

# TITLE - ASSOCIATION BETWEEN PRESENCE OF MULTISEGMENTED NEUTROPHILS AND ITS POSSIBLE CAUSATIVE FACTORS: A PROSPECTIVE COHORT STUDY

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### Abstract:

**Background:** Hypersegmentation of neutrophils is defined as presence of 5% or more neutrophils with five or more lobes or single neutrophil with 6 lobes. It is usually associated with deficiency of or failure to utilize cobalamin or folate and impaired DNA synthesis. **Aims & Objectives:** to find out any abnormality in the neutrophils of those subjects having hypersegmented neutrophils, to assess the manifestations in peripheral smear of these patients and to find out any association of the presence of hyper segmented neutrophils in peripheral smear with Vitamin B12 level, folic acid

level and platelet count. **Methodology:** A prospective study was carried out from November 2021 to June 2022 in the department of pathology of a tertiary care teaching hospital of Government medical college, Betiah, Bihar among 136 study participants by total consecutive sampling. Patients having hypersegmented neutrophils were administered for data collection and were subjected to further tests. Analysis of data was done using SPSS software version 22. Multivariate regression analysis was performed and the value of  $p < 0.5$  was taken as statistically significant. **Results:** Hypersegmented neutrophils were present more commonly in females (75 out of 136, 55%) as compared to those of males (61 out of 136, 45%). Most of the study participants 81 out of 136 (59.56%) were in the age group of 31-60 years and educated upto primary level (52 out of 136, 38.24%). The various abnormalities in neutrophils in addition to hypersegmentation seen were cytoplasmic toxic granules, cytoplasmic vacuoles and dohle bodies. The peripheral smear has macrocytic normochromic appearance in about 35.29% of the cases. Lower vitamin B12 levels was significantly associated ( $p < 0.01$ ) with presence of hypersegmented neutrophils.

**Conclusion:** The presence of hypersegmented neutrophils were significantly associated with lower vitamin B12 levels which is one of its possible causative factor.

**Keywords:** Hypersegmented neutrophil, causative factor.

## Introduction

Granulocytes, a class of white blood cells, include neutrophils. As they are included under granulocytes, they contain granules. Neutrophil granules generally tint pink or purple-blue after being exposed to a dye. Neutrophils make up between 50 and 80 percent of the white blood cells found in the human body. The neutrophils' diameter, which ranges from 9 to 15 micrometres, is quite uniform. The nucleus comprises of two to five lobes connected by filaments that resemble hairs. These cells are a crucial component of the immune system and aid the body in the battle against infection [1]. Neutrophils are among the first immune cells to react when bacteria or viruses enter the body. When they arrive at the infection site, they consume the germs and release enzymes that kill them. [2]

