

To Evaluate Peripheral Smears with Hypersegmented Neutrophils-an Etiological Analysis in Tertiary Care Center of North Bihar

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Abstract

Aim: To evaluate peripheral smears with hypersegmented neutrophils and classify the etiological factors.

Material & Method: This is a prospective study carried out over a period of one year conducted in Department of Pathology, Darbhanga Medical College, Laheriasarai, Darbhanga, Bihar, India. EDTA blood samples received in our hematology laboratory were analyzed for hypersegmentation of neutrophils using leishman stained peripheral smears.

Results: Although major cases were contributed by macrocytic anemia, 43.7% cases were having microcytic hypochromic anemia. Out of the 42 cases with normocytic normochromic blood picture; only 12 had subnormal levels of either Vit B12 or folic acid values. Rest of the 30 cases had normal Vit B12 and folic acid levels.

Conclusion: Microcytic hypochromic anemia, myelodysplastic syndromes and inflammatory conditions also can cause hypersegmented neutrophils in peripheral smears. Study also points to increased incidence thrombocytosis in pure Microcytic hypochromic anemia cases compared to other etiological factors which is still to be established with further detailed studies.

Keywords: Hypersegmented neutrophils, Peripheral smear

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Introduction

Hypersegmented neutrophils are classically seen with folate (vitamin B9) or cobalamin (vitamin B12) deficiency. These morphologic changes of the neutrophil nucleus occur due to impaired DNA synthesis from inadequate substrate or impaired replication from a toxin or

medication effect. Arrest of nuclear maturation, impaired cell division, and unbalanced cell growth results in characteristic large cells with immature nuclei with relative cytoplasmic maturity. Red blood cell macrocytosis often accompanies hypersegmented neutrophils

and can be seen in hypothyroidism, alcohol abuse, uremia, and myelodysplastic syndromes. [1]

Hyper segmentation of neutrophils is defined as presence of 5% or more neutrophils with five or more nuclear lobes or single neutrophil with 6 lobed nucleus. [2] It is usually associated with deficiency of or failure to utilize cobalamin or folate and impaired DNA synthesis is the accepted mechanism for the morphological changes seen in megaloblastosis. [3-4]

Pancytopenia is characterized by low levels of hemoglobin (Hb), Low ANC (Absolute neutrophil count) and low platelets. The list of differentials which can account for this abnormality is extensive, as are the potential investigations and associated treatments. [5]

The co-morbidities result from a variable degree of cytopenia and clonal instability with a tendency to progression mainly into acute myeloid leukemia (AML). [6] Uremia, hyperthermia, drugs including chemotherapy, steroids, G-CSF are also known to produce neutrophil hyper segmentation. [7]

Thus, this study aims to evaluate peripheral smears with hypersegmented neutrophils and classify the etiological factors.

Material & Method:

This is a prospective study carried out over a period of one year conducted in Department of Pathology, Darbhanga Medical College, Laheriasarai, Darbhanga, Bihar, India.

A total of 160 Patients classified according to the peripheral smear picture. Patients with microcytic hypochromic anemia were separately assessed for serum Vit B12 and folic acid values using ion capture assay and micro particle enzyme intrinsic factor assay. Presence of thrombocytosis in pure microcytic hypochromic anemia cases were checked separately and it was

compared with presence of thrombocytosis in cases with normal hemogram without microcytic hypochromic anemia.

Patients with known medical conditions like pregnancy, uremia, renal failure and exposure to drugs like chemotherapeutic agents, steroid and G-CSF were excluded.

Methodology

EDTA blood samples received in our hematology laboratory were analyzed for hyper segmentation of neutrophils using leishman stained peripheral smears. Neutrophils hyper segmentation is defined as the presence of five or more five-lobed neutrophils per 100, or any neutrophils with six or more lobes. 160 such cases which satisfied the inclusion criteria were taken as sample size. Complete blood count of individual cases was obtained using ERBA Mannheim H360 analyzer and peripheral smear picture was compared with blood counts.

Results:

Detailed analysis of all cases showed the following details. Age and gender distribution of cases are shown in Table 1. Majority of cases were males and majority of cases were in the age group 40-60.

Cases were further analyzed for associated peripheral smear picture. Although major cases were contributed by macrocytic anemia, 36.25% cases were having microcytic hypochromic anemia. Detailed picture is given in Table 2.

Table 3 clearly shows that out of the 25 cases with normocytic normochromic blood picture, only 10 had subnormal levels of either Vit B12 or folic acid values. Rest of the 15 cases had normal Vit B12 and folic acid levels. Out of the 160 cases with hyper segmented neutrophils in peripheral smear 36% cases were having pure microcytic hypochromic anemia without any vit B12 or folic acid deficiency.

