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Clinicohematological profile of anaemia with special emphasis on red blood cell parameters by five part cell counter - an observational cross sectional study

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Abstract

Introduction: Anaemia (Greek, "bloodlessness"), is defined as an abnormal reduction in the number of red blood cells or in their haemoglobin content.

Visual inspection of this graphic representation can be used to supplement and sometime even to replace conventional slide review.

The present study was being carried out to find out the morphological typing of anaemia based on RBC parameters obtained from five part hematology analyzer.

Method: It was an observational cross sectional study conducted in Department of Pathology, in NKP Salve Institute of Medical Science and LATA MANGESHKAR Hospital NAGPUR (M.H).

Patients of all age group suspected to have anaemia clinically were sent for investigation. A total of 300 patients were studied.

Result: Among the 300 cases studied, the most common age group affected were in the age group of 18 – 27 years (21%) followed by less than 18 years (19%) and 38-47 years (19.3%).

119 (39.7%) were male and 181 (60.3%) were female. 22 (7.3%) had mild anemia, 129 (43.0%) had moderate anemia and 149 (49.7%) had severe anemia in the study group.

36 (12.0%) had normocytic normochromic peripheral smear, 154 (51.3%) had microcytic hypochromic, 21 (7.0%) had microcytic hypochromic with sickle cells, 67 (22.3%) had macrocytic hypochromic and 22 (7.3%) had Dimorphic hypochromic peripheral smear in the

study group. 28 (9.3%) had normal curve, 179 (59.3%) had left shift, 72 (24.0%) had right shift and 22 (7.3%) had bimodal histogram findings in the study group.

28 (9.3%) were Normocytic normochromic, 159 (53.0%) were Microcytic hypochromic, 73 (24.3%) were Macrocytic hypochromic, 22 (7.3%) were Dimorphic and 18 (6.0%) had Microcytic hyperchromic in the red cell cytogram study group.

Conclusion: Iron deficiency anaemia (IDA) is most widespread nutritional deficiency and accounts for almost one half of anaemia cases.

Histograms and cytograms generated by five part analyzer is a practical working tool in the initial stage of morphological analysis. It is used in differentiating various types of anemia.

Keywords: Anaemia, histograms, cytograms

Introduction

Anaemia (Greek, "bloodlessness"), is defined as an abnormal reduction in the number of red blood cells or in their haemoglobin content.

Anaemia is defined as decreased concentration of haemoglobin and RBC mass as compared to the values in age-matched controls.[1]

According to the World Health Organization (WHO), there are 2 billion people affected with anaemia in the world, and half of the anaemia is due to iron deficiency. [2]Anaemia is an indicator of iron deficiency, it is estimated that the prevalence of iron deficiency is 2.5 times that of anaemia.[2] Iron deficiency is the most common in the developing world and the cause of nutritional anaemia in young children and women of reproductive age.

According to epidemiologic data from World Health Organization (WHO), 24.8% of the human population is currently suffering from anaemia.

Among the female population, almost 41% of all pregnant females suffer from anaemia while among nonpregnant pre-menopausal females 30% females are struggling with anaemia. The male population is usually resistant to anaemia due to circulating testosterone levels. However, 12.7% adult males are also globally affected with anaemia. [3]

After the female population, pre-school aged children suffer the most from anaemia. Human milk contains 0.3 mg/L iron which is not sufficient to provide enough iron. Cow milk contains double the amount of iron, but that iron has poor bioavailability.[3]

Peripheral blood film (PBF) is an important hematological tool at the clinician's screening, diagnosis and monitoring of disease progression and therapeutic response.

Its diagnostic relevance has not been lessened by advances in hematology automation and molecular techniques.[4]

In cases of anaemia, the peripheral smear permits interpretation of significant red blood cell (RBC) findings. These include assessment of RBC shape, size, color, inclusions, and arrangement. [5]

Blood indices and haemoglobin are of added value. A good idea can be obtained from reviewing histograms and cytograms obtained from hematology analyzers nowadays.

