Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

To Analyse the Differentiation and Grading of Non-Hodgkins Lymphoma by Immunohistochemical Expression of CD20, CD3 and Ki67 in Lymph Node.

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Received: February 2019
Accepted: February 2019

ABSTRACT

Background: Immunophenotyping, although has emerged indispensable in the diagnosis and classification of lymphoid neoplasms, has to be used cautiously with knowledge of the antibodies used. No antigen is totally lineage or lymphoma specific, and for this reason, immunostaining must be performed in the context of a panel. In addition, familiarity with the diagnostic criteria and differential diagnosis of each lymphoid tumor and ultimately correlation with morphology, and clinical history are essential to enhance the diagnostic accuracy and reproducibility. AIM: The present retrospective study aimed to analyse the differentiation and grading of Non-Hodgkin's lymphoma by immunohistochemical expression of CD20, CD3 and Ki-67 in lymph node. **Methods:** A total of 50 samples of NHL were included in the study. Written informed consent of the patient was taken where ever required in the vernacular. Relevant history of the patient was also taken as per the proforma attached along with. The tissues were stained with H and E staining, CD20, CD 3 and Ki-67 immunostaining. The positive immunostained slides were then evaluated and scored both qualitatively and quantitatively. **Results:** In the present study a total of 50 samples of NHL were included with an age range from 12 to 72 years and a mean age of 46.54 years and male predominance.45 cases showed immunopositivity for CD 20, showing that they belong to B cell phenotype and only 5 cases showed immunopositivity for CD 3, thus showing T cell phenotype. The mean Ki-67 for B cell lymphoma patients was 47.86 ±28.04,with a minimum score of 2 and a max score of 92 and for T cell lymphoma patients was 61.4±18.02, with a minimum score of 40 and a max score of 81, but there was no significant correlation between them (P=0.382). **Conclusion:** Ki-67 expression in NHL can help in monitoring of patients at risk and can to some extent also aid in detecting the degree of aggressiveness of the disease to give suitable treatment but Ki-67 alone cannot be a risk factor in NHL patients and other factors such as age, sex and type of NHL can be affective, too. The outcome of further analyzing the association between Ki-67 expression and the prognosis of various subtypes of lymphoma should be supported.

Keywords: Cancer, Hodgkins Lymphoma, Immunophenotyping.

INTRODUCTION

Lymphadenopathy, refers to nodes that are abnormal in either size, consistency or number. It is a common clinical presentation which may be local or generalised. Although the finding of lymphadenopathy, sometimes raises fears about serious illness, but, usually a result of benign infections. Common causes of lymphadenopathy are acute viral and bacterial infections, chronic infections like tuberculosis, autoimmune diseases and cancers. Tuberculosis is one of the main cause among infections. Besides infectious and granulomatous diseases, the lymphoproliferative disorders consisting of reactive hyperplasia, (RH), Hodgkin's disease (HD) and Non-Hodgkin's lymphoma (NHL) are also seen.[1,2]

The Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of lympho-proliferative malignancies, with distinct causes and showing distinctive patterns of behavior and responses to treatment. It consists of many subtypes, each with distinct epidemiology, etiology, and morphologic, immunophenotypic, and clinical features. It is the

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8th most commonly diagnosed cancer in men and the 11th in women. In terms of incidence, the disease accounts for 5.1% of all cancer cases and 2.7% of all cancer deaths. In India, as per the estimates, there are approximately 23,718 new NHL cases reported each year. NHL are commonly subclassified into B, T or natural killer on basis of cell of origin. It is widely accepted that many lymphomas arise from the B-cells at the different developmental stages of the germinal center reaction.[3-7]

The unique feature of lymphomas is the fact that these are considered as clonal proliferation of lymphocytes arrested at different stages of differentiation, thereby recapitulating stages of normal lymphocyte differentiation. Various diagnostic modalities used in lymphoproliferative disorder are fine needle aspiration, excision biopsy, flow cytometery and immunohistochemistry.