Platelet count of all cases was assessed. Results are shown in Table 4. 1.5-4.5

lakh/microliter is considered as normal platelet count. Out of the 160 cases, only 19 had Platelet count less than

1.5lakh/cmm. 141 cases had platelet count in the normal range.

Table 1: Age and gender distribution of all cases showing hyper segmented neutrophils in peripheral smears

Gender	Below 20	20-40	40-60	Above 60	Total
Male	8	18	44	27	97
Female	6	10	33	14	63
Total	14	28	77	41	160

Table 2: Peripheral smear picture of cases with hypersegmented neutrophils

Macrocytic anemia	70
Microcytic hypochromic anemia	58
Normocytic normochromic blood picture	25
Myelodysplastic syndrome	7
Total	160

Table 3: Serum Vit B12 and folic acid values of cases with neutrophil hyper segmentation in microcytic hypochromic blood picture

Vit B12(in pg /ml)	Observed frequency	Folic acid (in ng/ml)	Observed frequency
<200pg/ml	9	<2ng/ml	5
200-500pg/ml	28	2-8ng/ml	8
500-700pg/ml	13	8-15ng/ml	30
700-900pg/ml	5	15-20ng/ml	14
>900pg/ml	3	>20ng/ml	1
Total	58		58

Table 4: Correlation of neutrophil hyper segmentation and platelet count

Platelet count	Macrocytic anemia	Microcytic hypochromic Picture (Normal B12 and folic acid)	Microcytic hypochromic Blood picture (subnormalB12 and folic acid)	Myelodysplasia	Normocytic Normochromic Blood Picture
<1.5 lakh/microlitre	11	1	2	2	3
1.5-4.5 lakh/microlitre	54	19	12	5	13
>4.5lakh/microlitre	5	21	3	0	9
Total	70	41	17	7	25

Discussion:

Hypersegmented neutrophils without red blood cell macrocytosis, as in our patient, has been described in patients with hyperthermia, uremia, and concurrent megaloblastic and microcytic anemia from combined folate and/ or cobalamin

deficiency along with iron deficiency or thalassemia. As the finding of hypersegmented neutrophils precedes macrocytosis, neutrophil hyper segmentation without macrocytosis may represent early cobalamin and folate deficiency. [8]

The term “botryoid” refers to nuclei that appear like a cluster of grapes around a stem.[9]. Botryoid nuclei have been described in patients with hyperthermia due to cocaine and methamphetamine use, [10] malignant hyperthermia, neuroleptic malignant syndrome, [11] and autoimmune disorders such as rheumatoid arthritis, psoriatic arthritis, and systemic sclerosis. [12] In comparison, the multilobed nuclei in cobalamin and folate deficiency appear disorganized.

The importance of B12 as a cofactor in the body for varying reactions has been well studied. It plays an essential role in DNA synthesis, hematopoiesis and myelination. Given the hematological picture presented in both cases, the list of differentials was extensive. However, the characteristic finding of oval macrocytosis and hypersegmented neutrophils was key in both cases, ultimately resulting in the diagnosis of B12 deficiency. [13] A normal neutrophil has up to three to four lobes in its nucleus, and a hypersegmented neutrophil has five or greater. Microscopic criteria cite 1 neutrophils with six lobes or 5% with five lobes as a relevant finding. There were several other abnormalities in the blood profile, namely the elevated LDH and elevated bilirubin. The latter findings create a picture which is similar to a hemolytic anemia; however, in this case, the etiology is lysis of immature cells due to ineffective erythropoiesis and associated release of LDH. [14-15]

There are some explanations in previous studies that neutrophil changes in iron deficiency represent a recent event with only young red cells and not the overall population of red cells, showing a reduced folate content. [16-17] Some other studies shows that iron deficiency can affect the folate dependent degradation of FIGLU catalyzed by enzyme FIGLU transferase. [18-19] It is also possible that iron deficiency may directly influence DNA synthesis. [20-21]

Vitamin B12 deficiency is prevalent among the older population. The Framingham study demonstrated a prevalence of 12% among older people living in the community while some estimates suggest as many as 30%–40% in institutions, with the predominant pathology food-cobalamin malabsorption and pernicious anemia. [22] The presentation is usually insidious due to the gradual reduction in the stores of B12 rather than an acute decline. Symptoms are polymorphic and include hematological, neuropsychiatric, digestive including altered bowel habit and possibly gynecological. [22]

Neutrophil hyper segmentation and thrombocytosis can be expected as a part of megaloblastic anemia. But it may be also seen in iron deficiency anemia also. [23-25]

Conclusion:

Causes of neutrophil hyper segmentation, microcytic hypochromic anemia, myelodysplastic syndromes and inflammatory conditions also can cause hypersegmented neutrophils in peripheral smears. Study also points to increased incidence thrombocytosis in pure microcytic hypochromic anemia cases compared to other etiological factors which is still to be established with further detailed studies.

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