron insufficiency both exhibit a reduced MCV. Alcoholism, myelodysplastic syndrome, and folate or vitamin B12 deficiency can contribute to an elevated MCV. A minor but considerable degree of microcytosis or macrocytosis as well as the existence of anisocytosis can be missed because only the mean cell volume is examined. The presence of extremely large quantities of white blood cells, red blood cell agglutination, abnormalities in cell form, and unexpected osmotic swelling can all cause errors in the MCV, despite its accuracy and great reproducibility. Blood is referred to as microcytic when the MCV is low, macrocytic when the MCV is high, and normocytic when the MCV is normal. The MCV is often estimated from the erythrocyte count and hematocrit using the formula below but it can also be measured directly with automated devices.[22]Red cell count (10^{12}/L)/1,000/MCV = Hct (L/L)The typical MCV is in the 80–100 femtolitre.

MEAN CORPUSCULAR HEMOGLOBIN (MCH)

Is a good indicator of how much haemoglobin is present in each individual red blood cell. Patients with thalassemia or iron insufficiency who are unable to produce normal levels of haemoglobin exhibit a marked drop in the MCH. Small cells contain less haemoglobin than large cells, hence changes in the MCH typically follow changes in the MCV. Independent of the MCV, the MCH doesn't add much information. The following formula can be used to calculate it manually or automatically. [22]Hemoglobin (g/L)/red cell count (10^{12}/L) is known as MCH. Picograms, the mass unit, is used to express it (pg). The typical range is 27–32pg.

MEAN CORPUSCULAR HEMOGLOBINCONCENTRATION (MCHC)

The following formula can be used to determine the average haemoglobin concentration in a specified red cell volume, or MCHC:

Hemoglobin (g/dl)/Hct (L/L) = MCHC[22]

The MCHC is a measurement of the ratio of haemoglobin mass to red cell volume and is given in grammes of haemoglobin per deciliter of packed RBCs.

It is a measure of Hb content or cell chronicity in each RBC. The MCHC is almost twice the value for haemoglobin in whole blood and expressed in the same units since whole blood is approximately 50% cells by volume and all haemoglobin is contained within the cells. The range is between 32 and 36 g/dL. Cells are categorised as normochromic, hyperchromic, or hypochromic depending on their MCHC values.

As was already established, the MCV, MCH, and MCHC indicate average values and may not accurately depict blood samples when heterogeneous populations of red cells are present. For instance, despite the fact that the indices may be normochromic and normocytic, sideroblastic anemias may contain a dimorphic population of red blood cells that comprises both hypochromic and normochromic cells. It is essential to examine both the blood smear and the red cell histogram in order to identify such dimorphic populations.[29]Despite the fact that the MCV is a very useful value for categorising anemias, the MCH and MCHC typically do not offer further, clinically relevant information.[19,27,28] However, because their values will remain consistent over time for a particular specimen, the MCH and MCHC serve a significant role in laboratory quality control.[30]

RED BLOOD CELL DISTRIBUTION WIDTH

The variety of red cell sizes within a sample is reflected by the red cell distribution width (RDW), which quantitates cellular volume heterogeneity.[25,31,32]Since RDW displays abnormalities in nutritional deficiency anemias earlier than other red cell parameters, especially in cases of iron deficiency anemia, it has been suggested that RDW may be useful in the early categorization of anemia.

Due to its ability to distinguish between straightforward iron deficiency anemia (high RDW, normal to low MCV) and uncomplicated heterozygous thalassemia (normal RDW, low MCV), RDW is particularly useful in characterising microcytic anemia.[25,34,35,36]

Most of the time, more testing is required to confirm the diagnosis.[37]RDW can also be used to identify dimorphic, agglutinating, or fragmented red blood cell populations (including patients who have had transfusions, have sideroblastic anemias, or have been recently treated for a nutritional deficiency).[34,38]

Physiological conditions that lead to alteration of RDW values.[39]

RDW values	**Normal**	**Elevated**
Decreased MCV	(i) Anemia of chronic disease (ii) Hemoglobin E trait (iii) Heterozygous thalassemia	(i) Iron deficiency (ii) HbS/Beta thalassemia (iii) Hemolytic anemia (iv) Microangiopathic hemolytic anemia
Elevated MCV	(i) Chronic liver disease (ii) Aplastic anemia (iii) Chemotherapy/antivirals/alcohol	(i) Vitamin B12, folate deficiency (ii) Immune hemolytic anemia (iii) Hereditary spherocytosis
Normal MCV	(i) Anemia of chronic disease (ii) Acute blood loss or hemolysis	(i) Early vitamin B12, folate deficiency (ii) Sickle cell anemia (iii) Transfusions (iv) Chronic hepatobiliary disease

The normal value of red cell distribution width range from 11-15%. Counters compute this value using two different techniques. Since it is measured lower on the red cell volume distribution curve, the RDW-SD (Red Cell Distribution Width - Standard Deviation) is more susceptible to the development of small populations of macrocytes or microcytes. The RDW-CV (Red Cell Distribution Width - Coefficient of Variation) better captures the general change in size distribution seen in well-established macrocytic or microcytic anemia but is less sensitive to the development of small populations of microcytes, true macrocytes, or reticulocytes.

RETICULOCYTE COUNTS

Counting the number of reticulocytes, or immature, nonnucleated RBCs, that still carry RNA allows one to assess how well the bone marrow can produce and release red blood cells in response to a physiological challenge like anemia. Traditionally, reticulocytes have been counted manually using supravital staining with methylene blue, which will stain precipitated RNA as a dark blue meshwork of granules (at least two per cell).[40] Reticulocyte counts in adults should range from 0.5% to 1.5%, while in newborns they can range from 2.5% to 6.5%. By the second week of life, they should be between 0.5% and 1.5%. (dropping to adult levels).

Automated detection methods for staining enable the analysis of a much larger number of cells, boosting count accuracy and precision, which enhances reticulocyte counting precision.[41,42,43]

Since haematology analyzers feature automated reticulocyte counting as one of the testing options, most recently developed automated reticulocyte counts can be combined with typical whole blood count data. Using a fluorescent dye that binds to RNA, reticulocytes can be recognised.

PERIPHERAL BLOOD SMEAR

The results of an automated haematology analyzer may point to a specific diagnosis, however numerous disorders can have blood counts that are normal but abnormal cellular morphology. It takes careful consideration to prepare blood smears that are satisfactory, staining abilities, and knowledge of the morphologic characteristics of normal and pathological cells.

In the haematology laboratory, a flag is the signal to the operator that the analysed data may have a significant abnormality during analysing blood samples, necessitating confirmation of the results by microscopic examination of a well-stained PBS.44Hematology analyzers generate suspect flags in the presence of abnormal cells, and it is well known that false-positive rates are high.

However, it is claimed that in the majority of cases, haematological analyzers deliver prompt and precise results. Blasts, aberrant lymphocytes, immature granulocytes (IGs), huge PLTs, or other CBC characteristics may occasionally produce erroneous results that are connected to WBC or PLT problems. The majority of automated haematology analyzers are configured in various ways to "flag" or otherwise distinguish samples that may be abnormal. The automatic blood analyzer generates warning signals that allow the user (technician, nurse, or physician) to recognise positive samples and respond with the necessary follow-up activities as a result of the warnings. [44]

Leukemia, leukemoid reactions, and other illnesses causing leukocytosis and leukopenias are only a few of the WBC disorders known to produce signals (flags) in the analyzer report that should trigger a PBS check. Suspect flags are also generated by PLT count anomalies brought on by enormous PLTs or PLT clumps. Our lab uses a 5-part automated analyzer that generates a number of flag signals, including myeloblast (MO blast), immature granulocytes (IGs), left shift, eosinophilia, and big PLTs. This study aimed to compare PBSs with WBC and PLT questionable flags. RBC, WBC, and PLT questionable flags have been the subject of numerous independent studies, but very few have combined these three types of research.[44]

Blood films may be prepared on either glass slides or coverslips. Each method has specific advantages and disadvantages.[45,46,47,48] Blood smears are often prepared from Samples of anticoagulated blood remaining after automated hematology analyser prepared at the time of analysis. However, artifacts in cell appearance and staining may be induced by anticoagulant.[21]Optimal morphologyand staining are obtained from nonanticoagulated blood, most often from a finger prick procedure. Mechanical dragging of the cells across the glassslide or coverslip and uneven distribution ofblood might contort the cells; however, these artifacts may proper technique.[45](Annexure:Procedure and preparation)

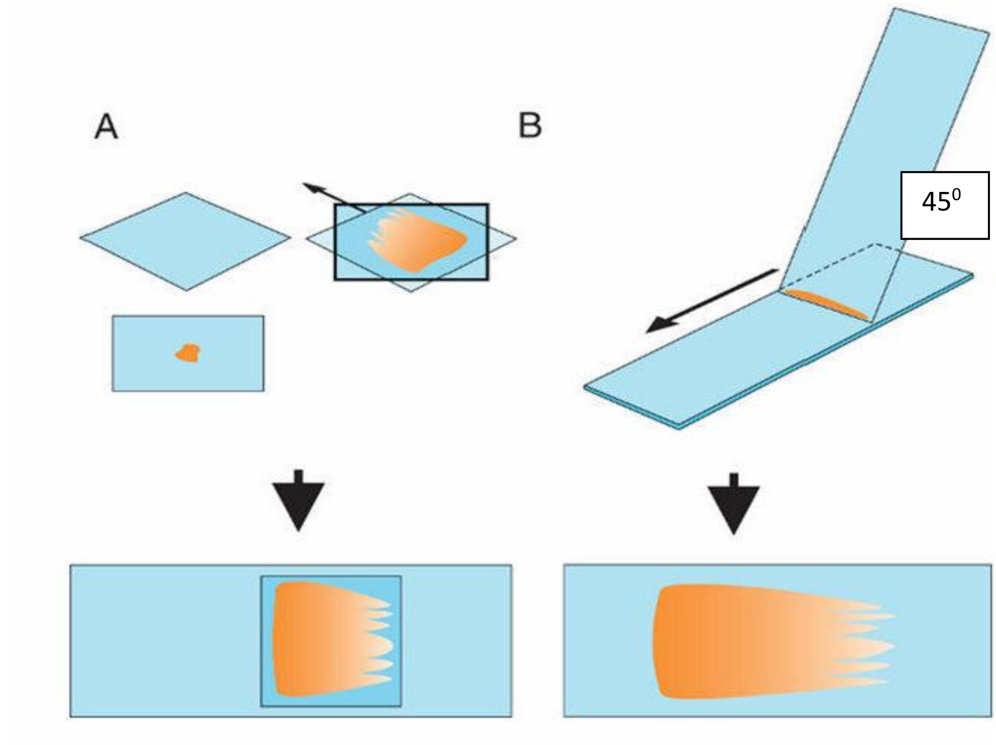


Fig 2: Preparation of Blood Smears[45]

Features of ideal Peripheral Smear:[45]

- Slides should measure 75*25 mm and be approximately 1mm thick.
- Ideally they should be frosted at one end to facilitate labeling
- The film should be about 3cm in length and finishes atleast 1cm before the end of the slide.
- The ideal thickness is one that allows for some red cell overlap along the majority of the film's length when viewed under a microscope.

Parts of Peripheral Smear:

- Head
- Body
- Tail

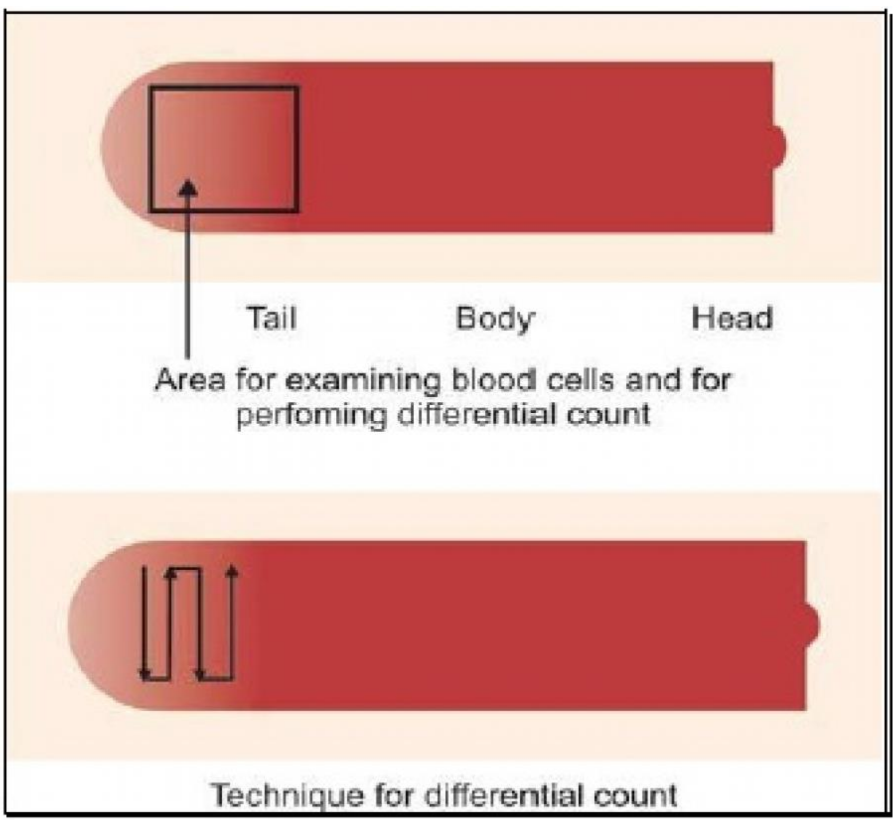


Fig 3: Parts of ideal peripheral smear[45]

Routine Staining of Blood Smears

Blood smears are generally stained with either Wright or Leishman stains. Both stains are modifications of the Romanowsky procedure.[49.50] The various Romanowsky stains are:

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

Methylene blue and eosin are the main components of the stain; water artefacts can be prevented by putting slides or coverslips in anhydrous methanol before staining; and water must be completely removed from all Romanowsky stains since it causes red blood cell artefacts. Romanowsky stains can all be dissolved in methyl alcohol but are insoluble in water. [49]

Using Eosin Y and oxidised methylene blue (azure) dyes, neutral stains were created.The blue-purple colour comes from the azures, which are basic dyes that adhere to the acid nuclei.Eosin, an acid dye, binds to the alkaline cytoplasm and causes it to turn red.Romanowsky staining functions best when several colours that can be used to distinguish different cellular components are created.

This skill is known as the Romanowsky effect or metachromasia.

The Romanowsky effect, also known as the Romanowsky-Giemsa effect, is when some white blood cells' cytoplasmic granules and nuclear chromatins take on a purple hue. The combination of active eosin Y and active methylene blue was blamed for this phenomena.

The Romanowsky effect can also be caused by the oxidative demethylation effect, which changes unmethylated methylene blue into multichromatic stains, some of which are responsible for the Romanowsky effect.

Polychrome methylene blue, which is methylene blue that has undergone oxidative demethylation, has about 11 different colours, including azure A, azure B, azure C, methylene blue, methylene violet Bernthesen, methyl thionoline, and thionoline.

Examination of the Blood Smear

To ensure that aberrant populations that may be localised at the blood smear's edge are not migrating within, it is crucial to thoroughly inspect the blood smear. To evaluate the effectiveness of cellular distribution and staining, the blood smear should first be examined at an intermediate power (10 to 20 objective). At this level of strength, one can make an educated approximation as to the white blood cell count. Use of an oil immersion lens (50 or 100×) or high-power dry lens (40×) is usually sufficient for performing leukocyte differential counts, although a 100× oil lens may be necessary for identification of cellular inclusions or abnormal cytoplasmic granules. Systematic evaluation of the blood smear is essential so that all cell types are examined and characterized. Each cell type should be evaluated for both quantitative and qualitative abnormalities.[51,52]

A red blood cell's (RBC's) size, shape, haemoglobin distribution, and presence of cellular inclusions should all be checked. This assessment is difficult because quantitative red cell abnormalities in a blood smear are frequently dispersed randomly throughout the blood film.[51]

Red blood cells that appear to adhere to one another are known as rouleaux, or stacks of RBCs, and they can be seen in blood smears. In areas of the smear where the red cells are clumped too closely together, this outcome may occur again in healthy individuals. However, the presence of Rouleaux even in the thinnest parts of the blood film indicates that it is abnormal.[51]

Anisocytosis, a fluctuation in red cell size, can be measured with a micrometre or by contrasting it with a microscopic lymphocyte nucleus, which is around the same size or slightly smaller. Macrocytes are cells with a diameter larger than 9 m and a high concentration of haemoglobin. Haemoglobin in macrocytic, less developed erythrocytes has a bluish tint.[51,52]

The red cell should have a border of red to orange haemoglobin and a core pallor that is normally pale; hypochromia implies inadequate hemoglobinization and causes the central pallor to be more pronounced; A target cell with an area of pallor and a centre spot of haemoglobin may develop as a result of aberrant haemoglobin distribution.

Aside from inclusions like Pappenheimer bodies[52], Howell-Jolly bodies, mitochondrial fragments, siderosome fragments, infectious organisms (malaria parasites, babesiosis), red cell fragments, or schistocytes that could be signs of mechanical red cell disintegration, red cells can also contain other things.[53,54]

Additionally, red cell fragments or schistocytes that indicate mechanical red cell death are simpler to discover with blood smear tests.[55]

Platelet numbers and morphology are then inspected. Typically 1 to 2 m in diameter, platelets are tiny blue cytoplasmic pieces with red to purple granules that can have various shapes. Blood film analysis can be used to measure platelet numbers. An oil immersion field should have a high platelet count (5 to 15), or 1 platelet for every 10 to 20 RBCs.[56]It should be emphasised that if blood is not anticoagulated, platelets may congregate.[56,57]

Leukocyte shape and distribution make up the final analysis, which can be carried out by scanning the blood film at an intermediate power to count the number of leukocytes present. By paying great attention to the blood film's boundaries, mechanical effects that could result in an irregular distribution of bigger cells should be ruled out. [45,51]

Immature myeloid cells such blasts, metamyelocytes, promyelocytes, and myelocytes are clearly abnormal.[52,58,59]

Before making a manual white blood cell differential, at least 100 cells should be counted and recognised.[51,52] The WBCs that are most frequently observed in a blood smear include neutrophils, eosinophils, basophils, lymphocytes, and monocytes

AUTOMATED~HEMATOLOGY CELL COUNTERS

According to a study by Siemel DL et al., "Visual inspection of blood films vs. automated analysis of red cell distribution width," beta thalassemia trait, which is distinguished from iron deficiency anemia by increased microcytosis and preserved RBC haemoglobin concentration, is characterised by increased microcytosis.[16] To distinguish between subclinical anemias, it is helpful to have a basic awareness of histogram patterns.

The first parameter to be automatically calculated was the number of red blood cells; subsequent parameters to be determined automatically were the number of white blood cells, the measurement of haemoglobin, and a complete blood count that included the number of platelets. The automated haematology analyzer based on Coulter's principle was created fifty years ago.