Evaluation of anaemia is frequently needed as the prevalence of anaemia is high. The diagnosis of red blood cell disorders is supplemented by histogram and cytograms as they provide guidance regarding red blood cell morphology on peripheral smear.

RBC histogram is a graphic representation of particle size distribution and routinely generated by automated cell analyzers as a standard part of automated CBC analysis. [6,7]

Histograms are helpful in monitoring and interpreting abnormal morphological changes. [8,9]

Visual inspection of this graphic representation can be used to supplement and sometime even to replace conventional slide review.[10]

Aims And Objectives

To study the clinico- hematological profile in cases of anaemia.

To assess the red blood cell parameters on five part cell counter in different types of anaemia.

Materials and method

It was an observational cross sectional study conducted in Department of Pathology

Patients of all age group suspected to have anaemia clinically were sent for Investigation.

Total duration of study was 2 years.

300 patients were included in the study. Blood sample collected in an Ethylene diamine tetra acetic acid (EDTA) anti-coagulated tube. The complete blood count was analyzed on the five part hematology analyzer.

Fresh peripheral blood smears stained by Leishman's stain was examined under the microscope.

All clinical details and systemic examination findings were correlated with morphological and Siemens Advia 2120 analyzer findings.

Informed written consent were taken from the patient for research purpose.

Complete history, important clinical details will be analyzed.

Various RBC parameters **(CHCM, HDW, CH, %Micro, %Hypo etc)** will be studied in anaemia cases.

Study instrument

Siemens Advia 2120 Hematology System – Five part hematology analyzer.

Olympus Cx21i binocular microscope

Peripheral blood smears stained with Leishman's stain or Haematoxylin and Eosin stain.

Inclusion criteria

1. All anemic patients with hemoglobin percentage less than 13 gm% for males and less than 11 gm% for females (ADULTS).

2. CHILDREN (4yrs to puberty): less than 11.5gm%

3. (6 months to 4 years) less than 11gm%.

Exclusion Criteria

1. Inadequate blood sample for automated analyzer will be excluded from the study.

2. Patients of leukemia will be excluded

3. Patients of (0-6 months) are excluded

Results

Age distribution of cases studied.

Of 300 cases studied, 57 (19.0%) had age less than 18 years, 63 (21.0%) had between 18 – 27 years, 52 (17.3%) had between 28 – 37 years, 58 (19.3%) had age between 38 – 47 years, 31 (10.3%) had age between 48 – 57 years, 28 (9.3%) had age between 58 – 67 years and 11 (3.7%) had age above 67 years.

The mean ± SD of age of cases studied was 34.09 ± 18.63 years and the minimum- maximum age range was 1 – 89 years.

Age group (years)	No. of cases	% of cases
<18	57	19.0
18 – 27	63	21.0
28 – 37	52	17.3
38 – 47	58	19.3
48 – 57	31	10.3
58 – 67	28	9.3
>67	11	3.7
Total	300	100.0

Table 1: age distribution

Sex distribution of cases studied.

Of 300 cases studied, 119 (39.7%) were male and 181 (60.3%) were female. The male to female sex ratio was 0.66 : 1.00.

Sex	No. of cases	% of cases
Male	119	39.7
Female	181	60.3
Total	300	100.0

Table 2: sex distribution

Distribution of grading of anemia among the cases studied.

Of 300 cases studied, 22 (7.3%) had mild anemia, 129 (43.0%) had moderate anemia and 149 (49.7%) had severe anemia in the study group.

Grading of anemia	No. of cases	% of cases
Mild	22	7.3
Moderate	129	43.0
Severe	149	49.7
Total	300	100.0

Table 3: Grading Of Anaemia

Distribution of peripheral smear among the cases studied.

Of 300 cases studied, 36 (12.0%) had normocytic normochromic peripheral smear, 154 (51.350 had microcytic hypochromic, 21 (7.0%) had microcytic hypochromic with sickle cells, 67 (22.3%) had macrocytic hypochromic and 22 (7.3%) had Dimorphic hypochromic peripheral smear in the study group.

