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Original Research Article

Hypersegmented neutrophils in peripheral smear –An etiological analysis

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ABSTRACT

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 is the accepted mechanism for the morphological changes seen in megaloblastosis. Other causes of neutrophil hypersegmentation (NH) listed include microcytic hypochromic anemia(MHA) but the evidence for this is based mainly on a limited number of case studies in which MHAs with vit B12 and folic acid deficiency also were included. More studies are needed to establish itiological factors other than already established megaloblastic anemia.

The present study was conducted in Department of Pathology in a tertiary care centre in Central Kerala, India. 100 cases peripheral smears with NH were evaluated. Aim of the study was to classify the itiological factors of NH in peripheral smears, study NH in microcytic hypochromic anemia and to check whether there is any connection between microcytic hypochromic anemia with NH and thrombocytosis. Complete blood count of all cases were taken using automated hematology analysers. Peripheral smear picture were correlated with the blood counts. Cases with MHA were examined for underlying vit B12 and folic acid deficiency. Study shows that 31% cases of peripheral smears with hypersegmentaion of neutrophils were pure microcytic hypochromic anemias with normal vitB12 and folic acid levels. Study also points to increased incidence thrombocytosis in pure MHA cases compared to other etiological factors.

1. Introduction

Hypersegmentation of neutrophils is defined as presence of 5% or more neutrophils with five or more lobes or single neutrophil with 6 lobes.[1] 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.[2,3] Other causes of NH listed include microcytic hypochromic anemia but the evidence for this is based mainly on a limited number of case studies in which patients in whom the IDA complicated by coexistent cobalamin or folate deficiency were also included.[4,5] Hypersegmented neutrophils are also seen as a part of myelodysplastic syndromes which is usually designated as bone marrow (BM) failure are a heterogeneous group of myeloid clonal disorders caused by a failure of blood cells maturation. 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, GCSF are also known to produce neutrophil hypersegmentation.[7] It is also known in a congenital condition (autosomal dominant) affecting 1% of the population.[8]

Here in the present study we evaluated 100 peripheral smears with hypersegmented neutrophils and classified the itiological factors. Patients with microcytic hypochromic anemia were further evaluated for underlying vit B12 and

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folic acid deficiency. This study also checks whether there is any association between neutrophil hypersegmentation in microcytic hypochromic anemia and thrombocytosis.

2. Material and Methods

This is a prospective study starting from 2020 January to 2020 December conducted in Department of Hematology in a tertiary care centre in central Kerala. EDTA blood samples received in our hematology laboratory were analysed for hypersegmentation of neutrophils using geimsa stained peripheral smears. Neutophils hypersegmentation is defined as the presence of five or more five-lobed neutrophils per 100, or any neutrophils with six or more lobes. 100 such cases which satisfied the inclusion criteria were taken as sample size. Complete blood count of individual cases were obtained using Sysmex SE9000 analyser and peripheral smear picture was compared with blood counts. These 100 cases were 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 microparticle 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 NH without microcytic hypochromic anemia. Patients with known medical conditions like pregnancy, uremia, renal failure and exposure to drugs like chemotherapy, steroid and GCSF were excluded.

3. 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 analysed for associated peripheral smear picture. Although major cases were contributed by macrocytic anemia, 41% cases were having microcytic hypochromic anemia. Detailed picture is given in Table 2.

Detailed analysis of peripheral smears were done to check whether neutrophil hypersegmentation was associated with any other neutrophil abnormalities. 22 cases showed cytoplasmic toxic granules along with nuclear hypersegmentation.18 cases showed cytoplasmic vacuoles. 5 cases showed both toxic granules and vacuoles. 8 cases showed Dohle bodies. One case among 3 myelodysplastic syndrome case showed hypogranular neutrophil.Rest of the 46 cases didn't show any neutrophil abnormality other than hypersegmentation.

Changes in the counts of white blood cells were also evaluated along with neutrophil hypersegmentation. 34% of cases showed neutrophilia. Eosinophilia was seen in 8% of cases. No cases showed lymphocytosis, monocytosis or basophilia.

41 cases of neutrophil hypersegmentation which showed microcytic hypochromic blood picture were checked for underlying vit B12 and folic acid deficiency. Normal range of Serum vit B12 and folic acid values are 200-900pg/ml and 2-20ng/ml.

Table 3 clearly shows that out of the 41 cases with normocytic normochromic bloodpicture, only 10 had subnormal levels of either Vit B12 or folic acid values. Rest of the 30 cases had normal Vit B12 and folic acid levels. So we can come to the conclusion that out of the 100 cases with hypersegmented neutrophils in peripheral smear 31% cases were having pure microcytic hypochromic anemia without any vit B12 or folic acid deficiency.

