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RED BLOOD CELL INDICES AND PLATELETS: ANALYSIS OF THEIR ONTOGENIC RELATIONSHIP IN ANEMIC PATIENTS

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Abstract

Background: *Anemia, often a sign of an underlying condition, is a significant global public health issue affecting people in both developed and developing countries. Assessing red blood cell (RBC) indices in conjunction with changes in platelet count in anemic patients is crucial for understanding their relationship and potential clinical significance.* ***Materials and Methods:*** *This hospital-based cross-sectional study was carried out between May 15 and July 15, 2023, among anemic patients (hemoglobin < 10 g/dL) attending G.S. Medical College and Hospital in Pilkhuwa, Hapur. The objective was to examine variations in RBC indices and platelet counts. Collected data included demographic information, RBC indices, platelet count variations, and classifications of anemia. Pearson's correlation coefficient was used to analyze the relationship between platelet count and RBC indices, while the Chi-square test assessed the association between different types of anemia and platelet count variations.* ***Results:*** *Among the 253 anemic patients studied, the female-to-male ratio was 2:1, with the highest prevalence observed in the 20–29-year age group. Marked differences were noted between the minimum and maximum values of individual RBC indices (Hb, MCV, MCH, MCHC) and platelet counts. Platelet count showed a statistically significant positive correlation with RBC values and a significant negative correlation with MCV. In contrast, its correlation with hemoglobin concentration and anemia type was positive but did not reach statistical significance.* ***Conclusion:*** *The findings indicate a notable correlation between platelet count variations, RBC indices, and anemia types in anemic patients. Understanding this association can aid clinicians in both diagnosis and treatment planning.*

INTRODUCTION

The development of human hematopoietic cells has been extensively studied and remains a key area of scientific interest. All three primary blood components—red blood cells (RBCs), white blood cells (WBCs), and platelets—originate from a shared progenitor cell known as the hematopoietic stem cell (HSC) [1].

Anemia is characterized by a decrease in either the total number of RBCs or the hemoglobin (Hb) content within these cells, leading to insufficient oxygen delivery to meet the body's physiological needs. These requirements vary depending on factors such as age, sex, altitude, ethnicity, and pregnancy status [2]. According to the World Health Organization, anemia is diagnosed when hemoglobin levels fall below specific thresholds: <13 g/dL in adult men, <12 g/dL in nonpregnant women over 15 years, <12 g/dL in adolescents aged 12–15 years, and <11.5 g/dL in children aged 5–

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12 years [3]. Severity in adult men is further categorized as mild (11–12.9 g/dL), moderate (7–10.9 g/dL), or severe (<7 g/dL) [4]. For nonpregnant women aged 15 and older, mild anemia corresponds to 11–11.9 g/dL, moderate to 8–10.9 g/dL, and severe to less than 8 g/dL. Globally, anemia is a significant public health challenge, affecting approximately 1.62 billion people—or about 24.8% of the world's population—across both developed and developing regions [5]. Its causes are diverse and often occur in combination, with variations depending on geographic location [6].

Mature RBCs and platelets exhibit similar physiological patterns and are frequently implicated together in various diseases. Several common features between these cells include:

i) Both derive from the megakaryocyte/erythrocyte progenitor (MEP);

ii) Both circulate in an enucleated form, while their nucleated precursors remain in the bone marrow; these nucleated forms appear in peripheral blood only in pathological conditions such as anemia (normoblasts) or myelodysplastic syndrome (micromegakaryocytes) [7];

iii) Each has an immature form in circulation—reticulocytes for RBCs and reticulated platelets for platelets [8];

iv) The growth factor erythropoietin influences the development of both cell types [9];

v) Hematology analyzers measure the volume of erythrocytes and platelets using the same aperture and dilution techniques.

Worldwide, iron deficiency is the predominant cause of anemia [10], although deficiencies in other micronutrients like vitamin B12, folate, riboflavin, vitamin A, and copper also increase the risk. Morphologically, anemia can be classified into:

- Microcytic hypochromic anemia: characterized by low mean corpuscular volume (MCV <80 fl) and low mean corpuscular hemoglobin concentration (MCHC <30 g/dL)
- Normocytic normochromic anemia: normal MCV values (82–100 fl)
- Macrocytic anemia: increased MCV (>100 fl) with normal MCHC [11]

The development of human hematopoietic cells has long been a subject of extensive research and significant scientific interest. All three major hematopoietic elements in the blood—red blood cells (RBCs), white blood cells (WBCs), and platelets—originate from a common progenitor known as the hematopoietic stem cell (HSC) [1].