Haematology analyzers can be broadly categorised into two groups based on their detecting method: 2) Optical and 1) Electric.[60,61]WH Coulter used a technique for measuring metal ions in suspension that was covered by a patent to count blood cells. Attempts to automate hematology analysis were started as a result. [61-72]

It is crucial that analyzers have the capability of doing precise WBC differential counts, especially those that can perform a five-part differential (counting neutrophils, lymphocytes, monocytes, and eosinophils). In addition to cell counting, the majority of automated haematology analyzers also do other hematologic parameters like leukocyte differentials, haemoglobin concentration (Hb), and red cell size.[41,73,74]

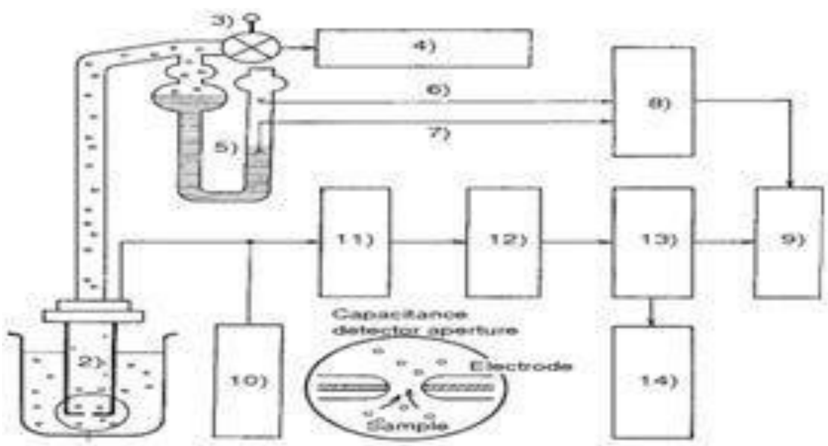
The Coulter principle is a technique for counting and sizing particles and cells. It is used to assess changes in electrical resistance brought on by non-conductive particles suspended in conductive isotonic electrolyte solution.

The Direct Current Impedance method and the Capacitance method, both of which have a similar structural makeup, are used to count blood cells. Both methods amplify and display on an oscillograph the change in electric impedance or capacitance of particles in a suspension passing through an aperture; each particle then generates a signal (pulse) that is subsequently tallied.[75,76]

Because a blood cell's electrical impedance differential is proportional to its volume as it passes through the aperture, the cell's volume can be determined by multiplying the impedance differential by a predetermined factor. Different blood cells have different behaviours. The typical RBC is quite malleable, taking on a fusiform shape, and prodding.

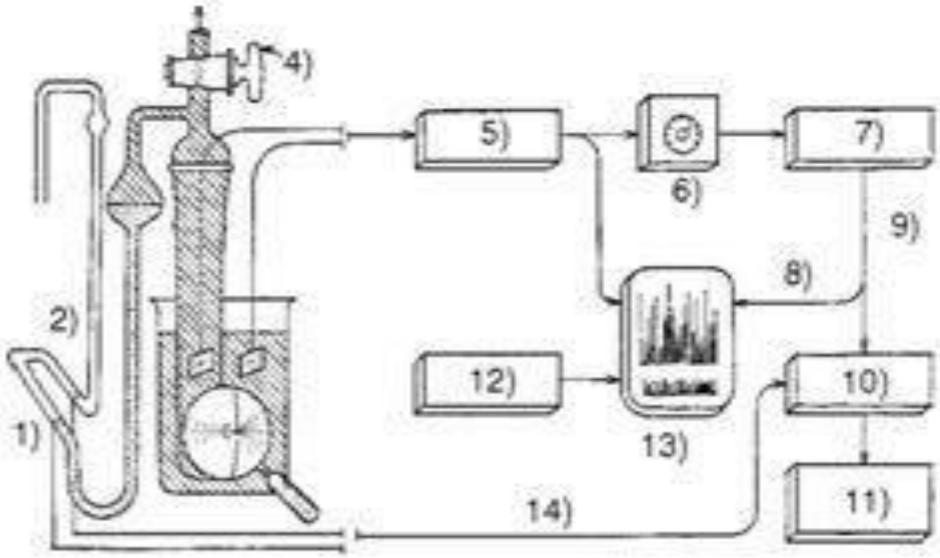
Effectively-mixed blood is considerably diluted/suspended in an isotonic electrolyte solution such that cell sizes are not altered. This isotonic electrolyte solution conducts electricity very well, whereas blood cells are non-conductive. This process is the basis for haematology counters.

The coincidence error occurs when two or more cells enter the aperture at the same time and can be measured and counted as one cell (as one high pulse), giving a high pulse indicating a very large cell. However, this error is greatly reduced by the massive dilution, the reduction in aperture dimensions, and the computer's statistical correction for this error.[75,76]



1) Start electrode
2) Stop electrode
3) To aspiration pump
4) Stop cock
5) Amplifier
6) Threshold circuit
7) Pulse amplifier
8) Pulse targeting signal exceeding threshold
9) Pulses exceeding given volume are takenand transferred to counter circuit
10) Counter driver
11) Numerical counter
12) Horizontal sweeps
13) Oscilloscope
14) Counter ··START·STOP··

Fig 4: Capacitance method used in automated counters[73]



1) Sample beaker
2) Detector
3) Cock
4) Aspiration pump
5) Manometer
6) Counting stop electrode
7) Counting start electrode
8) START·STOP(SU)
9) Count presentation (CU CUN)
10) Clog removal (AEC)
11) Detection (DU OU)
12) Amplification (HAU)
13) Discrimination/shaping (PU)
14) Monitoring (MU)

Fig 5: Electric impedance method used in automated counters[73]

A counting chamber is made up of the following parts: a beaker, two electrodes with direct current flowing through them, an orifice or small opening called an aperture with predetermined dimensions, and suspended cells or particles. Due to their non-conductivity between the two electrodes that are positioned on either side of the aperture, as the suspended cells or particles pass through the aperture, they will displace their own volume of the isotonic electrolyte solution and raise the electrical resistance (impedance).[75,76]

The impedance haematology counters, which work on the impedence principle, count and size the cells that are referred to here as particles rather than identifying them.[77]

Because white blood cells (WBCs) are larger than red blood cells and platelets, the RBC aperture is less than the WBC aperture.

The counter gives us the RBC, Platelet, and WBC counts, as well as a three-part differential that includes the counts of neutrophils, lymphocytes, and haemoglobin, and an extensive haematology menu. The counter computer receives the information mentioned above and uses it to do computations that provide useful data.[77]

In the modern world, automated blood counters have evolved, and many other cutting-edge equipment are in use.

A few examples include:

- Mindray- It uses the principle of impedence with low frequency direct current and fluorescence flow cytometry.
- Sysmex SE-It uses the principle of impedence with both low frequency direct current and radiofrequency current and fluorescence flow cytometry.
- Siemens Advia series- It uses the principle of light scattering and absorbance following peroxidase reaction.

Automated Hematology Counter Histograms

Visually scanning the histogram gives a strong initial understanding of the range, size, shape, and other salient characteristics of the red cell morphology, allowing judgments to be made quickly and economically. A histogram can occasionally reveal additional information that isn't always included in numerical data. The numerical data created automatically may not even show important information.[78,79]

The average sizes, frequency, and distribution of RBCs, WBCs, and platelets are all detailed in histograms. The computerised complete blood count (CBC) analysis now typically includes the RBC histogram, a graphic representation of particle size distribution, along with other CBC data.

It is commonly employed as a tool for monitoring and deciphering aberrant morphological changes, notably dimorphic red cells, together with the peripheral blood film. RBC distribution width (RDW) and mean corpuscular volume (MCV) are two metrics that have been reported to be aberrant in a variety of haematological illnesses and may offer important hints for the diagnosis and treatment of serious red cell disorders.

Monitoring the output of analyzers, investigating potential causes of inaccurate automated results, and aiding in the creation of a presumptive diagnosis can all be facilitated by a histogram.[81] Histograms can also be used to identify fragmented red blood cells, red blood cell agglutinations, and the gradual formation of new RBC populations in response to treatment.[82,83]

RBC HISTOGRAM

The histogram, a tool that is particularly useful in red cell morphological analysis, allows one to observe, assess, and grasp empirical data that visually represents morphological changes as peaks or valleys or as a line of frequency curve.[80]

The popular Coulter concept, which relies on the change in conductance that occurs as each red cell passes through an aperture to produce an electrical pulse whose amplitude is related to the cell volume, is used to create the histogram. The cells are divided into various size (volume) channels by an automatic analyzer using a set of criteria, from which the histogram is generated.

When the computer has finished processing the data, the RBC curve is smoothed using a moving average approach and shown on a data management system, allowing the relative number of cells (frequency) to be provided.

One can deduce either single or dimorphic populations of red cells from the histogram's shape. In single cell populations, the histogram may be extended with or without a left or right shift, skewed to one side or the other, or exhibit a mix of all these characteristics.[80]

Analysis and Interpretations of Histograms

A histogram can be comprehended by looking at the data's distributional pattern. The histogram of a single population will frequently be bell-shaped or Gaussian, expanded with or without a left or right shift, or exhibit any combination of all of these features. The histogram will have a traditional Gaussian or bell-shaped curve in dimorphic (many) red cell populations; it may be broadened with or without a left- or right-shift.[81]

The breadth and centering of the histogram, as shown by symmetric and/or bimodal, or bimodal but biased to the left or right, histograms, also affect the RBC variability.

Normal Histogram

The normal red cell distribution curve, which has a bell-shaped Gaussian distribution, should have an apex within the usual MCV range of 80 to 100 fl.When the distribution curve is narrow and wide, red cell populations are more diverse and are homogeneous when the distribution curve is narrow.

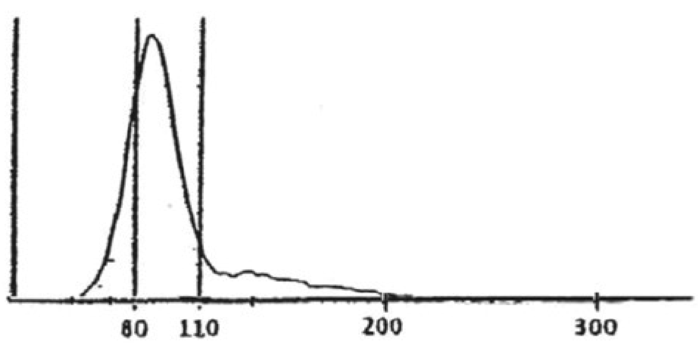


Fig 6: Normal RBCHistogram[80]

Histogram with Left Shift

The RBC histogram shifts to the left when microcytosis is present. The red cell distribution curves are pushed to the left and the percentage of microcytosis is elevated in beta thalassemia trait and iron deficiency anemia. The RDW metric of anisocytosis permits comparison despite similar histograms. Elevated RDW, which shows the heterogeneity in the acquired erythrocyte population, is a sign of iron deficiency anemia. The erythrocyte hereditary population is homogeneous, hence the RDW in the thalassemia trait is typically within normal limits. However, homogenous microcytosis can produce unreliable results in cases of severe anemia. [82]

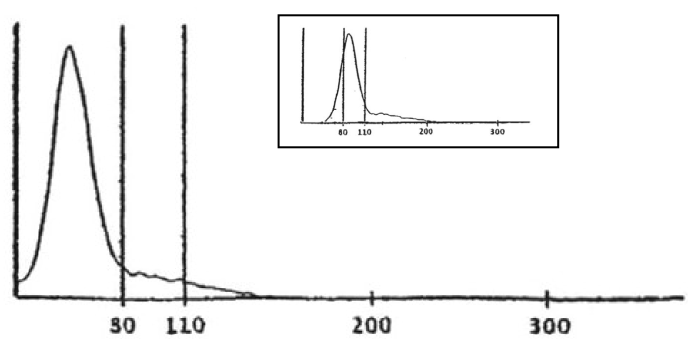


Fig 7: RBC Histogram inMicrocytosis[80]

Histogram with Right Shift

The rightward shift of the RBC Histogram indicates macrocytosis. A large portion of adult patients who have macrocytosis and an MCV greater than 100 fl and a normal RDW may not have anemia. This condition affects 3% to 5% of individuals.[84] Microscopically confirm the correlation between the MCV and the macrocytosis grading level.

It is occasionally possible to see a single, widely dispersed peak of cells in individuals with macrocytic anemia brought on by a vitamin B12 or folate deficiency. These cells range in size from microscopic cells and fragments of red blood cells on the left to macroytic cells approaching 250 fl on the right. The left's high takeoff and the trimline's 20% frequency show that there are many little cells.

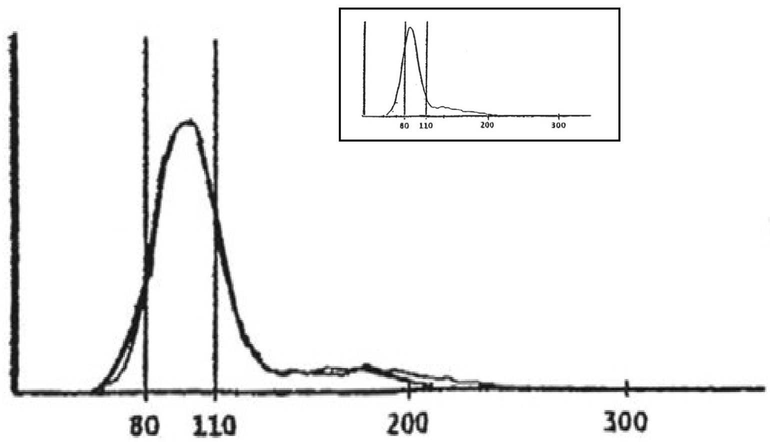
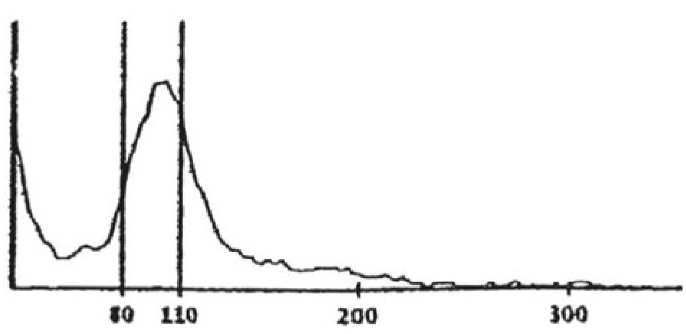


Fig 8: RBC Histogram in macrocytosis[80]

Histogram in Cold Agglutination[80]

The red cells agglutinate, giving the curve a U-shaped appearance, and interfere with their size and enumeration in response to high titer cold agglutinin. The histogram suggests the presence of two distinct populations, one on each side. The abnormal histogram is due to the high titer cold agglutinin that



induces red cells to agglutinate and obstructs their size and enumeration. The parameters most closely associated to red blood cells are denoted with an asterisk (*), indicating that the findings need to be reviewed and that the values for R D W should be substituted with dashes (—).

Fig 9: RBC Histogram in ColdAgglutination[80]

Histogram in SideroblasticAnemia[80]

As a result, there are two separate small cell populations in a bimodal distribution. Less than 15% must separate the two red cell populations in terms of volume. From a cluster of very small cells to a cluster of normal and macrocytic cells uniformly distributed on either side, the particle size distribution is incredibly varied. It is a mixture of small cells, regular cells, and gigantic cells in varying proportions.

Bimodal red cell histograms can be brought on by a variety of illnesses, but they often have something to do with therapeutic transfusion or the body's reaction to anemia. In a dual population image, there are truly two distinct red cell populations, whereas the histogram in an anisotropic image may contain two or more red cell populations. Red blood cells from a patient's own body or a mixture of cells from donors and the patient could make up these two groups. Dimorphic refers to either the dual cell population or the many red cell population.[85]

The important discoveries can be missed if only indices are employed since they fall within the predicted range. Under a microscope, the two red cell population can be seen, which explains the dimorphic appearance.

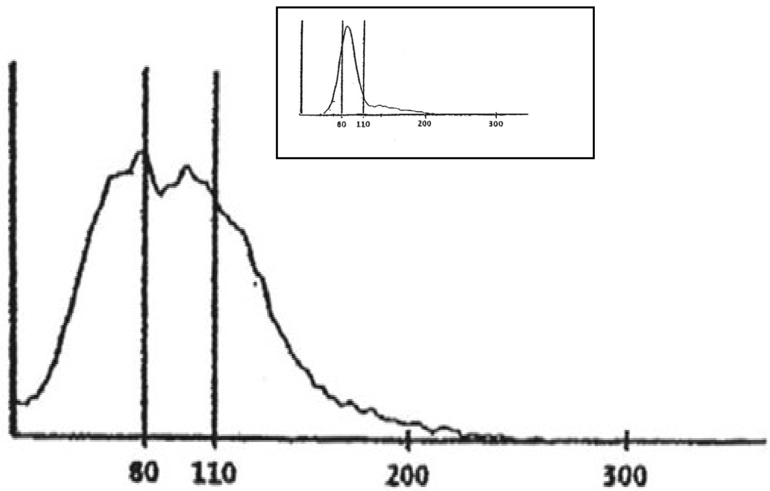


Fig 10: RBC Histogram in Sideroblastic Anemia[80]

BETA THALASSEMIA MAJOR[80]

Due to the tiny particles in this disorder, such as red cell fragments, nucleated R B C, Microspherocytes, and microcytic red cells, which provide an erroneous mean cell volume for the overall cell population, a considerable frequency of small cells can be seen in the beginning of the histogram. The curve appeared to be unimodal, but the instrument perceived it to be dimorphic because different cell populations were mixed together. The MCV, RDW, and histogram results will be impacted by any error cell population that is greater than 1% of the red cell population.[86]

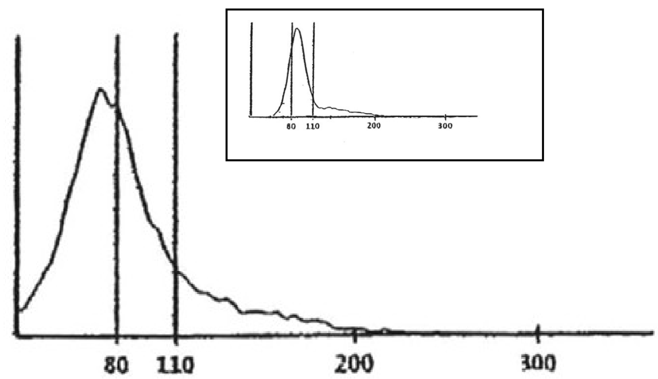


Fig 11: RBC histogram in Beta Thalassemia Major[80]

RETICULOCYTOSIS

Reticulocytosis has symptoms that are present in the right shoulder. The histogram is right-handed and bimodal. A reticulocytosis of this magnitude may arise three to eight days after effective iron therapy, resulting in a secondary peak that is associated with this new cell population. According to some writers, reticulocytosis is defined as a volume difference between two populations that is less than 15% and/or a reticulocytosis of more than 15%. Some claim that even at levels as low as 5% of the entire red cell population, it is possible to detect the emergence of a new cell population by skewing the histogram.[89]

The red cell histogram helps with IDA diagnosis and tracking the progress of treatment. Although individuals with IDA-induced microcytosis usually display a unimodal frequency curve, most patients experience the creation of a second population of normocytic cells with the right medication.

There is a tiny subset of reticulocytes that exclusively contributes to the right skew of the graph after three days of iron treatment.[90]

While the RDW keeps rising, the MCV now starts to rise.[87]A unique population of recently emerged normocytic cells with some polychromasia is visible on the graph and in the blood film after 2 to 4 weeks of therapy, but a secondary peak is still growing. Two symmetric peaks with almost equal proportions of hypochromic microcytic and normochromic normocytic cells emerged five to six weeks later.[91]The proportion of normocytic cells begins to rise seven to fifteen weeks later, and the histogram begins to skew to the left. After 17 weeks of successful therapy, a population of primarily normochromic normocytic cells with normal MCV and RDW predominates. In the absence of any problems or co-existing folate/vitamin B12 deficiencies, it appears that the histograms alter over time after iron therapy. However, the progression from initial reticulocytosis to growing normocytosis does not alter the pattern of red cell size changes.

