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Original Research Paper

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DIAGNOSTIC SIGNIFICANCE OF NEUTROPHIL HYPERSEGMENTATION IN MACROCYTIC ANEMIA: A PROSPECTIVE STUDY

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ABSTRACT Neutrophil hypersegmentation is characterized by the presence of ≥5% neutrophils exhibiting five or more nuclear lobes, or even a single neutrophil containing six lobes. It represents a significant hematological finding commonly associated with deficiencies of cobalamin or folate. In the present study, we assessed the occurrence of neutrophil hypersegmentation across all peripheral blood smears received in our hematology laboratory. A total of 350 samples were included, which demonstrated peripheral smear evidence of neutrophil hypersegmentation in the absence of macrocytosis. Complete blood counts were performed using automated hematology analyzers, and the peripheral smear findings were correlated with these parameters. The smears were stained with Leishman stain, and the observations were systematically recorded. Hypersegmentation was observed more frequently in male patients and was predominantly associated with normocytic, normochromic red blood cell indices. Neutrophilia emerged as the most common associated hematological finding. Our findings indicate that neutrophil hypersegmentation is not exclusively specific to vitamin B12 or folate deficiency anemia and is often encountered in cases of iron deficiency anemia. However, in patients with iron deficiency anemia exhibiting neutrophil hypersegmentation, the possibility of a concurrent folate or vitamin B12 deficiency should always be considered and excluded.

KEYWORDS : Peripheral Smears, Neutrophil Hypersegmentation, Anaemia

INTRODUCTION

Neutrophil hypersegmentation is defined by the presence of ≥5% neutrophils exhibiting five or more nuclear lobes, or the identification of a single neutrophil with six lobes. It represents a well-established hematological marker of cobalamin and folate deficiency. In the present study, all peripheral blood smears received in the hematology laboratory were systematically evaluated for the presence of neutrophil hypersegmentation.

MATERIALS AND METHODS

This prospective observational study was conducted over a one-year period from January 2025 to December 2025 at Government Medical College and its attached District General Hospital. EDTA-anticoagulated venous blood samples received in the hematology laboratory for routine complete hemogram analysis were considered for inclusion. Peripheral blood smears were prepared for all samples and systematically screened for the presence of neutrophil hypersegmentation. A total of 350 samples demonstrating neutrophil hypersegmentation, in the absence of morphological evidence of macrocytosis, were selected for further evaluation. Complete blood counts, including red cell indices and leukocyte parameters, were obtained using automated hematology analyzers and correlated with the corresponding peripheral smear findings. Peripheral smears were stained using Leishman and Field stains (field stain A-Methylene blue/azure whereas field stain B – Eosin) - according to standard laboratory protocols. Leishman stain protocol involves fixing an air-dried thin blood smear with methanol-based Leishman stain 8-10 drops(for 1-2 mins), diluting with buffered water (pH 6.8, 5-10 mins) to stain cell components, rinsed under running water and then dried and examined under light microscopy. Relevant hematological parameters and morphological features were recorded and documented for subsequent analysis.

RESULTS

In the present study of 350 peripheral smear samples, neutrophil hypersegmentation was observed more frequently in male patients (Figure 1). When correlated with red blood cell morphology (Figure 2), hypersegmentation was most commonly associated with microcytic hypochromic anemia (41%), followed by isolated hypochromia (28%), normocytic normochromic anemia (22%), and morphologically normal smears (9%). Analysis of leukocyte counts (Figure 3) revealed that neutrophilia was the predominant finding, present in 82% of cases, while eosinophilia (15%), lymphocytosis (2%), monocytosis (0.6%), and basophilia (0.4%) were less frequent. Age distribution analysis (Figure 4) demonstrated peak prevalence in the 41–50 year age group (26.34%), followed by 51–60 years (19.19%), with minimal occurrence in elderly patients above 70 years. Thrombocytosis was observed in 7% of cases with hypersegmented neutrophils. <1% of these patient's smears had thrombocytopenia.

