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Original Article

A Pilot Project- To find out the Causes of Hypersegmentation in Neutrophils apart from Existing Causes

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ABSTRACT

Background: Hypersegmented neutrophils are one of the earliest indicators of megaloblastic anemia rather than raised MCV. Hypersegmented neutrophils are also seen as a part of myeloproliferative and myelodysplastic syndromes. Uremia, hyperthermia, and many drugs are also known to produce neutrophilic hypersegmentation. Congenital condition which is autosomal dominant in inheritance is known to cause hereditary hypersegmentation. We aimed to evaluate causes of hypersegmentation in peripheral smear apart from already known causes.

Materials and methods: The study was conducted for three years. EDTA samples collected in the hematology lab were evaluated for hypersegmentation and other associated findings by examining the giemsa-stained peripheral smears. The study population was classified according to the size of red blood cells i.e. normocytic, microcytic, and macrocytic population. Other associated factors like chronic infections, drug history, and other significant history were also recorded on a predesigned proforma.

Results: After obtaining written, understandable, informed consent for this pilot project,410 volunteers were enrolled. Macrocytic red blood cells were seen in more than 50% of cases. Cases with normal peripheral smears were evaluated for hidden vitamin B12 deficiency and other associated factors. Approximately 90% of these had low vitamin B12 levels so neutrophilic hypersegmentation was the first evident sign of megaloblastic anemia. vitamin B12 levels less than 200 pg/ml had a significant correlation with neutrophilic hypersegmentation (p-value <0.05).

Conclusions: The study was planned to evaluate causes of neutrophilic hypersegmentation other than existing causes however the results were inconclusive.

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INTRODUCTION

Hypersegmentation of neutrophils is defined as the presence of 3% or more neutrophils with five or more lobes or a single neutrophil with 6 lobes.[1] It is a clinical laboratory finding & is visualized by drawing blood from a patient and viewing the blood smeared on a slide under a microscope

Peripheral blood smear is a crucial and powerful diagnostic tool for observing neutrophilic hypersegmentation (NH) but unfortunately with the advancement in automation examination of peripheral blood is becoming a “lost art”.

The smear examination offers a window to many hidden parameters like hypersegmentation so a review of the smear is an important adjunct to other clinical data.

Hypersegmented neutrophils are associated with deficiency of or failure to utilize cobalamin or folate which leads to impaired DNA synthesis,[2] so hypersegmented neutrophils are said to be pathognomonic of the megaloblastic anemia. Other listed causes of NH include microcytic hypochromic anemia however in most IDA cases, there is coexistent cobalamin or folate deficiency.[3,4] Hypersegmented neutrophils are also seen as a part of myeloproliferative and myelodysplastic syndromes. Uremia, hyperthermia, and drugs including chemotherapy, steroids, and GCSF are also known to produce neutrophil hypersegmentation.[5] It is also known as a congenital condition (autosomal dominant) affecting 1% of the population,[6] however the data supporting such cases of hereditary hypersegmentation is very limited and under ongoing research.

We observed while working in the hematology Lab of MBGH that hypersegmented neutrophils were noted in many cases without accompanying RBC findings. This led us to plan a pilot study in this field to evaluate our hypothesis that hypersegmentation does exist in normal peripheral smears and to evaluate such cases to find out causes of hypersegmentation apart from known existing causes.

Aims and objectives

1. To test the hypotheses that hypersegmented neutrophils are seen in patients who do not have specific hematological abnormalities.
2. To relate these findings with any other hematological parameters (If a correlation exists)
3. To relate these findings with diet, drug intake, geographical area, and familial predisposition.

MATERIALS AND METHODS

The study design was cross-sectional, observational and was conducted from October 2019 to October 2022 for 3 years in the Department of Pathology RNT Medical College Udaipur. EDTA samples collected in our hematology lab were evaluated for hypersegmentation and other associated findings by examining the Giemsa-stained peripheral smears. Complete blood counts of individual cases were obtained using HoribaYumizen H550 automated analyzer and peripheral smear pictures were compared with blood counts. After obtaining informed understandable consent regarding the project 410 willing participants were enrolled in the study. The study population was classified according to the size of RBC i.e. normocytic, microcytic, and macrocytic RBC populations. Other associated factors like chronic infections, drug history, and other significant history were also recorded on a predesigned Performa. Cases where no known cause of hypersegmentation was evident on history or smear examination were evaluated for vitamin B12/folate levels on ADVIA centaur CP Automated immunoassay analyzer to rule out masked cases of megaloblastic anemia. Out of these cases, patients with normal vitamin B12/ folate levels were further enquired about their demography, dietary habits, and any significant drug history. First-degree relatives of such patients were also screened for the presence of hypersegmentation. Diagnosed cases of megaloblastic anemia were excluded from the study as hypersegmented neutrophils are a pathognomic finding in megaloblastic anemia. Also, cases who presented with hypersegmentation but were lost to follow-up for detailed workup and patients on vitamin B12 supplements (being effect modifier) were excluded.

