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Original Research Article

Association of Ki-67 Proliferative Index with Clinico-Pathological Features of Non-Hodgkin's Lymphoma Classified According to WHO (2008)

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ABSTRACT

Objective: To assess the value of Ki-67 proliferative index (PI) in non-hodgkin's lymphoma (NHL) in relation to clinico-pathological features.

Materials and Methods: Immunohistochemical (IHC) method was used to detect the expression of Ki-67 in 103 formalin-fixed, paraffin-embedded tissue sample of NHL according to WHO classification (2008). Ki-67 PI with cutoff 45% assessed in relation to age, gender, site of involvement, B symptoms, Ann Arbor stage, lactate dehydrogenase (LDH) level, performance status (PS) and international prognostic index (IPI).

Results: Significant correlation was present between high Ki-67 index and B symptoms, increase LDH level and IPI. Mean Ki-67 index included 26 for CLL/SLL, 30.6 for plasma cell neoplasm, 45 for follicular lymphoma, 28.75 for mantle cell lymphoma, 25 for MALT lymphoma. The mean Ki-67 index was highest in burkitt's lymphoma (94.3). Mean Ki-67 index were 58.02 for diffuse large B cell lymphoma, 74.2 for lymphoblastic lymphoma, 46.9 for T cell lymphoma (NOS) and 51.6 for anaplastic large cell lymphoma.

Conclusions: Ki- 67 index is a valuable IHC marker to distinguish indolent from aggressive lymphomas. High Ki-67 PI has a significant role to establish the proliferative activity of tumor as prognostic index marker along-with clinical parameters.

Keywords: Ki-67 proliferative index, Non-hodgkin's lymphoma.

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INTRODUCTION

Lymphomas are divided into two categories, Hodgkin's lymphoma and Non-Hodgkin's Lymphoma (NHL).[1] NHL is a broad category consisting of B-cell and T-cell type, comprises 85% and 15% respectively. Clinically NHL is categorized into indolent group & aggressive group. Indolent group are slow growing tumours with relative resistance to chemotherapy. It includes the small lymphocytic lymphoma, low grade follicular lymphoma and mantle cell lymphoma. Aggressive group comprises Burkitt's lymphoma, lymphoblastic lymphoma and diffuse large B cell lymphoma.[1] Clinical variables like age, sex, Ann Arbor stage, B symptoms, site of involvement and lactate dehydrogenase together with proliferative index are important in diagnosis, grading and determination of prognosis of patient's outcome in NHL. Proliferation is a key feature of the progression of Tumours. Proliferation is estimated by the Immunohistochemical assessment of the nuclear antigen Ki-67.[2] Ki-67 is expressed during the active phases of cell cycle in G1, S, G2, and mitosis. During interphase it can be detected within the nucleus while in mitosis it is detected on the chromosomal surface. Therefore Ki-67 is an excellent marker for determining the growth fraction of a given cell population.[3] Tumour progression and malignancy are associated with increased angiogenesis and higher Ki-67 PI is associated with poor prognosis in solid and haematological malignancies.[4] Ki-67 PI is helpful in the prediction of disease stage and progression. Todays the Ki-67 positivity by IHC is the most popular method to determining the growth fraction of benign & malignant cell population.[4] Moreover, a positive correlation exist between prognosis and proliferation rates in chronic B cell and T cell lympho-proliferative disorders.[5] The diagnostic criteria of Ki-67 PI approaches 100% in Burkitt's and atypical Burkitt's lymphoma.[6] Aggressive lymphoma are associated with higher expression of Ki-67 proliferation index in comparison to indolent lymphoma. Hence Ki-67 PI has clinical significance in diagnosis and determination of prognosis of NHL. This study was conducted to assess Ki-67 PI in tissue sections for NHL with correlation to clinical and pathological parameters in NHL patients, to predict the behavior of disease at the time of presentation.

