Original Research Article

Nuclear morphometric study of Non-Hodgkin's Lymphoma (NHL)

Sridhar Reddy Erugula[1], P. Sujatha[2], Ayesha Sameera[3], B. Suresh Reddy[4], Jesudass Govada[5], G. Sudhakar[6], Kandukuri Mahesh Kumar[7*]

[1]Senior Lecturer, Department of Oral Pathology, MNR Dental College and Hospital, Sanga Reddy, Telangana State, India
[2]Professor, Department of Pathology, Malla Reddy Institute of Medical Sciences, Hyderabad, Telangana State, India
[3]Consultant, Oral Pathologist, Hyderabad, Telangana State, India
[4]Assistant Professor, Department of General Medicine, Maheswara Medical College, Telangana State, India
[5]Associate Professor, Department of Pedodontics and Preventive Dentistry, Government Dental College and Hospital, RIMS, Kadapa, India
[6]Associate Professor, Department of Oral and Maxillofacial Surgery, Government Dental College and Hospital, RIMS, Kadapa, India
[7]Assistant Professor, Department of Pathology, Malla Reddy Institute of Medical Sciences, Hyderabad, Telangana State, India
[*]Corresponding author email: **doctormaheshgoud@gmail.com**

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Abstract

Background: Assessment of nuclear morphology is crucial for the diagnosis of non-Hodgkin's lymphoma. The non-Hodgkin's lymphomas (NHL) constitute a heterogenous group of lymphoid neoplasms that vary in clinical behavior, histology, immunology and genetic profile. The diversity makes it difficult to diagnose accurately and reproducibly under light microscopy. The nuclear features play a major role in categorizing non-Hodgkin's lymphomas.

Materials and methods: The present study was conducted for a period of two years in the Department of Oral Pathology, MNR dental College and Hospital, Sangareddy, Telangana, India. We encountered only three cases of Non-Hodgkin Lymphomas and so we have borrowed the some

specimens of NHL from other hospitals in Hyderabad, India. Hematoxylin and Eosin (H and E) stained histological sections were selected and assessed for nuclear area, perimeter and nuclear size of 40 nuclei of tumour cells were measured using the Windows® based image analysis software

Results: Total number of cases in our study was 15. The mean nuclear area, perimeter and nuclear size of neoplastic cells were studied. In our study, the mean nuclear area, perimeter and nuclear size of neoplastic cells were significantly high in large cell lymphomas as compared to intermediate cells and small cell lymphoma. P value was significant.

Conclusion: Our study supports the assumption that nuclear morphometry offers a more objective and reproducible diagnostic method for subcategorizing lymphoid tumors than is currently possible by conventional histopathological techniques.

Key words

Non Hodgkin Lymphomas, Morphometry, Image analysis, Large Cell Lymphoma, Small Cell Lymphomas, Intermediate Cell Lymphomas, Nuclear features.

Introduction

The non-Hodgkin's lymphomas (NHL) constitute a heterogenous group of lymphoid neoplasms that vary in clinical behavior, histology, immunology and genetic profile. The diversity makes it difficult to diagnose accurately and reproducibly under light microscopy. The appearance and size of the nuclei are of prime diagnostic importance but tedious and subjective. Nuclear features play a major role in categorizing non-Hodgkin's lymphomas, little attention has been focused on cellular events that influence or directly contribute to nuclear size and shape or the configuration and distribution of condensed chromatin masses within the nucleus in normal and neoplastic lymphocytes. Though it is acknowledged that the nucleus is the repository for genetic information controlling cellular differentiation, it is generally not appreciated that the nucleus is an organelle that itself undergoes modifications during differentiation. Quantitative assessments of nuclear organization are now being increasingly reported, some of which involve lymphocytes. Such investigations may have relevance to the understanding of the morphologic heterogeneity of the lymphomas, particularly those studies dealing with large-scale alterations in chromatin organization during differentiation and the cell cycle. Mechanisms involved in the differentiation of normal lymphocytes and the resulting morphologic increase in nuclear size and disaggregation of condensed chromatin masses precedes and is independent of DNA synthesis. Since the full range of morphologic alterations observed in lymphocyte transformation can occur in the G, phase of this process, modifications to the above hypothesis are required. Assessment of the nuclear contour index following mitogenic stimulation indicates that at least in this in vitro system, there is no cleaved or convoluted phase during the transformation of human peripheral T lymphocytes. Expressions of this dynamic process in the cell and nucleus have important implications for the study of non-Hodgkin's lymphoma. An alternative approach, but not explored much is the use of image analyzers for quantitative morphometrical analysis of neoplastic lymphoma cells.

Aim

The objective of the study was to evaluate a computerized morphometric analysis of paraffin-embedded lymph node sections of non-Hodgkin's lymphomas (NHL) as an accurate diagnostic tool for lymphoid tumours.