Peripheral smear	No. of cases	% of cases
Normocytic normochromic	36	12.0
Microcytic hypochromic	154	51.3
Microcytic hypochromic with sickle cells	21	7.0
Macrocytic hypochromic	67	22.3
Dimorphic hypochromic	22	7.3
Total	300	100.0

Table 4: Peripheral Smear Distribution

Distribution of Histograms among the cases studied.

Of 300 cases studied, 28 (9.3%) had normal curve, 179 (59.3%) had left shift, 72 (24.0%) had right shift and 22 (7.3%) had bimodal histogram findings in the study group

Histogram findings	No. of cases	% of cases
Normal curve	28	9.3
Left shift	179	59.3
Right shift	72	24.0
Bimodal	22	7.3
Total	300	100.0

Table 5: Histograms Distribution

Distribution of Mentzer's index among the cases studied.

Of 300 cases studied, 12 (4.0%) had Mentzer's index below 13 (thalassemia), 288 (96.0%) had Mentzer's index more than or equal to 13 in the study group.

Mentzer's index	No. of cases	% of cases
<13	12	4.0
≥13	288	96.0
Total	300	100.0

Table 6: Mentzer's Index

Distribution of %M/%H (%micro / %hypo) ratio among the cases studied.

Of 300 cases studied, 165 (55.0%) had %M/%H ratio less than 0.9 and 135 (45.0%) had %M/%H ratio more than 0.9 in the study group.

%M/%H Ratio	No. of cases	% of cases
<0.9	165	55.0
≥0.9	135	45.0
Total	300	100.0

Table 7: %Micro/%Hypo Ratio

Distribution of RBC parameters among the cases studied.

Of 300 cases studied, 132 (44.0%) MCV <80, 135 (45.0%) had MCV between 80 – 100 and 33 (11.0%) had MCV above 100 in the study group.

Of 300 cases studied, 155 (51.7%) MCH <27, 101 (33.7%) had MCH between 27 – 32 and 44 (14.7%) had MCH above 32 in the study group.

Of 300 cases studied, 136 (45.3%) MCHC <32, 126 (42.0%) had MCHC between 32 – 35 and 38 (12.7%) had MCHC above 35 in the study group.

Of 300 cases studied, 48 (16.0%) CHCM <29, 187 (62.3%) had CHCM between 29 – 34 and 65 (21.7%) had CHCM above 34 in the study group.

Of 300 cases studied, 102 (34.0%) CH <24, 175 (58.3%) had CH between 24 – 35 and 23 (7.7%) had CH above 35 in the study group.

Of 300 cases studied, none had HDW below 1.9, 47 (15.7%) had HDW between 1.9 – 3.0 and 253 (84.3%) had HDW above 3.0 in the study group.

Of 300 cases studied, none had RDW below 11, 26 (8.7%) had RDW between 11 – 14.5 and 274 (91.3%) had RDW above 14.5 in the study group.

RBC parameter		No. of cases	% of cases
MCV (fl)	<80	132	44.0
	80 – 100	135	45.0
	>100	33	11.0
MCH (pg)	<27	155	51.7
	27 – 32	101	33.7
	>32	44	14.7
MCHC (gm/dl)	<32	136	45.3
	32 – 35	126	42.0
	>35	38	12.7
CHCM (gm/dl)	<29	48	16.0
	29 – 34	187	62.3
	>34	65	21.7
CH (pg)	<24	102	34.0
	24 – 35	175	58.3
	>35	23	7.7
HDW (gm/dl)	<1.9	0	0.0
	1.9 – 3	47	15.7
	>3	253	84.3
RDW (%)	<11	0	0.0
	11 – 14.5	26	8.7
	>14.5	274	91.3

Table 8: RBC parameters distribution

Distribution of cytogram among the cases studied.

Of 300 cases studied, 28 (9.3%) were Normocytic normochromic, 159 (53.0%) were Microcytic hypochromic, 73 (24.3%) were Macrocytic hypochromic, 22 (7.3%) were Dimorphic and 18 (6.0%) had Microcytic hyperchromic in the study group.