RESULTS

A total of 2180 cases presented with neutrophilic hypersegmentation, but after obtaining written, understandable, informed consent for this pilot project 410 volunteers in the age group of 20-70 were enrolled for the study which resulted in an effective reduction in sample size. Out of these 53% of cases were males and 47% were females with most of the patients in the 30-50 years of age group i.e. 64.8%. (Table 1)

Table 1: Age and sex-wise distribution of neutrophilic hypersegmentation

Age group	Males	Females
20-30 years	18	11
31-40 years	92	62
41-50 years	48	64
51-60 years	26	30
61-70 years	33	26
Total	217(53%)	193 (47%)

Macrocytic RBC picture was seen in 53.6% of cases as expected and only 6.8% of cases had microcytic blood picture where coexisting nutritional deficiency of iron and vitamin B12 was considered as a cause of hypersegmentation However 162 cases i.e. 39.5% had normocytic blood picture.

Table 2: RBC morphology and etiology of neutrophilic hypersegmentation

RBC morphology	Etiology	Percentage
Macrocytic(n=220)	Vitamin B12 deficiency	91.8%
	Folate deficiency	0.45%
	Drug history	0.90%
	MPD/MDS	6.81%
Normocytic (n=162)	Vitamin B12 deficiency	43.02%
	Drug history	12.34%
	Infections	29.22%
	MPN/MDS	9.25%
	Others	6.17%
Microcytic(n=28)	Vitamin B12 deficiency	28.5%
	Iron deficiency	71.4%

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Cases with normocytic populations associated with NH were further divided according to possible known causes like Myeloproliferative Disease, Chronic infection, and drug history. (Table 2)

NH was associated with neutrophilia in 60 out of 162 cases (37.03%) with evidence of toxic granulation in 21 cases, cytoplasmic vacuolation in 8 cases, and dohle bodies in 2 cases. However, no significant correlation with lymphocytosis, monocytosis, or eosinophilia was noted in our study. Similarly, no correlation with thrombocytosis or thrombocytopenia was noted in the smear examination.

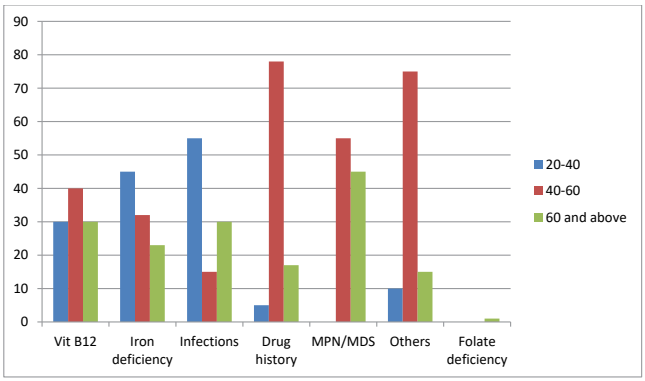

Figure 1: *Bar diagram representing age-wise distribution of various causes of hypersegmentation*

62.3% of cases were there that had normal peripheral smear findings. This was our population of interest, we followed these cases and evaluated them for hidden B12 deficiency and other associated factors but on investigating approximately 90% of these cases had low Vit B12 levels so NH was the first evident sign of megaloblastic anemia. Vit B12 levels <200 pg/ml had a significant correlation with NH(p-value <0.05). (Table 3)

We tried to follow up on rest 10 cases however only 3 were traced and their first-degree relatives were screened for hypersegmentation though the results were found to be negative. There was no significant demographic or dietary association in these cases, hence the results in the present study were inconclusive.

Table 3: Vitamin B12 levels in cases with normocytic and microcytic red cells

Vitamin B12 levels	Observed frequency	p-value
<200 pg/ml	104	<0.05
200-400 pg/ml	08	
400-900 pg/ml	10	
>900 pg/ml	01	

DISCUSSION

Neutrophilic hypersegmentation is defined as the presence of more than 5 lobes in neutrophils. NH is considered to be an important finding in megaloblastic anemia & is caused due to deficiency or failure of utilization in vitamin B12/Folate. Vitamin B12 and folic acid are essential for the maturation and DNA synthesis of neutrophils.