Anemia is defined as a condition in which either the total number of RBCs or the hemoglobin (Hb) content within them falls below normal levels, resulting in an inadequate oxygen-transport capacity to meet the physiological demands of the body. These physiological requirements differ depending on age, sex, altitude of residence, ethnicity, and pregnancy status [2]. According to the World Health Organization, anemia is diagnosed in adult men when hemoglobin levels are <13 g/dl, in nonpregnant women over 15 years when levels are <12 g/dl, in adolescents aged 12–15 years when <12 g/dl, and in children aged 5–12 years when <11.5 g/dl [3].

In adult men, anemia severity is further classified as mild (11–12.9 g/dl), moderate (7–10.9 g/dl), or severe (<7 g/dl) [4]. For nonpregnant women aged 15 years or older, the thresholds are 11.9–11 g/dl for mild, 10.9–8 g/dl for moderate, and <8 g/dl for severe anemia.

Anemia remains a global public health issue, affecting populations in both developed and developing nations, with an estimated 1.62 billion individuals (about 24.8% of the global population) affected [5]. The condition arises from numerous causes, which may occur alone or more often in combination [5], with the specific etiological factors differing by geographic region [6].

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Mature red blood cells (RBCs) and platelets share comparable physiological rhythms and are often simultaneously involved in various pathological conditions. Several similarities exist between these two cell types:

i) Both originate from a common progenitor known as the megakaryocyte/erythrocyte progenitor (MEP);

ii) In peripheral blood, both are present in an enucleated form, while their nucleated precursors reside in the bone marrow. These nucleated forms appear in peripheral circulation only under pathological conditions, such as normoblasts in anemia or micromegakaryocytes in myelodysplastic syndrome [7];

iii) Each cell type has an immature stage in the peripheral blood—reticulocytes for RBCs and reticulated platelets for platelets [8];

iv) The cytokine growth factor erythropoietin influences the development of both lineages [9];

v) Hematology analyzers assess erythrocyte and platelet volume using the same aperture and dilution method.

On a global scale, iron deficiency is the leading cause of anemia [10]. Other micronutrient deficiencies, such as vitamin B12, folate, riboflavin, vitamin A, and copper, also contribute to its risk. Morphologically, anemia can be categorized into:

- Microcytic hypochromic anemia: reduced MCV (<80 fl) and reduced MCHC (<30 g/dl)
- Normocytic normochromic anemia: normal MCV (82–100 fl) values
- Macrocytic anemia: elevated MCV (>100 fl) with normal MCHC [11]

Since anemia often indicates an underlying medical condition, evaluating hemogram parameters alongside platelet count variations may help uncover potential associations [12]. Normally, platelet counts range between 1.5 to 4 lakhs/mm^3 \ [4]. Produced in the bone marrow, platelets are essential for forming platelet plugs, aiding in clot retraction, and facilitating vascular repair [13]. However, there is limited data regarding the relationship between hemoglobin levels and platelet counts. One study did find a statistically significant inverse linear correlation between hemoglobin concentration and platelet count \ [14].

Although many anemic patients also exhibit platelet abnormalities, only a few studies have explored the correlation between platelet indices and red blood cell (RBC) parameters. Therefore, the present study aims to investigate the relationship between various RBC indices and platelet count variations in anemic individuals with hemoglobin levels below 10 g/dL.

MATERIAL AND METHODS

The study employed a convenience (non-probability) sampling technique, involving the examination of peripheral blood smears from 253 patients, irrespective of age or gender. Inclusion criteria: All patients were eligible for inclusion regardless of their presenting symptoms, sex, age, or reason for visiting either the outpatient (OPD) or inpatient (IPD) departments. Exclusion criteria: Patients who were advised to undergo a complete blood count (CBC) but did not receive a peripheral smear examination were excluded from the study.

For each participant, 3 ml of venous blood was drawn using disposable syringes under strict aseptic conditions. The samples were then transferred to EDTA vacutainers containing anticoagulant at a concentration of 1.5 mg/ml and gently mixed. CBC testing was conducted using the Mindray BC-5150 automated hematology analyzer. Peripheral blood smears were prepared, stained with Leishman

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stain, and analyzed microscopically for cellular morphology. Data analysis included both descriptive and inferential statistical methods. The Chi-square test was used to evaluate the association between different types of anemia and variations in platelet count, with a significance level set at $p < 0.05$. Pearson's correlation coefficient was also calculated to assess the relationship between platelet count and red blood cell (RBC) indices.

RESULTS

Among the total study population of 253 participants, females (n = 168; 66.5%) outnumbered males (n = 85; 33.5%), with the highest occurrence seen in individuals in their third decade of life (Table 1). Minimum, maximum, mean, and standard deviation values were calculated for hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC count, and platelet count (Table 2).

