ORIGINAL RESEARCH PAPER

Pathology

COMPARATIVE STUDY OF BLOOD SMEAR EXAMINATION WITH RED CELL INDICES IN VARIOUS TYPES OF ANEMIAS

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ABSTRACT

Anemia is the most prevalent disorder found throughout the world ,hence its proper diagnosis and correct differentiation is necessary for the accurate treatment of its various types. Peripheral blood smear examination has been used since decades as a diagnostic tool in the etiopathological diagnosis of anemias. Cell counters have come up with the advantage of increased efficacy, less time consuming and are cost effective tools. The present study was conducted to compare the findings of peripheral smear examination with cell counter generated indices and to analyse their usefulness and limitations in the diagnosis of anemias.

Material And Methods- Blood samples collected from 500 patients over a period of one year were analysed and compared by both cell counter generated indices { RBC indices and RDW } as well as by peripheral smear examination.

Results- Microcytic hypochromic anemia {39.6% } was the most common followed by normocytic normochromic anemia { 32.8% } ,macrocytic { 13.6% }, dimorphic anemia { 12% } and hemolytic anemia { 2% } as per peripheral smear examination. RBC indices showed {42% }, {33.8% }, {16.8% } ,{6.4%} and { 1% } of microcytic ,normocytic, macrocytic, dimorphic and hemolytic anemia respectively. Thus discrepancy in results by two methods was seen in dimorphic, macrocytic and hemolytic anemias.

Conclusion- For the accurate diagnosis of anemia red cell indices should be always correlated with the peripheral smear examination, which remains to be the gold standard and a major diagnostic tool in anemia.

INTRODUCTION

Anaemia is one of the most common health issue that is prevalent in our part of society and is also quite significant in the developed world . Anemia is a condition in which there is decreased oxygen carrying capacity of blood due to lowered hemoglobin level or RBC counts. Pre-school children, pregnant women and adolescents are the most vulnerable groups.[1]

Most common cause of anaemia is nutritional deficiency, other causes such as hemolysis are also common.[2] Anemia is associated with significant morbidity and mortality.Its prevalence among different age groups in the developing countries has been studied as 39% in less than 5yrs, 48% in 5-14yrs, 42% in women 15 to 59 years, 30% in men 15 to 59 years and 45% in adults > 60-years.[3]

Analysis of peripheral blood smears for interpretation of various haematological disorders has been a basic and informative tools since decades.[4] Peripheral blood film examination has always been the main diagnostic tool in the work up of etio pathogenesis of different forms of anaemia.[5] Automated haematology analysers have come up with the advantage of increased accuracy precision and are cost-effective. Despite of these advantages PBF examination is an essential and a primary tool for the evaluation of morphological abnormalities of blood cells for example pencil cells, Sickle cells, teardrop cells ,schistocytes etc that are not provided by automated analyzers.[6] and also for the diagnosis of type of anemia, leukemias and other hematological disorders[7]. These two diagnostic tools have complemented each other in providing the complete and a reliable interpretation of blood samples. [8,9,10] Results from both the methods should correlate with each other to validate the reports.[6]

AIMS AND OBJECTIVES

To correlate the findings of peripheral blood film examination and cell counter generated red cell indices in different kinds of anaemia.

MATERIALS AND METHODS

This study was conducted in the department of haematology of a tertiary care hospital for a period of 1 year from April 2020 to March 2021. This study was carried in a total of 500 patients with anemia {defined as per who criteria 2011} . As per who 2011 reference range the recommended levels of hemoglobin levels to diagnose anemia in various age groups is as follows[11, 12]

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Children {1/2 – 5 years}...... Hb    <11gms/dl
Children  {5-11years}......... Hb    <11.5gms/dl
Children {11-15years}.........Hb    <12gms/dl
Pregnant females      ...........Hb    <11gms/dl
Non- pregnant females ..........Hb    < 12gms/dl
Men                   .........Hb    <13gms/dl
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All the anemic patients attending opds as well as in patient departments who were advised PBF examination as well as CBC were studied. The patients with hematological malignancies were excluded from the study.