Platelet count of all cases were assessed. Results are shown in Table 4.

1.5-4.5 lakh/microliter is considered as normal platelet count. Out of the 100 cases, only 2 had thrombocytopenia.73 cases had platelet count in the normal range. 25 cases had thrombocytosis. Out of the 31 cases with microcytic hypochromic anemia and neutrophil hypersegmentation, 19 cases had thrombocytosis (61.29%). In all other cases majority were in normal range group.

4. Discussion

In this study of 100 cases with peripheral smears, showed that there are many causes for neutrophil hypersegmentation other than already established macrocytic anemia. Microcytic hypochromic anemia, melodysplastic syndrome and normocytic normochromic blood picture show neutrophil hypersegmentation in peripheral smear. Deficiency of or failure to utilize cobalamin or folate and impaired DNA synthesis is the accepted mechanism for the morphological changes seen in megaloblastosis.[2,3]

The underlying mechanism of neutrophil hypersegmentation in microcytic hypochromic anemia is not fully understood. There are several studies explaining this as undetected Vit B12 and folic acid deficiency.[4,5] But that is unlikely as 31% cases of NH were pure microcytic hypochromic anemia without Vit B12 and folic acid deficiency. As per previus studies which excluded underlying Vit B12 and folic acid deficiency there are significant association between iron deficiency(presenting as microcytic hypochromic anemia) and neutrophil hypersegmentaion.[3] There are some explanations in previous studies that neutrophil changes in iron defiency represent a recent event with only young red cells and not the overall population of red cells, showing a reduced folate content.[9,10] Some other studies shows that iron deficiency can affect the folate dependant degradation of Figlu catalysed by enzyme Figlu transferase.[11,12] It is also possible that iron deficiency may directly influence DNA synthesis.[13,14]

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	9	11	19	15	54
Female	5	9	20	12	46
Total	14	20	39	27	100

Table 2: Peripheral smear picture of cases with hyper segmented neutrophils

Macrocytic anemia	45
Microcytic hypochromic anemia	41
Normocytic normochromic blood picture	11
Myelodysplastic syndrome	3
Total	100

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

Vit B12(in pg /ml)	Observed frequency	Folic acid (in ng/ml)	Observed frequency
<200pg/ml	7	<2ng/ml	3
200-500pg/ml	22	2-8ng/ml	8
500-700pg/ml	9	8-15ng/ml	19
700-900pg/ml	2	15-20ng/ml	11
>900pg/ml	1	>20ng/ml	0
Total	41		41

Table 4: Correlation of neutrophil hypersegmentation and platelet count

Platelet count	Macrocytic anemia	Microcytic hypochromicPicture (Normal B12 and folic acid)	Microcytic hypochromic Picture (subnormal B12 and folic acid)	Myelodysplasia	NormocyticNormochromic Blood picture
<1.5 lakh/microlitre	1	0	0	1	0
1.5-4.5 lakh/microlitre	41	12	9	2	9
>4.5lakh/microlitre	3	19	1	0	2
Total	45	31	10	3	11

There are several studies showing that hypersegmented neutrophils can be seen as a part of trauma and chronic infections. It is also said to be recruited to bloodstream during inflammation.[15] In our study 22 cases showed neutrophil toxic granules along with hypersegmentation.18 cases showed vacuoles also.Both toxic granules and vacuoles are known to be the response to infection, inflammation and stress.[16] The presence of toxic granules and vacuoles along with hypersegmentaation in our study also point towards the apperance of hypersegmentation as a part of inflammation.

3 cases in our study were diagnosed as myelodysplastic syndromes. There are several earlier studies demonsrating that neutrophil hypersegmentation can be seen as a part of myelodysplasia.[17,18] One case of myelodysplastic syndrome in this study showed hypolobated neutrophil along with hyper segmented neutrophils.

Neutrophil hypersegmentation and thrombocytosis can be expected as a part of megaloblastic anemia. But it may be also seen in iron deficiency anemia also.[19,20] In the present study out of the 25 cases having thrombocytosis (>4.5lakhs /ml), 19 cases are having microcytic hypochromic anemia with normal vit B12 and folic acid values. But further detailed studies are to be done to know whether there is any association between neutrophil hypersegmentation and thrombocytosis.

5. Conclusion

The present study indicates that other than the already established causes of neutrophil hypersegmentation, 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 MHA cases compared to other etiological factors which is still to be established with further detailed studies.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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