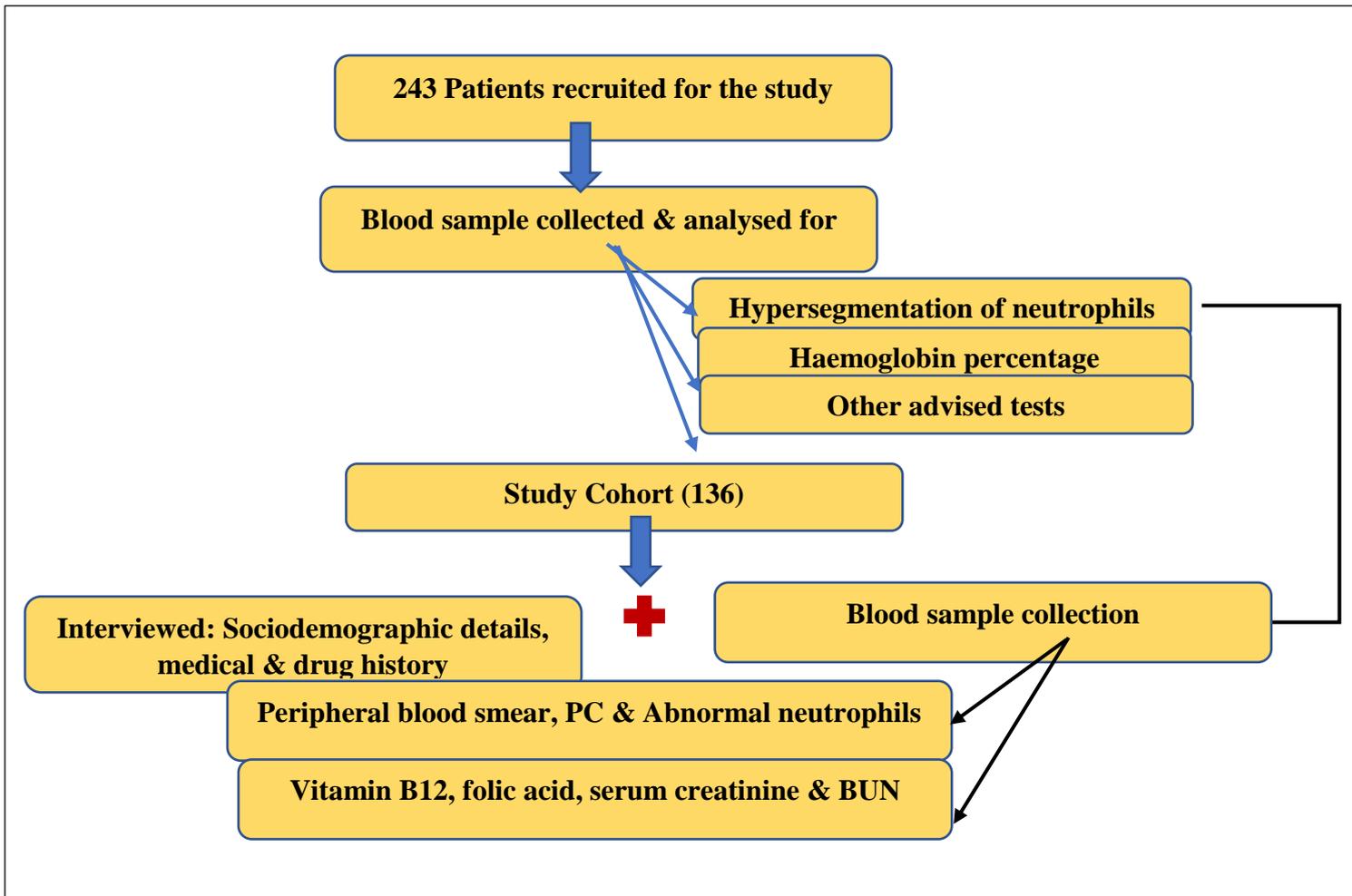
A neutrophil is said to be hyper segmented if more than 5% of them exhibit lobes with less than five, or even if only one exhibits lobes with more than six. [3] It can be recognised even before anaemia sets in and is one of the earliest symptoms of megaloblastic hematopoiesis. An significant haematological sign of cobalamin or folate insufficiency is neutrophil hyper segmentation. [4] The predicted observation in anemias caused by folic acid and vitamin B12 deficiency is neutrophil hypersegmentation. But prior research [5] indicated that hypersegmentation could also be present in iron deficiency anaemia, and subsequent research [6,7] supported this finding. It is unclear how iron deficiency anaemia causes neutrophil hypersegmentation [8]. Iron may or may not have an impact on how neutrophil DNA is synthesised. For the metabolism of folate and/or the production of granulocyte DNA, iron may be a crucial cofactor [5]. A severe iron deficiency may also make it difficult for cells to use folate or vitamin B12[9]. Another case of the Boucher-Neuhäuser syndrome in an Arabian family included the presence of hypersegmented neutrophils (BNS)[10]. Therefore, it may be claimed that hypersegmentation of neutrophils can arise owing to a variety of additional conditions, including iron deficiency anaemia, myelodysplastic syndrome, Boucher-Neuhauser syndrome (BNS) and cobalamine or folic acid insufficiency. Neutrophil hypersegmentation is also

a known side effect of uremia, hyperthermia, and other medications, including chemotherapy, steroids, and GCSF. [11] It is also recognised as an autosomal dominant congenital disease affecting 1% of the general population.[12]

In order to determine any abnormalities in the neutrophils of those subjects who had hypersegmented neutrophils, to evaluate the manifestations in peripheral smear of these patients, and to determine any relationships between the presence of hyper segmented neutrophils in peripheral smear and Vitamin B12 level, folic acid level, and platelet count, this study was conducted.

