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Original article

RBC INDICES, PERIPHERAL SMEAR AND PLATELET INDICES CORRELATION FOR MICROCYTIC ANEMIA ASSESSMENT: A CROSS-SECTIONAL STUDY.

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ABSTRACT

Aim
The current study aims to explore the relationship among platelet indices, RBC indices and peripheral blood smears in the assessment of microcytic hypochromic anemia.

Methods
A cross sectional study was conducted at the Indira Gandhi Institute of Medical Sciences, Patna, Bihar over the duration of a February 2021 to March 2022, which included 4560 cases comprising indoor and outdoor patients. Peripheral blood smear stained with Leishman were made using blood samples obtained in EDTA tubes. Automated cell counters were for analysis of complete blood counts including hemoglobin, RBC indices and other hematological parameters.

Results
Among 4560 cases, 51.63% were male and 48.37% were female, and adults in their thirties (19.35%) were the group most affected. The most prevalent morphological type was microcytic hypochromic anemia (48.10%), mostly of intermediate severity (63.47%), affecting females and children. Normocytic normochromic anemia (41.01%) was primarily mild (61.52%) and common among the elderly. Macrocytic anemia (11.54%) showed no significant age or severity variations. Only 4.08% cases had dimorphic anemia, with 63.7% being mild.

Conclusion
Anemia's prevalence and severity vary across age groups due to diverse underlying causes. As individuals age progresses, anemia becomes more common, linked to infections, inflammation, chronic diseases, and reduced bone marrow response. Accurate diagnosis and treatment planning require identifying cause, type, and severity of anemia.

Recommendations
According to the study, healthcare providers should monitor age-related anemia prevalence and severity. Consider infections, inflammation, chronic illnesses, and diminished bone marrow response when examining patients, especially as they age. To optimize patient outcomes, accurate diagnosis and individualized treatment regimens should focus on anemia etiology, type, and severity.

***Keywords**: Microcytic Hypochromic Anemia, Red Cell Distribution Width (RDW), Platelet Distribution Width (PDW), Peripheral Smear, Anemia, Hematology Indices.*
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INTRODUCTION
Anemia is a hematological disorder categorized by a deficiency in the quantity of erythrocytes (red blood cells) or hemoglobin within the circulatory system [1, 2]. This insufficiency impairs the capability of the blood to effectively transport oxygen throughout the body. Anemia encompasses various classifications, one of which involves the examination of red blood cell dimensions and hemoglobin content as a means of categorization. When the size of red blood cells is diminutive, it is referred to as microcytic anemia [1]. If the RBCs have a noticeable increase in size, this is a sign of macrocytic anemia. In the event that the individuals in question exhibit regular dimensions, the condition can be classified as normocytic anemia [1]. The hue of the erythrocytes, regardless of being within the range of normalcy or exhibiting a pallid appearance, additionally provides insights into the extent of hemoglobinization [2].
The WHO estimates that anemia affects around 2 billion people worldwide, and that low iron levels are responsible for about half of the instances of anemia [3]. Children under

five years old, children between five and fourteen years old, women between the ages of 15 and 59, men between the ages of fifteen and 59, and senior citizens above the age of sixty are affected, in differing amounts within each specific cohort [2].

Anemia, a prevalent condition among the elderly population, exerts a substantial impact on their overall well-being and life satisfaction [3]. However, it is imperative to acknowledge that anemia should not be passively embraced as an inherent consequence of the aging process. The results of research show that the incidence of anemia rises gradually with age. This condition is more common in women than in men among the population under 75 years old. However, in individuals aged 75 and above, the prevalence of this condition is higher among the male population [3]. Anemia among the elderly population can arise from a multitude of etiologies, encompassing chronic illnesses, inflammatory processes, and insufficient iron levels.

Microcytic hypochromic anemia typically arises from impaired hemoglobin synthesis, frequently attributed to insufficient iron stores [4]. Macrocytic anemia frequently arises due to aberrations in the maturation process of erythrocytes within the confines of the hematopoietic tissue, specifically the bone marrow. Normocytic normochromic anemia encompasses a multitude of etiologies.

In order to ascertain the specific classification of anemia and establish a definitive diagnosis, medical practitioners must conduct comprehensive physical examinations and analyze pertinent hematological parameters [5]. These include of hemoglobin concentrations, hematocrit readings, and a number of red blood cell indices, including red cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) [6]. In addition, it is imperative to conduct microscopic examination of the erythrocytes' morphological characteristics. The determination of anemia severity is predicated upon adherence to the hemoglobin level guidelines as prescribed by the WHO [6].

Therefore, in the present study, a comprehensive analysis was conducted to investigate the association between platelet indices, RBC indices, and peripheral smears in the assessment of microcytic hypochromic anemia.

MATERIAL AND METHODS

Study design

A cross sectional study was designed and conducted.