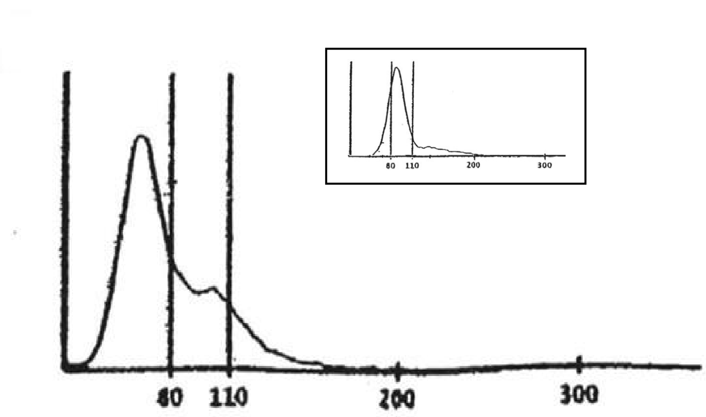


Fig 12: RBC Histogram in Reticulocytosis[80]

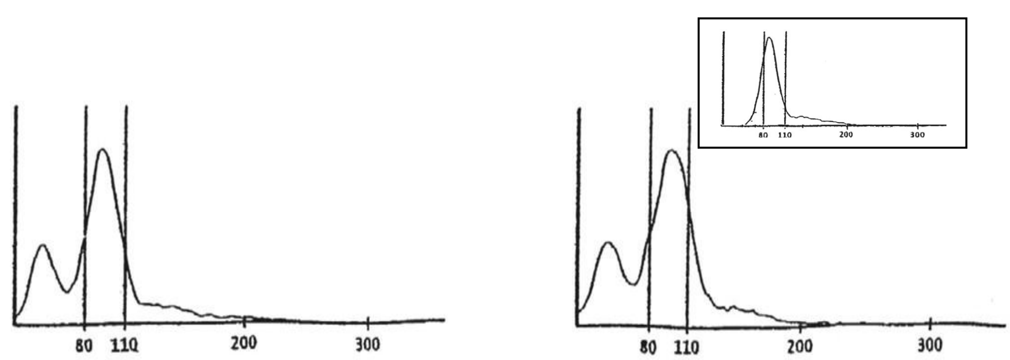


Fig13: RBC Histogram in Post Iron Therapy[80]

INTERPRETING RDW USING HISTOGRMAS

By lowering the denominator of the ratio, the MCV, microcytosis has a tendency to raise RDW-CV. For microcytic red cell disorders as opposed to macrocytic ones, RDW is a more sensitive test. 85 Macrophages undergo macrocytosis, which alters SD and lowers RDW CV. As a result, populations with big MCVs may appear broader on the histogram and produce a typical RDW-CV, whereas populations with extremely small MCVs may produce a huge RDW-CV while simultaneously appearing smaller.[92]

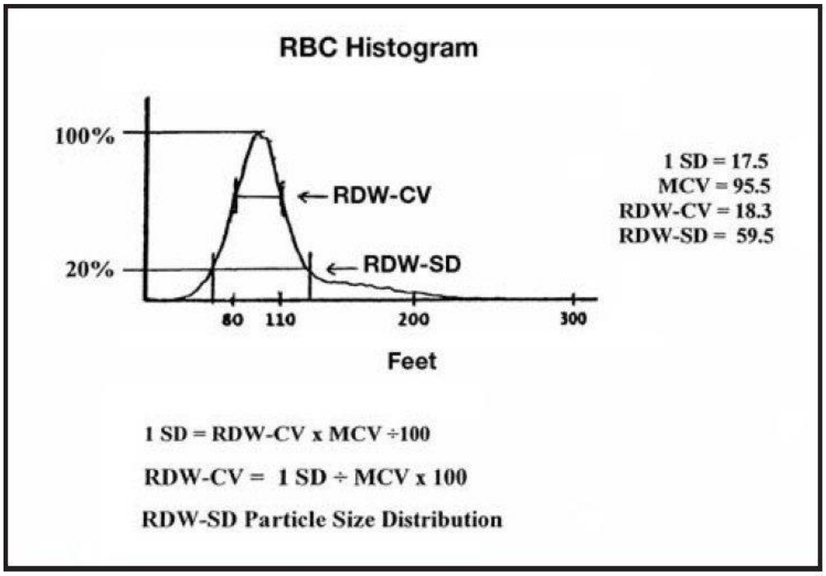


Fig14: Calculation of RDW Using Histograms[80]

The RDW-SD calculates the scatter or dispersion of values around the mean. Depending on how widespread the data is, the SD rises and vice versa. RDW-CVs are one type of measurement for data dispersion around the mean. The 1SD to the mean ratio is shown.

The RDW-SD is a superior and more accurate method to measure anisocytosis since it is a direct measure across the RBC histogram. Given that it contains the entire range of MCV values, it correctly represents the patients' actual morphological and pathological status.

The measurement, in contrast to RDW-CV, includes both large and small aberrant cells outside the 1SD with varying degrees of anisocytosis.

When the MCV is in the low normal range and anisocytosis is challenging to detect, the RDW - CV correlates better as an indicator of the condition. The level of anisocytosis or poiklocytosis is raised by RDW.[88]

THE PERIPHERAL BLOOD FILM: VERIFICATION OF AUTOMATED RESULTS IN THE CLINICAL PATHOLOGY LABORATORY[93]

For some flag inspection of the blood film is sufficient to confirm that the value of the parameter in question was measured correctly. For example, when thrombocytopenia is noted, a pathologist should scan the blood film for evidence of platelet clumping before concluding that the parameter's value was measured correctly.

When the WBC or platelet count is flagged, the automated value will be compared with an estimate obtained manually by counting the average number of platelets present in 5–10 high power (1000) fields (hpf) and/or the number of leukocytes in a comparable number of low power (100X) fields (hpf).

Evaluation of red cell abnormalities[93]

Blood film inspection helps in detection of abnormal cells in the early stages of iron deficiency before red cell indices and the RDW become abnormal. The film plays a more important role in the evaluation of other types of anemia. While detection of anisocytosis, poikilocytosis, or polychromasia often does not lead to a specific diagnosis, their severity usually reflects the seriousness of the disorder. More important· is the recognition of characteristic abnormalities such as sickled cells, dacrocytes, spherocytes, schistocytes, echinocytes, acanthocytes, or intracellular parasites. These can critically influence the direction of an anemia evaluation, and in some cases the findings are diagnostic.Blood cell differential counting is a crucial diagnostic technique for effective patient management and treatment. Therefore, accurate and effective examination of patient samples is essential. Current automated cell counters provide a leucocyte, red cell, and platelet count as well as a five-part leucocyte differential and a panel for screening red cell abnormalities. They are based on laser light scattering and flow cytochemical principles. Automated cell counters have the disadvantage of providing very little morphological information and being unable to accurately identify immature and diseased cells. Results are automatically indicated when anomalies are suspected in a sample. A blood film is generated for microscopic inspection and cell classification in these situations or if quantification of the diseased cells is required.

MATERIALS AND METHODS

Source of data:

Two hundred eighty patients with anemia in the age group 1-18 years as determined by decreased haemoglobin levels of less than the lower limit of the normal range for the particular age and sex at the diagnostic laboratory of Jawaharlal Nehru Medical College& KLEs Dr Prabhakar Kore Hospital and MRC were included in the present study.

Study design:

Cross sectional study

Study period: 1 year prospective (1st January 2021 to 31st December 2021)

Study population: Patients in pediatric age group (1-18years) who are clinically diagnosed as anaemia in JNMC and KLE'S DR. PRABHAKAR KORE HOSPITAL and MRC from 1st January 2021 to 31st December 2021.

Inclusion criteria:

All patients withhaemoglobin levels of less than the lower limit of the range for the particular age and sex were included in the study.Patients of age group 1-18 years were included in the study.

Exclusion criteria:

- Patients with haemoglobin levels greater than upper limit of the range for the particular age and sex
- Patients with leukaemia and platelet disorders.

Sample size: 280

The minimum sample size based on prevalence size is

$$n=4pq/d^2$$

Where p is the percentage of Prevalence, q= (100-p) and “d” is the relative error.

With p=80% and d=6% of p

$$=4.8$$

Thus the sample size comesupto approximately 280.

Method of Collection:

Hb levels were determined in EDTA sample by the 5-part differential autoanalyzer Sysmex XN 350. Peripheral blood smear of patients having lower limit of the range for the particular age and sex were made by push or wedge technique and Leishman staining is done(Procedure-Annexure). Then the slide is studied under microscope and provisional impression is made without referring to the histogram. The RBC histogram generated by the impedance principle and the RBC indices – MCV, MCHC, RDW-CV/RDW-SD were analysed. The impression derived by peripheral smear examination were then correlated with histogram patterns and RBC indices.

Data collection:

The present study will be conducted on cases with haemoglobin less than the lower limit of the normal range for the particular age and sex admitted at JNMC and KLES Dr. Prabhakar Kore Hospital Charitable Hospital and MRC, Belagavi.

After obtaining permission from the hospital authorities, the participants will be briefed about the study and informed consent will be taken from the prospective cases.

DATA Analysis:

The peripheral smear findings were evaluated for RBC morphology- RBC Anisocytosis and poikilocytosis were noted.

Anisocytosis

Anisocytosis is defined as variation in size greater than the normal. When the degree of variation is with in normal limits,cells are called as normocytic.When red cells are smaller than the nucleus of small lymphocytes they are called as microcytes. When they are larger than normal more than the nucleus of small lymphocytes, they are called macrocytes.

Grading the degree of anisocytosis

1+ Anisocytosis-Most red cells in the average oil immersion field appear uniform in size but5-10 cells per field are easily distinguished from the predominant cell line.

2+ Anisocytosis -10-15 cells per field are easily distinguished from the predominant cellline.

3+ Anisocytosis-15-25 cells per field are easily distinguished from the predominant cellline.

4+Anisocytosis-26-50 cells per field are easily distinguished from the predominant cellline.

Poikilocytosis

When the variation in shape is greater than normal, known as poikilocytosis.

If a particular abnormal shapes exceeds 5% of the cells percent, grading is done. Grading the degree of poikilocytosis

RESULTS AND ANALYSIS

This prospective study was conducted from January 2021 to December 2021.The RBCindices,histogramandperipheral smearof 280patients having hemoglobin levels less than the lower limit of the normal range for the particular age and sex were analyzed.

Age of the study group

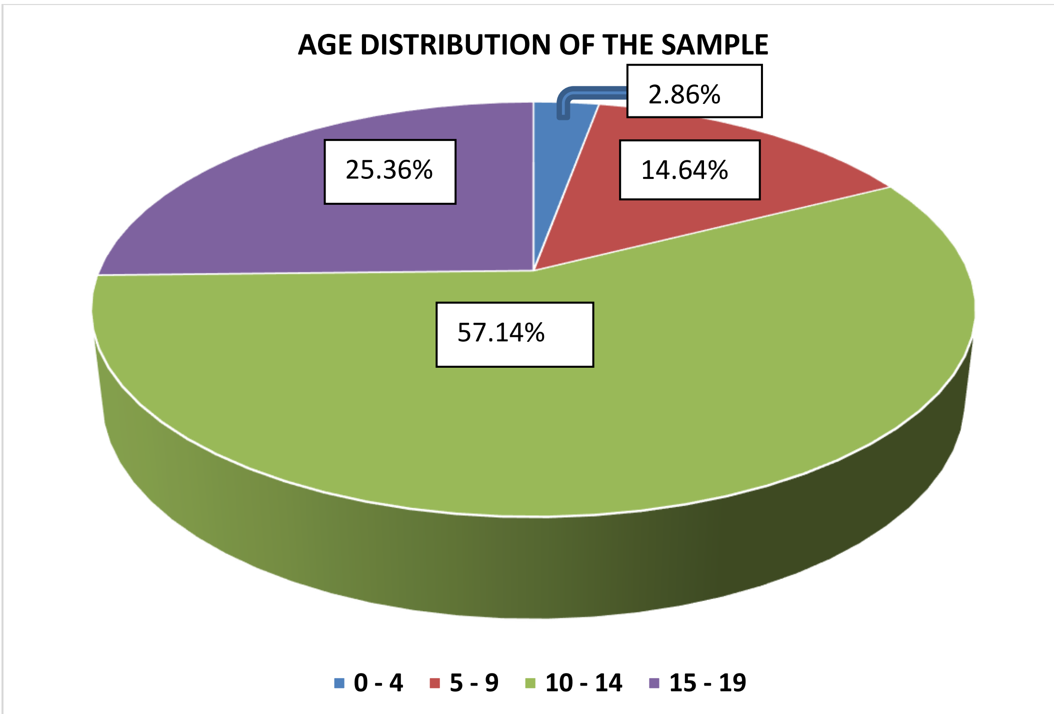
The age group of patients taken in this study ranged from 1 to 18 years.Majority of patients i.e57.14%(160 cases) were within 10 to 14 years of age followed by 25.36% (71 cases)in 15 to 19 years, 14.64%(41 cases) in 5 to 9 years and 2.86%(8 cases) in 1-4 years of age as shown in the table below**.**

Table No 2: Age distribution of cases with anemia

AGE	NUMBER	%
0 – 4	8	2.86
5 – 9	41	14.64
10 – 14	160	57.14

15 – 19	71	25.36
TOTAL	280	100.00

Graph No 1: Age distribution of cases with anemia



Sex distribution

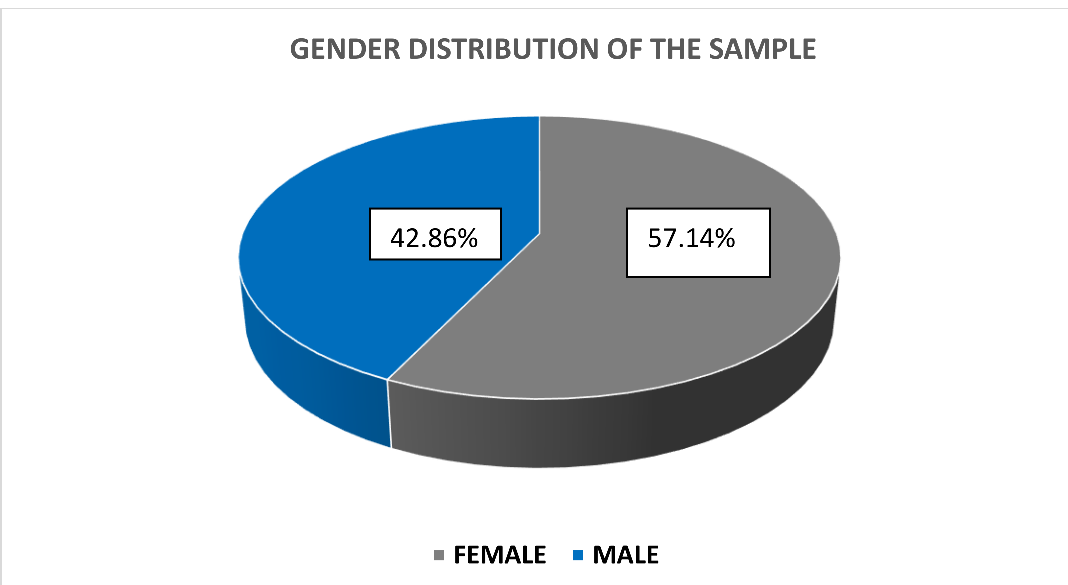
The bulk of the patients in the study were females. Out of 280 patients57.14% (160 cases) were females and 42.86% (120 cases)were males.

Table no 3: Sex distribution of cases with anemia

GENDER	NUMBER	%

FEMALE	160	57.14
MALE	120	42.86
TOTAL	280	100.00

Graph No 2: Sex distribution of cases with anemia



Age and gender wise distribution of anemia

Majority of females i.e 61.97% (44 cases) were in the age group from 15 to 19 years. Females are more commonly affected than males in this study as depicted in the table below.

Tables no 4: Age and gender wise distribution.

AGE	FEMALE	%	MALE	%	TOTAL
0 - 4	3	37.50	5	62.50	8
5 - 9	19	46.34	22	53.66	41
10 - 14	94	58.75	66	41.25	160
15 - 19	44	61.97	27	38.03	71
TOTAL	160	57.14	120	42.86	280

HISTOGRAM ANALYSIS:

Position of curve on histogram analysis.

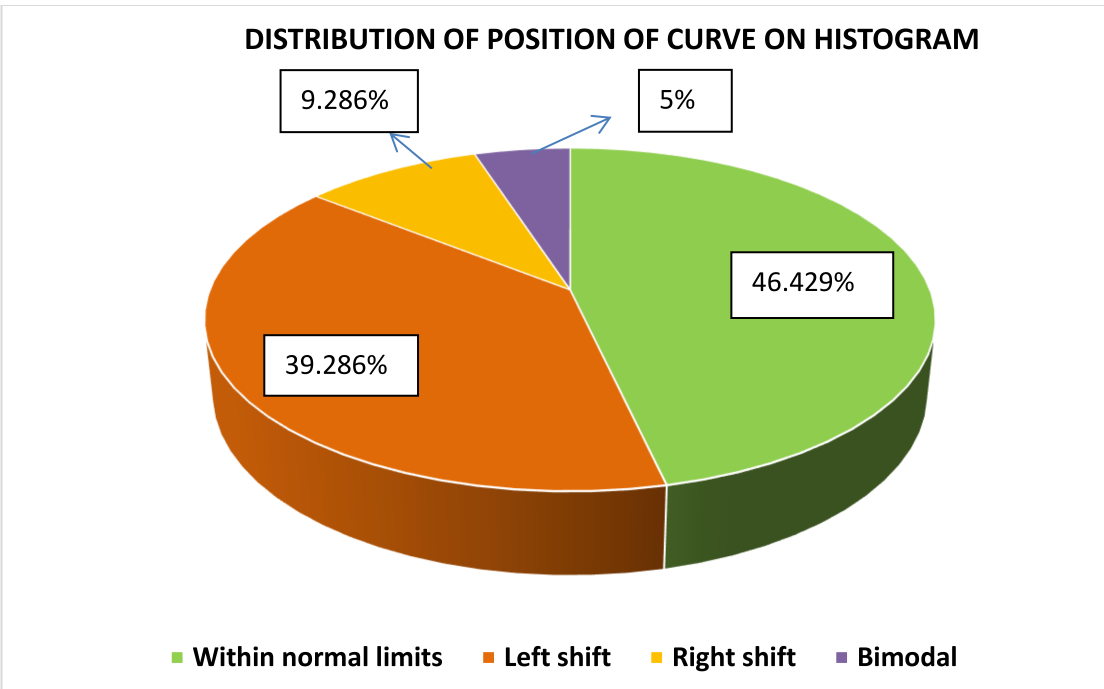
Majority of histograms showed curves within normal limits 46.429% i.e 130 cases followed by shift to left in 39.286% (110 cases), 9.286% (26 cases) showed shift to right and only 5% (14 cases) showed bimodal pattern.

Table no 5: Position of curve on histogram analysis.

POSITION	NUMBER	%
Within normal limits	130	46.429
Left shift	110	39.286

Right shift	26	9.286
Bimodal	14	5.000
TOTAL	280	100.000

Graph no 3: Position of curve on histogram analysis



Shape of curve on histogram analysis.