Microcytic hypochromic anemia emerged as the most prevalent type, followed by normocytic and macrocytic anemia. Platelet count variations were assessed using an automated blood cell counter, with flagged results cross-verified manually through peripheral blood smear examination.

Pearson's correlation coefficient was used to analyze the relationship between RBC indices and platelet count variations. A statistically significant positive correlation was observed between platelet count and RBC count ($r = 0.15$, $p = 0.01$). Conversely, platelet count showed a significant negative correlation with MCV ($r = -0.16$, $p = 0.01$) and a weaker but still significant negative correlation with MCH ($r = -0.12$, $p = 0.05$). Although a positive correlation was found between platelet count and hemoglobin levels, it was not statistically significant ($r = 0.021$, $p = 0.73$) (Table 4).

Table 1: Frequency of age groups of the patients

Age group (years)	Frequency n (%)
0-9	17 (6.7%)
10-19	21 (8.3%)
20-29	67 (26.4%)
30-39	47 (18.6%)
40-49	42 (16.6%)
50-59	17 (6.7%)
60-69	27 (10.7%)
≥70	16 (6.3%)

Table 2: RBC indices and platelet count range

RBC indices and platelet counts (units)	Minimum	Maximum	Mean	Std. Deviation
Hb Level (gm/dl)	**1.7**	**9.9**	**8.39**	**1.28**
MCV (fl)	51	116.9	79.19	12.43
MCH (pg)	13.4	37.7	25.06	4.99
MCHC (gm/dl)	21.6	37	31.28	2.03
RBC (million/mm3)	0.62	5.72	3.47	0.77
Platelet (lakh/mm3)	0.28	7.11	2.42	1.32

Table 3: Variation in anemia & platelet count

Types of anemia	Platelet count variation		
	Thrombocytopenia	Normal count	Thrombocytosis
Microcytic hypochromic	16(11.3%)	100(70.92%)	25(11.3%)
Normocytic normochromic	8(8.7%)	27(29.34%)	57(61.95%)
Macrocytic anemia	5(25%)	5(25%)	10(50%)

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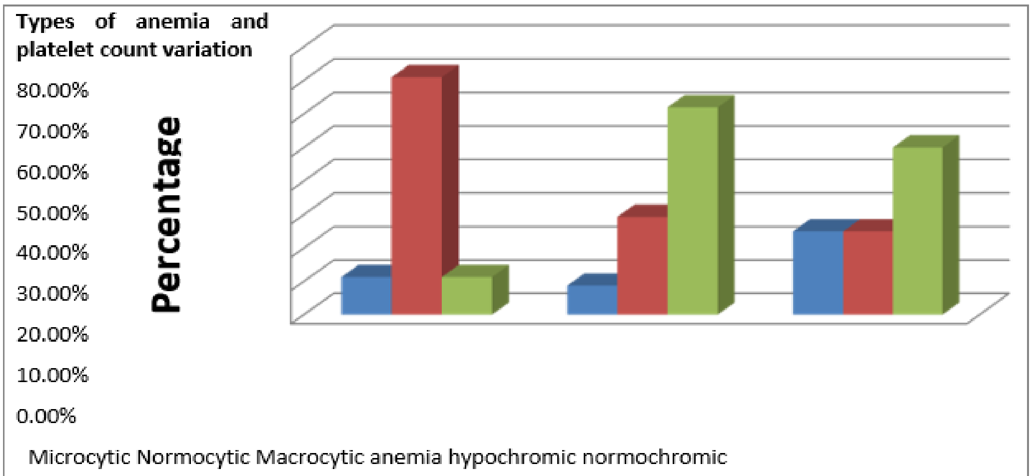

Thrombocytopenia	11.30%	8.70%	25%
Normal count	70.92%	29.34%	25%
Thrombocytosis	11.30%	61.95%	50%

Table 4: Correlation of RBC indices with platelet count variation

	RBC and indices values				
Platelet count variation	**Hb**	**MCV**	**MCH**	**MCHC**	**RBC**
Pearson's correlation coefficient (r)	0.021	-0.16	-0.12	-0.05	0.15
p-value	0.73	0.01	0.05	0.42	0.01