Study setting

The study was conducted at the Indira Gandhi Institute of Medical Sciences, Patna, Bihar over the duration of February 2021 to March 2022.

Participants

The study encompassed a diverse cohort of participants, representing various age groups, and including both outpatient and inpatient cases. A total of 4560 individuals were specifically chosen based on their hemoglobin levels, quantified in grams per deciliter (g/dl), in accordance with the diagnostic criteria outlined by the WHO for identifying anemia.

Inclusion criteria

All cases of anemia from both the sexes with age more than 15 years were included in the study.

Exclusion criteria

Cases with leukocytosis, leukemoid reaction, leukemia, parasite, platelet disorders were excluded from the study.

In our study, the normal values of hemoglobin are considered according to the age and sex of the individual cases concerning WHO criteria for anemia. According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men.

Data Collection and Analysis

The process of blood sample collection was meticulously carried out using EDTA tubes, followed by immediate automated analysis utilizing a hematology analyzer. This comprehensive examination encompassed the assessment of hemoglobin concentration and an array of parameters associated with red blood cells, including Mean Cell Hemoglobin (MCH), Mean Cell Volume (MCV), Mean Cell Hemoglobin Concentration (MCHC), Red Blood Cell count, hematocrit (PCV), total white blood cell count, platelet count including indices, differential count, and the scrutiny of peripheral blood smears.

To identify anemia and assess its severity, the study utilized the criteria defined by the World Health Organization, specifically focusing on hemoglobin concentrations (g/dL) and RBC indices. The reference ranges for the following parameters were taken into consideration:

- Mean Cell Volume (MCV) = 80-100 femtoliters (fl)
- Mean Cell Hemoglobin (MCH) = 27-32 picograms (pg)
- Mean Cell Hemoglobin Concentration (MCHC) = 32-36 grams per deciliter (g/dl)

Microcytic anemia was diagnosed when the MCV value fell below 80fl, and MCH was less than 27. On the other hand, macrocytic anemia was confirmed when MCV exceeded 100fl. The classification of normocytic normochromic anemia was applied when all hematological indices remained within the specified reference ranges.

Statistical analysis

The data are presented as percentages and mean ± SD for continuous variables. P value< 0.05 was considered statistically significant.

RESULTS

The study encompassed a total of 4560 participants, whose observations and findings were acquired via the analysis of hematological characteristics, RBC indices and peripheral blood smear examinations. These results have been compiled and presented in Table 1. A total of 2354 individuals were identified as male, while 2206 individuals were identified as female. Notably, no statistically significant disparity was observed between the genders.

Table 1: Gender distribution of participants

	Total number	Percentage
Male	2354	51.62%
Female	2206	48.38%

In the present study, the prevalence of mild anemia was found to be the highest, followed by moderate and severe anemia. Males exhibited a higher prevalence of mild anemia, constituting 55.79% of the affected population, whereas females displayed a greater incidence of moderate and severe anemia. Anemia was most prevalent in the adult population, affecting 45.14% of the subjects, with a secondary peak in the age group of 30-41 (19.35%), and the lowest prevalence in another age group (Table 2).

Table 2: Grading of anemia

Severity of anemia	Number of participants	Percentage
Mild	2120	46.50%
Moderate	1884	41.30%
Severe	556	12.2%
Total	4560	100%

Out of the total 4560 individuals presenting with anemia, a notable majority of 549 patients were identified as having microcytic hypochromic anemia (Table 3). This particular subtype emerged as the most prevalent form within the study, accounting for approximately 48.14% of all cases observed. The majority of the participants, 530 exhibited a mild degree of anemia, followed by 471 individuals presenting with moderate anemia, and 139 subjects displaying severe anemia. The predominant demographic of individuals included in this cohort comprised of females within the reproductive age range and pediatric patients

Table 3: The incidence of morphological anemia as determined by peripheral smear analysis

Peripheral smear examination	Total participants	Percentage
Microcytic hypochromic	2194	48.12%
Normocytic normochromic	1870	41.0%
Macrocytic	310	6.80%
Dimorphic	186	4.08%

Additionally, 1870 out of the 4560 participants were found to have normocytic normochromic anemia, making up 41.0% of the cases. Among the normocytic group, 61.52% had mild anemia, with a predominance of adult males. Macrocytic anemia was observed in 6.8% of the 310 patients in this group. Among the cases classified under the macrocytic category, a significant majority of 62.3% exhibited moderate anemia, while 70 individuals presented with severe anemia, and an additional 198 individuals displayed mild anemia. Notably, the macrocytic cohort did not exhibit a statistically relevant correlation with the degree of anemia (Table 4).