All 280 cases showed bell shaped curve (100%). Curve did not have broad base in majority 64.286% (180 cases), and broad base was present in only 35.714% (100 cases).

Table no 6: Shape of curve on histogram analysis.Bell shape

BELL SHAPE	NUMBER	%
PRESENT	280	100.000

ABSENT	0	0.000
TOTAL	280	100.000

Table no 7: Base of curve (Broadbase)

BROAD BASE OF CURVE	NUMBER	%
PRESENT	100	35.714
ABSENT	180	64.286
TOTAL	280	100.000

RBC parameters in study population:

Hemoglobin in study population ranged from 1.6 to 12.7 gram % with mean of 9.05gram%. MCV in study population ranged from 46.4 fl to 133.6 fl with mean of 82.54 fl.MCHCinstudypopulationrangedfrom23.2gm/dlto39.2g/dlwith meanof31.43 g/dl. MCH ranged from 11.7 pg to 41.4 pg with mean of 26.06 pg. RDW in ranged from 11.9 % to 40.8 %with mean of 18.31%.PCV ranged from 6.9% to 45.8% with mean of 29.30 %.And the age ranged from 2 to 18 years mean age being 12.14.

Table no 8: RBC parameters in study population.

	MEAN	S.D.	MIN	MAX
Hb	9.05	1.80	1.6	12.7
MCV	82.54	14.24	46.4	133.6
MCHC	31.43	1.83	23.2	39.2
MCH	26.06	5.10	11.7	41.4
RDW	18.31	10.35	11.9	40.8
PCV	29.30	5.95	6.9	45.8

RBC parameters in Normocytic Normochromic anemia

In cases with normocytic normochromic anemia, hemoglobin ranged from 4.1 to 12.5gm/dl, mean being 9.31 g/dl. MCV ranged from 87.4 fl to 100.7 fl with mean of 88.73 fl . MCHC ranged from 27.5 g/dl to 39.2g/dl with mean of 32.37g/dl. MCH in study population ranged from 25 pg to 37.9 pg with mean of 29.46pg.

RDW in ranged from 11.9 %to 17.2 %with mean of 17.09%. PCV in study population ranged from 12.8 % to 45.8%with mean of 29.45%. The age group in this type of anemia ranged from 2-18 years mean being 11.05 years.

Table no 9: RBC parameters in normocytic normochromic anemia.

	MEAN	S.D.	MIN	MAX
Hb	9.31	1.66	4.1	12.5
MCV	88.73	6.77	87.4	100.7
MCHC	32.37	1.18	27.5	39.2
MCH	29.46	5.87	25	87.9
RDW	17.09	15.05	11.9	17.2
PCV	29.45	5.84	12.8	45.8
AGE	11.05	3.70	2	18

Histogram pattern in normocytic normochromic anemia.

Histogram analysis showed bell shaped curve in all the cases. No shift toright or left was seen, and was within normal limit.

RBC parameters in Normocytic Hypochromic anemia

In cases with normocytic hypochromic anemia, haemoglobin ranged from 7.2 to 11.8 gm/dl, mean being 9.92 gm/dl.MCV ranged from 80fl to 88.1 fl with mean of 82.74 fl . MCHC ranged from 30.1 g/dl to 33.6g/dl with mean of 31.87g/dl. MCH in study population ranged from 24.2 pg to 26.9 pg with mean of 26.35pg.

RDW in ranged from 12.3 %to 21.6 %with mean of 15.92%. PCV in study population ranged from 22.5 % to 41.5%with mean of 31.77%. The age group in this type of anemia ranged from 9 to 18 years mean being 14.21 years.

Table no 10: RBC parameters in normocytic hypochromic anaemia

	MEAN	S.D.	MIN	MAX
Hb	9.92	1.29	7.2	11.8
MCV	82.74	2.50	80	88.1
MCHC	31.87	0.87	30.1	33.6
MCH	26.35	0.69	24.2	26.9
RDW	15.92	2.25	12.3	21.6
PCV	31.77	4.71	22.5	41.5
AGE	14.21	2.07	9	18

RBC parameters in Microcytic Hypochromic Anemia

In cases with Microcytic Hypochromic Anaemia, haemoglobin ranged from1.6 to 12.6 gm/dl, mean being 8.89gm/dl.MCV ranged from 46.4 fl to 79.9 fl with mean of 69.31 fl. MCHC in study population ranged from 23.2 g/dl to 35.2 g/dl with mean of 30.44 g/dl. MCH in study population ranged from 11.7 pg to 29.7 pg with mean of 21.30 pg. RDW in study population ranged from 13.2% to 38.3 %with mean of 18.90%. PCV in study population ranged from 6.9% to 41.2% with mean of 29.22%. The age group in this typeof anemia is from 2-18 years mean being 12.75 years.

Table no 11: RBC parameters in microcytic hypochromic amimia.

	MEAN	S.D.	MIN	MAX
Hb	8.89	1.83	1.6	12.6
MCV	69.31	7.77	46.4	79.9
MCHC	30.44	2.01	23.2	35.2
MCH	21.30	3.43	11.7	29.7
RDW	18.90	3.97	13.2	38.3
PCV	29.22	5.44	6.9	41.2
AGE	12.75	3.35	2	18

Histogram analysis in microcytic hypochromic anemia.

The histogram pattern seen in microcytic hypochromic was shift to left.

RBC parameters in Macrocyticanemia.

In cases with Macrocytic anemia, hemoglobin ranged from 3.9 to 12.7 gm/dl, mean being 8.58 gm/dl. MCV ranged from 100.1fl to 133.6 fl with mean of 109 fl.

MCHC in study population ranged from 28.8 g/dl to 33.9 g/dl with mean of 31.92 g/dl. MCH in study population ranged from 29.2 pg to 41.4 pg with mean of 34.08 pg. RDW in study population ranged from 12.3% to 27.3 % with mean of 16.53%.

PCV in study population ranged from 12.1% to 45% with mean of 28.85%. The age group in this type of anaemia ranges from 5-17 years mean being 12.81 years.

Table no 12: RBC parameters in macrocytic anemia.

	MEAN	S.D.	MIN	MAX
Hb	8.58	1.98	3.9	12.7
MCV	109.00	8.54	100.1	133.6
MCHC	31.92	1.16	28.8	33.9
MCH	34.08	3.04	29.2	41.4
RDW	16.53	3.33	12.3	27.3
PCV	28.85	7.85	12.1	45
AGE	12.81	2.58	5	17

Histogram analysis of Macrocyticanemia

Histogram pattern showed shift to right in all cases. Most of the cases showed broad base curve.

RBC parameters in Dimorphic anemia

In cases with Dimorphic anemia, hemoglobin ranged from 5.4 to 12.7gm/dl, mean being 7.97gm/dl. MCV ranged from 66.1 fl to 122.9 fl with mean of 86.63 fl. MCHC in study population ranged from 27.4 g/dl to 35.7 g/dl with mean of 30.54 g/dl.MCH ranged from 21.2 pg to 35.1 pg with mean of 26.29 pg. RDW ranged from 17.9% to40.8% with mean of 28.58%. PCV in study population ranged from 15.6 %to 42.6 %with mean of 26.33% The age group in this type of anaemia is from 5-16 years mean age being 10.92 years.

Table no 13: RBC parameters in dimorphic anemia.

	MEAN	S.D.	MIN	MAX
Hb	7.97	2.19	5.4	12.7
MCV	86.63	14.71	66.1	122.9
MCHC	30.54	2.06	27.4	35.7
MCH	26.29	4.50	21.2	35.1
RDW	28.58	7.57	17.9	40.8
PCV	26.33	7.59	15.6	42.6
AGE	10.92	3.15	5	16

Histogram analysis of dimorphic anemia:

Bimodal pattern was seen in majority of cases with broad based curve and shift to left predominantly.

Impression based on histogram analysis/RBC indices

Further in our study, cases were analysed based on histogram and RBC indices. Majority 113 (40.357%) were normocytic normochromic anemia followed by Microcytic anaemiai.e110 (39.285%)cases. Macrocytic and dimorphic were9.286% and 5% respectively.

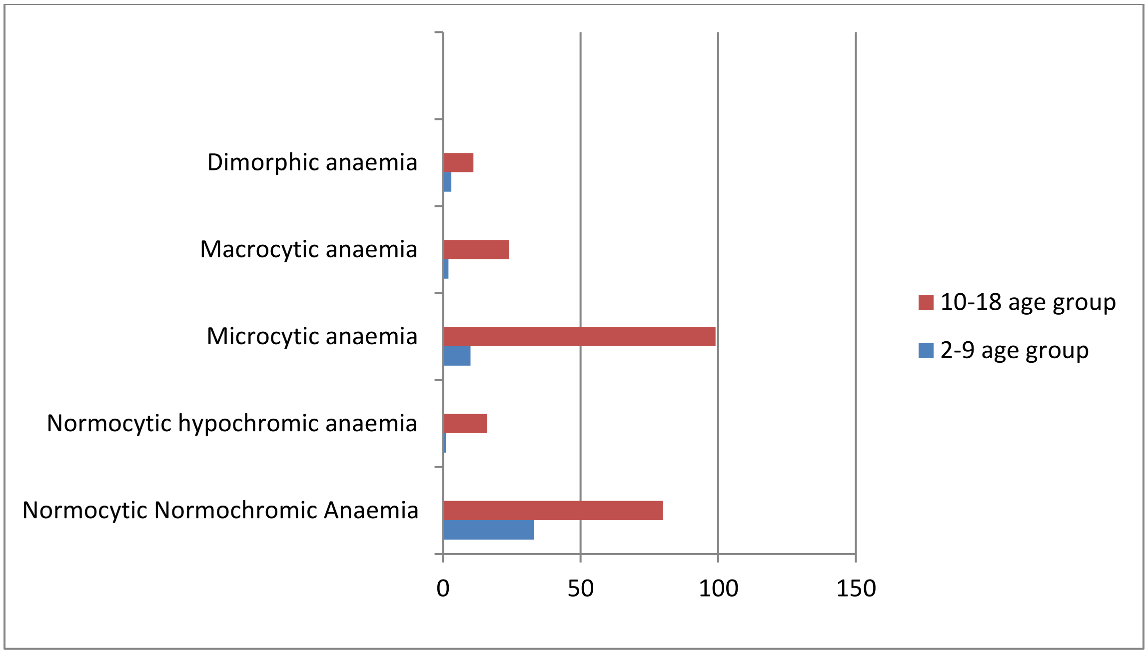
Table no 14: Impression based on histogram analysis/RBCindices.

IMPRESSION ON HISTOGRAM ANALYSIS AND RBC INDICES	NUMBER	%
NORMOCYTIC NORMOCHROMIC ANEMIA	113	40.357
NORMOCYTIC HYPOCHROMIC ANAEMIA	17	6.071
MICROCYTIC HYPOCHROMIC ANEMIA	110	39.285
MACROCYTIC ANEMIA	26	9.286
DIMORPHIC ANEMIA	14	5.000
TOTAL	280	100.000

Table no 15: Impression on histogram and RBC indices according to age group

	AGE		
IMPRESSION ON HISTOGRAM ANALYSIS AND RBC INDICES	**2 - 9**	**10 - 18**	**TOTAL**
NORMOCYTIC NORMOCHROMIC ANEMIA	33	80	113
NORMOCYTIC HYPOCHROMIC ANAEMIA	1	16	17
MICROCYTIC HYPOCHROMIC ANEMIA	10	100	110
MACROCYTIC ANEMIA	2	24	26
DIMORPHIC ANEMIA	3	11	14
TOTAL	**49**	**231**	**280**

Graph 4: Impression on histogram and RBC indices according to age group



On analysis of histogram and RBC indices, we found that microcytic hypochromic anaemia is predominantly seen in the age group of 10-18 years followed by normocytic normochromic anaemia.In 2-9 years of age, normocytic normochromic anaemia is more common in our study.For the above table, the value of p has been calculated using chi square test. P value is 0.0015 which is statistically significant in this study.

PERIPHERAL SMEAR EXAMINATION

Anisocytosis on peripheral smear examination.

Anisocytosis score 1 was seen in majority of cases 144 cases(51.42%) followed by 103 cases(36.78%)which showed score 2. Score 3 was seen in least no of cases 22(9.286%). Remaining 11(3.9%) cases showed score 4.

Table No 16: Anisocytosis on peripheral smear examination

Anisocytosis Score	NUMBER	%

1	144	51.42
2	103	36.78
3	22	7.8
4	11	3.9
Total	280	100.000

Poikilocytosis on peripheral smear examination.

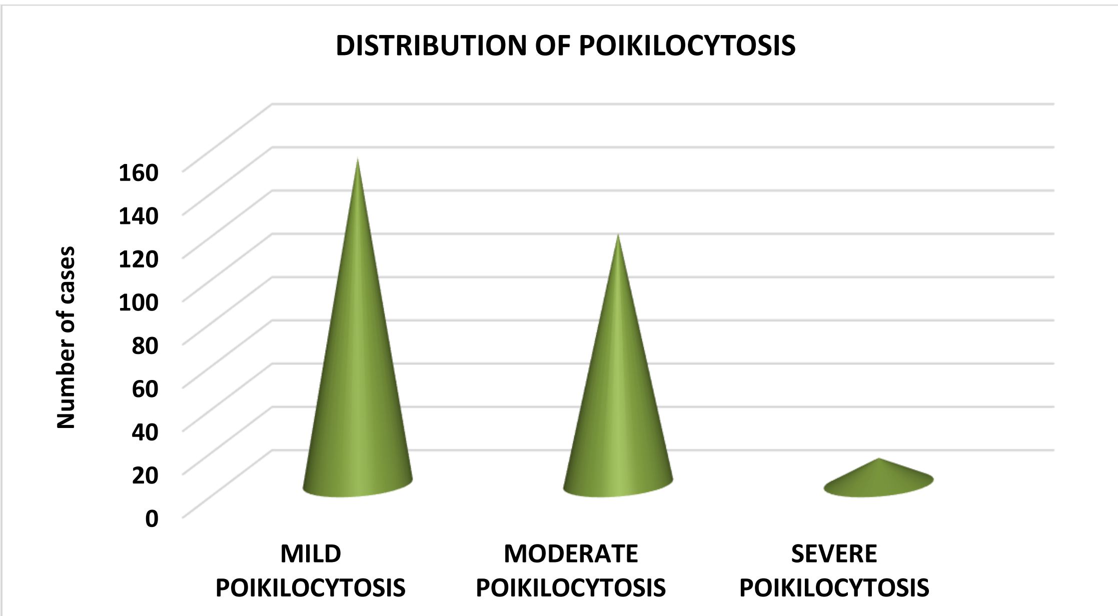
Majority of cases showed mild poikilocytosis 151 cases(53.929%). Moderate poikilocytosis was seen in 117(41.78%) of cases. Only few 11 cases (3.92%) showed severe poikilocytosis.

Table no 17: Poikilocytosis on peripheral smear examination.

POIKILOCYTOSIS	NUMBER	%
MILD POIKILOCYTOSIS	151	53.929
MODERATE POIKILOCYTOSIS	117	41.78
SEVERE POIKILOCYTOSIS	11	3.92

TOTAL	280	100.000

Graph no 5: Poikilocytosis on peripheral smear examination.



Impression based on peripheral smear examination:

Majority 129 cases (40.357%) were normocytic normochromic anemia. Microcytic, macrocytic and dimorphic were 36.78%,9.286% and 5% respectively.

MORPHOLOGICAL TYPE OF ANAMEIA ON PERIPHERAL SMEAR	NUMBER	%

NORMOCYTIC NORMOCHROMIC ANEMIA	129	46.071
NORMOCYTIC HYPOCHROMIC ANAEMIA	15	5.357
MICROCYTIC HYPOCHROMIC ANEMIA	103	36.78
MACROCYTIC ANEMIA	22	7.857
DIMORPHIC ANEMIA	11	3.929
TOTAL	280	100.000

Table no 18: Morphological type of anemia based on peripheral smear

Graph no 6: Morphological type of anemia based on peripheral smear

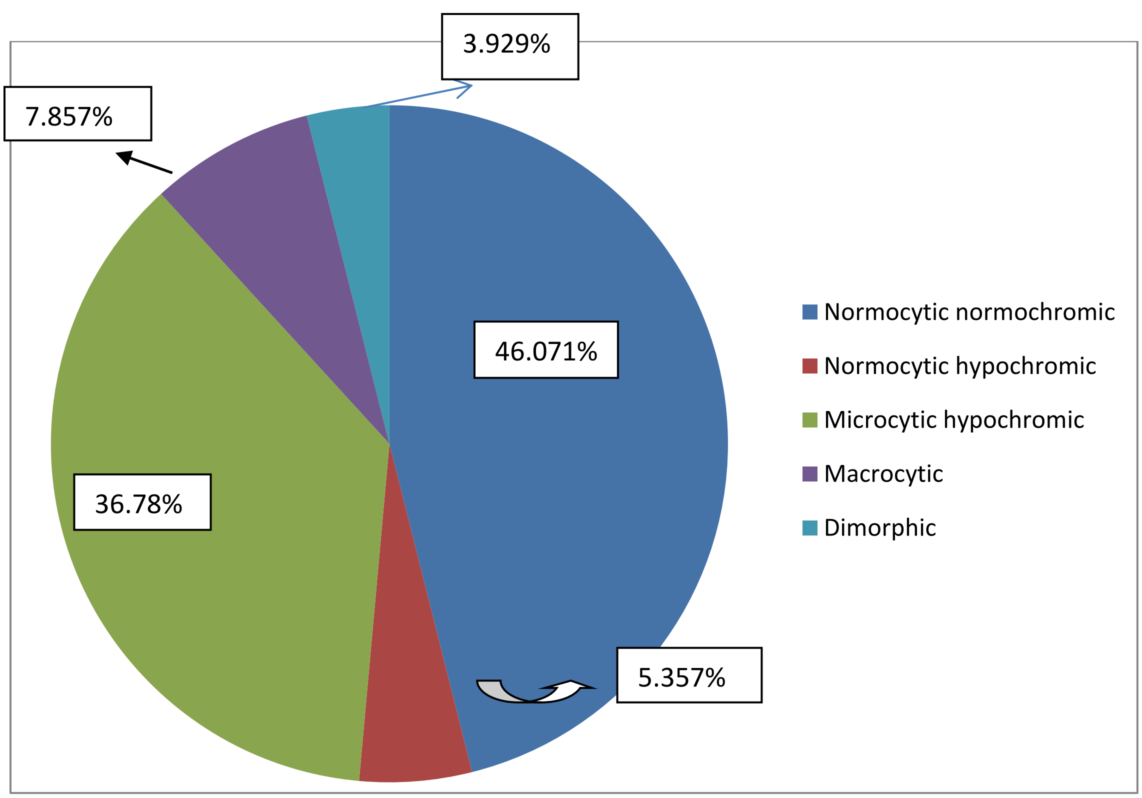


Table no 19: Morphological type of anemia according to age group

	AGE		
MORPHOLOGICAL TYPE OF ANAEMIA BASED ON PERIPHERAL SMEAR	**2 - 9**	**10 - 18**	**TOTAL**
NORMOCYTIC NORMOCHROMIC ANEMIA	29	100	129
NORMOCYTIC HYPOCHROMIC ANAEMIA	1	14	15
MICROCYTIC HYPOCHROMIC ANEMIA	10	93	103
MACROCYTIC ANEMIA	3	19	22
DIMORPHIC ANEMIA	4	7	11
TOTAL	**47**	**233**	**280**

On peripheral smear analysis we found that normocytic normochromic anaemia is predominantly seen in the age group 10-18 years followed by microcytic hypochromic anaemia. In 2-9 years, normocytic normochromic anaemia is more common in our study.