Correlation with mean cell volume (MCV) (Figure 5) showed that the majority of hypersegmentation cases (66.68%) occurred within the normal MCV range (80–100 fL), while 28.12% were seen in microcytic states (<80 fL) and only 5.20% in macrocytic states (>100 fL). Similarly, mean cell hemoglobin (MCH) values (Figure 6) revealed that hypersegmentation was most frequent in the normal range (27–32 pg, 53.91%), followed by low MCH (<27 pg, 41.54%), with only 4.55% in the high range (>32 pg). Mean cell hemoglobin concentration (MCHC) analysis (Figure 7) demonstrated that 76.19% of cases occurred within the normal range (30–35 gm/dL), while 18.26% were associated with low MCHC (<30 gm/dL) and 5.55% with high MCHC (>35 gm/dL).

Taken together, these findings highlighted that hypersegmented neutrophils are a hallmark diagnostic feature of macrocytic anemia. Neutrophilia emerged as the most consistent hematological association, while the majority of cases occurred even when MCV, MCH, and MCHC values were within normal ranges. This highlights the specificity of hypersegmentation as a sole diagnostic marker for macrocytic anemia and emphasizes the need for biochemical correlation to exclude concurrent vitamin B12 or folate deficiency.

DISCUSSION

In the present study of 350 peripheral smear samples, neutrophil hypersegmentation was observed not only in iron deficiency anemia but also in cases with normocytic, normochromic red cell morphology. The underlying mechanism of hypersegmentation in megaloblastic states is attributed to impaired DNA synthesis due to cobalamin or folate deficiency, leading to defective nuclear maturation. None of the patients in this study had a history of drug exposure or underlying malignancy that could explain these findings.

Previous studies have highlighted the association between neutrophil hypersegmentation and iron deficiency anemia. Düzgün S et al. reported the occurrence of neutrophil hypersegmentation along with thrombocytosis in children with iron deficiency anemia. Similarly, Westernman D.A. et al. conducted a case–control study evaluating neutrophil hypersegmentation in iron deficiency anemia. The association between iron deficiency and neutrophil hypersegmentation was first described by Chanarin I et al. in 1965. Furthermore, Beard J.L. and Weintraub L.R. documented cases of iron deficiency anemia with neutrophil hypersegmentation in the absence of overt cobalamin or folate deficiency, although one patient demonstrated borderline cobalamin levels.

In addition, studies have shown variability in folate status among patients with hypersegmentation. Das K.C. et al. reported patients with iron deficiency anemia exhibiting neutrophil hypersegmentation without biochemical evidence of cobalamin or folate deficiency, with persistence of these changes even after iron therapy in most cases. Siphahi E. et al. also observed a high prevalence of neutrophil hypersegmentation, identifying it in 81% of pediatric patients with iron deficiency anemia. These findings suggest that neutrophil hypersegmentation is not exclusively limited to megaloblastic anemia and may be encountered in a broader spectrum of hematological conditions.

CONCLUSION

The findings of the present study indicate that neutrophil hypersegmentation is not a specific morphological marker confined to vitamin B12 or folate deficiency anemia, but is also frequently observed in cases of iron deficiency anemia. This highlights the limited specificity of hypersegmentation as an isolated diagnostic indicator of megaloblastic anemia. Nevertheless, the presence of neutrophil hypersegmentation in patients with iron deficiency anemia warrants careful evaluation, as it may signify an underlying or concurrent deficiency of vitamin B12 or folate. Therefore, comprehensive biochemical assessment is essential to accurately identify and address any coexisting nutritional deficiencies, ensuring appropriate clinical management.

