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# CORRELATION BETWEEN THE PLATELET INDICES, RBC INDICES AND PERIPHERAL SMEARS IN EVALUATING THE MICROCYTIC HYPOCHROMIC ANEMIA.

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**ABSTRACT:**

**INTRODUCTION:**

Anaemia is a condition in which the number of red blood cells or the haemoglobin concentration within them is lower than normal. According to WHO, Anemia affects 1.62 billion people worldwide, equivalent to 24.8 percent of the population making it a major public health problem in both developing and developed countries. According to India's NFHS-5 (National Family Health Survey), 57 % of women and 25 % of men age 15-49 in India are anaemic.Anemia is diagnosed using automated hematology analyzer that analyzes parameters such as Hb, RBC counts, RBC indices like MCV,MCH,MCHC,RDW and confirmed by peripheral blood smears. Platelet indices like Mean Platelet volume (MPV), Platelet Distribution Width (PDW) and plateletcrit (PCT) are potentially useful markers for the early diagnosis of thromboembolic diseases. Both MPV and PDW are increased during platelet activation. Plateletcrit is an effective screening tool for detecting platelet quantitative abnormalities**.**

**AIMS:** To study the correlation between the Platelet indices, RBC indices and Peripheral smears in evaluating the Microcytic Hypochromic Anemia.

**MATERIAL AND METHODOLOGY:** It is a prospective study conducted in the Department of pathology from May 2022 to July 2022, a period of three months. A total of 200 cases was studied. Out of 200 100 were control (normal Hb) and 100 were microcytic hypochromic anemia cases. The RBC and platelet parameters were correlated with peripheral smears. The data are presented as mean ± SD for continuous variables. A student's t- test was applied for comparison

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of group means. P value <0.05 was considered statistically significant. **RESULTS:** In this study the difference in mean Hb, RBC count, Hct, MCV, MCH, MCHC, RDW between MHA cases and control was statistically significant (p<0.05). Similarly, the difference in mean platelet count, PDW, MPV and PCT between MHA cases and control was statistically significant (p<0.05).

**CONCLUSION:** This study shows a significant relationship between RBC and platelets parameters in microcytic hypochromic anemia.

**KEYWORDS:** Microcytic hypochromicanemia, Mean corpuscular volume(MCV), Mean corpuscular hemoglobin concentration(MCHC), Red cell distribution width (RDW). Mean Platelet volume (MPV), Platelet Distribution Width (PDW) and plateletcrit (PCT).

**INTRODUCTION:** Anaemia is a condition in which the number of red blood cells or the haemoglobin concentration within them is lower than normal. Haemoglobin is needed to carry oxygen and if you have too few or abnormal red blood cells, or not enough haemoglobin, there will be a decreased capacity of the blood to carry oxygen to the body's tissues. This results in symptoms such as fatigue, weakness, dizziness. The optimal haemoglobin concentration needed to meet physiologic needs varies by age, sex, elevation of residence, smoking habits and pregnancy status. Anemia is defined when hemoglobin less than11.0 g/dl in Non-pregnant women (age 15-49yrs) , 12.0 g/dl in Pregnant women (age 15-49yrs) and 13.0 g/dl in Men (age 15-49yrs). The NFHS's classify anaemia as Mildly anaemic when hemoglobin is 10.0-10.9g/dl , Moderately anaemic 7.0-9.9 g/dl and Severely anaemic <7.0 g/dl.

According to WHO, Anemia affects 1.62 billion people worldwide, equivalent to 24.8 percent of the population making it a major public health problem in both developing and developed countries. Over the time, total cases of anemia increased from 1.42 (1.41–1.43) billion in 1990 to 1.74 (1.72–1.76) billion in 2019. Anemia was responsible for 58.6 (40.14–81.1) million years lived with disability. The regions with the highest burden were Western Sub-Saharan Africa, South Asia, and Central Sub-Saharan Africa. The most common contributing causes globally were Dietary iron deficiency, Vitamin A deficiency, and Beta-thalassemia trait, respectively. Anemia affects all stages of the life cycle but is more prevalent in pregnant women and children. In 2019, global anaemia prevalence was 29.9% (95% uncertainty interval (UI) 27.0%, 32.8%) in women of reproductive age, equivalent to over half a billion women aged 15-49 years. Prevalence was 29.6% (95% UI 26.6%, 32.5%) in non-pregnant women and 36.5% (95% UI 34.0%, 39.1%) in pregnant women. Global anaemia prevalence was 39.8% (95% UI 36.0%, 43.8%) in children aged 6-59 months, equivalent to 269 million children with anaemia. The prevalence of anaemia in children under five was highest in the African Region, 60.2% (95% UI 56.6%, 63.7%)[1].