For the above correlation, p value has been calculated using chi square test which showed statistically significant result with p value less than 0.0001.

Table no 20: Anisocytosis score in morphological type of anaemia

	MORPHOLOGICAL TYPE OF ANAEMIAON PERIPHERAL SMEAR					
Anisocytosis	**NNA**	**NHA**	**MHA**	**Macrocytic anaemia**	**Dimorphic anaemia**	**TOTAL**
1	125	15	2	0	2	144
2	2	0	100	0	0	102
3	0	0	0	23	0	23
4	2	0	0	0	9	11
Total	129	15	102	22	11	280

The score of anisocytosis was correlated with the morphological type of anaemiaon peripheral smear and was statistically analysed. Majority of the cases of normocytic normochromic anaemia showed an anisocytosis score of 1. Score 2 was predominantly seen in cases of microcytic hypochromic anaemia.Score 3 and 4 were mostly seen in cases of macrocytic and dimorphic anaemia.The value of p has been calculated using chi square test which is less than 0.0001. There is highly significant association between score ofanisocytosis and morphological type of anaemia on peripheral smear.

Table no 21: Severity of poikilocytosis in morphological type of anaemia

	MORPHOLOGICAL TYPEOF ANAEMIA ON PERIPHERAL SMEAR					
POIKILOCYTOSIS	**NNA**	**NHA**	**MHA**	**Macrocytic anaemia**	**Dimorphic anaemia**	**TOTAL**

MILD	92	9	37	13	0	151
MODERATE	37	6	62	9	4	117
SEVERE	0	0	4	0	7	11
TOTAL	129	15	102	22	11	280

The severity of poikilocytosis was also correlated with peripheral smear finding. Majority of the cases of normocytic normochromic anaemia show mild poikilocytosis. Moderate poikilocytosis is mostly seen in cases of microcytic hypochromic anaemia. Severe poikilocytosis is seen in cases of dimorphic anaemia in this study.

The value of p using chi-square test is less than 0.0001.

There is highly significant association between morphological type of anaemia on peripheral smear and severity of poikilocytosis.

Table no 22: Correlation between anisocytosis score and severity of poikilocytosis

POIKILOCYTOSIS				
Anisocytosis Score	**MILD**	**MODERATE**	**SEVERE**	**TOTAL**
1	100	43	1	144
2	38	59	5	102
3	13	10	0	23
4	0	5	6	11
Total	151	116	13	280

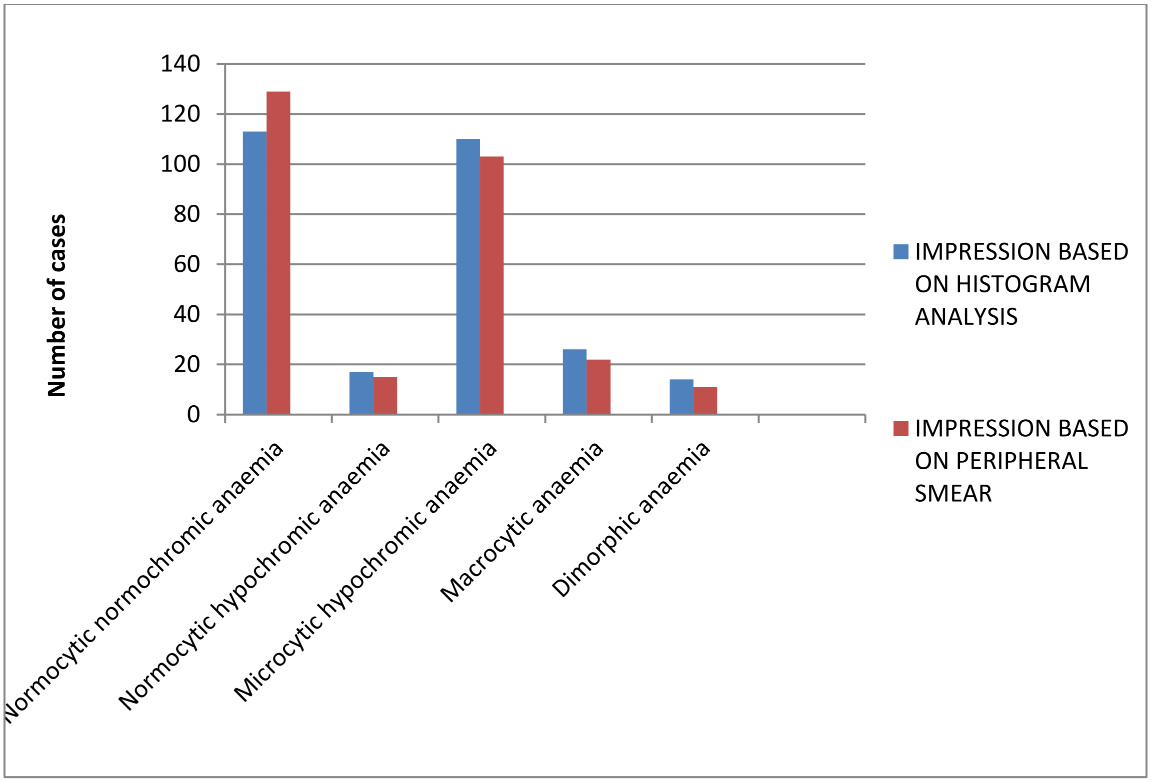
The correlation was seen between the score of anisocytosis and severity of poikilocytosis also.Most of the cases with anisocytosis score of 1 show mild poikilocytosis. Moderate poikilocytosis are seen in cases with anisocytosis score 2. Severe degree of poikilocytosis correspond to the score 4 of anisocytosis.This was statistically analysed and p value was calculated.The value of p using chi square test is less than 0.0001(hs). There is highly significant association between score of anisocytosis and severity of poikilocytosis.

Table No 23: agreement between the two procedures of assessment

				MORPHOLOGICAL TYPE OF ANAEMIA ON PERIPHERAL SMEAR FINDING			
	IMPRESSION ON HISTOGRAM ANALYSIS AND RBC INDICES	**NNA**	**NHA**	**MHA**	**Macrocytic anaemia**	**Dimorphic anaemia**	**TOTAL**
1	**NORMOCYTIC NORMOCHROMIC**	113	0	0	0	0	113

	ANEMIA						
2	**NORMOCYTIC HYPOCHROMIC ANAEMIA**	2	15	0	0	0	17
3	**MICROCYTIC HYPOCHROMIC ANEMIA**	8	0	102	0	0	110
4	**MACROCYTIC ANEMIA**	4	0	0	22	0	26
5	**DIMORPHIC ANEMIA**	2	0	1	0	11	14
	TOTAL	129	15	103	22	11	280

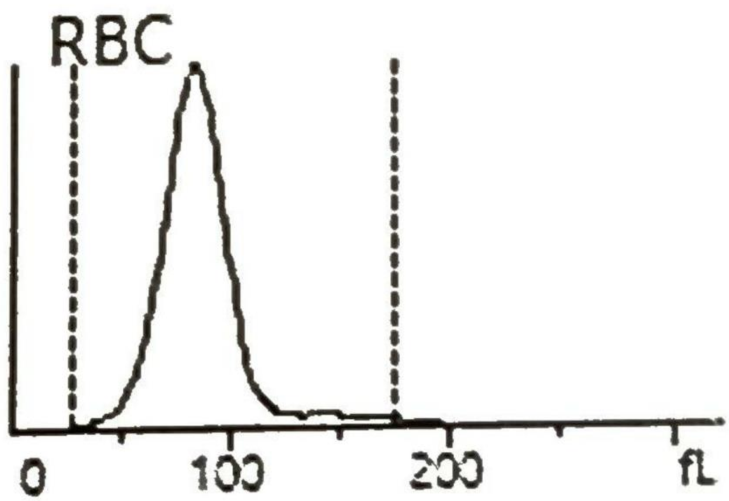
Graph no 7: Correlation between impression on histogram analysis and on peripheral smear



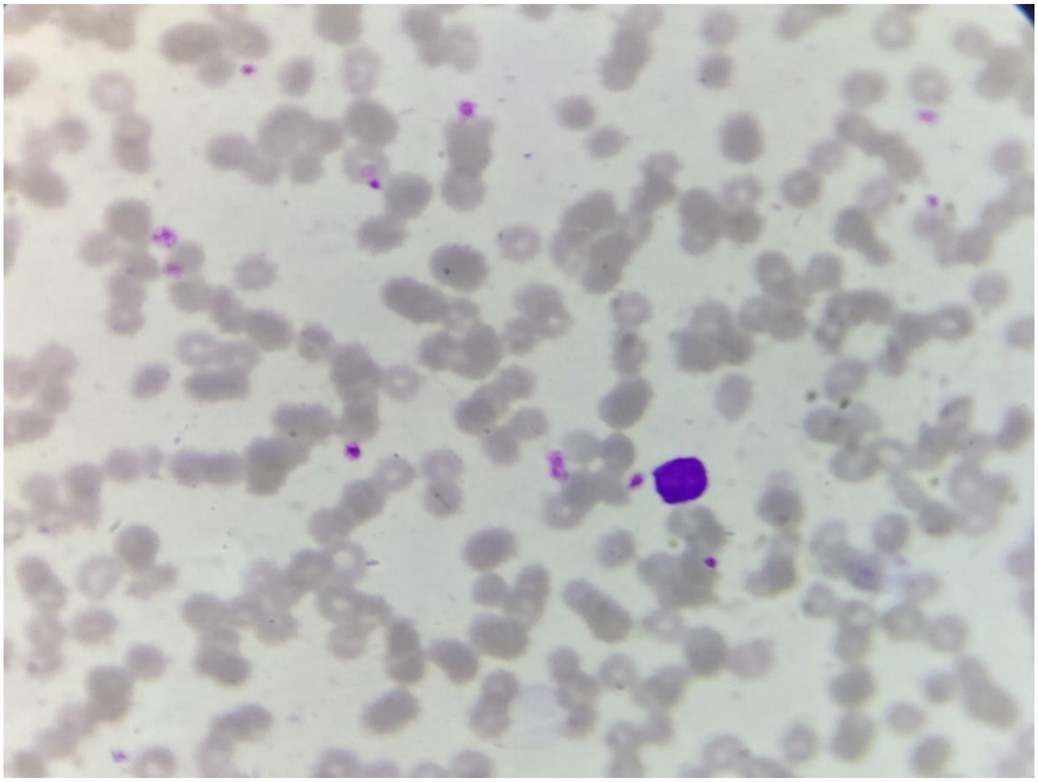
Further impression made on the morphological type of anaemia by Peripheral smear vs RBC histogram and indices were statistically analysed. The correlation between the morphological types of anaemia based on Peripheral smear vs RBC histogram and indices was analyzed using Cramer's V(0.523) where the value is 0.9302 with p <0.0001, which was statistically significant. Kappa statistics for agreement between the two methods=0.9078(moderate agreements),p<0.0001.Both these suggest there is high degree of agreement between the two procedures of assessment.

PHOTOS

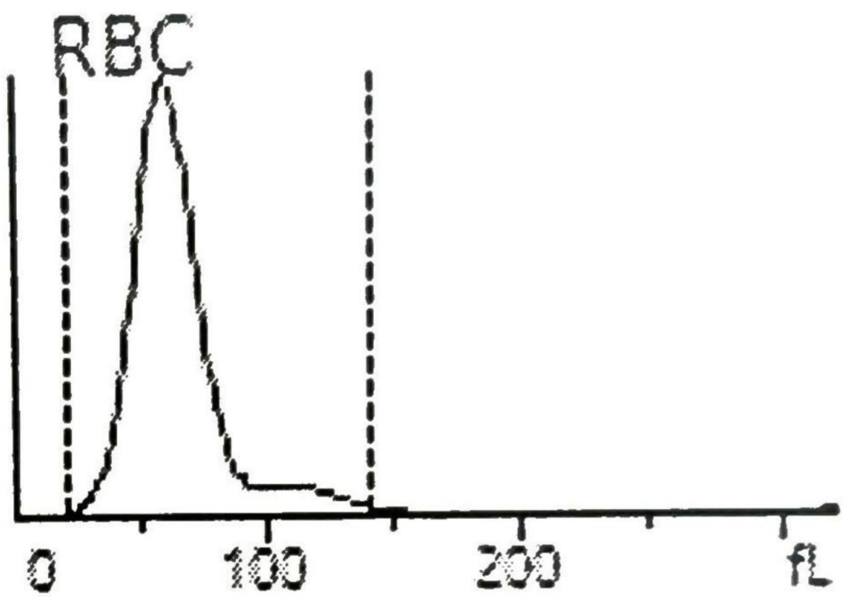
Picture 1: RBC histogram of normocytic normochromic anemia



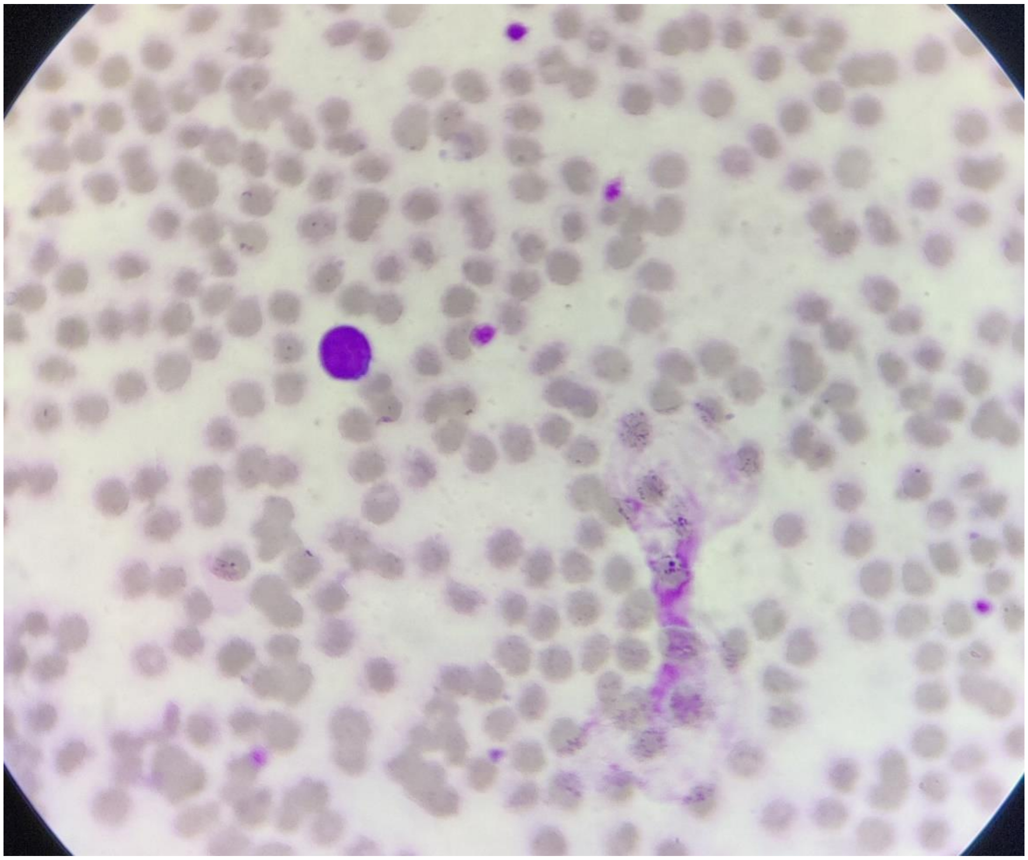
Picture 2: Peripheral smear of Normocytic normochromic anemia



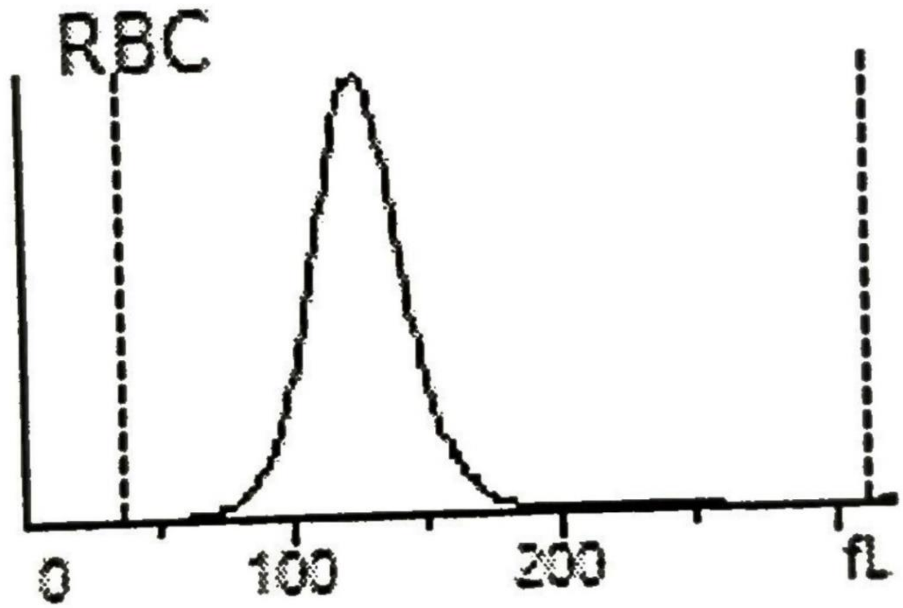
Picture 3: RBC histogram of Microcytic hypochromic anemia



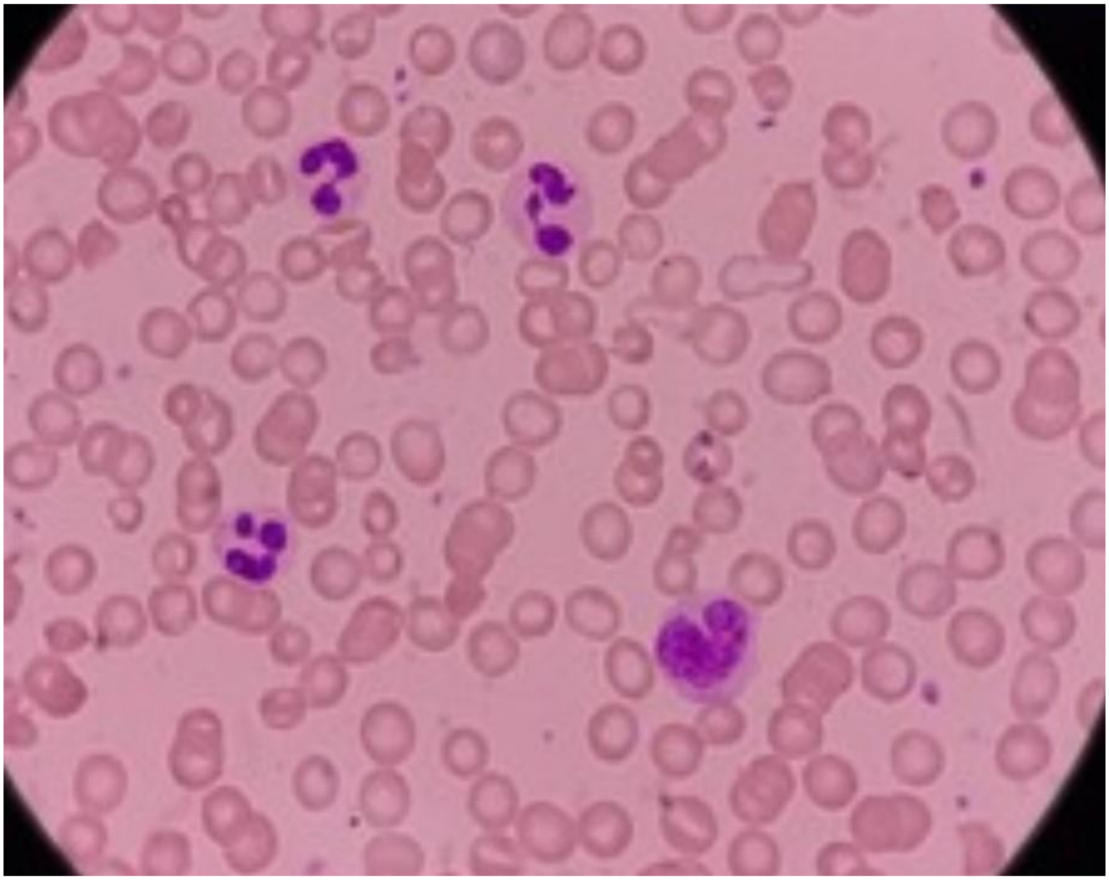
Picture 4: Peripheral smear of Microcytic hypochromic anemia