MATERIAL & METHODS

The present study was carried-out in 103 patients with NHL, which were categorized according to WHO classification (2008) of lymphoid neoplasms by immunohistochemistry from the period of January 2014 to December 2015 at department of pathology, SMS Medical College Jaipur. Patients with available records are included in our study. Previous diagnosed cases of NHL, HIV positive patients & patients with previous history of low grade lymphoma are excluded. Routinely stained hematoxylin and eosin (H&E) slides were reviewed. Histopathological sub-typing &

grading was performed based on the criteria described in the WHO (2008) working formulation. Immunohistochemical (IHC) stains using antibodies against CD 20, CD 3, CD 10, CD 5, CD 23, CD 21, BCL2, Ki-67 were done. Other markers including CD 79a, ALK, CD 43, CD 30 and CD 15, PAX 5, MUM1, CD38, Kappa and Lamda were done in selected cases.

Immunohistochemistry[7]

Serial 3 to 4 µm sections were cut from the paraffin block, mounted on positively charged slides. Keep the slides at 60°-70° c temperature in oven for 30-40 min. Put the slides in xylene for 2 changes, each change for 10 min. Then put the slides in alcohol for 2 changes, each change for 10 min. Keep the slides in distilled water for 5 min. Then put the slides in peroxidase solution for 10-15 min. (methanol 97 ml & H_2O_2 3 ml). Keep slides in tris buffer solution for 2 changes; each change for 5 min. Antigen retrieval is done in following steps.

- Take 600 ml distilled water in declocking chamber
- The slides are arranged in slide holder and put in (retrieval box) citrate buffer
- Slides are kept in a de-clock chamber and the chamber is closed.
- The temperature of the chamber is set at 125° c.
- First beep comes after 15-20 minutes when temperature reaches 125°c.
- Then, after first beep, push start/off button.
- After second beep, de-clocking chamber is opened, at that time temperature is near 89°-90° c.

Slides are kept at room temperature (20 minute) for cooling. Wash the slides with tris buffer. Mark the sections by PEP pen. Apply background snipper 3-4 drops for 15 minutes. Wash slides with tris buffer 2 times with the help of wash bottle. Apply primary antibody for 1 hour. Wash slides with tris buffer. Apply probe for 15 minutes. Wash with tris buffer. Apply polymer for half an hour. Wash with tris buffer. Apply DAB for 5 minutes (1drop DAB chromogen along with 1 ml of DAB chromogen solution). Wash with de-ionised water. Slides kept in haematoxylin for 2 minutes. Slides kept in running tap water for 5 minutes. Alcohol – 2 changes – each change for 5 minutes. Xylene – 2 changes – each change for 10 minutes. The slides are mounted with DPX.

Quality control of IHC[8]

Negative control for immunostaining consisting of histological section of each case processed without the addition of primary antibody were prepared for each antigen, along with a positive control section prepared with each IHC run than staining result were evaluated.

Assessment of Ki-67 indices[9]

Immunohistochemical results were scored semi-quantitatively. For Ki-67, nuclear pattern were considered positive. Hot spots of neoplastic cells are observed and Proportion of Ki-67 positive cells were counted in 1000 cells & percentage is taken out. A cut-off value of 45% was used to differentiate high versus low proliferative activity.

Statistical analysis

The data was analyzed using SPSS version 20 for windows software program. Chi-square test used for statistical analysis to assess the relationship of clinical parameters. Ki-67 PI using cut-off value of 45% and the mean Ki-67 PI within the NHL groups was tested by one way analysis of variances (ANOVA). P value <0.05 was considered significant.