### **Methodology:**

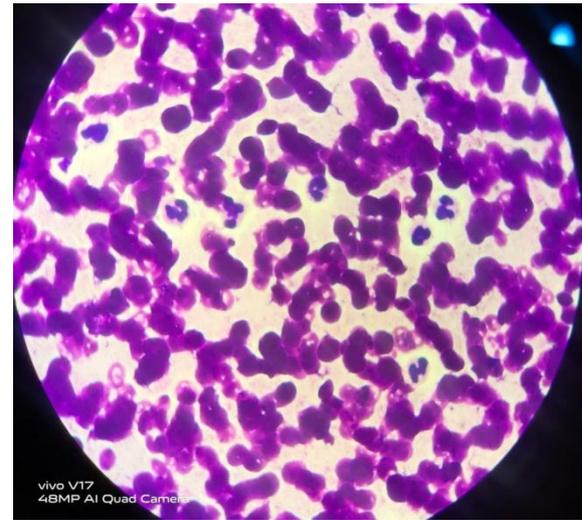
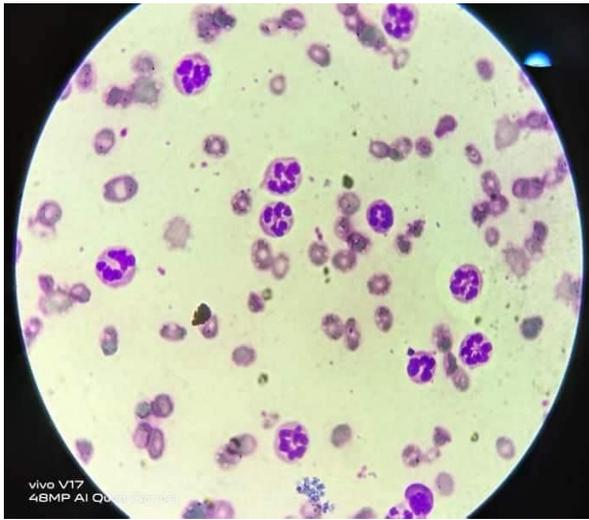
A prospective cohort study was carried out for a total duration of 08 months (November 2021 to June 2022) in the department of pathology of a tertiary care teaching hospital of Government medical college, Betiah, Bihar. All the patients attending the pathology department for undergoing tests after referral from the OPDs of the same hospital were briefed about the research and explained the purpose of the study. Those patients who gave consent to be a part of study were recruited in the study. The total sample size attained was 243. The sampling technique used was total consecutive sampling. Blood samples were collected and analysed for presence of hypersegmented neutrophils, hemoglobin estimation along with other tests that the patient was advised. Those patients who had hypersegmented neutrophils were taken as study cohort and were further subjected to 2<sup>nd</sup> episode of blood sample collection and were interviewed using a pretested structured questionnaire constructed in google forms and containing the following sections- socio demographic profile, medical and drug history. Blood sample collected during the 2<sup>nd</sup> episode was examined for the following- peripheral smear examination, platelet count, assessment of vitamin B12, folic acid levels, serum creatinine and blood urea nitrogen (BUN). The peripheral smear was also examined for any other abnormality with the neutrophils. Analysis of data was done using SPSS software version 22. Variables were presented as frequency and percentages and multivariate regression analysis was used to find out the association between variables and the value of  $p < 0.05$  was taken as statistically significant. The study was approved by the ethics committee of the institution.



