STUDY OF NEUTROPHIL NUCLEAR LOBULATIONS ON PERIPHERAL BLOOD SMEARS AND DISTINGUISHING THE BILOBED NEUTROPHILS FROM PELGER HUET CELLS

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ABSTRACT

BACKGROUND

Myeloid cells in the peripheral blood are classified into neutrophils, eosinophils and basophils depending on the staining characteristics of the cytoplasmic granules.[1] Neutrophils are lobulated granulocytes. The mature human neutrophil, unlike most other cells exhibits a distinctly non-spherical nucleus, which is segmented into three to five lobes.[2]

MATERIALS AND METHODS

A prospective study of 1000 Peripheral Blood Smears (PBS) was undertaken. The EDTA venous sample was run on cell counter. NDC was calculated, the data was tabulated and analysed statistically.

RESULTS

All smears showed bilobed neutrophils. Maximum (89.9%) smears showed 11-30 % bilobed neutrophils in their smears. Classical Pince-Nez nuclear morphology was found in 808 cases (80.8%) (Pince-Nez morphology- two well defined symmetrical lobes connected with thin strand of nuclear material).

CONCLUSION

The pince-nez morphology is frequent in neutrophils. Detecting rod like, dumb-bell shapes, peanut shaped nuclei are more diagnostic of PHA/PPHA. Important distinguishing features are smooth outline of nuclear lobes and coarseness of chromatin.

KEYWORDS

Neutrophil Nuclear Lobulations, Neutrophil, Differential Count, Pince-Nez.

BACKGROUND

Myelocytes mature to a metamyelocyte with appearance of nuclear indentation. Metamyelocyte cannot divide. When a promyelocyte transforms to myelocyte, there is disappearance of nucleoli and appearance of secondary granules.[1] When myelocyte transforms into metamyelocyte, the most pronounced change is indentation of nucleus. Metamyelocytes cannot synthesize DNA but myelocytes can synthesize DNA. Fortunately, the distinction between a myelocyte and metamyelocyte is of little clinical importance and the ability of the cell to synthesize DNA is only assayed for research.[1] The band form has deeply indented nucleus, like a band, with parallel borders. The mature neutrophil has segmented nucleus; the segments are connected by thin strands of nuclear material.

Neutrophils usually have 2-5 segments.[1]

The mechanism and purpose of nuclear lobulation are the subject of speculation.[2] Current hypothesis are 1) enhances cell deformability and movement through vessel walls and into inflammatory area. 2) Nucleolar emptying leads to segmentation.

The previous concept is that granulocytes with 3 or more lobes are more mature than those with only 2 lobes. The number of lobes a neutrophil develops appears to be determined in the band stage or earlier.[2]

Pelger-Huët Anomaly (PHA) is a rare benign autosomal-dominant anomaly with an incidence of ~1 in 6000.[3] PHA can cause a false rise in band forms.[3] Pseudo - Pelger Huet anomaly has similar morphology but different aetiology like Myelodysplastic syndrome, infections, drugs etc.[3] The cause is mutations of the Lamin B receptor (LBR) gene,[4,5] which is important for maintaining nuclear structure. Bone Marrow in homozygote shows normal morphologic maturation. Electron Microscopy shows persistence of nucleoli in the mature neutrophils which had a round nucleus.

P-H heterozygote shows a) Bilobed, pince-nez nuclei in 69 to 93% b) <10% cells with 3 lobes c) rare cells with 4 lobes. Acquired P- H also known as Pseudo P-H[6] have been observed in association with myxoedema, acute enteritis, agranulocytosis, multiple myeloma, malaria, leukemoid

reactions secondary to bone metastases, drug sensitivity or CLL.

Pseudo P-H of MDS and acute as well as chronic myeloid leukaemia appear late in the disease, often after administration of chemotherapy. Most of the nuclei are single and oval.