A panel of markers is decided based on morphologic differential diagnosis (no single marker is specific) which includes leukocyte common antigen (LCA), B-cell markers (CD10, CD19, CD20 and CD79a), T-cell / NK cell markers (CD2, CD3, CD4, CD5, CD7, CD8, CD34, and CD56) and other markers like CD23, bcl-2, CD10, cyclinD1, CD15, CD30, ALK-1, CD138 (based on cytoarchitectural pattern). For subclassification of lymphomas, monoclonal antibodies can be used.[6,7]

Thus, the present retrospective study was undertaken to analyse the differentiation and grading of non-hodgkin's lymphoma by immunohistochemical expression of CD20, CD3 and Ki-67 in lymph node. Immunohistochemical marker CD20 and CD3 were applied to differentiate between B and T cell lymphomas and subsequent grading was ascertained by utilizing Ki-67 marker.

MATERIALS AND METHODS

The present study was conducted in 50 specimens of lymph nodes received in the Department of Pathology and Department of Radiotherapy and Oncology, Government Medical College, Amritsar, after approval from the Institutional Ethics Committee, Government Medical College, Amritsar. All the cases of Non-Hodgkin's lymphoma were included in the study, irrespective of their age. Normal lymph node was considered as control in the study. In the lymph node Ki-67 and CD20 positivity was seen in germinal centre and CD3 positivity in inter follicular area. Cases of Hodgkin's lymphoma, granulomatous lesions, metastatic carcinomatous deposits and patient not willing to give consent were excluded from the study. The tissues were stained with H and E staining, CD20, CD 3 and Ki-67 immunostaining. The positive immunostained slides were then evaluated and scored both qualitatively and quantitatively.

Statistical Analysis:

Means and standard deviations were calculated. The correlation between variables with Ki67 index was done by SPSS software (Chi-square test) and also correlation between Ki67 index with type of NHL was done with T-test.

RESULTS

In the present study a total of 50 samples of NHL were included with an age range from 12 to 72 years and a mean age of 46.54 years. Majority of the patients belonged to 25-50 yrs of age range (62%). Gender distribution showed that 48% were females and 52% were males and the male to female ratio came to be 1.1:1. As more than one group of lymph nodes involvement was observed in some patients, a total of 76 sites of lymph nodes were observed in total of 50 patients. Out of the studied sample maximum cases of cervical lymph node enlargement were observed which was seen in 88%, followed by submandibular lymph nodes, axillary lymph nodes, inguinal lymph nodes, abdominal lymph nodes and lastly supraclavicular lymph nodes in descending order.

All cases of NHL when subjected to detailed immunochemistry employing CD3, CD20. Among the total cases of 50 NHL studied, 45 cases showed immunopositivity for CD 20, showing that they belong to B cell phenotype and only 5 cases showed immunopositivity for CD 3, thus showing T cell phenotype. Thus all the cases were classified accordingly.

The mean Ki67 for B cell lymphoma patients was 47.86 ±28.04,with a minimum score of 2 and a max score of 92 and for T cell lymphoma patients was 61.4±18.02, with a minimum score of 40 and a max score of 81, but there was no significant correlation between them (P=0.382).

Table 1: Mean Ki67 Score In Study Population

	Number of cases	KI-67 score (mean± SD)	Min	Max
B Cell Lymphoma	45 (90%)	47.86 ±28.04	2	92
T Cell Lymphoma	5 (10%)	61.4±18.02	40	81

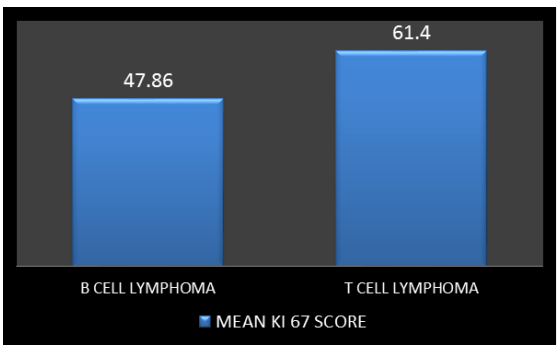

Figure 1: overall mean ki-67 scoring in b cell and t cell lymphomas in the sample.

Further, the Patients were divided into <65% (low) and ≥65% (high) KI index, it was observed that out of total 33 (66%) cases of low KI index, 30 (90.9%) belonged to B cell lymphomas and 3 (9.09%) were T cell lymphomas. Similarly, out of total 17 (34%) cases of high KI index, 15 (88.23%) belonged to B cell lymphomas and 2 (11.76%) were T cell lymphomas.