A 2ml of venous blood was collected in EDTA and gently mixed well and was run and processed on the cell counter {3 part}for CBC and determination of red cell indices like MCV,MCH,MCHC,RDW.[13, 14]

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MCV [76 – 96FL]
MCV [27-32pg ]
MCHC [32-36 gm/dl ]
RDW [11.5 -13.5% ]
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Simultaneously a blood smear was also prepared from the same blood sample and was stained using Leishmans stain. The smear was then examined for the morphological diagnosis of anemia and results were noted down .The results from both the methods {PBF and Cell counter} were analysed and compared and then a diagnosis was made.

RESULTS

In our study a total of 500 patients were studied from age

group of 1 year infant to 80 years old. Most of the cases were in the age group of 21-50 years. Among total of 500 cases 275 were females and 225 were males as shown in table 1

Table 1 Age And Gender Wise Distribution Of Cases Of Anemia

AGE GROUP	MALE	FEMALE	TOTAL
<15 years	12	14	26
15 – 30 years	37	78	115
31 -40 years	31	58	89
41 -50 years	38	54	92
51 -60 years	38	25	63
61 – 70 years	41	27	68
>70 years	28	19	47
TOTAL	225	275	500

As depicted from table 2, 164 cases were reported as normocytic normochromic anemia and 198 were showing small sized RBCs and increased central pallor ie, microcytic hypochromic anemia on peripheral smear examination.68 cases showed RBCs bigger than normal size suggesting macrocytic anemia and 60 cases were having dual cell population of microcytes as well as macrocytes ie, dimorphic anemia.

Table: 2 Distribution Of Cases As Per Morphological Type Of Anemia

TYPE OF ANEMIA	MALE	FEMALE	TOTAL
NORMOCTIC NORMOCHROMIC ANEMIA	96	68	164
MICROCYTIC HYPOCHROMIC ANEMIA	62	136	198
MACROCYTIC ANEMIA	29	39	68
DIMORPHIC ANEMIA	32	28	60
HEMOLYTIC ANEMIA	6	4	10
TOTAL	225	275	500

Table 3; Distribution Of Cases As Per Red Cell Indices

TYPE OF ANEMIA	MALE	FEMALE	TOTAL
NOMOCYTIC NORMOCHROMIC ANEMIA	99	70	169
MICROCYTIC HYPOCHROMIC ANEMIA	66	144	210
MACROCYTIC ANEMIA	39	45	84
DIMORPHIC ANEMIA	18	14	32
HEMOLYTIC ANEMIA	3	2	5
TOTAL	225	275	500

As per cell counter generated red cell indices there were 169 cases of normocytic normochromic anemia and 210 cases of microcytic anemia.Macrocytic anemia was reported in 84 cases and 32 cases were given as dimorphic anemia.Out of total of 500 cases only 5 were of hemolytic anemia type.

Table :4 Comparison Of Findings Of Peripheral Smear Examination And RBC Indices Generated Via Cell Counter

TYPE OF ANEMIA	NO. OF CASES ON SMEAR EXAMINATION	NO OF CASES ON CELL COUNTER
NORMOCYTIC NORMOCHROMIC ANEMIA	164	169
MICROCYTIC HYPOCHROMIC ANEMIA	198	210
MACROCYTIC ANEMIA	68	84
DIMORPHIC ANEMIA	60	32
HEMOLYTIC ANEMIA	10	5
TOTAL	500	500

Table 4,Shows the comparison between results of PBF examination and cell counter generated RBC indices. It was observed that there was a significant correlation in results generated by both of these methods in normocytic normochromic anemia, microcytic hypochromic anemia while in macrocytic anemia , dimorphic anemia and hemolytic anemia these two methods showed discrepancy in the results.

DISCUSSION

In our study peripheral blood smears of 500 patients with anemia were evaluated and then correlated with the automated cell counter generated red cell indices. Majority of patients in our study fall in the age group of 21 to 50 years, among these 225 were males and 275 were females and among 275 females majority of patients were in their reproductive age .Same findings were observed in the study of Kumar et al [15], Cook et al[16],Japheth et al [17]and Singhal et al[11] . All this can be explained by the fact that in the age group of 21-50 years multiple factors are responsible such as inadequate iron intake ,increased iron demands in adolescents, chronic blood loss , malabsorption ,infections etc along with lifestyle factors, dietary habits and social and behavioral factors [7 ,18]. Frequent blood loss [menstruation] in the women of reproductive age explains the high risk of developing iron deficiency iron deficiency in this age group .After 40 yrs males were seen to be more affected than females.