Aims and Objectives

1) To calculate the Neutrophil Differential count (NDC) i.e., the exact percentage of neutrophils showing 2, 3, 4, 5 & more lobes.
2) To correlate NDC with other haematological parameters.
3) To find the range of bilobed neutrophil percentage and their detailed morphology with emphasis on distinguishing from Pelger Huet Anomaly and Pseudo Pelger Huet Anomaly.

MATERIALS AND METHODS

This was a prospective study in which one thousand (n= 1000) Peripheral Blood Smears (PBS) were analysed. Random selection of cases was done, at the same time, ensuring inclusion of normal smears too. EDTA venous sample was collected. The blood sample was 'run' on System KX21 cell counter. Routine assessment of PBS using Field stain was done. NDC was calculated i.e., in a smear, out of 100 neutrophils encountered, how many had 2 or 3 or 4 or 5 or more lobes on all 1000 PBS. The criteria for nuclear lobe enumeration were standard and were applied strictly. A separate lobe was counted only if there was a clear filament or the isthmus between two lobes contained no nuclear material. If there was any doubt a separate lobe was not regarded as present. Cells with folded nuclei where the number of nuclear segments was uncertain were excluded. In every case, Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet count, Red Cell Distribution width (RDW) and Platelet count was noted. NDC was correlated with other haematological parameters like Total Leucocyte Count (TLC), Haemoglobin, MCV, MCH, MCHC, Platelet count in lacs. The range of bilobed neutrophil percentage and their detailed morphology was studied with emphasis on distinguishing from Pelger Huet Anomaly and Pseudo Pelger Huet Anomaly. The data was meticulously tabulated in an excel sheet. The Statistical analysis was done using Primer of biostatistics software (7th edition). Chi-square test & p value was calculated.

RESULTS

The following results were obtained.

% Range of Bilobed N	% of PBS
0 - 10	10
11 - 20	43.9
21 - 30	46
31 - 32	0.1

Table 1. Frequency and Range of Bilobed Neutrophils (Cases Examined 1000)

Range	% of PBS
0%	93
1%	5.7
2%	1.2
3%	0.1

Table 2. Hypersegmented Neutrophils > 5 lobes

Range	% of PBS
0 - 4%	98.2
5 - 6%	1.8

Table 3. Hypersegmented Neutrophils (Equal to 5 Lobes)

MCV (in fl)	NDC				
	2 Lobes	3 Lobes	4 Lobes	=5 Lobes	>5 Lobes
Normal with Normal RDW	23	32	29	3	0.5
Normal with High RDW	33	31	22	3	1
<80	18	34	32	2	0.5
>100	20	38	29	3	1.5

Table 4. Showing Correlation of NDC with MCV

Hb Status	2 Lobes	3 Lobes	4 Lobes	=5 Lobes	>5 Lobes
Normal	19	33	27	5	1
Mild Anaemia	15	33	31	9	1
Moderate anaemia	19	34	28	3	1.5
Severe anaemia	17	17	32	3	1

Table 5. Showing Correlation between NDC and Haemoglobin (Hb) Status

TLC	2 Lobes	3 Lobes	4 Lobes	=5 Lobes	>5 Lobes
Normal	18	33	32	15	1
TLC till 25,000	20	28	31	14	1
TLC>25,000	20	33	27	3	0.5
Leucopenia	13	25	30	3	1.5

Table 6. Showing Correlation of NDC with Total Leucocyte Count (TLC)

| MCH	2 Lobes	3 Lobes	4 Lobes	=5 Lobes	>5 Lobes
<27	18	32	32	2.5	0.5
27 − 33	17	33	31	3	1
>33	21	24	30	3	1.5

Table 7. Showing Correlation of NDC with Mean Corpuscular Haemoglobin (MCH)

MCHC	2 Lobes	3 Lobes	4 Lobes	=5 Lobes	>5 Lobes
<33	18	32	31	3	1.5
33 – 36	19	31	31	3	1
>36	20	34	27	2.5	1