Normocytic normochromic	28(9.3%)
Microcytic hypochromic	159(53%)
Macrocytic hypochromic	73(24.3%)
Dimorphic hypochromic	22(7.3%)
Microcytic hyperchromic	18(6%)

Table 9: Distribution of Cases of Cytograms

Discussion

Age Group: In the present study , the most common age group affected were in the age group of 18 – 27 years (21%) followed by less than 18 years (19%) and 38-47 years (19.3%). The findings of our study were similar to findings of Ramana Sastry CP et al [11](5-12 years) ,Nakate DP et al[12](5-15 years) Marken P et al [13] (1-6 years)and P Durga Prasad et al [14] (15-30years) and (31-45 years).

In the present study, in the age group of less than 18 years, males (32males, 25 females) were commonly affected. In the age group of 18-27 years (52 females,11 males) and 38 – 47 years(38 females,20 males), females were commonly affected. In the age group of 18-27 years females were most commonly affected as mostly the women were in child bearing age and also there is blood loss due to menstruation .

Iron requirements are high in pregnancy, and iron deficiency is associated with maternal death, preterm delivery, and low birthweight.[15,16].In the age group of 38-47 years females were commonly affected. The women mostly are in peri-menopausal age so bleeding is mostly seen in this age group. Strict vegetarians women who do not consider animal protein is also one of the cause of anemia in this age group.[17]Nutritional deficiency anemia is the commonest cause of anemia among children aged 5 years to 12 years.

Sex Distribution: In the present study females presented commonly with anaemia (60.3%) which was similar and concordance with the findings of P Durga Prasad et al[25] and Ingale SV et al [26] indicating that anemia is still prevalent more commonly in females. The high proportion of microcytic hypochromic anaemia in women indicates iron deficiency as the main cause of anemia. Women in childbearing age have important health implication. Iron requirements are higher in pregnancy and its deficiency is associated with maternal death, preterm delivery and low birth weight. [15,16]

Grading Of Anaemia: In the present study, the cases of severe anaemia were more followed by moderate and mild cases of anemia which was similar to study conducted by Choudhary S et al.[17]

Peripheral Smear: The result was concordance to findings of Maqsood S et al[18], Sandhya V et al[19], Chavada J et al[20] and Shrivastava A et al[21].

The study indicates that microcytic hypochromic as the most common cause of anemia.

Peripheral blood film (PBF) is a basic and informative haematological tool at the clinician's initial screening, diagnosis and monitoring of disease progression and therapeutic response. An understanding of peripheral blood interpretation is important for a successful clinical practice.[4]

In cases of anaemia, the peripheral smear permits interpretation of significant red blood cell (RBC) findings. These include assessment of RBC shape, size, color, inclusions, and arrangement. [5]

Histogram Distribution: In our study,28 (9.3%) had normal curve, 179 (59.3%) had left shift, 72 (24.0%) had right shift and 22 (7.3%) had bimodal histogram findings in the study group which are in concordance with study conducted by, Maqsood S et al[18], SandhyaV et al [19], Chavada J et al[20] and Shrivastava A et al [21].

When compared with the findings of Microcytic hypochromic anaemia on peripheral smear we found additional **25 cases (8.3%)** of left shift on histograms. Additional **5 cases (1.6%)** of right shift were found on histograms suggesting that histograms help us in knowing red blood cells morphology at the earliest.

In above findings we can interpret that histograms generated by analyzer are very useful in interpreting various anemia's when compared with peripheral smear examination.

Mentzer's Index

In our study we found **12 cases (4%)** cases having MENTZER'S INDEX less than <13. This parameter is calculated using MCV and RBC indices. Value less

than 13 is helpful in assessing cases of beta thalassemia trait.

On performing Hb electrophoresis, 6 out of 12 cases were found to have Hb A2 pattern and were diagnosed as having beta thalessemia trait

%Micro/%Hypo Ratio

In the present study of 300 cases studied, 165 (55.0%) had %M/%H ratio less than 0.9 and 135 (45.0%) had %M/%H ratio more than 0.9 in the study group.