According to India's NFHS-5 (National Family Health Survey), 57 percent of women and 25 percent of men age 15-49 in India are anaemic. 26% of women are mildly anaemic, 29 % are moderately anaemic, and 3 % are severely anaemic. 20% of men are classified as mildly

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anaemic, 5 % as moderately anaemic, and 0.4 % as severely anaemic.Anaemia prevalence has increased between NFHS-4 and NFHS-5, from 53 percent in 2015-16 to 57 percent in 2019-21 among women and from 23 percent in 2015-16 to 25 percent in 2019-21 among men. Anaemia is more prevalent among children under age 35 months than among older children, with a peak prevalence of 80 percent observed among children age 12-17 months[2].

Anemia is diagnosed using automated hematology analyzer that analyzes parameters such as Hemoglobin, RBC counts, RBC indices like MCV,MCH,MCHC,RDW and confirmed by peripheral blood smears. Hemoglobin values with MCV (80 -100fl), MCH (27 - 32pg), MCHC (30-35%), RDW-CV% (9 - 14.5%) determine the anaemic state. Morphologically anemia can be classified as microcytic hypochromic anemia which characteristically presents with reduced MCV (mean corpuscular volume) values (<80fl) and reduced MCHC (mean corpuscular hemoglobin concentration) values (30gm/dl), normocytic normochromic which has normal MCV (82-100 FL) values, macrocytic hypochromic anemia which shows characteristic increased MCV values (>100fl) and normal MCHC. On peripheral smear, a normal sized red cell is comparable to the size of the nucleus of a small lymphocyte. Microytes are smaller than nucleus of small lymphocytes. Normally, red cells exhibit narrow variations insize as reflected by normal red cell distribution width (RDW). A wide variation in cell size is described as anisocytosis. Hypochromia reflects low haemoglobin content in the red cell and commonly results from iron deficiency. Severely hypochromic and large cells with thin border are termed leptocytes and may also be seen in liver diseases[3].

The etiological method of classification involves anemias due to impaired red cell production, hemolytic anemia due to increased red cell destruction and anemia due to blood loss in cases of trauma or injuries. Iron deficiency is most common cause of microcytic hypochromic anemia. In 2002, iron deficiency anemia (IDA) was considered one of the most important contributors to the global burden of disease[4].

Platelet indices like Mean Platelet volume (MPV), Platelet Distribution Width (PDW) and plateletcrit (PCT) are potentially useful markers for the early diagnosis of thromboembolic diseases. Both MPV and PDW are increased during platelet activation. Plateletcrit is an effective screening tool for detecting platelet quantitative abnormalities[4].

This study tried to explore the correlation of different platelet parameters like platelet counts,Mean Platelet volume (MPV), Platelet Distribution Width (PDW) and plateletcrit (PCT) with RBC parameters and peripheral smears of microcytic hypochromic anemia

**AIMS:** To study the correlation between the Platelet indices, RBC indices and Peripheral smears in evaluating the Microcytic Hypochromic Anemia

**MATERIAL AND METHODOLOGY:** It is a prospective study conducted in the Department of pathology from May 2022 to July 2022, a period of three months. A total of 200 cases was

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studied. Out of 200 100 were control (normal Hb) and 100 were microcytic hypochromic anemia cases. Hb<10.5 g/dL with MCV<76.1fl, MCH <25.05pg, MCHC <31.35%, RDW-CV% >16.35% and RBC count <4.18 million/cumm were considered as microcytic hypochromic anemia cases.

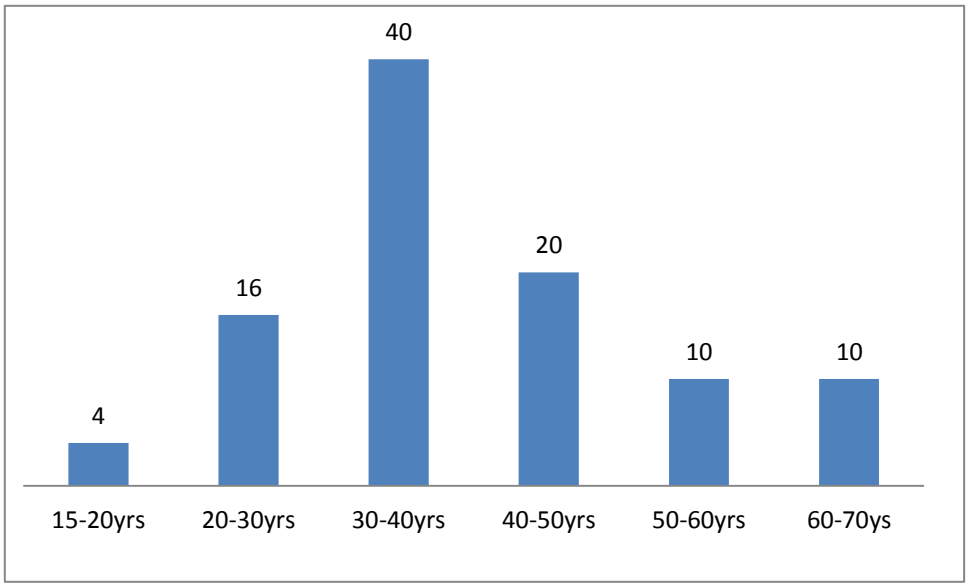
The venous blood samples were collected from patients in Ethylene Diamine Tetracetic Acid (EDTA) vacationers. The samples were run in the five part haematology analyser CELL-DYN Ruby 5 part. RBC and platelet parameters were documented. Peripheral smears were prepared using Leishman stain. The RBC and platelet parameters were correlated with peripheral smears.