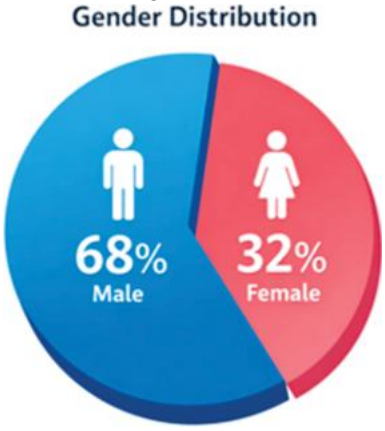


Figure 1: Gender Distribution of Hypersegmentation in Patients with Anemia

Morphological Pattern	Percentage of Cases
Microcytic hypochromic morphology	41%
Isolated hypochromia	28%
Normocytic normochromic anemia with hypersegmentation	22%
Morphologically normal smears	9%

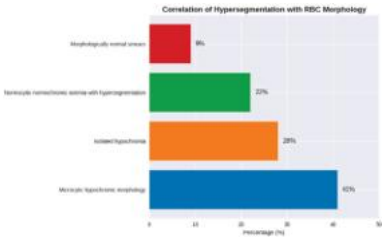


Figure 2: Correlation of Hypersegmentation with RBC Morphology

WBC Type	Percentage of Cases
Neutrophilia	82%
Eosinophilia	15%
Basophilia	0.4%
Monocytosis	0.6%
Lymphocytosis	2%

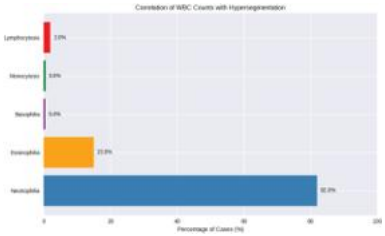


Figure 3: Correlation of WBC Counts with Hypersegmentation

Age Group (years)	Percentage of Cases
≤10	6.28%
10–20	10.58%
21–30	11.39%
31–40	12.73%
41–50	26.34%
51–60	19.19%
61–70	7%
71–80	0.39%

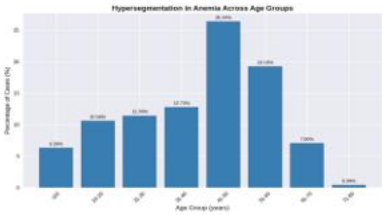


Figure 4: Hypersegmentation in Anemia Across Different Age Groups

MCV Range	Percentage of Cases
<80fL	28.12%
80–100 fL	66.68%
>100 fL	5.20%

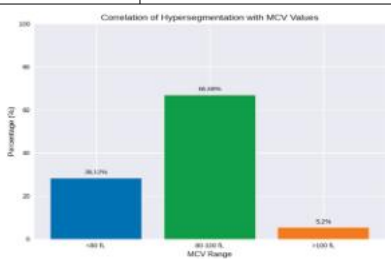


Figure 5: Correlation of Hypersegmentation with Mean Red Cell Volume

MCH Range	Percentage of Cases
<27pg	41.54%
27–32 pg	53.91%
>32 pg	4.55%

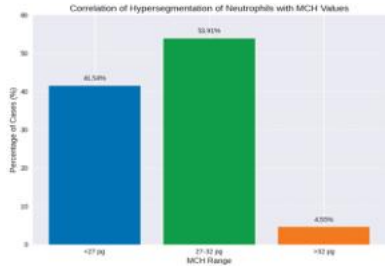


Figure 6: MCH with Hypersegmentation of Neutrophils

MCHC Range (gm/dL)	% with Hypersegmentation
<30	18.26%
30–35	76.19%
>35	5.55%

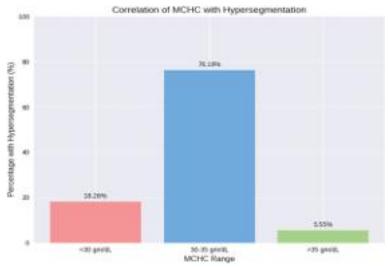
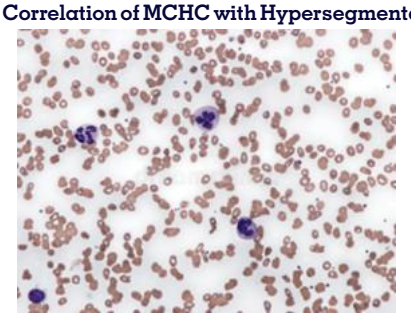


Figure 7: Correlation of MCHC with Hypersegmentation



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