Cytogram Distribution: With the help of red cell cytograms which is generated by analyzers we can help to detect anaemia. Red cell cytogram patterns from automated analyzers provide useful information that acts as an adjunct to the numerical parameters &peripheral smear and at times is even diagnostic of some hematological conditions.

Red blood cell size and hemoglobin distribution cytograms provide very useful qualitative information, which is an adjunct to the numerical data, but can sometimes be a very important pointer to a hematological condition not otherwise suspected.[22]

In patients with iron deficiency on treatment, sequential histograms can show the progressive appearance of a new well erythrocyte population.[22]

Dual populations of red cells may exist in patients on hematinic treatment, following blood transfusion or in dual (vitamin B12/folate and iron) deficiency anemia. These populations can be identified on the RBC cytogram with the most classic representation seen during RBC transfusions.

RBC Parameters: In the present study, most of the cases fall under low MCH and MCH with raised RDW suggesting higher number of microcytic hypochromic anemia.

The study findings were almost similar in cases of **Shrivastava A et al [21]and Chavada J[20].**

In our study the Normocytic normochromic anemia cases show MCV, MCH and MCHC within the normal limit with slight variation seen in RDW.

In Microcytic anemia MCV, MCH, MCHC were low with increased RDW due to anisopoikilocytosis.

In Macrocytic anemia there is high MCV,MCH,MCHC and RDW. This is due to high degree of anisopoikilocytosis.

In Dimorphic anemia a MCV, MCH and MCHC were normal with high RDW suggesting variation in size and shape due to marked anisopoikilocytosis.

Anemia has been classified by two main methods. [23]

(I) **Morphologic** – The classification is based on RBC size where MCV is used.

- Normocytic, (Normal MCV)
- Microcytic (Low MCV)
- Macrocytic (Higher MCV).

MCV as a classifier can be deceptive in condition like mixed nutritional deficiency where the average MCV may be in the normocytic range.

(II) **Physiologic** – Bone marrow studies, biochemical markers and reticulocyte count are used in this classification

(A) Hypoproliferative or bone marrow production defect

(B) RBC maturation defect or ineffective erythropoiesis

(C) Decreased RBC survival (hypoproliferative)

Biochemical markers like serum iron, ferritin, TIBC, folate and vit. B12 help to distinguish between other maturation disorders.

Reticulocyte production distinguishes between hypoproliferative and hemolytic anemia.

The modern classification is based on two indices – MCV and RDW was proposed by Bessman.

There are six possible combinations which classify almost possible causes of anemia. [23]

- Low MCV with Normal RDW(Anemia of chronic disease • Thalassemia (heterozygous)) or High RDW (• Iron deficiency Anemia • RBC fragmentation • HbH • Thalassemia intermedia • G6PD deficiency)
- Normal MCV with Normal RDW(Anemia of chronic disease • Acute blood loss • Hemolysis • Normal variant) or High RDW (RDW increases before MCV becomes abnormal • Early iron deficiency anemia • Early vitamin B12 deficiency • Early folate deficiency).
- High MCV with Normal RDW (Aplastic anemia • Preleukemia • Myelodysplastic syndrome • Anemia of chronic disease • Thalassemia (heterozygous)or High RDW(• Vitamin B12 deficiency • Folate deficiency • Immune hemolytic anemia • Liver disease • Cold agglutinins • Alcoholism)

Newer RBC parameters generated on 5 part analyzers –

HDW,CHCM, CH:

Very few studies have been conducted on these parameters.

HDW (Hemoglobin Distibution Width) [24]

Few analyzers determine hemoglobin concentration of individual red cells .

In the present study, of 300 cases studied, none had HDW below 1.9, 47 (15.7%) had HDW between 1.9 – 3.0 and 253 (84.3%) had HDW above 3.0 in the study group.

The normal (95%) range is 1.82-2.64.