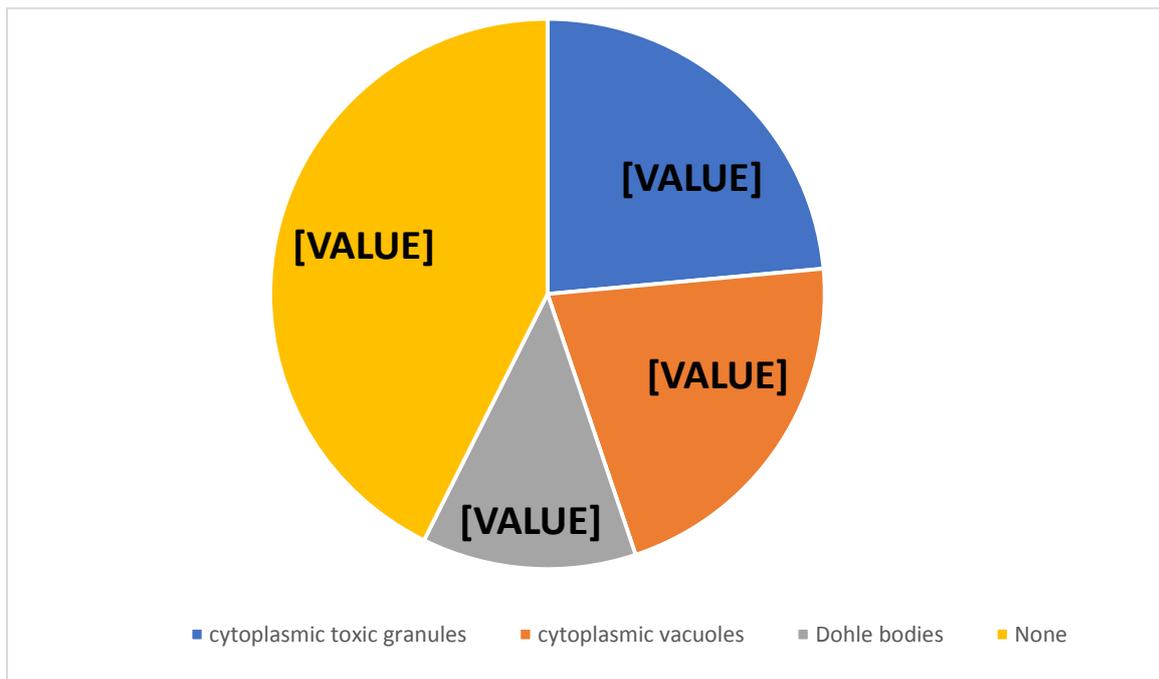
**Results:** At the end of the study conducted among 136 study participants to see the association between presence of hyper segmented neutrophils and abnormal cytological profile of blood smear, the sociodemographic profile of the patients is presented in Table 1 which reveals that hyper segmented neutrophils were present more commonly in females (75 out of 136, 55%) as compared to those of males (61 out of 136, 45%). Most of the study participants 81 out of 136 (59.56%) were in the age group of 31-60 years and educated up to primary level (52 out of 136, 38.24%). Figure 1 presents the appearance of a normal and a hyper segmented neutrophils under the microscope. The various abnormalities in neutrophils in addition to hyper segmentation is depicted in figure 2 where it is seen that more than half of the cases 57.35% have some or the other abnormality in addition to hyper segmentation. The peripheral smear has macrocytic normochromic appearance in about 35.29% of the cases which can be seen in figure 3. Lower vitamin B12 levels was significantly associated ( $p < 0.01$ ) with presence of hyper segmented neutrophils as depicted in Table 2.