**Inclusion criteria:** All cases of anaemia (Haemoglobin <10.5gm%) from both the sexes with age more than 15 years were included in the study as microcytic hypochromic anemia cases. CBC sample with normal Hb (according to WHO) were included as control samples.

**Exclusion criteria:** Cases with leucocytosis, leukemoid reaction, leukemia,parasite, platelet disorders were excluded from the study.

The data are presented as mean ± SD for continuous variables. A student's t- test was applied for comparison of group means. P value <0.05 was considered statistically significant.

**RESULTS:** Of 100 MHA cases, 85 % (n = 85) were female patients and 15 % (n = 15) were male patients. Most common age group among females was between 20 to 30 years and 45 to 65 years in males.




Graph age distribution

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Graph sex distribution

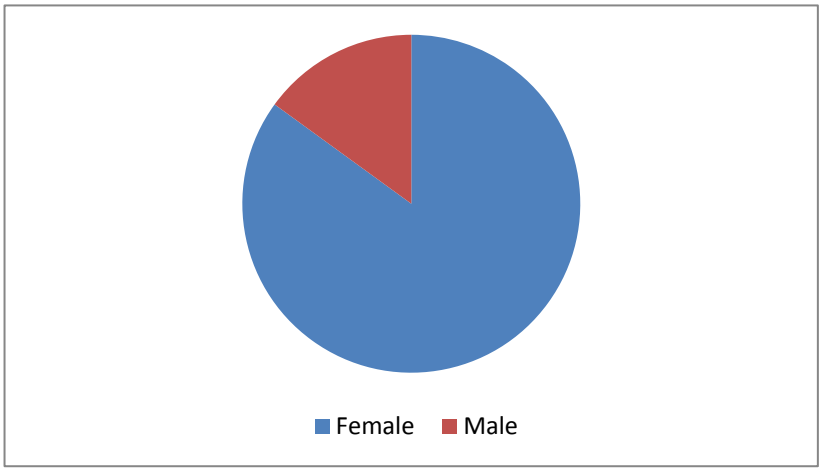
RESULTS Table-1: Comparison of red cell parameters between MHA cases and control groups.

| RBC PARAMETERS | CONTROL | MHA CASES | t values | P values |
|---|---|---|---|---|
| Hb | 13±1.5 | 7.19±1.6 | 26.4 | 0.0001 |
| RBC COUNT | 4.5±0.5 | 3.78±0.73 | 8.13 | 0.0001 |
| MCV | 90±10 | 64.5± 9.03 | 18.9 | 0.0001 |
| MCH | 29.5±2 | 19 ± 4.07 | 23 | 0.0001 |
| MCHC | 33.5±2 | 27.7±3.13 | 15.6 | 0.0001 |
| RDW - CV | 12.5±2 | 15.3± 3.00 | 7.7 | 0.0001 |

In this study the difference in mean Hb, RBC count, Hct, MCV, MCH, MCHC, RDW between MHA cases and control was statistically significant ($p<0.05$).

T able-2: Comparison of platelet parameters between microcytic hypochromic anemia cases and control

| PLATELET PARAMETERS | CONTROL<br>MHA CASES | t values | t values | P values |
|---|---|---|---|---|



ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 03, 2023

| | | | | |
|---|---|---|---|---|
| **Platelet count** | **2.0±1.0** | **3.6 ± 1.39** | **9.3** | **0.0001** |
| **MPV** | **7.2±1.0** | **11.4±1.21** | **26.75** | **0.0001** |
| **PDW** | **10.2±2.0** | **19.3±0.94** | **41.1** | **0.0001** |
| **PCT** | **0.18±0.01** | **0.34±0.07** | **22.6** | **0.0001** |

Similarly, the difference in mean platelet count, PDW, MPV and PCT between MHA cases and control was statistically significant ($p<0.05$).

**DISCUSSION**: Microcytic hypochromic anemia is morphological characterized by significant reduction in red cell size combined with increased central pallor of the red cells. Iron deficiency anemia and thalassemia trait were the commonest causes of a microcytic hypochromic blood picture. RBC parameters which are easily available on automated cell counters plays an significant role in diagnosing anemia.