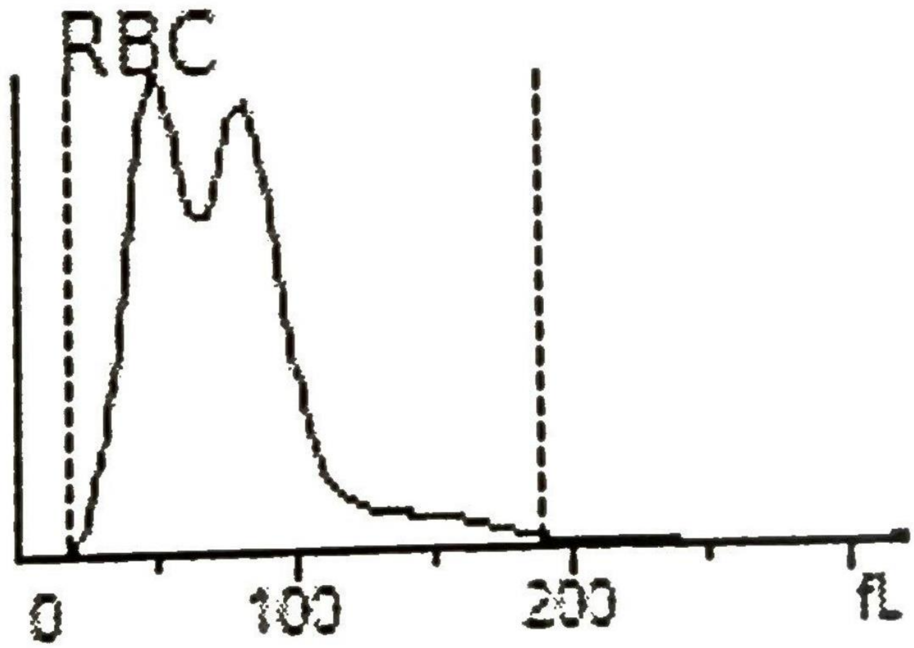
Picture 5: RBC histogram of Macrocytic anemia



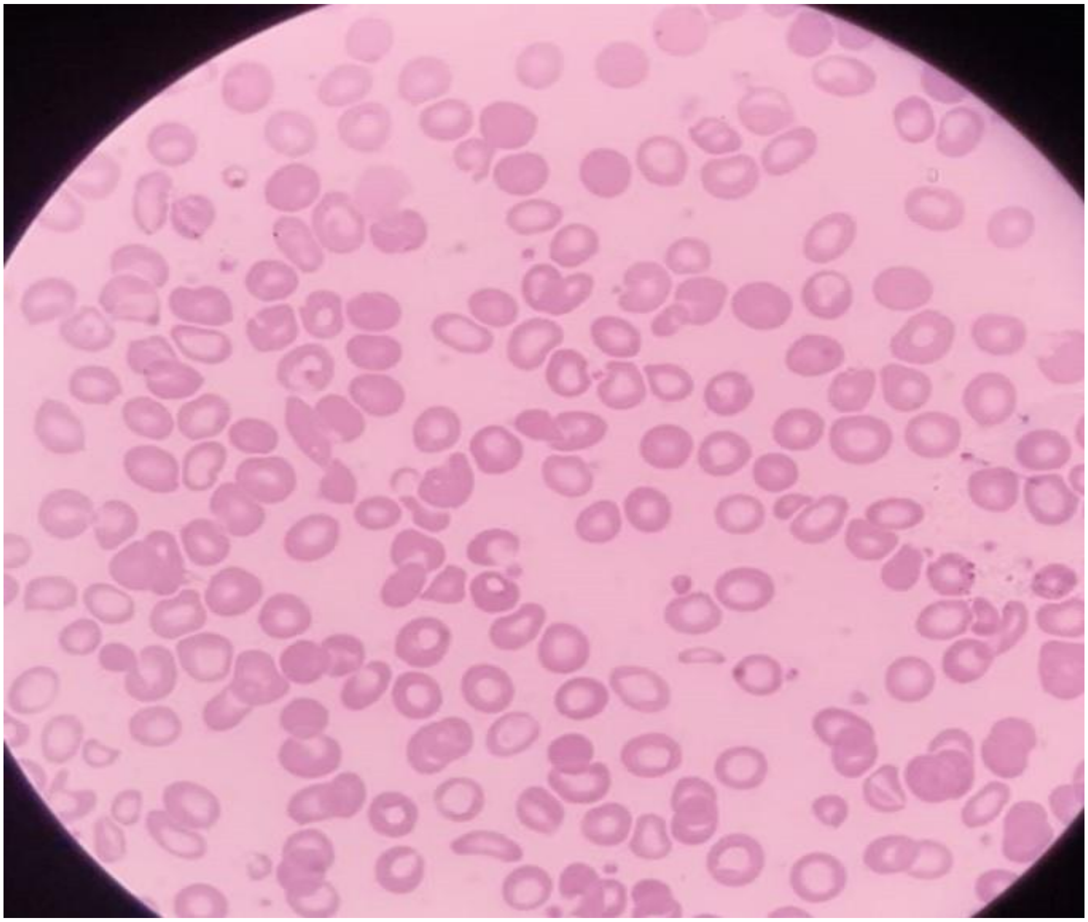
Picture 6: Peripheral smear of Macrocytic anemia



Picture 7: RBC histogram of Dimorphic anemia



Picture 8: Peripheral smear of Dimorphic anemia



Age:

In our study the population taken into consideration is 2-18 years mean age being 12.14. It can be compared to similar study done by Prakash A et al, Bhushan D Kamble et al and Rosemerry Ferreira et al

Table No 24: Comparision of patients in different age group

<table>
<tr><th>Age Group</th><th>Prakash A et al[94]</th><th>Bhushan D Kamble et al[95]</th><th>Present study</th></tr>
<tr><td>0-4</td><td>40.7%</td><td rowspan="2">61.7%</td><td>2.86%</td></tr>
<tr><td>5-9</td><td>30.7%</td><td>14.64%</td></tr>
<tr><td>10-14</td><td>8.6%</td><td rowspan="2">46.3%</td><td>57.14%</td></tr>
<tr><td>15-18</td><td>20%</td><td>25.36%</td></tr>
</table>

Table No 25: Comparison of patients under 5 years

AGE	Rosemerry Ferreira et al[96]
6 months-1 year	70.9
1 -2 year	65.1
2- 3 years	54.8
3-5 years	36.2

In our study we have found out that majority of the cases having anaemia are in the age group 10-14 years whereas in the study by Prakash A et al showed the predominance of anaemia is in the age group 0-4 years. The reason for the discordance might be the small sample size of the former study which included only 150 participants. Our present study is also in concordance with a study by BhushanDKamble et al which showed that majority of the anaemia is below the age group of 15 years.

Another study by Rosemerry Ferreira et al showed most of the anaemic cases are less than one year. But limitation of the study is that it considered population only under 5 years. So this study was discordant with our study.

Sex distribution

The total numbers of cases of anemia in our study was 280. Out of these majority were females 160 (57.14%) and 120 (42.86%) were males. This is comparable to the previous studies done as shown in table below.

Table no 26: Comparison of sex distribution

Sex	Anisetti&Komuravelli (2021)[97]	Ingale SV et al (2017)[98]	Present Study
Female	52.81%	61.5%	57.14%
Male	47.19%	38.5%	42.86%

In our study it was seen that majority were female patients which are in the age group 10-18 years. These results were in concordance with the studies conducted by Anisetti&Komuravelli and Ingale SV et al. The prevalence of anaemia was higher among females than males, as would be expected because of blood loss during these reproductive age.

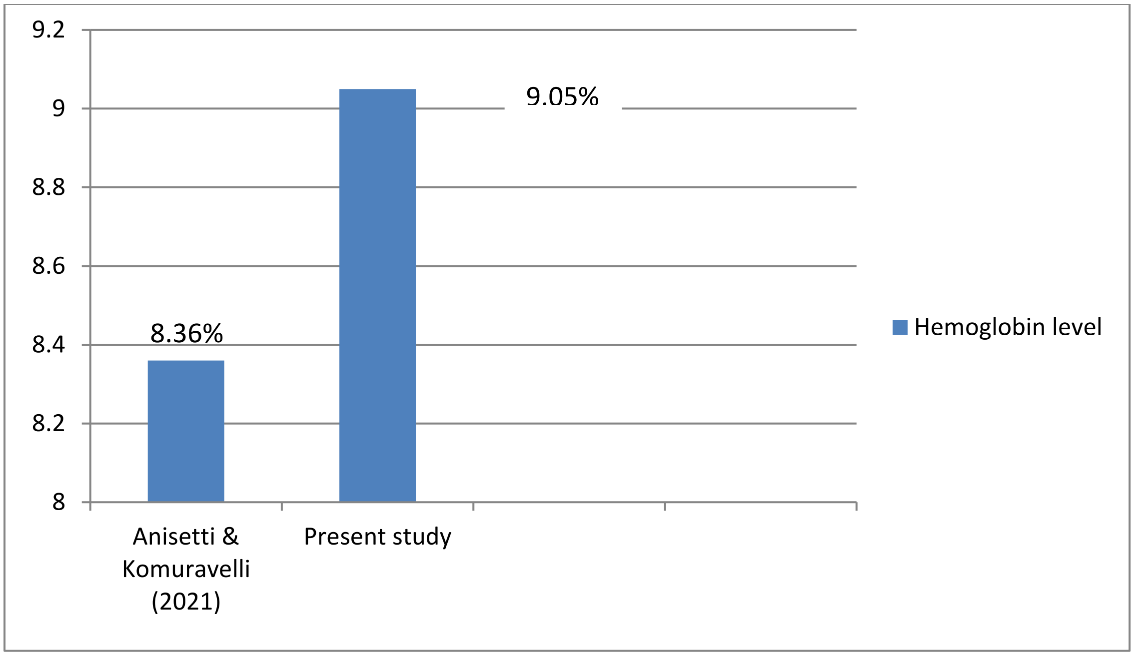
Mean hemoglobin

In our study all cases with hemoglobin less then 13gm% in males and less than 12gm% in females were included. Hemoglobin ranged from 1.6 gram% to 12.7gram%. Mean hemoglobin level of the study group was 9.05grams%. This was compared with similar study byAnisetti and Komuravelli.

Table no 27: Comparison of mean hemoglobin

Hemoglobin level	Anisetti&Komuravelli (2021)[97]	Presentstudy
Mean	8.36gm%	9.05gm%

Graph No 8: Comparison of mean hemoglobin



In our study,the mean hemoglobin as per the different diagnostic groups showed minor variations.The difference was indicative that severity of anemia is not a function of the type of anemia but depends on the severity which can be seen at any grade.[99]

Mean MCV

In the present study MCV in different types of anemia was studied. Mean MCV of the study population was 82.54fl. Mean MCV in different types of anemia was noted and is presented in table below. This is comparable to previous study done by Kumar et al[88] as shown in table below.

Table no 28: Comparison of mean MCV.

Types of Anemia	Mean MCV ofpresent study	Mean MCV of study done by Kumar et al(2013)[99]
Normocytic normochromic Anemia	88.73fl	87.5fl
Microcytic hypochromic Anemia	69.31fl	68.83fl

Macrocytic Anemia	109fl	99.4fl
Dimorphic Anemia	86.63fl	87.5fl

For distinguishing Macrocytic anaemia from Hemolytic anaemia and Macrocytic anaemia from Microcytic anaemia, MCV was an anticipated reliable criterion. The results were consistent with research by Kumar et al.[99]and on the use of RBC indices in the differential diagnosis of microcytic anemias to prospectively evaluate RBC indices as a diagnostic tool.[99]

Due to the antagonistic effects of micro and macrocytosis on the MCV, Constantino et al. demonstrated in their study that MCV is an average value and a measure of central tendency; hence, it does not reflect population heterogeneity as seen in dual cell populations.[2] Since MCV represents the mean of the distribution curve and is insensitive to the presence of tiny populations of cells, peripheral smear was therefore more sensitive than RBC indices for recognising early microcytic or macrocytic alterations.[99]

MCH

With MCH, a similar pattern was observed. Our study's mean MCH, which distinguished between macrocytic and microcytic anaemia, was 26.06pg. This study agreed with the study conducted by Kumar et al. [99]

MCHC

Although a marker for low haemoglobin, MCHC did not produce the same results as MCH, but it was useful in distinguishing between macrocytic and microcytic anaemia. This study is comparable to one by Rathod et al. on the usefulness of cell counter-based parameters in the identification of Thalassemia, which found that MCHC was not significantly reduced in hemolytic anaemia as compared to microcytic anaemia.[100]

However, MCH and MCHC don't provide much data for MCV-independent anaemia classification

RDW

The majority of instances of dimorphic anaemia in the current study had high RDW, which confirms that RDW is a very sensitive indicator of anisocytosis. RDW is significant in cases of microcytic hypochromic anaemia as well. In their study on red cell distribution width, Constantino et al. found that RDW is a marker for anisocytosis when MCV is below the normal range and that anisocytosis is difficult to detect in cases of iron deficiency anaemia.[2] However, on treatment, the histogram curve widens, increasing RDW. Thus, both treated and untreated nutritional deficiencies can affect RDW.When comparing the acquired disease Iron deficiency anaemia to the inherited disorder Thalassemia minor, a homogeneous red cell population is typically defined as having red cells that are morphologically the same size, visually narrow on the histogram, and numerically within a normal RDW range.

Peripheral Smear Examination

Present study showed out of 280 cases, majority were normocytic normochromic patients. This was compared to other studies as shown in table below.

Table no 29: Comparison of peripheral smear findings with similar studies.

Peripheral smear findings	Anisetti&Komurav-elli (2021)[97]	Prakash A et al[94]	Kumar et al(2013)[99]	Saba et Al (2014)[101]	Present study(2021)
Normocytic normochromic anemia	32.20%	-	31.7%	22%	46.42%

Microcytic hypochromic anemia	40.82%	98.7%	33.3%	49%	36.07%
Macrocytic anemia	3.74%	1.3%	21.7%	4%	7.857%
Dimorphic anemia	23.22%	-	13.3%	24%	3.93%

In the present study, the maximum cases were of normocytic normochromic anaemia.This is in discordant with the other studies due to lesser sample size in the former studies.

The other morphoplogical types of anaemia found in the study was in corcordance with the referred studies in the table.

The above comparison shows that the second most common morphological type of anemia was Microcytic hypochromic anaemia.Iron deficiency is the most common cause of microcytic hypochromic blood picture. The reason for this includes chronic iron deficiency, malabsorption, parasitic infestations etc. Furthermore in adolescent girls, menstruation may be the major contributing factor for the microcytic hypochromic anaemia. Prevalence of anemia in all groups is higher in India as compared to other developing countries.

Iron nutrition is a matter of continuing concern in both developing and developed countries of the world. A reduction in the amount of dietary iron and its availability has made iron a borderline nutrient in modern society[102].

Correlation of diagnosis of anemia based on peripheral smear vs Histogram and RBC indices analysis.

The current study was double-blind study. First, peripheral blood smear analysis was performed without consulting the histogram or indices. Later, without conducting an independent peripheral smear examination, the diagnosis is determined using the RBC histogram and RBC indices.

Table no 30: Showing diagnosis of anemia made on Peripheral Smear vs Histogram and indices

Type of Anemia	Onperipheral smear analysis	On histogram and RBC indices analysis
Normocytic normochromic anemia	129	113
Normocytic hypochromic anemia	15	17

Microcytic hypochromic anemia	103	110
Macrocytic anemia	22	26
Dimorphic anemia	11	14

Correlation of Peripheral smear vs RBC histogram and Indices in Microcytic Hypochromic Anemia

Only 36.429% of cases had microcytic anaemia on peripheral smear examination, despite the fact that histogram and RBC indices analysis revealed that 39.28% of cases shifted to the left with MCV range from 46.4fl-79.9fl supporting this. This can be explained because automated analyzers are known to make mistakes when giant platelets are present, when hemolytic diseases cause fragmented RBCs, when platelet clumps are present, and when microerythrocytes are present because the machine misinterprets them as microcytes and produces falsely high values. Peripheral smear rules out these errors.

This study was in agreement with Poonam et al study's from 2015 "Automated Red Blood Cell Analysis Compared with Routine RBC Morphology by Smear Review."[103]

Correlation studies in Normocytic Normochromic Anemia.

On peripheral smear examination 129 out of 280 cases were normocytic normochromic anemia. On histogram analysis 130 cases showed position within normal limits and MCV ranged from 87.4-100.7fl. So diagnosis based on RBC indices and histograms were comparable to diagnosis with peripheral smear examination.It is comparable to study done by Kumar

Aetal(2013)[99].The causes for this might be early iron deficiency or any subclinical infection.The other causes are nutritional deficiencies,acute blood loss, hemolysis or any renal diseases.

Correlation studies in Macrocytic Anemia:

Macrocytic anemia was seen in 22 out of 280 cases on peripheral smear examination. But histogram and RBC indices showed 78 cases as macrocytic anemia. Although the MCV measured by an automated blood cell counter is rarely wrong, conditions including hyperglycemia, marked leukocytes, and cold agglutinins might cause the MCV to be incorrectly elevated. Furthermore, partial instrument obstruction and many hours of room temperature storage of the blood samples might potentially cause a misleading rise of the MCV value. This study is consistent with a study conducted by Florence et al. in that a small number of individuals identified as macrocytic on histogram and RBC indices analysis were identified as dimorphic on peripheral smear examination.[104]

Correlation studies in DimorphicAnemia:

Out of 280 cases, only 11 cases were diagnosed as dimorphic anaemia inperipheral smear.Dimorphic anaemia showed a variety of histogram patterns, from simple curve to complex curve. The majority of the curves were tilted to the left and had wide bases. The broad base may be explained by the presence of numerous populations of cells of differing sizes (i.e.normocytic, microcytic and macrocytic). Peripheral smears of the majority of patients showed a combination of microcytes and macrocytes, with microcytes predominating. Our study agreed with Constantino et alfindings'. from 2010.[2]

The dimorphic blood picture will appear as a dual population of microcytic and normocytic or normocytic and macrocytic red cells or as a mixture of small, normal, and large cells of different sizes and forms with or without normal red blood cell indices, so it is crucial to examine the peripheral smear to look at all the cell populations. Practically speaking, morphological findings should be coupled with the graphical and numerical data for better interpretation of results since the dimorphic blood picture is often associated with aberrant red cell populations.