The identification of an increased percentage of hyperchromic cells may be caused by the presence of spherocytes or sickle cells.

In the present study, **21 cases (7%)** on peripheral smear examination had sickle cell anaemia. There haemoglobin distribution width was found to be more than 3.

On further performing electrophoresis **8 out of 21 cases** had AS pattern.

CHCM(Corpuscular Haemoglobin Concentration Mean):[24]

It gives the true estimate of hypochromia in iron deficiency anemia.

This parameter is estimated in some instruments (ADVIA 120). Normal value (29-34 gm/dl).

In the present study of 300 cases studied, 48 (16.0%) CHCM <29, 187 (62.3%) had CHCM between 29 – 34 and 65 (21.7%) had CHCM above 34 in the study group.

Among the 48 cases which had CHCM less than 29 gm/dl , **42 cases(14%)** had microcytic hypochromic anaemia ,**2 cases(0.6%)** had microcytic hyperchromic anaemia, **3 cases(1%)** had macrocytic anaemia and **1 case(0.3%)** had dimorphic anaemia in the cytogram.

Cellular Hemoglobin(CH):[24]

It represents the distribution of RBCs by amount of Hb present in each cell independent of cell volume.

Normal value (24-35 pg) of 300 cases studied, 102 (34.0%) CH <24, 175 (58.3%) had CH between 24 – 35 and 23 (7.7%) had CH above 35 in the study group.

Distribution of RBC parameters according to grading of anemia

MCHC , CHCM ,HDW, RDW are sensitive parameters in detecting morphological types and hemoglobin concentration. The p value was found to be (<0.05) which was statistically significant in the study.

RDW is degree of variation of red cells size and can be determined by many hematology analyzer. It especially helpful in differentiating iron deficiency anaemia (high

RDW) with beta thalassemia trait (normal RDW). It ranges from 11.5-14.5.

HDW is degree of variation in red cell hemoglobinization is estimated as the hemoglobin distribution width or HDW; which is the coefficient of variation of the measurements of hemoglobin concentration of individual cells.

The normal (95%) range is 1.82-2.64.

CHCM gives the true estimate of hypochromia in iron deficiency anemia.

This parameter is estimated in some instruments (ADVIA 120). Normal value (29-34 gm/dl).