Further the mean KI index for the cases that belonged to low KI index category came to be 33.8±23.46 for B cell lymphomas and 49.33 ± 10.06 for T cell lymphomas. Also the mean KI index for high KI index category was 76±7.66 for B cell lymphomas and 79.5±2.12 for T cell lymphomas.

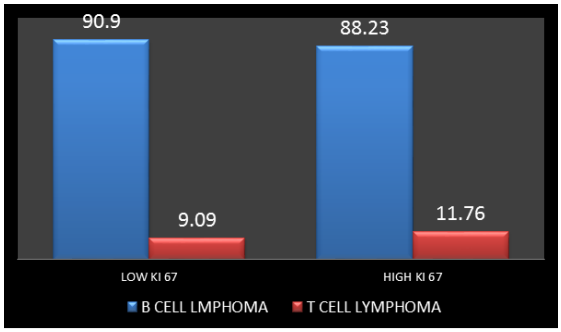

Figure 2: Distribution Of Sample Based On Ki67 Index

When we studied the correlation between age, type of NHL, type of lymphadenopathy and size of lesion with Ki67 index, no significant correlation was observed between variables with Ki67 index (P>0.05).

DISCUSSION

Lymphoid malignancies are a heterogeneous group of disorder that may arise in lymph node or extra-nodal sites. Morphological identification and classification of lymphomas based on immunophenotyping is of paramount importance for the management of patients and determiningthe prognosis because each type and subtype exhibits distinct clinicopatholgic features. Histopathological examination remains fundamental for diagnosis and classification of malignant lymphomas, but poses problems when characteristic features of specific lymphoma are not present. This raises the value of immunohistochemistry.

Therefore we conducted this study including a total sample of 50 specimens of lymph nodes from patients with Non Hodgkins Lymphomas (NHL).

Although, there are many T-cell-specific markers CD2, CD3, CD7, CD8, and CD45RO. Only CD3 was used in the present study to differentiate between B cell and T cell population of the Non-Hodgkins lymphomas. Antibody to CD3 is currently a key member of such panels, indicating T-cell phenotype. Notable, exceptions to this includes some of the more aggressive, large T cell lymphomas and anaplastic large cell lymphomas, which may not express detectable antigen. However, it was then suggested that polyclonal antibody CD3 is useful and more reliable to distinguish between T-cell and B cell lymphomas when used in conjunction with CD20. Therefore in the present study we used both these antibodies.All the samples were subjected first to CD20 to evaluate for B cells and all the samples which were negative for CD 20,on them CD3 was applied as it is specific for T cells.[8-10]

The mean age of diagnosis in the present study was 46.54 years. Majority of the patients belonged to 25-50 yrs of age range (62%). Our results favours the fact that NHL is more common in older adults than younger adults, hence age presents to be strong risk factor for this disease. Incidence data obtained from the United States National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program accounted that the incidence of total lymphoid neoplasms increased monotonically with age in all race and sex subgroups. Steep increases in incidence with age were observed for most subtypes. Our results are also in accordance with various other studies like that by Roy et al., Sharma et al and Padhi et al.[4,11-13]

It's been seen worldwide that the occurrence of NHL is higher in men then women .The results of this study also favour this finding. Various other authors like Sharma et al, Kalyan K et al, Vallabhajosyula S et al, Sengar M et al, Padhi S et al, and Roy A et al have also reported male preponderance in their studies, which are also in accordance with our study.[4,12-16]

Among all the cases of NHL studied in the current study, immunophenotypic analysis using CD3 and CD 20, revealed 90% NHL to be of B – cell type while only 10% were of T – cell type, and B cell: T cell ratio being 9:1. These findings were in agreement with studies conducted by various authors both in India and worldwide: Sharma et al reported 89.3% cases of B cell lymphomas, Roy A et al reported 54% cases, Padhi et al reported 96% cases, Naresh KN et al 79.1% cases, Mushtaq S et al reported 86% cases and lastly kalyan K et al reported 72% cases of B cell lymphomas.[4,12,13,17,18,14]