DISCUSSION

This study was founded on two key observations: first, the clear ontogenic link between erythrocytes and megakaryocytes, which is evident in their maturation processes; and second, that RBCs and platelets are analyzed using the same detector with hydrodynamic focusing, producing comparable and parallel parameters. Our research focused on anemic patients with hemoglobin levels below 10 g/dL, irrespective of age or sex. Females made up the majority (66.5%) compared to males (33.5%), with the most common age group being 20–29 years (Table 1). A similar study by Jadhav SU et al. [15] also included patients with Hb < 10 g/dL, though their sample was limited to individuals aged 20–40 years. In our study, RBC indices and platelet counts were analyzed independently (Table 2). Jadhav SU et al. [15] reported that among their participants, hemoglobin levels most commonly ranged from 9.1 to 10 g/dL in 59 females, while 41 males predominantly had levels between 6.1 and 7 g/dL. In our dataset, the majority of patients (n = 167; 66%) had platelet counts within the normal range of 1.5–4.5 lakhs/mm³, followed by 57 patients (22.53%) with thrombocytopenia (<1.5 lakhs/mm³) and 29 patients (11.47%) with thrombocytosis (>4.5 lakhs/mm³). By contrast, Jadhav SU et al. [15] identified thrombocytopenia as the most common platelet abnormality.

In our study, anemia was classified according to MCV and MCH values, with microcytic hypochromic anemia being the most prevalent subtype (141 cases; 55.73%), followed by normocytic normochromic and macrocytic anemia. These results are consistent with observations reported by Jadhav SU et al. \ [15] and Kafle SU \ [16]. When examining platelet count variations, normal platelet counts were the most common, followed by thrombocytopenia and thrombocytosis. This pattern aligns with findings by Kafle SU \ [16], but differs from the study by Jadhav SU et al. \ [15]. Thrombocytopenia and thrombocytosis were also noted in patients with normocytic anemia (27 cases; 29.34%) and microcytic

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hypochromic anemia (16 cases; 11.3%). These changes in platelet counts among anemic patients may be related to the interaction between erythropoietin (EPO) and thrombopoietin (TPO). EPO, the hormone that regulates red blood cell production, shares significant structural similarity with TPO, particularly in the first 155 amino acids. This similarity, combined with elevated EPO levels, might contribute to alterations in platelet counts. Bilic E et al. \ [17] explained the occurrence of thrombocytosis in children with iron deficiency anemia through this mechanism.

Our correlation analysis revealed a statistically significant negative correlation between MCV and platelet count ($r = -0.16$, $p = 0.01$), which is in agreement with findings from Kafle SU [16]. However, Ray S et al. [18] reported no significant correlation ($p = 0.197$). Additionally, we observed a significant positive correlation between RBC count and platelet count ($r = 0.15$, $p = 0.01$), supporting Kafle SU's [16] results and suggesting a direct association influenced by the EPO–TPO relationship. On the other hand, hemoglobin concentration showed a positive but statistically insignificant correlation with platelet count ($r = 0.021$, $p = 0.73$) in our study. This contrasts with the findings of Ray S et al. \ [18], who reported a significant negative correlation ($r = -0.157$, $p = 0.042$), and Ram Mohan A et al. \ [19], who also noted inverse correlations. Similarly, Jadhav SU et al. \ [15] found that platelet counts tended to decrease as hemoglobin levels declined. Berad AS et al. \ [20] reported comparable results, indicating an inverse relationship between platelet counts and hemoglobin levels.Other RBC indices in our study, including MCH and MCHC, showed negative correlations with platelet count (MCH: $r = -0.12$, $p = 0.05$; MCHC: $r = -0.05$, $p = 0.42$), but these were not statistically significant. An inverse linear relationship between MCV and platelet count has been described in inflammatory bowel disease [21]. This relationship has clinical relevance in plateletpheresis, where higher donor platelet counts are associated with reduced MCV, suggesting a greater likelihood of donor iron deficiency in high-yield procedures [22]. Likewise, an inverse relationship between Hb and platelet count has been reported in multiple sclerosis [23], inflammatory bowel disease [21], and other medical conditions.

CONCLUSIONS

The observed correlations between RBC indices, anemia subtypes, and platelet counts in anemic patients underscore the importance of this relationship. Our results showed a statistically significant negative correlation between MCV and platelet count ($r = -0.16$, $p = 0.01$), which could have clinical implications, particularly in plateletpheresis. Since iron deficiency anemia can cause thrombocytosis, a better understanding of this mechanism might aid in developing pathophysiology-driven treatments to prevent complications like thrombosis. Additionally, RBC count exhibited a statistically significant positive correlation with platelet count ($r = 0.15$, $p = 0.01$), highlighting the close connection between erythropoietin (EPO) and thrombopoietin (TPO). Both RBCs and platelets play roles in inflammatory processes seen in diseases such as inflammatory bowel disease and rheumatoid arthritis. Awareness of these links may help clinicians improve diagnosis and management, especially since anemia often occurs alongside platelet abnormalities.

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