Materials and methods

The present study was conducted for a period of two years in the Department of Oral Pathology, MNR dental College and Hospital, Sangareddy, Telangana, India. We encountered only three cases of Non-Hodgkin Lymphomas and so we have borrowed the some specimens of NHL from

other hospitals in Hyderabad, India. Hematoxylin and Eosin (H and E) stained histological sections were selected and assessed for nuclear area, perimeter and nuclear size of 40 nuclei of tumour cells were measured using the Windows® based image analysis software (image j 1.43 from the institute of health, USA) [1].

Results

Total number of cases in our study was 15. The mean nuclear area, perimeter and nuclear size of neoplastic cells were studied. All these NHL cases were first diagnosed on light microscopic examination and classified based on the size of the cells (**Table - 1**) and confirmed by Immuno-histochemistry (IHC) markers such as Bcl-6, CD 10, CD 23 and CD 5 to know about the cell of origin.

Table - 1: Type and number of Non-Hodgkin's Lymphoma cases selected.

Histomorphological Variants	No. of cases
Diffuse Large cell lymphoma (LCL/DLCL)	7
Diffuse small cell lymphoma (SCL/DSCL)	6
Intermediate lymphocytic/ Mantle cell Lymphoma(ICL)	2
Total	15

Different types of Non-Hodgkin's Lymphomas (NHL) and their positivity with different IHC markers were as per **Figure – 1** to **Figure – 6.**

In our study, the mean nuclear area, perimeter and nuclear size of neoplastic cells were significantly high in large cell lymphomas as compared to intermediate cells and small cell lymphoma. P value was significant. (**Table – 2**)

Discussion

The gold standard in the diagnosis of non-Hodgkin's lymphoma (NHL) is the microscopic examination of tumor tissue samples. The appearance and size of nuclei are of prime diagnostic importance. Nuclear features play a major role in categorizing non-Hodgkin's lymphomas, little attention has been focused on cellular events that influence or directly contribute to nuclear size and shape or the configuration and distribution of condensed chromatin masses within the nucleus in normal and neoplastic lymphocytes. However, subjective (qualitative) assessment of nuclear features is tedious and prone to considerable inter- and intra-observer variation [2].

Quantitative assessments of nuclear organization are now being increasingly reported, some of which involve lymphocytes. Such investigations may have relevance to the understanding of the morphologic heterogeneity of the lymphomas, particularly those studies dealing with large-scale alterations in chromatin organization during differentiation and the cell cycle. The results of cellular morphometric study can be affected by the fixation solution, fixation time, cutting thickness of the tissue, and staining solution .In our study, the traditional routine tissue processing was adopted, including 10% neutral formalin fixation, paraffin embedding, 4-µm section thickness, and hematoxylin-eosin staining, to obtain relatively representative results [3, 4]. Image-analyzer machines (such as Zeiss microvideomat or various software formats) had been attempted to obtain more objective and reproducible assessment of nuclear features. However, aside from being costly and even with sections cut at conventional thickness, there is considerable overlap or contact between cells or nuclei. Thus cell clusters are "read" by the machine as a single, large object and the end result may be inaccurate [5]. The progressive alterations in nuclear size and distribution of condensed chromatin masses occur due to mitogenic stimulation. A significant proportion of the mature lymphocytes with relatively small nuclei and prominent aggregates of condensed chromatin in the unstimulated sample and early phases of the post-stimulation population show a gradual transition to intermediate stages with nuclei that are slightly to moderately increased in size and in which there is progressive separation

and disaggregation of chromatin masses. At these early times after stimulation, some nuclei show irregularity of the nuclear contour, but only small numbers of profiles have a deep narrow indentation greater than 1 in length. In one study, subsequent to 24 hours of incubation there are increasing numbers of large, round, smoothly contoured nuclei with prominent nucleoli and almost complete disaggregation of the large masses of condensed chromatin. Reference to the unstimulated population shows that the majority of mature lymphocyte nuclei (79%) occur in the small-volume class, only approximately one-fifth of this sample occurring in the intermediate-sized class. After the application of the mitogen, there is no shift in the population of lymphocyte nuclei in the intermediate and large-volume classes until 24 hours of incubation, when this combined group rises to 32.5%, the principal increase occurring in the nuclei of the large-volume class. However, at this stage the mean nuclear volume of the 24-hour post-mitogen sample is not significantly greater than control nuclei, and it is not until 36 hours that a major alteration in the percentage of intermediate and large-volume nuclei occurs and the mean nuclear volume becomes significantly increased. Subsequently, the mean nuclear volume rises at 72 hours after continuous stimulation.

Figure – 1: Microphotograph showing Diffuse Large cell Lymphoma (DLCL).