Ki-67, a nuclear nonhistone protein, is synthesized at the beginning of cell proliferation, and it is expressed in all phases of the cell cycle except during G0 phase. Its strict association with cell proliferation and its co-expression with other well-known markers of proliferation indicate a pivotal role in cell division. Ki-67 expression has been widely used in clinical practice as an index to evaluate the proliferative activity of lymphoma. The percentage of Ki67-positive cells reflects the proportion of actively proliferating tumor cells. Its been shown that Ki-67 Proliferative index may add in both diagnostic (assessing lymphoma grade) and prognostic value.[19]

To evaluate the Ki 67 expression earlier, both Kim SJ et al and Mehrdad Payandeh et al used the criteria of <65% (low) and ≥65% (high) KI-67 expression. Therefore in the present study we also similar criteria for evaluating the expression of Ki-67 in our sample of NHL.[20,21]

The results of immunoexpression of Ki-67 revealed that the mean distribution value of Ki67 expression for B cell lymphoma patients was 47.86 ±28.04 and for T cell lymphoma patients was 61.4±18.02. Though there was a difference in the expression, but there was no significant correlation between them (p=0.382). Grogan et al reported that Ki-67 PI ranged from 3 to 91%, with a mean of 39.7%. A high Ki-67 PI (>60%) was found to be a strong predictor of poor survival (p< 5 0.003).[22]

Broyde A et al that the Ki-67 PI in NHL ranged from 3 to 100%, with a mean of 51.7% (SD ± 29.9). They noted a statistically significant increase in the mean Ki-67 index with an increase in tumor grade (P < 0.001).Mehrdad Payandeh divided Ki67 index to two groups reporting that 67.9% had Ki-67<60% and 32.1% had Ki-67≥65%. The Mean value observed was 47.33±16.50 in their study.[23,21]

Thus from the results it is observed that this heterogeneity in results is caused by the different cut-off point of high Ki-67 expression. The high inter-observer variability still restricts the use of the Ki-67 index in experimental as well as clinical practice relatively.

Spyratos et al. suggested to choose the cut-off point according to the clinical objective. That is to exclude patients with slowly proliferating tumors, low cut-off point should be set to avoid overtreatment, while high cut-off point is suitable to be used to identify patients sensitive to chemotherapy schedule.[24]

Further it was observed that 66% NHL expressed low KI index, while 34% expressed high KI index. In low Ki-67 category 90.9% of NHL were of B cell phenotype and 9.09% were T-cell phenotype. While in high Ki-67 index category, 88.23% of NHL were of B cell phenotype and 11.76% were T-cell phenotype. The mean KI index for the cases that belonged to low KI index category came to be 33.8±23.46 for B cell lymphomas and 49.33 ± 10.06 for T cell lymphomas. The mean value Ki index for high KI index category was 76±7.66 for B cell lymphomas and 79.5±2.12 for T cell lymphomas.

On comparision of age, type of NHL with Ki67 index it was seen that no significant correlation was observed between all these variables.

Mehrdad Payandeh also reported that there is no significant correlation between age (≤50 years vs. >50 years, sex and type of NHL with Ki-67 index. Also, Kim SJ showed that there is no correlation between age with Ki-67.[21,20]

CONCLUSION

Our data suggest that the detection of differentially expressed lymphoma markers, such as CD3 and CD20, may offer a simpler approach to phenotypic classification of T-cell and B cell lymphomas. Ki-67 expression in NHL can help in monitoring of patients at risk and can to some extent also aid in detecting the degree of aggressiveness of the disease to give suitable treatment but Ki-67 alone cannot be a risk factor in NHL patients and other factors such as age, sex and type of NHL can be affective, too. The outcome of further analyzing the association between Ki-67 expression and the prognosis of various subtypes of lymphoma should be supported.

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How to cite this article: Singh J, Saini S, Mehra V, Paul S, Jyoti H, Singh T, Neki NS. To Analyse the Differentiation and Grading of Non-Hodgkins Lymphoma by Immunohistochemical Expression of CD20, CD3 and Ki67 in Lymph Node. Ann. Int. Med. Den. Res. 2019; 5(2):PT28-PT32.

Source of Support: Nil, **Conflict of Interest:** None declared