Figures

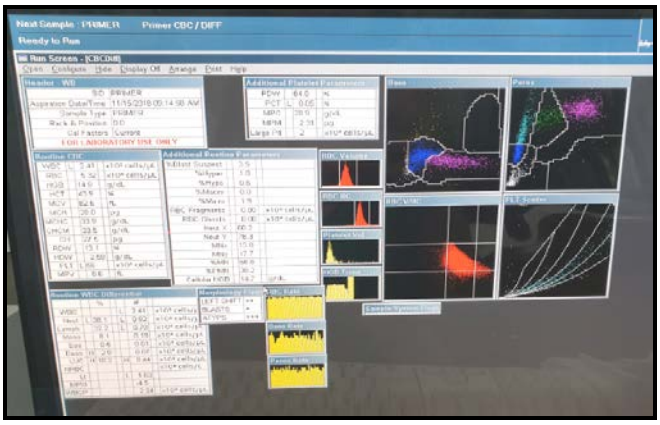

Figure 1: Normocytic Normochromic Anaemia

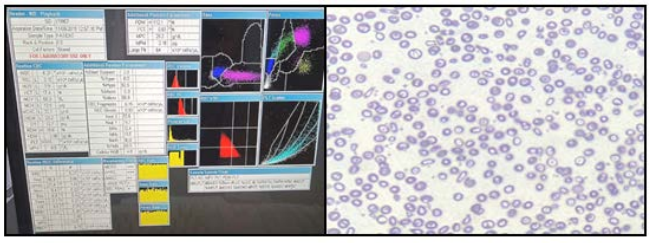

Figure 2: Microcytic Hyochromic Anaemia

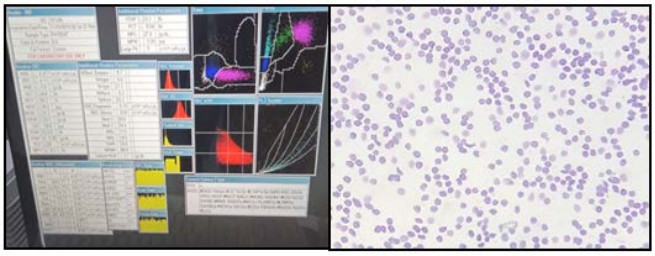

Figure 3: Sickle Cell Anaemia

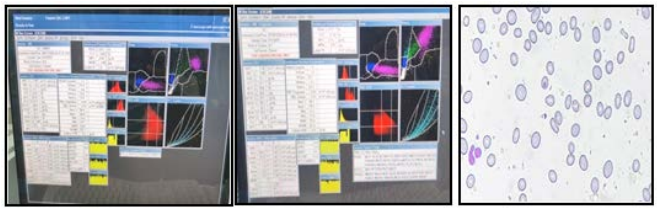

Figure 4: Bimodal Population

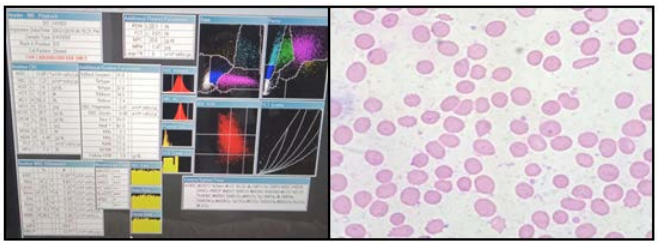

Figure 5: Macrocytic Anaemia

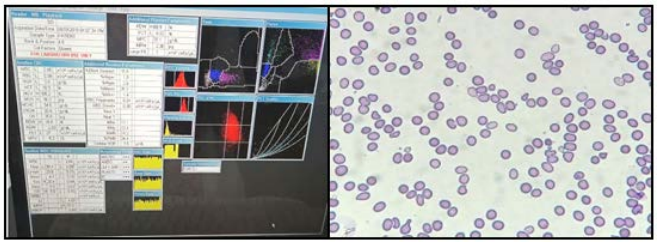

Figure 6: Pancytopenia

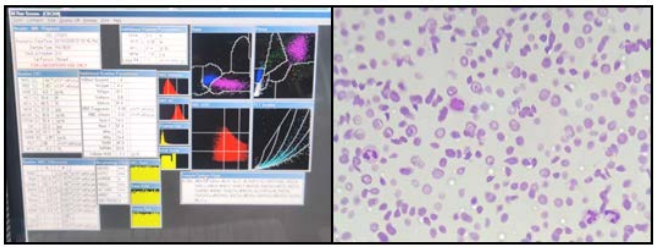

Figure 7: Beta Thalassemia Trait

Conclusion

Iron deficiency anaemia (IDA) is most widespread nutritional deficiency and accounts for almost one half of anaemia cases. It is common in young women and is due to an imbalance between iron intake and iron loss. It could be reduced by prophylactic measures and active case finding. Nutritional deficiency anemia is the commonest cause of anemia among children aged 5 years to 12 years. Iron deficiency is most common nutritional deficiency in children followed by megaloblastic anemia.

The diagnosis of red blood cell disorders is supplemented by histogram and cytograms as they provide guidance regarding red blood cell morphology on peripheral smear. Histograms and cytograms generated by five part analyzer is a practical working tool in the initial stage of morphological analysis. It helps us regarding RBC morphology. It provides idea about Hemoglobin, RBC count, MCV and of RDW. It is used in differentiating various types of anemia. It is also very helpful in detecting subclinical cases of anemia. The flags generated by them will help us to know whether to review a peripheral smear.Cases that are not picked up in peripheral smears & histograms can better be analyzed in cytograms. The novel parameters generated by hematology analyzers like HDW,CHCM,CH provide an extra aid in cases of anaemia. Cytograms are helpful in monitoring and interpreting abnormal morphological changes with accuracy.

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