**Table 1: Socio demographic profile of the study participants (N = 136)**

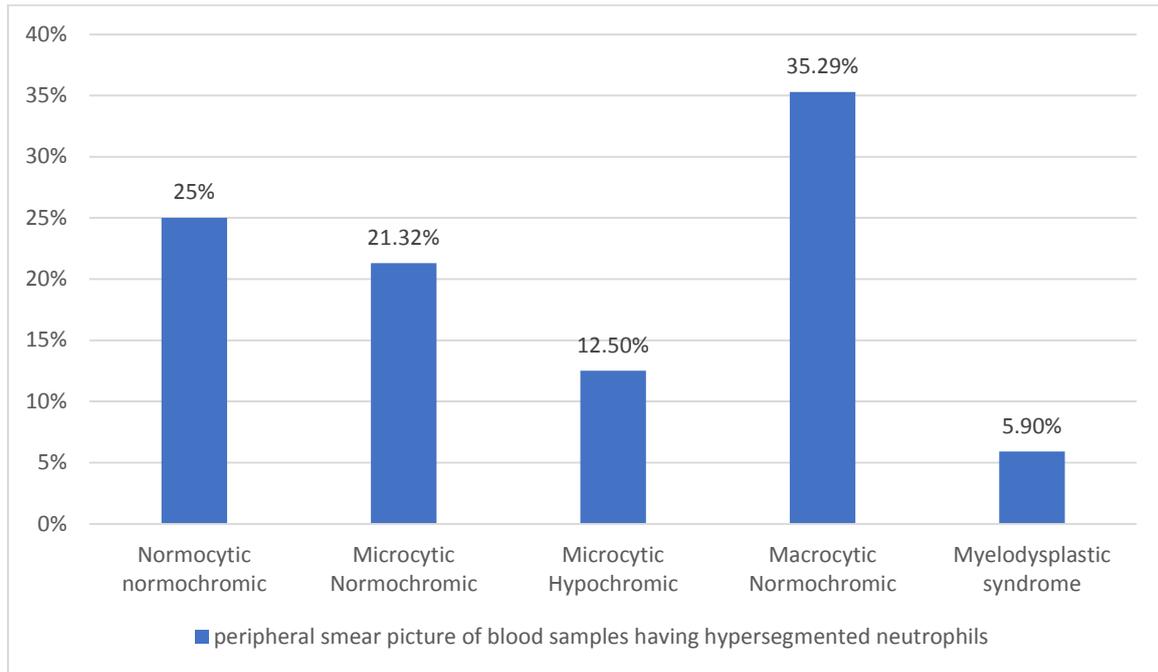
S. No.	Variables	Frequency	Percentage	
1	Gender	Male	61	45%
		Female	75	55%
2	Age group	≤ 15 Years	17	12.5%
		16 – 30 Years	28	20.59%
		31 – 45 Years	39	28.68%
		46 – 60 Years	42	30.88%
		≥ 60 Years	10	7.35%
3	Occupation	Professional/semi-professional/clerical	19	13.97%
		Skilled/semiskilled/unskilled	73	53.68%
		Unemployed	35	25.74%
		Retired	09	6.6%
4	Education	Graduate & above	18	13.24%
		Upto Intermediate	37	27.21%
		Upto High/middle school	21	15.44%
		Upto Primary	52	38.24%
		illiterate	8	5.88%
5	Marital status	Married	107	78.68%
		Single	26	19.12%
		Separated	3	2.2%
6	Socio economic status	Class 1	09	6.62%
		Class 2	15	11.03%
		Class 3	37	27.20%
		Class 4	53	38.97%
		Class 5	22	16.18%



**Figure 2: Hypersegmented neutrophil and a normal neutrophil as seen in peripheral smear under the microscope**



**Figure 3: Abnormality in neutrophils in addition to hypersegmentation (N = 136)**



**Figure 4: Peripheral smear picture of blood samples having hyper segmented neutrophils (N = 136)**

**Table 2: Association of the presence of hypersegmented neutrophils in peripheral smear with Vitamin B12 level, folic acid level and platelet count (N = 136)**

S. No.	Blood Parameters	No. of hypersegmented neutrophils	P value
1	<b>Vitamin B12 levels</b>		< 0.001
	< 200pg/ml	48 (35.29%)	
	200 – 500 pg/ml	29 (21.32%)	
	500 – 700 pg/ml	08 (5.9%)	
	700 – 900 pg/ml	34 (25%)	
	>900 pg/ml	17 (12.5%)	
2	<b>Folic acid levels</b>		3.125
	<2ng/ml	22 (16.18%)	
	2 – 8 ng/ml	29 (21.32%)	
	8 – 15 ng/ml	45 (33.09%)	
	15 – 20 ng/ml	37 (27.21%)	
	>20 ng/ml	03 (2.21%)	
3	<b>Platelet count</b>		1.53
	<1.5 lakh/ $\mu$ l	12 (8.8%)	
	1.5 – 4.5 lakh/ $\mu$ l	88 (64.71%)	
	>4.5 lakh/ $\mu$ l	36 (26.47%)	

## Discussion:

The results of the suggested study were contrasted with those of previous studies. In comparison to males, hyper segmented neutrophils were found more frequently in females (75 out of 136, or 55 percent) than in males (61 out of 136, 45 percent ). The majority of study participants (59.56%) were in the 31–60 age range and had only completed elementary school (52 out of 136, 38.24 percent ). It is evident from the several abnormalities in neutrophils that more than half of cases—57.35 percent—have some aberration in addition to hyper segmentation. About 35.29 percent of the time, the peripheral smear displays a macrocytic normochromic look.