The most common morphological type of anemia in our study was microcytic hypochromic anemia ,198 cases [39.6%] followed by normocytic normochromic anemia ,164 cases [32.8%]. Various factors can lead to microcytic hyoochromic anemia like decreased intake of iron, poor absorption, increased demands as well as chronic blood loss. This is in concordance with the study conducted by Khan et al [19] and Patel et al [20] in which most common type of anemia was microcytic hypochromic .In children there is more requirement of iron for growth and increased blood loss is the reason for high risk of development of iron deficiency in the women of reproductive age [7].

In our study out of 500 cases, 198 cases [39.6%] were of microcytic hypochromic anemia on smear examination and low MCV was seen in 210 cases [42%] as per analyser .This mild difference can be due to giant platlets, platlet clumps, fragmented RBCs in which analyser takes them as microcytic. These findings were in concordance with the findings of study conducted by Singhal etal 49.8% [11] and Sandhya I et al 46% [21].

In a study conducted by Alvarez uria G et al [3] most prevalent anemia in children and women of reproductive age were were found to be microcytic hypochromic anemia and frequency of normocytic normochromic anemia increased with age in adults men and in women after menopause all these findings were in concordance with the findings of our study.This can be explained due to increased frequency of chronic diseases and inflammatory conditions and decreased bone marrow functioning which is common in occurrence in this age group. Macrocytic anemia was seen in 68 cases[13.6%] on smear examination and raised MCV was found in 84 [16.8%] of cases as per analyser readings.This considerable difference could be due to interpretation of hemolytic anemia as macrocytic anemia by counters because of polychromasia same was observed by Chavda et al [22] and Rao et al [23] in their study.Other factors can also lead to false elevation of MCV values like hyperglycemia, cold agglutinins, and leucocytosis.

Dimorphic anemia was diagnosed in 60 cases [12%] on smear examination while as RBC indices showed this only in 32 [6.4%] of cases.This significant difference could be explained due to the presence of cells of varying sizes { normocytes, microcyte, macrocytes] which could mislead the diagnosis if we rely on automated values alone which show some cases of dimorphic anemia as normal MCV .Thus it is very important to examine the peripheral smear to see all

sizes of cell populations. These findings were concordant with study done by Chavda et al [22] and Sandhya et al [21].Hence it shows that MCV is an insensitive indicator to classify anemia.The reason being that MCV is an average value and does not reflect the presence of different red cell population[24].Therefore in all patient with decreased hemoglobin peripheral smear examination is a must for identifying early RBC changes.

Dimorphic anemia is usually associated with therapeutic transfusions and or hematinic agent response to microcytic and macrocytic anemia, but they may also indicate other hematological disorders such as early iron deficiency with megaloblastic anemia developing microcytic population and folate/vit b12 defciency developing macrocytic population, post iron treatment of iron deficiency anemia with megaloblastic anemia [25].

Elevated RDW with low hematocrit and relatively high RBC counts points to hemolytic anemia, only few cases were diagnosed as hemolytic anemia on indices { 1% } as compared to peripheral smear examination {2% } because fragmented RBCs were counted as microcytes and polychromatophills were counted as macrocytes, same was observed by Radadiya et al [26] Sandhya I et al [21] ,Poonam et al[27] and Sandhya V et al [28]. These findings point to the limitation of RBC indices in the diagnosis of hemolytic anemia .

In our study we found that cell counter parameters should always be interpreted in the light of peripheral smear examination as this could help us to get to the accurate diagnosis mainly in dimorphic , macrocytic and in hemolytic anemia where cell counter indices can misled us to the erroneous diagnosis.

Hence we conclude that peripheral smear examination remains the gold standard test for accurate diagnosis and differentiation of anemias whereas the automated analysers are good for screening purposes as well as are also the cost effective tools.

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