In our study there was an significant variation in the RBC parameters compared to the control groups. Apart from RBC parameters, platelet parameters showed a significant difference between the Microcytic Hypochromic Anemia cases and control group.

Our study results show a statistical significant increase in platelet count and platelet crit in microcytic hypochromic anemia cases when compared with control (Table 2). This coincides with the results of lavanya Rajagopal et al[5], Yuce S et al[6], Kodikoylu G et al[7], Park MJ et al[8] but contradicts with results of Gupta et al[9], Perlman MK et al[10], Morris VK et al[11].

Munker M et al suggest that both thrombopoietin and erythropoietin belonging to the same hematopoietic growth factor subfamily, are majorly produced in the kidney and act similarly by activating the JAK/STAT pathway and Ras signal transduction on their respective precursors. GATA-1, a transcription factor is expressed in primitive and definite erythroid and megakaryocytic cells and expression of both lineages are dependent on the presence of an intact 40 41 GATA site. Thus a large body of data supports the concept that megakaryocytic and erythrocytic cell lineages share a common progenitor. Megakaryocytes have been shown to express erythroid-specific transcription factors, such as 42 43 GATA factors, a specific DNA-binding protein or a nuclear factor involved in the regulation of 44 globin transcription. Some of the features that are similar to both RBCs and platelets are: I) both develop from a common Megakaryocyte/Erythroid progenitor cell (MEP). A population of probable erythrocytic and megakaryocytic cell lineage precursors coexpressed glycophorin A and glycoprotein IIIa, ii)Both red blood cells and platelets are anucleate form in the peripheral blood, iii) Both the cells have an immature peripheral blood stage called as reticulocyte for RBCs and reticulated platelets for

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platelets .Erythropoietin and thrombopoietin share a high degree of amino acid sequence homology (first 155 amino acids are common)[12].

Iron deficiency is the most common nutritional disorder worldwide, regardless of age, gender and socioeconomic status. About one third of anemic patients have iron deficiency. The annual incidence of iron deficiency anemia is 7.2-13.9 per 1000 people per year. More than 90% of the Microcytic Hypochromic case in our study were found to have iron deficiency anemia.There are few studies on the relationship between iron deficiency anemia and altered platelet parameters, and the relationship between them has long been a subject of debate in the literature.

Iron is postulated to play a key role in the synthesis of platelets and in the regulation of thrombopoiesis.However, exact mechanism leading to these pathological changes is not known. The duration and the degree of IDA may play a role in determining the mechanism of platelet production. In moderate IDA, the causes of thrombocytosis may be: 1) shortening of megakaryocyte maturation 2) increased rate of influx of precursor cells into the megakaryocyte compartment with an increased rate of efflux 3) stimulator effect of transferrin on megakaryopoiesis 4) inhibition of 52-54 iron on megakaryocyte maturation. However, when iron deficiency becomes very severe, megakaryocyte numbers decreased, megakaryocyte size increased, and platelet counts tended to normalize. This may be due to the shortening of megakaryocyte maturation. This could, however, also be consistent with the previously described diphasic pattern of increased stimulation by endogenous Epoprecursors. Bilic and Bilic reported that an amino acid sequence homology between erythropoietin and thrombopoietin may explain thrombocytosis in children with IDA[5].

Our study results show a statistically significant increase in MPV in microcytic hypochromic anemia cases when compared with control (Table 2). This coincides with the results of Park MJ et al[8].

Similarly a statistically significant increase in PDW in microcytic hypochromic anemia cases when compared with control (Table 2). This coincides with the results of Timuragaoglu et al[13].

Several recent studies and meta-analyses suggest that higher MPV and PDW values indicate platelets, which are metabolically and enzymatically more active with a great prothrombotic potential and can be used as an alternative marker for platelet activity. The activated platelets differ in size from non-activated ones mainly due to a change from a discoid to a spherical shape and pseudopodia formation, leading to a change in the Platelet Distribution Width (PDW). The differences in platelet volume vividly correlates with differences in density, platelet aggregation to adenosine diphosphate and serotonin uptake and release, supporting the relevance of the Mean Platelet Volume (MPV) as a measure of platelet function. Thus Platelet size has become an important marker of platelet function and also a physiological variable of hemostatic importance[5].

ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 03, 2023

**CONCLUSION**: This study shows a significant relationship between RBC and platelets parameters in microcytic hypochromic anemia. This study showed microcytic hypochromic anemia especially iron deficiency anemia can cause thrombocytosis, high MPV and PDW with an increased risk of thrombosis.This understanding should pave the way for pathophysiology-oriented therapy to help prevent numerous side effects such as thrombosis associated with iron deficiency anemia and the importance of careful monitoring of these patients.

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