Table 8. Showing Correlation of NDC with Mean Corpuscular Haemoglobin Concentration (MCHC)

Plt Counts in Lacs	2 Lobes	3 Lobes	4 Lobes	=5 Lobes	>5 Lobes
<1.5	17	33	33	3	1
1.5 – 4.5	19	32	32	3	1.5
>4.5	21	33	32	3	1

Table 9. Showing Correlation of NDC with Platelet (Plt) Counts (in Lacks)
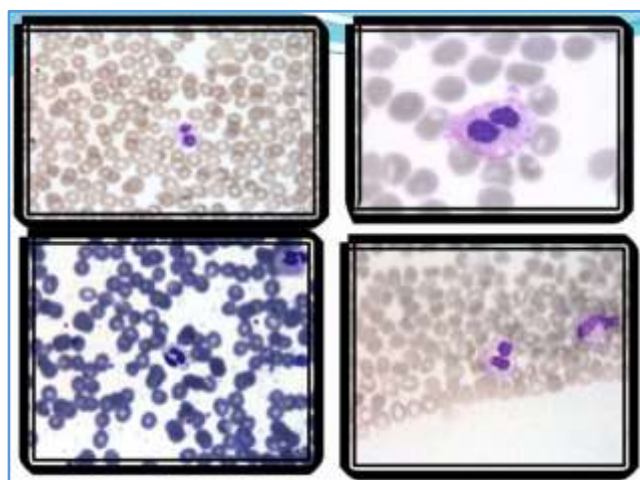
Figure 1. Peripheral Smears showing Bilobed Neutrophils

DISCUSSION

- Frequency of bilobed neutrophils found in our study is similar to literature reports. Pince-nez nuclear shape is only with reference to PHA and PPHA.
- We found Pince-nez shape frequent, but it was unaccompanied by smooth contours and coarse chromatin.
- In the homozygous PHA, all the neutrophils are-
    1. Mononucleated
    2. Round/ oval nuclei
    3. No evidence of segmentation
    4. Cytoplasm appears mature
- Increased nuclear lobulation is a feature of megaloblastic anaemia, iron deficiency anaemia,[7,8] uraemia, infection, myelodysplastic syndromes and hereditary neutrophil hypersegmentations. An association of iron deficiency with NH was first reported by Chanarin, I., et al in1965.[9] Hypersegmentation due to megaloblastic anaemia recovers in about two weeks after initiation of therapy.[10] They are tetraploid (have 96 chromosomes instead of 48 chromosomes). Hypersegmentation in macropolycytes reflects increased DNA.[1]

- We expected a correlation between MCV and NDC as we expect a macrocytic anaemia to have hyper-segmented neutrophils and vice versa. But no correlation was found because hyper-segmented neutrophils was seen in other conditions as well like microcytic anaemia, so actually there is no correlation.
- Statistical analysis was unable to demonstrate a 'statistically significant' correlation between the variables like haemoglobin, MCV, MCH, MCHC, Platelet counts and neutrophil differential counts.

CONCLUSION

- The pince-nez morphology is frequent in neutrophils.
- Detecting rod like, dumb-bell shapes, peanut shapes[11] are more diagnostic of PHA/PPHA. Important distinguishing features, morphologically, are smooth outlines of nuclear lobes, coarseness of chromatin.
- The practical importance is distinguishing this defect (PHA/PPHA) morphologically from the shift to left which occurs in infection. Two human homozygotes of PHA have been reported.
- Presence of similar abnormalities in PBS of other family members is helpful.[12]
- In Pseudo–Pelger-Huët anomaly, the percentage of abnormal neutrophils is usually less than 20% and often around 5%. Coarser clumping of nuclear matter may be seen. Therapy may be started, but this is usually transient and resolves after 10-14 days of chemotherapy administration.
- Neutrophil differential counts and red cell variables were not found to be correlated in our study, however more biochemical studies are required that would establish correlation of neutrophil nuclear lobes and PHA/PPHA/nutritional deficiencies.

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