Table 4: Peripheral smear examination

Peripheral smear examination	Percentage		
	Mild	Moderate	Severe
Microcytic hypochromic	22.53%	63.47%	47.50%
Normocytic normochromic	61.52%	19.82%	8.88%
Macrocytic	6.94%	10.68%	38.25%
Dimorphic	5.01%	2.03%	1.40%

Within the dimorphic anemia classification, a total of 118 individuals (63.7%) exhibited mild anemia, while 52 individuals presented with moderate anemia. Furthermore, 16 individuals were classified as having severe anemia. The frequency of mild anemia was observed to be greater in males, constituting 51.21% of the cases, whereas moderate and severe anemia exhibited a greater occurrence in females. A statistically relevant disparity was observed in the platelet count, PDW, MPV, and PCT values when comparing patients diagnosed with MHA to the group comprising of anemia other than microcytic hypochromic (Table 5).

Table 5: Platelet characteristics across patients with microcytic hypochromic anemia

Platelet Parameters	MHA cases (Mean)	p-values
Platelet count	3.0	0.0001
MPV	6.2	0.0001
PDW	9.2	0.0001
PCT	0.17	0.0001

DISCUSSION

The study identified a significant prevalence of anemia in both adults and young children, primarily characterized by microcytic hypochromic anemia. Notably, a high incidence of microcytic hypochromic anemia was observed among women, particularly those who had reached menarche, suggesting iron deficiency as a primary cause. The study's findings emphasize the substantial burden of anemia, particularly microcytic hypochromic anemia, in the studied population. The prevalence of anemia in adults and children is indicative of a public health concern that warrants attention. The observed association between iron deficiency and microcytic anemia, especially among post-menarcheal females, underscores the significance of addressing iron deficiency as a key factor contributing to anemia. These results are consistent with previous research conducted by Qureshi *et al.* [5], which also reported a high prevalence of anemia among adults and young children. Similarly, various Indian studies [6-9] have highlighted the substantial occurrence of microcytic anemia linked to iron deficiency in young females. This aligns with our study's outcomes, reinforcing the notion that iron deficiency is a common driver of anemia in this demographic. Moreover, the study's identification of iron deficiency-related microcytic anemia among women of childbearing age has critical public health implications. Anemia is a recognized contributor to maternal mortality in Asia [7, 10], and addressing this issue through targeted health programs and surveys aimed at mitigating iron deficiency could lead to significant reductions in maternal, infant, and child mortality rates. This underscores the importance of developing and implementing effective interventions to combat anemia, particularly in high-risk populations such as young females. The study observed that the male gender constituted 51.63% of the total participants, while the female gender accounted for 46.36%. This gender distribution is similar to the findings reported by Kaur *et al.* [9], where males comprised 37% and females constituted 33% of the sample population. However, these results differ significantly from the study conducted by Choi *et al.* [10], where the male population accounted for only 11.4%, and the female population accounted for merely 2.1%. When comparing our study's gender distribution to that of Kaur *et al.* [9], we find a relatively consistent representation of males and females. This consistency suggests that the gender distribution in anemia studies can vary across different regions and populations. In contrast, the study by Choi *et al.* [10] deviates significantly from our findings, indicating a considerable gender imbalance. This discrepancy emphasizes the need for caution when generalizing findings from one study to another, as the demographic characteristics of the study population can significantly influence the results. Overall, while estrogen's potential role in anemia is an intriguing concept, our study primarily contributes to the understanding of gender distribution in anemia cases. Further research is warranted to explore the

complex interplay of hormonal factors, age, and anemia prevalence in different populations and contexts [8].

The study included 4,560 cases, with a male-to-female ratio of 51.63% to 48.37%. The most affected age group was adults in their thirties (19.35%). Microcytic hypochromic anemia was the most prevalent morphological type (48.10%), primarily of intermediate severity (63.47%), and it predominantly affected females and children. Normocytic normochromic anemia was common (41.01%), primarily mild (61.52%), and more prevalent among the elderly. Macrocytic anemia was observed in 11.54% of cases and showed no significant age or severity variations. Dimorphic anemia was present in 4.08% of cases, with 63.7% being mild [11]. The findings suggest that anemia is a prevalent condition among adults, with varying degrees of severity. Microcytic hypochromic anemia appears to be the most common type, especially among females and children. This could be indicative of factors such as iron deficiency or other underlying causes that affect red blood cell size and hemoglobin content. Additionally, the study highlights that anemia's prevalence and severity vary with age, which is consistent with previous research. Anemia in older adults may be linked to factors like infections, inflammation, chronic diseases, and reduced bone marrow response [11]. Moreover, the study's emphasis on the importance of hematopoietic growth factors in regulating red blood cell production is consistent with existing literature [12-14]. The involvement of renal regulation of erythropoietin (EPO) corresponds with prior research, which underscores the role of the renal system in EPO production [13]. Therefore, these results corroborate and build upon existing knowledge regarding the etiology and factors contributing to anemia in adults.