According to a study by G. S. Thiriveni Balajji, only 11 people in the control group showed hypersegmented neutrophils in their peripheral smears, compared to 67 alcoholic patients who had them. This implies that peripheral smear analysis might be regularly performed in rural areas without access to more sophisticated equipment. The results revealed a significant difference (p 0.0001) between the test and control groups in the MCV levels and hypersegmented neutrophils.[13] Neutrophil hypersegmentation (NH), which was discovered in another investigation by D. A. WESTERMAN & his colleagues, is a significant haematological characteristic of cobalamin or folate insufficiency. It is crucial to determine whether iron deficiency alone is a cause of NH because iron deficiency and folate insufficiency frequently affect the same target populations. We present a case-control research that deals with this problem. The study involved two patient populations at hospitals. 50 patients with iron deficiency anaemia made up Group 1. (IDA). 50 control patients in Group II were age- and sex-matched, haematologically normal, and the iron investigations revealed no signs of iron deficiency. Patients with other conditions (cobalamin/folate insufficiency, renal failure, illness, and drug exposures) that might have an impact on the degree of neutrophil segmentation were not included in the study. A total of 10,000 neutrophils, 100 from each patient, were analysed. The presence of neutrophils with five or more lobes per 100, or any neutrophils with six or more lobes, was referred to as NH. These were the outcomes: Controls, mean neutrophil lobe count 2'96, number of patients with NH 2/50; IDA, mean neutrophil lobe count 3'36; number of patients with NH 31/50 (62%) (4 percent ). These variations were statistically noteworthy. The NH is widespread in IDA, we conclude. It is unclear how iron deficiency contributes to NH.[14]

In contrast to a study by RALPH CARMEL, which found that the diagnostic process for megaloblastic anaemia involves four typically sequential steps, it was found in the proposed study that lower vitamin B12 levels were significantly (p 0.01) associated with the presence of hyper segmented neutrophils. It is important to pay attention to altered blood cell size and morphology in order to identify megaloblastosis in the first place. These alterations can occasionally be undetectable or subtle. Assays of serum vitamin B2, and folic acid levels are the cornerstone of the second stage, diagnosis of the specific vitamin shortage, however they can occasionally be deceptive. Tests of absorption and stomach function typically form the basis of the third phase, which identifies the precise disease entity causing the vitamin deficit. Reevaluation after replacement therapy, the fourth stage, is frequently overlooked as a diagnostic step but has significant diagnostic consequences and is perhaps the only opportunity to unmask and identify coexisting disorders.[15] The investigation of 50 patients with hypochromic iron deficiency anaemia by P. D. Roberts et al. revealed evidence that suggested a high frequency of folate

depletion. Three patients (70%) had a high mean neutrophil lobe count, five patients (80%) had peripheral blood films that revealed more than 3% of neutrophils with five nuclear lobes, 20 patients (40%) had hypersegmented neutrophils, and 19 patients (38%) had large metamyelocytes in the bone marrow. 12 individuals had serum folate levels below 3 ng/ml, whereas 18 had levels between 3 and 6 ng/ml. IS percent had subnormal red-cell folate, and 45% had a positive Figlu test. Apart from an association between red-cell folate levels and morphological alterations in the neutrophils, correlations between different tests for folate deficiency were not discovered. When the blood folate level was low, the increase in haemoglobin after intravenous iron therapy was less. Although the numbers tested were tiny, there was probably a comparable link to the red-cell folate level. The response to iron therapy was unaffected by the existence of neutrophil multilobing in the peripheral blood film, large metamyelocytes in the bone marrow, or aberrant Figlu excretion. Both neutrophil multilobing and marrow giant metamyelocyte numbers were considerably decreased after intravenous iron therapy. Six weeks after starting this therapy, Figlu excretion did not change appreciably, while serum and red-cell folate levels decreased. The serum vitamin B levels did not significantly change as a result of intravenous iron therapy. It is highlighted how iron deficiency itself may contribute to the appearance of folate loss.[9]

**Conclusion:** After completion of the study, it was concluded that more than half of total cases of hypersegmented neutrophils had certain other abnormalities also in the neutrophils. The peripheral smear appeared macrocytic normochromic in more than one fourth of the cases and the hypersegmented neutrophil status was significantly associated with the lower vitamin B12 levels.

**Conflict of Interest:** None

**Funding:** Nil

**Acknowledgement:** We express our sincere thanks to all the faculty and staff who helped us conduct this study. We also thank the study participants for giving their consent and time to be a part of this study.

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