In the case of vitamin B12 deficiency or folate deficiency-related megaloblastic anemia, macrocytosis is a usual finding. Hypersegmented neutrophils and raised MCV generally occur hand in hand. However several studies have reported that one of the earliest indicators of megaloblastic change is hypersegmented neutrophils rather than raised MCV.

Hence, in many cases, where anemia and raised MCV have not manifested, hypersegmented neutrophils can be reported. According to Thompson et al, in 91% of 515 patients, hypersegmented neutrophil was a more sensitive indicator as compared to MCV.[7] In the present study also NH has been proven to be the earliest and more sensitive indicator of vitamin B12 deficiency than macrocytosis as >50% cases of normocytic blood picture having Neutrophilic hypersegmentation had significantly low vitamin B12 levels with normal MCV levels i.e.<90 femtoliter.

NH though considered to be a hallmark of megaloblastic anemia yet is also observed in varying clinical scenarios like iron deficiency, chronic infection, myelodysplastic syndrome, hypogonadism, and chronic myelogenous leukemia.[8]

The pathogenesis of neutrophil hypersegmentation in microcytic hypochromic anemia is not fully understood. Some previous studies claim this to be due to underlying vitamin B12 and folic acid deficiency while some studies have found NH in cases of microcytic blood picture with pure iron deficiency anemia.[4,9] In our study 20 out of 28 cases of microcytic anemia had low or borderline vitamin B12 levels which is in concordance with a hypothesis of coexisting iron and vitamin B12 deficiency.

The half-life of neutrophils in peripheral blood is around 6-8 hrs however it increases in cases of inflammation as neutrophils become activated. The nuclear segmentation typical for neutrophils has been used as an indicator of their age. In a multitude of inflammatory conditions, immature neutrophils with a non-segmented banded nucleus are released from the bone marrow, causing a so-called left shift.[10] However, unexpectedly, hypersegmented neutrophils are also mobilized into the peripheral blood during acute inflammation.[10] In our study NH was observed in 20 cases of acute infections.

The origin of neutrophils with a hypersegmented nucleus in inflammation is still obscure, but the phenotype does not seem to be induced by activation, because the proteome is completely different and the cells are characterized by a marked downregulation of the protein translation machinery.[11]

Studies have documented a distinct human neutrophil phenotype during acute inflammation with an immune-suppressive capacity. These cells display a hypersegmented nuclear morphology, which implies increased maturation compared with that of normal blood neutrophils. In addition, this subset is characterized by a unique phenotype ($CD62L^{dim}$/$CD16^{bright}$/$CD11b^{bright}$/$CD54^{bright}$), which distinguishes them from classically short-term activated neutrophils ($CD62L^{dim}$/$CD16^{bright}$/$CD11b^{bright}$/$CD54^{dim}$).[12]

Drug-induced NH is also a well-documented entity. Many drugs like steroids, GCSF, and chemotherapeutic agents are found to be associated with hypersegmentation. Chemotherapeutic agents may result in transient marked dysplasia of all lineages. Methotrexate and other drugs that affect deoxyribonucleotide synthesis can cause dysgranulopoiesis specifically hypersegmentation.[13]

Many rare causes of hypersegmentation are also reported like heat stroke, uremia, and even with the Covid-19 infections but supporting evidence for the significant association is not sufficient .[5,14] Similarly hereditary hypersegmentation is a rare entity with autosomal dominant inheritance[15] where asymptomatic individuals present with neutrophilic hypersegmentation but usually these patients are at high risk of hypercoagulability. Hereditary hypersegmentation can be due to certain defects in DNA metabolism. This defect in DNA synthesis can also provoke coagulation activity and therefore hypercoagulability-associated presentations. [16]

CONCLUSIONS

The study was planned to evaluate causes of neutrophilic hypersegmentation other than existing causes however we ended up with already known causes. Only in a small group i.e. 10 cases out of 410 of NH, no definite cause could be identified. Considering the known possible causes of NH and the prevalence of megaloblastic anemia, the study sample size was very small and follow-up of cases during the pandemic era was a tedious job so our study findings were inconclusive about other causes causing neutrophilic hypersegmentation apart from the already known causes.

However, large multicentric studies with a scientific temper and regular follow-up with dedicated manpower for field work are needed to evaluate causes of hypersegmented neutrophils apart from the known causes. It would help in better understanding the fact that neutrophilic hypersegmentation does exist in peripheral smears apart from known existing causes.

LIMITATION

Small sample size and loss of follow-up cases as 2 year long pandemic period was going on during the study duration.

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