Microcytic hypochromic anemia was the most prevalent morphologically classified anemia in the current study, with a prevalence rate of 48.10%. Normocytic normochromic anemia accounted for 41.01% of the cases. The findings from our study reveal that microcytic hypochromic anemia is the predominant morphological type of anemia in our study population, affecting nearly half of the cases. This aligns with the observations made in previous studies conducted by Gerardo *et al* [14] and Patel *et al* [15], where microcytic hypochromic anemia was also reported as the most common type of anemia, with a prevalence rate of 72%. These consistent findings suggest that microcytic hypochromic anemia may be a prevalent form of anemia across different populations. In contrast, our study results differ from those of the Kaur *et al* [9] study, where normocytic normochromic anemia was the predominant type, with a prevalence rate of 56%. This variation in results may be attributed to differences in the study populations, geographical locations, or other factors that influence the prevalence of different anemia types. It highlights the importance of considering regional or population-specific variations when assessing the prevalence of anemia subtypes.

In the present study, normocytic normochromic anemia was observed in 41.01% of the cases. A majority of these cases (61.52%) exhibited mild anemia. The elderly population was the predominant age group within the normocytic normochromic anemia category. Adults comprised 45.14% of the total sample, with individuals in their third decade of life accounting for 19.35% of the participants. The study's key results indicate a significant occurrence of normocytic normochromic anemia, with a majority of cases being of mild severity. This type of anemia was particularly prevalent among the elderly population. The distribution of normocytic normochromic anemia cases across different age groups aligns with patterns observed in previous research. Comparing these findings with those of previous studies reveals some variations. For instance, Kaur *et al*.'s study [9] reported a substantial proportion (55%) of subjects aged 60-69 years in their investigation, which contrasts with our study's distribution pattern. In contrast, Patel *et al*. [15] found that the age group with the highest incidence of normocytic normochromic anemia was 21-30 years, comprising 46% of the cases, which differs from our study's results. These variations highlight the potential influence of demographic factors and geographic location on the prevalence of normocytic normochromic anemia. Further research may be needed to explore the underlying causes of these differences and their clinical implications.

A statistically significant increase in MPV in microcytic hypochromic anemia cases when compared with control (Table 5) was noted in our study which was in concordance with the results of Park MJ *et al* [16]. Similarly, a statistically significant increase in PDW in microcytic hypochromic anemia cases when compared with control (Table 5). This coincides with the results of Timuragaoglu *et al* [17].

CONCLUSION

The present study revealed a significant incidence of microcytic anemia, succeeded by normocytic normochromic anemia, as determined by the evaluation of hematological parameters and examination of peripheral blood smears. These findings exhibited variations across different age groups and genders, indicating diverse underlying causes for such anemia. Microcytic hypochromic anemia, characterized by reduced red blood cell size and decreased hemoglobin content, is the predominant type observed in women and younger individuals. This type of anemia is primarily attributed to iron deficiency.

In the adult population, the most commonly observed type of anemia is normocytic normochromic anemia. This specific form of anemia may manifest as a result of multiple etiological factors, encompassing chronic illnesses, inflammatory states, hemorrhage, neoplastic disorders,

reduced bone marrow reactivity and cellularity, or the physiological process of senescence. A considerable proportion of individuals presenting with microcytic anemia demonstrated a moderate manifestation of the disorder, whereas those with normocytic normochromic anemia exhibited a less severe variant.

Anemia in India presents a substantial public health issue, leading to increased mortality rates among pediatric and reproductive-age females. Anemia, although not an independent ailment, presents as a clinical manifestation of various pathological processes.

Limitations

The study has limitations. A Patna, Bihar, single-center study may limit its applicability to varied cultures. Exclusion criteria may have excluded problematic hematology cases. Despite its large sample size, the study may not represent all anemia subtypes in larger populations. It used consistent hemoglobin criteria and ignored iron deficiency causes. Anemia and platelet parameters differed by gender without clinical relevance. These constraints should be considered when assessing and planning anemia prevalence and cause research.

Recommendations

Based on the study's findings, it is recommended that healthcare providers pay close attention to age-related variations in the prevalence and severity of anemia. When assessing patients, particularly as they age, consider a diverse range of underlying causes such as infections, inflammation, chronic diseases, and reduced bone marrow response. Accurate diagnosis and tailored treatment plans should prioritize identifying the cause, type, and severity of anemia to improve patient outcomes.

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List of abbreviations

RBC- Red Blood Cells
WHO- World Health Organization
MCV- Mean Corpuscular Volume
RDW- Red Cell Distribution Width
MCH- Mean Corpuscular Haemoglobin
MCHC- Mean Corpuscular Haemoglobin Concentration

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Conflict of interest

The authors report no conflicts of interest in this work.

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