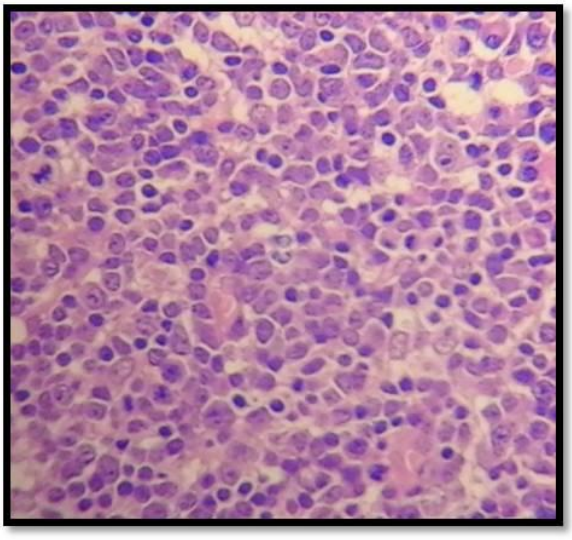


Figure – 2: Microphotograph showing Intermediate/Mantle Cell Lymphoma.

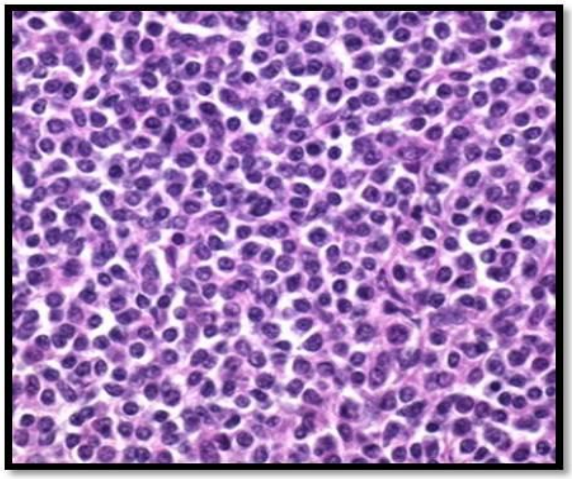


Figure – 3: Microphotograph showing Diffuse Small cell Lymphoma (DSCL).

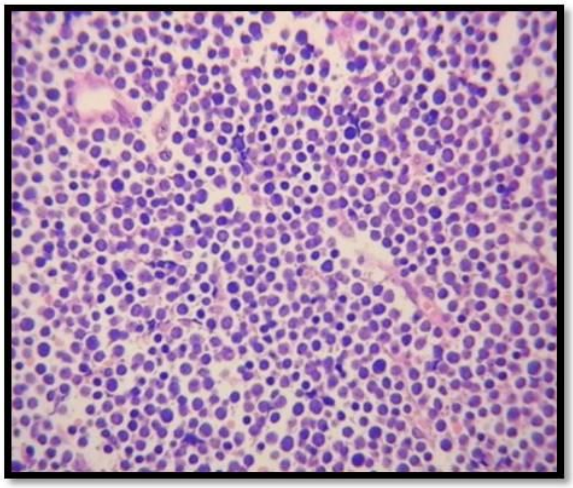


Figure – 4: Microphotograph showing Bcl-6 expression in Large cells.

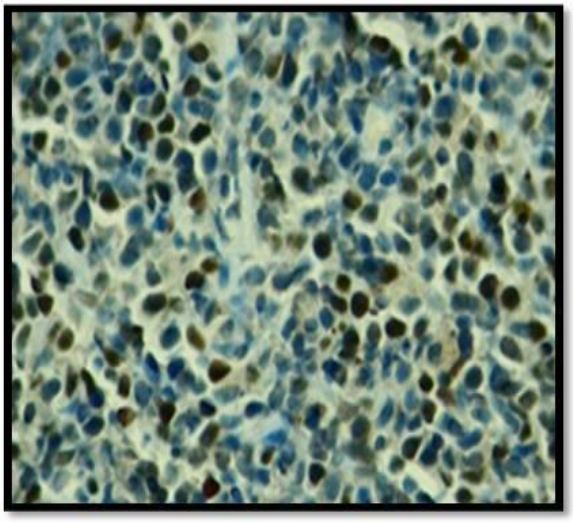


Figure – 5: Microphotograph showing CD 5 expression in Intermediate cells.