In 2013 Naveen et al. presented similar findings for the dual population of RBCs. He asserted that the dual RBC population can be recognised using the histogram's most striking representation.[105]

CONCLUSION

The automated haematology analyzer's red cell histograms and RBC indices offer important insights into haematological diseases like anaemia. RBC histograms have received very little attention in research, which have placed more emphasis on WBC histogram and leukemic blast populations. In identifying different morphological kinds of anaemia, our study found a substantial association between RBC histograms and RBC indices versus peripheral smear diagnosis.

Bimodal peaks in the histograms suggested the presence of two RBC populations.However, a peripheral smear was required to assess the same. The RBC indicators didn't increasehelp with the identification of these situations, as MCV showed that the sizeof the current RBC populations.

Thus, it was determined that peripheral smear test, together with clinical history, is a significant diagnostic tool when treating patients in the era of molecular analysis and automation. The visual evaluation of peripheral smears is supplemented by the use of red cell histograms and numerical metrics such MCV, MCH, MCHC, and RDW.

SUMMARY

1. A total of 280 patients whose haemoglobin levels were less than the lower limit of the normal range for the particular age and sex were included in the present study

2. Females were 57.14%which outnumbered the males 42.86%.

3. Hemoglobin value in study population ranged from 1.6-12.7gm% with mean of 9.05gm%

4. The majority of the cases showed normalhistogram (46.43%) followed by left shift histogram (39.28%). Right shift histograms and bimodal pattern were only 9.286% and 5% respectively.

5. MCV in study population ranged from 46.4fl to 133.6fl with mean of 82.54fl.MCH ranged from 11.7pg to 41.4pg with mean of 26.06pg and MCHC from 23.2 to 39.2gm/dl with mean31.43.

6. RDW shows a wide range of 11.9% to 41.4% with mean being 18.31.

7. On peripheral smear analysis, the cases consisted of Normocytic normochromic anemia 46.071%, Normocytic hypochromic anaemia 5.357%, Microcytic hypochromic anemia 36.429%, Macrocytic anemia 7.85%, Dimorphic anemia 3.93%.

8. The histogram patterns and RBC Indices correlated with the morphological type of anaemia on peripheral smear in majority of cases of microcytic hypochromic, macrocytic anemias and normocytic normochromicanemia. The correlation was statistically analysed and was statistically significant with p value less than 0.0001.

9. Disconcordance between histogram patterns and RBC indices VS peripheral smear was seen in few cases of microcytic hypochromic anemia and dimorphic anemia.

10. Few dimorphic anaemiacases were falsely categorised as microcytic anemia and a few as macrocytic anemia by histograms and RBC indices. This was because of the presence of platelet clumps and polychromatophils which were analysed as microcytes and macrocytes respectively by

the histograms. Thus peripheral smear examination plays an important role in dimorphic anaemias.

11. On analysis of histogram and RBC indices, microcytic hypochromic anaemia is predominantly seen in the age group 10-18 years followed by 2-9 years which shows normocytic normochromic anaemia. The p value was calculated for the correlation which was statistically significant with p value 0.0015.

12. On peripheral smear analysis, normocytic normochromic anaemia is mostly seen in the age group 10-18 years followed by microcytic hypochromic anaemia. The age group between 2-9 years show predominantly normocytic normochromic anaemia. The p value was less than 0.0001 showing statistically significant result.

13. Majority of the cases of normocytic anaemia show anisocytosis score of 1. Score 2 is seen in cases of microcytic hypochromic anaemia. Score 3 and 4 were mostly seen in cases of macrocytic anaemia and dimorphic anaemia.The p value has been calculated which is statistically significant in this study.

14. Majority of normocytic normochromic anaemia cases show mild poikilocytosis.Moderatepoikilocytosisis predominantly seen in cases of microcytic hypochromic anaemia. Severe poikilocytosis is seen mostly in dimorphic anaemia. The correlation has been found to be statistically significant with p value less than 0.0001.

Most of the cases with anisocytosis score 1 shows mild poikilocytosis. Score 2 was seen mostly in cases of microcytic hypochromic anaemia. Severe poikilocytosis was seen to be associated with score 4 of anisocytosis. This correlation was also statistically significant with p value less than 0.0001

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ANNEXURE I

STAINING PROCEDURE AND PREPARATION

Procedure

- The well dried thin blood smear is covered with undiluted Leishman Stain solution by counting the drops of Leishman stain.
- It is made to stand for 2 minutes. The methanol present in the stain fixes the smear onto the glass slide
- After 2 minutes, twice the amount of distilled water or phosphate buffer solution is added and the content is mixed by swirling or blowing gently and allowed to stand for 5-7 minutes.
- The slides are air dried in a tilted position so that the water easily remove out of the slides.
- Then the slides are air dried and the slides are observed under the lens of microscope.

Preparation:

- Dissolve 0.15 gm of Leishman stain powder in 100 ml of absolute methanol
- The stain is then filtered into stock bottle.
- Place at 50^{0}C for 15 minutes in water bath.

- Again filter into clean brown borosilicate glass bottle and store in dark at room temperature

बालरोगवयोगटामध्येअनेमियाच्याविविधप्रकारांमध्येलालरक्तपेशीच्याहिस्टोग्रामचीउपयुक्तता (1-18 वर्षे)

प्रधानअन्वेषक: डॉ. आयूसीएनपूजा

मार्गदर्शक : डॉ. विजयालक्ष्मीधोरीगोल

अभ्यासाचाउद्देश:

आपणयाअभ्यासामध्येसहभागीहोण्यासाठीपात्रआहातम्हणूनआपल्यालायाअभ्यासामध्येनावनोंदणीकरण्याससांगितलेजाते. याअभ्यासाचाहेतूबालरोगवयोगटातील (1-18 वर्षे) विविधप्रकारच्यारक्तक्षयातीलपरिघीयरक्तधब्बाच्यानिष्कर्षांसहलालरक्तपेशीच्याहिस्टोग्रामशीसंबंधितआहे .

प्रक्रियाः

याअभ्यासादरम्यान, आपल्यासंपूर्णरक्ताचीगणना (सीबीसी) आणिपरिघीयरक्तस्मीयर (पीबीएस) चेमूल्यांकनकेलेजाईल. याअभ्यासाचेमुख्यअन्वेषकडॉ.आयूसीएनपूजा(पीजी) डॉ. विजयालक्ष्मीधोरीगोल (मार्गदर्शक) यांच्यामार्गदर्शनाखालीआहेत. आपणयाअभ्यासामध्येआपलीनावनोंदणीकरण्याससहमतीदर्शविल्यासआपलासीबीसीआणिपीबीएसअहवालतसेचआपल्यारक्तगटाच्याअहवालाचाउपयोगसंशोधनासाठीकेलाजाईल .

जोखीमआणिफायदे

याअभ्यासामध्येभागघेण्यातकोणतीहीजोखीमनाही.
बालरोगवयोगटातीलविविधरक्तक्षेत्रांमधीललालरक्तपेशीच्याहिस्टोग्रामचीउपयुक्तता (1-18 वर्षे) जाणूनघेणेहात्याचाफायदाआहे .

विकल्पः

याअभ्यासामध्येभागघेणेऐच्छिकआहे.

आपणयाअभ्यासामध्येभागनघेण्याचीनिवडकरूशकताकिंवाआपणआताभागघेण्याचानिर्णयघेतल्यासआपणनंतरआपलेमतबदलू आणिअभ्यासापासूनदूरजाऊशकता. अभ्यासडॉक्टरकिंवाप्रायोजकयाअभ्यासामधीलआपलासहभागकधीहीरद्दकरूशकतात.

गोपनीयता

याअभ्यासाच्यादरम्यानआपल्याबद्दलसंकलितकेलेलीसर्वमाहितीकायद्याद्वारेपरवानगीअसलेल्यामर्यादेपर्यंतगोपनीयठेवलीजाईल. कोडनंबरआपल्यालायासंशोधनरेकॉर्डमध्येओळखतील.

याअभ्यासाचीमाहितीप्रकाशितकेलीजाईलपरंतुआपलीओळखकोणत्याहीप्रकाशनातगोपनीयअसेल.

आपल्याबद्दलकिंवासंशोधनादरम्यानप्रदानकेलेलीमाहितीकिंवाइतरमाहितीआपल्यालिखितपरवानगीशिवायइतरांनाउघडकेलीजाणारनाहीः

1. आपत्कालीनपरिस्थितीतआपलेहक्कआणिकल्याणसंरक्षितकरण्यासाठी.
2. कायद्यानेआवश्यकअसल्यास.

सहभागासाठीआर्थिकप्रोत्साहनः

याअभ्यासामध्येभागघेण्यासाठीआपल्यालाकोणतीहीभेट / प्रोत्साहनदिलेजाणारनाही.

परिणामप्रकाशितकरण्यासाठीअधिकृतताः

याअभ्यासाचेनिकालएमएडीपदवी, आढावाआणिप्रकाशनपूर्णकरण्याच्याआवश्यकतेचाभागम्हणूनकाहेर, बेलागावीकडेपाठविलाजाईल.

प्रश्नों

भविष्यातअभ्यासाशीसंबंधितकाहीप्रश्नअसल्यासआपणसंपर्कसाधूशकताः

1. डॉ. आयूसीएनपूजा, पॅथॉलॉजीविभाग, जे.एन. मेडिकलकॉलेज, फोननंबर- 8731095142

2. डॉ. विजयालक्ष्मीधोरीगोल, पॅथॉलॉजीविभाग, जे.एन. मेडिकलकॉलेज, फोननंबर- 9964318734

3. आपल्याकडेअभ्यासाचाविषयम्हणूनआपल्याहक्कांबद्दलकाहीशंकाअसल्यासआपणडॉ. रूपाबेल्लद, प्रोफेसर, बालरोगशास्त्रविभाग, मानवीविषयसंशोधनाचीसंस्थानैतिकसमिती, अध्यक्षजे.एन. मेडिकलकॉलेजबेलागावी. कॉलकरूशकता. फोननंबर 9448113403,

संमतीविधान

मीखालीस्वाक्षरीकरूनयाअभ्यासातभागघेण्यासस्वेच्छेनेसहमतआहे. मीकेव्हाहीमाघारघेऊशकतो. याफॉर्मवरसहीकरूनमीकोणतेहीकायदेशीरहक्कसोडतनाही.
खालीमाझीस्वाक्षरीसूचितकरतेकीमीहासंपूर्णसंमतीफॉर्मवाचलाआहेकिंवामलावाचलागेलाआहेआणिमाझ्यासर्वप्रश्नांचीउत्तरेदिलीआहेत

प्रधानअन्वेषक: डॉ. आयूसीएनपूजा

मार्गदर्शक: डॉ. विजयालक्ष्मीधोरीगोल

सहभागीचेनाव: (स्वाक्षरी / अंगठाचाठसा)

साक्षीदाराचेनाव: (सही / अंगठाचाठसा)

चौकशीचेनाव: (स्वाक्षरी)

तारीख:

ತಿಳುವಳಿಕೆಯುಳ್ಳಒಪ್ಪಿಗೆ

ಮಕ್ಕಳವಯಸ್ಸಿನಗುಂಪಿನಲ್ಲಿ (1-18 ವರ್ಷಗಳು) ವಿವಿಧರೀತಿಯರಕ್ತಹೀನತೆಗಳಲ್ಲಿಕೆಂಪುರಕ್ತಕಣಹಿಸ್ಟೋಗ್ರಾಮ್‌ಗಳಉಪಯುಕ್ತತೆ

ಪ್ರಧಾನತನಿಖಾಧಿಕಾರಿ: ಡಾ.ಐಯುಸಿಎನ್ಪೂಜಾ

ಮಾರ್ಗದರ್ಶಿ: ಡಾ. ವಿಜಯಲಕ್ಷ್ಮಿಧೋರಿಗೋಲ್

ಅಧ್ಯಯನದಉದ್ದೇಶ:

ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುನೀವುಅರ್ಹರಾಗಿರುವುದರಿಂದಈಅಧ್ಯಯನಕ್ಕೆಸೇರಲುನಿಮ್ಮನ್ನುಕೇಳಲಾಗುತ್ತದೆ.

ಈಅಧ್ಯಯನದಉದ್ದೇಶವುಕೆಂಪುರಕ್ತಕಣಗಳಹಿಸ್ಟೋಗ್ರಾಮ್‌ಗಳನ್ನುಮಕ್ಕಳವಯಸ್ಸಿನ (1-18 ವರ್ಷಗಳು) ವಿವಿಧರೀತಿಯರಕ್ತಹೀನತೆಗಳಲ್ಲಿನಬಾಹ್ಯರಕ್ತದಸ್ಮೀಯರ್ಸಂಶೋಧನೆಗಳೊಂದಿಗೆಪರಸ್ಪರಸಂಬಂಧಿಸುವುದು.

ವಿಧಾನ:

ಈಅಧ್ಯಯನದಸಮಯದಲ್ಲಿ, ನಿಮ್ಮಸಂಪೂರ್ಣರಕ್ತದಎಣಿಕೆ (ಸಿಬಿಸಿ) ಮತ್ತುಬಾಹ್ಯರಕ್ತದಸ್ಮೀಯರ್ (ಪಿಬಿಎಸ್) ಅನ್ನುಮೌಲ್ಯಮಾಪನಮಾಡಲಾಗುತ್ತದೆ. ಡಾ. ವಿಜಯಲಾಕ್ಸಮಿಧೋರಿಗೋಲ್ (ಗೈಡ್) ಅವರಮಾರ್ಗದರ್ಶನದಲ್ಲಿಅಧ್ಯಯನದಪ್ರಮುಖತನಿಖಾಧಿಕಾರಿಡಾ.ಅಯುಸಿಎನ್ಪೂಜಾ (ಪಿಜಿ). ಈಅಧ್ಯಯನಕ್ಕೆನಿಮ್ಮನ್ನುಸೇರಿಸಲುನೀವುಒಪ್ಪಿದರೆ, ನಿಮ್ಮರಕ್ತಗುಂಪುವರದಿಯೊಂದಿಗೆನಿಮ್ಮಸಿಬಿಸಿಮತ್ತುಪಿಬಿಎಸ್ವರದಿಯನ್ನುಸಂಶೋಧನಾಉದ್ದೇಶಕ್ಕಾಗಿಬಳಸಲಾಗುತ್ತದೆ.

ಅಪಾಯಮತ್ತುಪ್ರಯೋಜನಗಳು

ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸುವುದರಿಂದಯಾವುದೇಅಪಾಯಗಳಿಲ್ಲ. ಮಕ್ಕಳವಯಸ್ಸಿನ (0-18 ವರ್ಷಗಳು) ವಿವಿಧರಕ್ತಹೀನತೆಗಳಲ್ಲಿನಕೆಂಪುರಕ್ತಕಣಗಳಹಿಸ್ಟೋಗ್ರಾಮ್‌ಗಳಉಪಯುಕ್ತತೆಯನ್ನುತಿಳಿದುಕೊಳ್ಳುವುದುಇದರಪ್ರಯೋಜನವಾಗಿದೆ.

ಪರ್ಯಾಯಗಳು:

ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸುವುದುಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ. ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸದಿರಲುನೀವುಆಯ್ಕೆಮಾಡಬಹುದುಅಥವಾನೀವುಈಗಭಾಗವಹಿಸಲುನಿರ್ಧರಿಸಿದರೆ, ನೀವುನಂತರನಿಮ್ಮಮನಸ್ಸನ್ನುಬದಲಾಯಿಸಬಹುದುಮತ್ತುಅಧ್ಯಯನದಿಂದಹಿಂದೆಸರಿಯಬಹುದು. ಅಧ್ಯಯನದವೈದ್ಯರುಅಥವಾಪ್ರಾಯೋಜಕರುಈಅಧ್ಯಯನದಲ್ಲಿನಿಮ್ಮಭಾಗವಹಿಸುವಿಕೆಯನ್ನುಯಾವುದೇಸಮಯದಲ್ಲಿಕೊನೆಗೊಳಿಸಬಹುದು.

ಗೌಪ್ಯತೆ

ಈಅಧ್ಯಯನದಸಮಯದಲ್ಲಿನಿಮ್ಮಬಗ್ಗೆಸಂಗ್ರಹಿಸಲಾದಎಲ್ಲಾಮಾಹಿತಿಯನ್ನುಕಾನೂನಿನಿಂದಅನುಮತಿಸುವಮಟ್ಟಿಗೆಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ. ಈಸಂಶೋಧನಾದಾಖಲೆಯಲ್ಲಿಕೋಡ್ಸಂಖ್ಯೆಗಳುನಿಮ್ಮನ್ನುಗುರುತಿಸುತ್ತವೆ. ಈಅಧ್ಯಯನದಮಾಹಿತಿಯನ್ನುಪ್ರಕಟಿಸಲಾಗುವುದುಆದರೆಯಾವುದೇಪ್ರಕಟಣೆಯಲ್ಲಿನಿಮ್ಮಗುರುತುಗೌಪ್ಯವಾಗಿರುತ್ತದೆ.

ನಿಮ್ಮಲಿಖಿತಅನುಮತಿಯಿಲ್ಲದೆನಿಮ್ಮಬಗ್ಗೆಯಾವುದೇಮಾಹಿತಿಅಥವಾಸಂಶೋಧನೆಯಸಮಯದಲ್ಲಿನೀವುಒದಗಿಸಿದಮಾಹಿತಿಯನ್ನುಇತರರಿಗೆಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ:

1. ನಿಮ್ಮಹಕ್ಕುಗಳುಮತ್ತುಕಲ್ಯಾಣವನ್ನುರಕ್ಷಿಸಲುತುರ್ತುಪರಿಸ್ಥಿತಿಯಲ್ಲಿ.
2. ಕಾನೂನಿನಪ್ರಕಾರಅಗತ್ಯವಿದ್ದರೆ.

ಭಾಗವಹಿಸುವಿಕೆಗೆಆರ್ಥಿಕಪ್ರೋತ್ಸಾಹ:

ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುನಿಮಗೆಯಾವುದೇಉಡುಗೊರೆ / ಪ್ರೋತ್ಸಾಹಧನನೀಡಲಾಗುವುದಿಲ್ಲ.

ಫಲಿತಾಂಶಗಳನ್ನುಪ್ರಕಟಿಸಲುಅಧಿಕಾರ:

ಈಅಧ್ಯಯನದಫಲಿತಾಂಶಗಳನ್ನುಎಂಡಿಪದವಿ, ವಿಮರ್ಶೆಮತ್ತುಪ್ರಕಟಣೆಯಪೂರ್ಣಗೊಳಿಸುವಿಕೆಯಅವಶ್ಯಕತೆಯಭಾಗವಾಗಿಬೆಳಗವಿಯಕಾಹೇರ್‌ಗೆರವಾನಿಸಲಾಗುತ್ತದೆ.