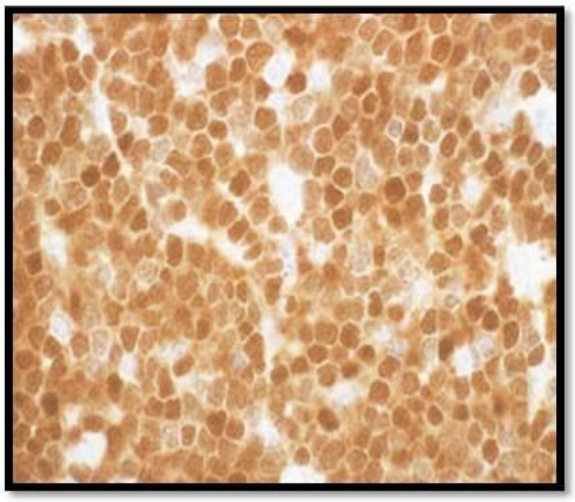
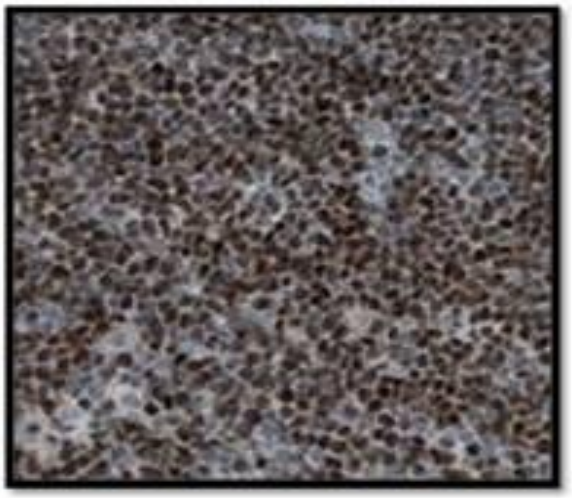


Figure – 6: Microphotograph showing CD10 expression in Small Cell Lymphoma.



Our findings agreed with Abbott, et al. who reported that the nuclear area correlates with the histopathological subtype of NHL [6]. Our findings are also correlating to the study conducted by Aiad Abdullah, et al. [7] where 28 cases were studied **(Table - 3, 4, 5)**.

Conclusion

In conclusion, our study supports the assumption that nuclear morphometry offers a more objective and reproducible diagnostic method for subcategorizing lymphoid tumors than is currently possible by conventional histopathological techniques.

References

1. Crocker J, Curran RC. A study of nuclear diameters in lymph node imprints using the Zeiss Microvideomat. J ClinPathol., 1979; 32: 670-4.
2. A. E. Martinez, et al. Grading of follicular lymphoma: Comparison of routine histology. Arch of Pathol. Lab Med., 2007; 131(7): 1084-8.
3. Jaffe ES, Harris ML, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. Blood, 2008; 312: 4384-99.
4. Crocker J, Curran RC. A study of nuclear diameters in lymph node imprints using the Zeiss Microvideomat. J Clin Pathol., 1979; 32: 670-4.
5. Crocker J. Morphometric and related quantitative techniques in the study of lymphoid neoplasms. A review. J pathol., 1984; 143: 69-80.
6. Abbott Cr, Blewitt Rw, C Bird CC. Quantitative analysis of non-Hodgkin's lymphoma. J Clin Pathol., 1982; 35: 135-138.
7. Aiad A. A. Non-Hodgkin's Lymphoma: a Preliminary Morphometric Study. Anb Med J, 2012; 11(1): 62-68.

Table - 2: Pooled Morphometric Data for Nuclear Parameters.

Lymphoma (subtype)	Area (μm²)	Perimeter(μm)	Nuclear size
DLCL	23.21 ± 4.33	16.4 ± 2.5	5.26 ± 0.7
ICL	13.88 ± 3.7	12.87 ± 2.34	4.129 ± 0.65
DSCL	13.17 ± 3.03	11.81 ± 2.1	3.40 ± 0.46
	p<0.01	p<0.01	p<0.01

Table - 3: Correlation of the results with study conducted by Aiad Abdullah, et al. in regards to the Diffuse Large Cell Lymphomas (DLCL).

	Area (µm)	**Perimeter (µm)**	**Nuclear size**
Aiad Abdullah, et al. study [7]	23.25±6.47	16.93±2.33	5.4±0.74
Our study	23.21±4.33	16.4±2.5	5.26±0.7

Table - 4: Correlation of the results with study conducted by Aiad Abdullah, et al. in regards to the Intermediate Cell Lymphomas (ICL).

	Area (µm)	**Perimeter (µm)**	**Nuclear size**
Aiad Abdullah, et al. study [7]	16.22±3.29	14.29±1.47	4.55±0.46
Our study	13.88±3.7	12.87±2.34	4.129±0.65

Table - 5: Correlation of the results with study conducted by Aiad Abdullah, et al. in regards to the Diffuse Small Cell Lymphomas (DSCL).

	Area (µm)	**Perimeter (µm)**	**Nuclear size**
Aiad Abdullah, et al. study [7]	13.45±4.56	12.83±2.1	4.08±0.67
Our study	13.17±3.03	11.81±2.1	3.40±0.46