ಪ್ರಶ್ನೆಗಳು

ಭವಿಷ್ಯದಲ್ಲಿನೀವುಅಧ್ಯಯನಕ್ಕೆಸಂಬಂಧಿಸಿದಯಾವುದೇಪ್ರಶ್ನೆಗಳನ್ನುಹೊಂದಿದ್ದರೆನೀವುಸಂಪರ್ಕಿಸಬಹುದು:

1. ಡಾ.ಐಯುಸಿಎನ್ಪೂಜಾ, ರೋಗಶಾಸ್ತ್ರವಿಭಾಗ, ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯಕಾಲೇಜು, ದೂರವಾಣಿಸಂಖ್ಯೆ - 8731095142

2. ಡಾ.ವಿಜಯಲಕ್ಷ್ಮಿಧೋರಿಗೋಲ್, ರೋಗಶಾಸ್ತ್ರವಿಭಾಗ, ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯಕಾಲೇಜು, ದೂರವಾಣಿಸಂಖ್ಯೆ - 9964318734

3. ಅಧ್ಯಯನದವಿಷಯವಾಗಿನಿಮ್ಮಹಕ್ಕುಗಳಬಗ್ಗೆನೀವುಯಾವುದೇಪ್ರಶ್ನೆಗಳನ್ನುಹೊಂದಿದ್ದರೆ, ನೀವುಪೀಡಿಯಾಟ್ರಿಕ್ಸ್ವಿಭಾಗದಪ್ರಾಧ್ಯಾಪಕಡಾ.ರೂಪಾಬೆಲ್ಲದಜೆ.ಎನ್. ವೈದ್ಯಕೀಯಕಾಲೇಜುಸಾಂಸ್ಥಿಕನೈತಿಕಸಮಿತಿಯಮಾನವವಿಷಯಗಳಸಂಶೋಧನೆ, ಅವರನ್ನುಕರೆಮಾಡಬಹುದು, ಪಿಎಚ್ಸಂಖ್ಯೆ- 9448113403, ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯಕಾಲೇಜು, ಬೆಳಗವಿ

ಒಪ್ಪಿಗೆಯಹೇಳಿಕೆ

ಕೆಳಗೆಸಹಿಮಾಡುವಮೂಲಕಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುನಾನುಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದಒಪ್ಪುತ್ತೇನೆ.
ನಾನುಯಾವುದೇಸಮಯದಲ್ಲಿಹಿಂತೆಗೆದುಕೊಳ್ಳಬಹುದು.
ಈಫಾರ್ಮ್ಗೆಸಹಿಮಾಡುವಮೂಲಕನಾನುಯಾವುದೇಕಾನೂನುಹಕ್ಕುಗಳನ್ನುಬಿಟ್ಟುಕೊಡುತ್ತಿಲ್ಲ.
ಕೆಳಗಿನನನ್ನಸಹಿನಾನುಓದಿದ್ದೇನೆ, ಅಥವಾಅದನ್ನುನನಗೆಓದಿದೆ,
ಈಸಂಪೂರ್ಣಒಪ್ಪಿಗೆಯರೂಪಮತ್ತುನನ್ನಎಲ್ಲಾಪ್ರಶ್ನೆಗಳಿಗೆಉತ್ತರಿಸಿದೆಎಂದುಸೂಚಿಸುತ್ತದೆ

ಪ್ರಧಾನತನಿಖಾಧಿಕಾರಿ: ಡಾ.ಐಯುಸಿಎನ್ಪೂಜಾ

ಮಾರ್ಗದರ್ಶಿ: ಡಾ. ವಿಜಯಲಕ್ಷ್ಮಿಧೋರಿಗೋಲ್

ಭಾಗವಹಿಸುವವರಹೆಸರು: (ಸಹಿ / ಹೆಬ್ಬೆರಳು)

ಸಾಕ್ಷಿಯಹೆಸರು : (ಸಹಿ / ಹೆಬ್ಬೆರಳು)

ತನಿಖಾಧಿಕಾರಿಯಹೆಸರು : (ಸಹಿ)

ದಿನಾಂಕ :

PROFORMA

PATIENT HISTORY

Name : Sample Id:

Age :

Sex :

IP no. :

Department:

Date of Analysis:

Brief clinical history

INVESTIGATIONS: -

1- Haemoglobin
2- WBC Total
3- WBC Differential
4- PCV
5- Platelet Count
6- Reticulocyte Count
7- RBC Count
8- Peripheral Smear

KEY TO MASTER CHART

On Peripheral Smear examination Column of Anisocytosis

1+ Anisocytosis-Most red cells in the average oil immersion field appear uniform in size but5-10 cells per field are easily distinguished from the predominant cell line.

2+ Anisocytosis -10-15 cells per field are easily distinguished from the predominant cellline.

3+ Anisocytosis-15-25 cells per field are easily distinguished from the predominant cellline.

4+Anisocytosis-26-50 cells per field are easily distinguished from the predominant cell line.

Column of Poikilocytosis

1) mild poikilocytosis

2)moderate poikilocytosis

3)severe poikilocytosis

Column of impression on Peripheral Smear

1)Normocytic normochromic anemia

2)Normocytic hypochromic anaemia

3)Microcytic hypochromic anemia

4)Macrocyticanemia

5)Dimorphic anemia

Histogram analysis:

Column of position of histogram

1)within normal limits

2)Shift to left

3)Shift to right

4)Bimodal

Shape of Histogram Column of Bell shape

1) present

2)absent

Column of Broad base

1)Present

2)Absent

Column of Impression on Histogram analysis and RBC indices

1)Normocytic normochromic anemia

2)Normocytic hypochromic anaemia

3)Microcytic hypochromic anemia

4)Macrocytic anemia

5)Dimorphic anemia

Sl.No	Name	Age	Sex	IP/OP	Hb	On Peripheral Smear Examination			
						Anisocytosis	Poikilocytosis		
1	x	8	m	21219499	11.7	1	1		
2	x	6	m	21219524	9.7	1	1		
3	x	14	m	21219615	12.5	2	2		
4	x	5	f	21219735	8.1	3	2		
5	x	13	f	21219404	10.8	1	1		
6	x	14	f	21219457	8.9	4	2		
7	x	4	f	21220449	11.2	1	1		
8	x	13	f	21220539	10.3	2	1		
9	x	15	m	21220594	11.5	1	1		
10	x	6	f	21220624	8.2	1	4		
11	x	4	m	21223639	10.5	1	1		
12	x	2	m	21223541	8.2	1	1		
13	x	12	f	21225884	10.2	2	2		
14	x	5	f	21227450	5.4	1	2		
15	x	2	f	21227772	6.6	2	2		
16	x	12	m	21231848	10.8	1	1		
17	x	10	f	21231933	8.0	1	1		
18	x	11	f	21219360	8.5	4	2		
19	x	7	m	22000866	4.3	1	1		
20	x	13	f	22002398	10.5	2	2		
21	x	13	f	22002682	11.2	1	1		
22	x	13	f	22003364	6.5	2	1		
23	x	14	m	22004768	9.4	3	1		
24	x	12	f	22004994	5.5	2	1		
25	x	10	f	22005081	9.9	2	2		
26	x	16	f	22006773	6.9	2	2		
27	x	11	f	22006883	9.2	2	1		
28	x	13	f	22007717	10.3	1	1		
29	x	5	m	21219384	7.1	1	1		
30	x	18	f	22007885	10.2	2	1		
31	x	10	m	22011347	7.8	1	1		

32	x	12	f	22013090	9.4	2	1		
33	x	10	f	22014210	11.2	2	2		
34	x	13	m	22014871	11.7	1	1		
35	x	10	f	22019083	8.0	1	1		
36	x	11	f	22018723	11.3	1	1		
37	x	12	f	22019762	7.5	2	1		
38	x	10	f	22019787	10.1	2	1		
39	x	6	m	22020064	10.0	1	1		
40	x	18	m	22020410	8.5	1	1		
41	x	16	f	22020413	8.9	1	1		
42	x	14	f	22020421	10.0	2	1		
43	x	10	m	22020580	9.5	4	2		
44	x	11	m	22021005	10.8	1	2		
45	x	16	m	22021061	9.1	1	1		
46	x	15	f	22021136	9.8	1	1		
47	x	18	f	22021639	10.0	1	1		
48	x	4	m	22021842	9.6	1	1		
49	x	6	m	22021825	8.7	1	1		
50	x	10	f	22021826	7.5	1	2		
51	x	12	f	22021831	9.2	1	1		
52	x	10	f	22021832	10.5	1	1		
53	x	17	f	22022204	9.7	2	1		
54	x	17	f	22035865	7.7	1	2		
55	x	16	f	22036244	9.3	2	2		
56	x	13	m	22036357	7.4	1	1		
57	x	12	m	22036250	10.7	2	2		
58	x	13	f	22036573	7.7	2	1		
59	x	12	f	22036559	7.7	2	2		
60	x	9	m	22036520	10.6	1	1		
61	x	7	f	22036851	5.6	2	2		
62	x	12	m	22037538	9.6	1	2		
63	x	3	m	22037562	6.4	1	1		
64	x	10	f	22037529	5.7	1	2		
65	x	16	f	22037790	9.1	2	2		
66	x	15	m	22038898	10.6	1	1		
67	x	18	f	22038921	8.8	2	2		
68	x	16	m	22038999	10.1	1	1		
69	x	15	f	22141040	6.5	1	2		
70	x	14	f	22142605	11.5	1	1		
71	x	15	m	22142577	3.9	3	1		
72	x	13	f	22141515	5.6	4	3		
73	x	15	m	22142035	8.9	1	2		

74	x	14	m	22141659	7.6	3	1		
75	x	12	f	22141065	8.4	1	1		
76	x	5	f	22244639	6.8	4	2		
77	x	10	f	22245514	5.7	2	2		
78	x	13	m	22246150	9.5	1	1		
79	x	12	f	22246225	8.7	1	2		
80	x	11	f	22246249	11.4	2	2		
81	x	18	f	22246303	5.5	2	2		
82	x	3	m	22246304	4.1	1	1		
83	x	18	f	22246377	10.9	2	1		
84	x	17	f	22246379	11.7	2	2		
85	x	17	f	22246381	9.7	2	1		
86	x	16	f	22246382	9.3	2	2		
87	x	16	f	22246384	11.8	1	1		
88	x	12	m	22246660	10	2	2		
89	x	18	m	22246679	11.1	1	2		
90	x	14	f	22246687	11.3	1	1		
91	x	18	m	22246689	8.3	1	1		
92	x	12	f	22246692	10.8	1	2		
93	x	12	m	22246720	10.9	1	2		
94	x	8	f	22246723	8.4	1	2		
95	x	12	m	22246756	7.4	3	1		
96	x	13	m	22246758	11.1	1	1		
97	x	10	f	22246764	8.3	1	2		
98	x	18	f	22246817	10.5	4	3		
99	x	16	f	22246801	8.5	1	2		
100	x	18	f	22246837	10.6	1	2		
101	x	15	f	22247099	10	1	1		
102	x	10	m	22247757	9.1	2	1		
103	x	12	m	22247933	8.8	2	2		
104	x	8	f	22248109	6.3	1	1		
105	x	10	m	22248264	9.6	2	2		
106	x	9	f	22247919	8.9	1	2		
107	x	13	f	22247927	9.5	1	1		
108	x	15	f	22247824	7.6	2	2		
109	x	13	m	22248017	10.9	1	1		
110	x	18	f	22248039	9	2	3		
111	x	18	f	22248044	8.7	1	2		
112	x	17	f	22248049	8.8	2	1		
113	x	18	f	22248054	8.7	2	2		
114	x	15	m	22248090	12.7	3	1		
115	x	12	f	22248141	10.5	2	2		
116	x	16	m	22248185	5.5	2	2		

117	x	15	m	22248280	11.8	2	2		
118	x	8	m	22248323	8.8	2	1		
119	x	15	m	22248330	11.6	1	2		
120	x	7	m	22248572	9.9	1	1		
121	x	7	f	22248575	8	1	1		
122	x	10	f	22248586	7.6	1	2		
123	x	12	m	22248613	8.1	1	2		
124	x	13	m	22248659	7.2	3	2		
125	x	12	m	22248663	7.4	1	2		
126	x	12	m	22248687	9	1	2		
127	x	10	m	22248714	8.6	4	2		
128	x	13	f	22248752	8.2	1	2		
129	x	8	m	22248766	9.1	1	2		
130	x	12	m	22248743	8.5	1	1		
131	x	16	m	22248771	10	1	1		
132	x	8	f	22248800	8.8	1	2		
133	x	10	m	22228503	8.9	1	1		
134	x	12	m	22228501	10.8	3	2		
135	x	10	m	22228549	8.6	1	1		
136	x	14	m	22229162	11	1	1		
137	x	12	f	22229163	10.5	2	1		
138	x	15	m	22229108	12.1	1	1		
139	x	16	f	22229275	10.8	1	1		
140	x	14	f	22229239	11.1	2	1		
141	x	15	f	22229344	11.4	1	1		
142	x	14	m	22229310	9	1	2		
143	x	5	f	22229374	9.9	2	2		
144	x	12	m	22229587	10.8	1	2		
145	x	13	m	22229611	11.4	2	2		
146	x	12	m	22229679	6.3	4	3		
147	x	18	m	22229800	12.6	2	2		
148	x	10	f	22229834	1.6	2	2		
149	x	7	m	22229889	8.7	2	2		
150	x	11	m	22229573	6.9	2	3		
151	x	9	m	22229614	7.7	1	1		
152	x	16	m	22229570	11.9	1	1		
153	x	10	f	22229682	10.5	2	1		
154	x	11	m	22229789	8.6	1	2		
155	x	5	m	22229931	7.6	1	1		
156	x	15	m	22229914	12.2	1	1		
157	x	10	f	22229970	9.8	2	1		
158	x	12	f	22242904	9.3	2	2		
159	x	14	f	22243089	10.3	1	1		
160	x	9	m	22243098	10.4	1	1		

161	x	14	m	22243103	11.3	1	1		
162	x	11	f	22243602	9.3	2	2		
163	x	13	m	22244052	12.7	4	3		
164	x	10	m	22244081	10.9	1	2		
165	x	12	f	22244077	10.4	2	2		
166	x	14	m	22244203	7.6	2	2		
167	x	11	f	22244205	9.8	2	1		
168	x	12	m	22244210	9.1	2	1		
169	x	6	f	22244213	7.6	1	1		
170	x	14	m	22244215	11.2	2	1		
171	x	15	m	22244219	11.4	1	2		
172	x	9	m	22244223	10.6	1	1		
173	x	10	f	22228578	10.9	2	3		
174	x	11	m	22228580	10.7	1	1		
175	x	11	f	22228582	9.7	1	1		
176	x	12	m	22228594	9.6	2	2		
177	x	10	f	22228623	9.9	1	1		
178	x	7	f	22228629	8.5	1	1		
179	x	8	f	22228631	9.4	1	1		
180	x	14	m	22228718	8	1	1		
181	x	12	f	22228656	8.2	3	1		
182	x	6	m	22228667	9.6	1	2		
183	x	10	f	22228683	10.8	1	1		
184	x	16	m	22228687	10.9	1	1		
185	x	12	f	22228786	9.6	1	1		
186	x	13	f	22228787	6.2	4	3		
187	x	14	m	22228788	8.7	1	2		
188	x	12	f	22228776	7.9	1	1		
189	x	12	m	22228543	9.2	3	2		
190	x	13	m	22228966	7.7	1	1		
191	x	10	f	22229156	9.9	1	1		
192	x	11	m	22229305	8.9	2	2		
193	x	16	f	22229398	4.7	2	2		
194	x	13	m	22229456	10.8	1	2		
195	x	11	f	22229975	10.2	2	1		
196	x	14	m	22229996	12.7	3	1		
197	x	10	f	22230044	11.1	2	1		
198	x	12	f	22230362	9.7	2	2		
199	x	15	f	22230338	7.9	2	2		
200	x	13	f	22230345	9.4	3	2		
201	x	10	f	22228789	9.1	1	1		
202	x	18	m	22228967	8.3	1	2		
203	x	17	f	22230567	8.3	3	2		

204	x	16	f	22230345	6.2	2	3		
205	x	10	m	22224356	9.2	2	1		
206	x	11	f	22223654	9.3	2	2		
207	x	12	f	22229157	8.8	2	2		
208	x	14	m	22236978	5.7	3	1		
209	x	8	m	22230786	6	4	3		
210	x	10	f	22236956	9.3	1	1		
211	x	10	m	22220564	9.4	1	1		
212	x	9	f	22234768	9.5	1	2		
213	x	4	f	22228543	9.4	2	2		
214	x	14	m	22226241	9.4	1	1		
215	x	12	f	22234856	9.4	1	1		
216	x	6	f	22223067	5.4	2	2		
217	x	10	f	22237045	7.3	2	2		
218	x	13	m	22234076	7.4	3	2		
219	x	13	f	22231735	7.2	1	1		
220	x	14	f	22221709	9.5	3	2		
221	x	10	f	22226321	9	2	1		
222	x	8	m	22248761	9.4	1	2		
223	x	18	f	22247345	6.7	2	2		
224	x	12	f	22248234	9	2	1		
225	x	13	f	22246324	10	1	1		
226	x	15	f	22248456	9.4	2	2		
227	x	10	f	22235823	9.5	3	1		
228	x	9	m	22231623	9	2	2		
229	x	13	f	22237342	5.3	3	1		
230	x	12	f	22230234	5.7	1	2		
231	x	8	f	22235823	7.4	3	1		
232	x	14	m	22230734	5.7	1	2		
233	x	12	f	22245023	8.7	2	2		
234	x	11	f	20225415	8.4	2	2		
235	x	16	m	20223439	9.2	2	1		
236	x	12	f	20222346	8.9	2	1		
237	x	18	f	22223654	9.4	1	1		
238	x	15	f	22224588	9.5	2	2		
239	x	18	m	22220448	9.4	1	1		
240	x	18	f	22220498	9	2	1		
241	x	14	f	20223607	9.5	1	1		
242	x	15	f	20226159	6.7	2	2		
243	x	12	m	20227230	8.7	1	1		
244	x	17	f	22223756	9.1	3	1		
245	x	18	f	20224454	5.2	2	3		
246	x	15	f	20223564	7.5	2	1		
247	x	13	f	20228019	9.1	1	1		

248	x	11	f	20227957	5.8	2	1		
249	x	14	m	20227549	9.4	2	2		
250	x	17	m	20229858	6.3	2	1		
251	x	18	f	22220849	7.2	4	3		
252	x	12	f	22230402	10.7	1	1		
253	x	15	f	22230566	8.9	1	1		
254	x	11	f	22230604	10.1	2	2		
255	x	9	m	22229995	9	1	1		
256	x	12	m	22230061	6.1	1	2		
257	x	7	f	22230088	10.2	1	1		
258	x	10	f	22230089	10.3	2	2		
259	x	8	m	22230090	10.1	1	1		
260	x	15	m	22230094	7.4	1	1		
261	x	14	f	22230327	9	1	2		
262	x	12	f	22230110	8.3	3	2		
263	x	10	m	22230108	8.3	3	1		
264	x	11	m	22230123	9.3	1	1		
265	x	12	m	22230124	11.7	1	1		
266	x	11	f	22230127	9.2	1	1		
267	x	14	f	22230128	10.7	1	2		
268	x	13	f	22230142	9.7	1	2		
269	x	12	m	22230185	10.6	2	1		
270	x	11	f	22230144	9.2	2	2		
271	x	10	m	22230188	9.4	1	1		
272	x	9	m	22230438	7.4	1	1		
273	x	18	m	22238174	11.2	1	1	1	1
274	x	16	f	22238175	9.3	2	2	1	1
275	x	10	f	22238176	7.7	2	2	1	1
276	x	8	f	22238177	10	2	1	1	1
277	x	18	f	22238179	7.8	2	2	1	1
278	x	12	f	22238181	10.5	2	1	1	1
279	x	14	m	22238182	11	1	1	1	1
280	x	14	f	22240310	10.9	1	1	1	1