Table 1: Ki-67 PI in relation to clinical parameters

		Ki-67 PI<45%(N)	Ki-67 PI>45%(N)	Total	P value
Age	<60 years	36	42	78	0.22
	>60 years	15	10	25	
Gender	Male	39	38	77	0.69
	Female	12	14	26	
Anatomical site	Nodal	29	21	50	0.09
	Extra-nodal	22	31	53	
LDH	Normal	34	3	37	0.001
Level	High	17	49	66	
Ann Arbor stage	I & II	33	28	61	0.25
	III & IV	18	24	42	
B symptoms	Present	12	36	48	0.001
	Absent	39	16	55	
Performance	0,1	38	40	78	0.77
Status	2,3,4,5	13	12	25	
International	Low risk	27	12	39	0.001
prognostic index (IPI)	Low intermediate risk	12	23	35	
	High intermediate risk	11	9	20	
	High risk	1	8	9	

Table 2: Ki 67 proliferative index (PI) in relation to subtypes of NHL

NHL type	Subtypes of NHL	Ki-67 PI		Total
		<45(%)	>45(%)	
B-cell	DLBCL	11(22.4)	38(77.6)	49
(n=94)	CLL/SLL	15(93.8)	1 (6.2)	16
	Plasma cell type	10(83.3)	2(16.7)	12
	Follicular	4(66.7)	2(33.3)	6
	Lymphoblastic	2(66.7)	1(33.3)	3
	Lymphoma B-Cell type			
	Mantle cell lymphoma	3(75)	1(25)	4
	Burkitt	0	3	3
	MALT	1	0	1
T-cell	Peripheral T-Cell Lymphoma NOS	3(75)	1(25)	4
(n=9)	Anaplastic Large Cell Lymphoma	2(40)	3(60)	5
	Total	51	52	103

Table 3: Mean Ki-67 proliferative index (PI) in NHL

NHL subtypes	No. (%)	Mean	SD(±)	Minimum	Maximum
DLBCL	49 (47.5)	58.02	15.74	25	90
CLL/SLL	16 (15.5)	26.0	10.68	10	50
Plasma cell type	12 (11.6)	30.6	25.15	15	95
Follicular	6 (5.8)	45.0	19.49	25	80
Lymphoblastic B-Cell type	3 (2.9)	74.2	29.6	40	90
Mantle cell lymphoma	4 (3.9)	28.75	17.01	15	50
Burkitt	3 (2.9)	94.3	4.04	90	98
MALT	1 (0.9)	25.0	-	25	25
Peripheral T-Cell Lymphoma NOS	4 (3.9)	46.9	29.25	25	90
Anaplastic Large Cell Lymphoma	5 (4.8)	51.6	9.76	40	63
Total	103	48.05	17.85	10	98

RESULTS

In this study, there were 77 males and 26 females with a male to female ratio of 2.9:1. Ki-67 is expressed >45% in 38 males and 14 females. Regarding age less than 60 years, Ki-67 expression >45% in 42 case and <45% in 36 case. Regarding the age, gender, anatomical site, performance status and stage, they show no significant association with Ki-67 PI at cutoff value 45% while the correlation of Ki-67 PI more than 45% with presence of B symptoms, increase LDH level and IPI was highly significant ($P, x^2=0.001$). In relation to site 50/103 (48.5%) patients present with nodal origin of NHL, while 53/103 (51.5%) cases present with extranodal origin of NHL. In nodal origin of NHL, 29/50 (58%) had PI less than 45%.31/53 (58.5%) cases of extranodal origin have PI more than 45%.

48 cases (46.6%) of NHL presented with B symptoms, out of which 36 (75%) cases showed higher expression of Ki-67. Ki-67 expression less than 45% is present in 39 (71%) cases without B symptoms. B symptoms are significantly associated with high expression of Ki-67 PI. 66 cases presented with increase LDH level. Among these 49 cases (74%) express Ki-67> 45%. Increase LDH level is significant with Ki- 67 expression more than 45%. In our study 78/103 (75.7%) patients presented with performance status zero & one, in which 40/78 (51.3%) cases had Ki-67 PI more than 45%. Only 25/103 (24.3%) cases present with restricted routine activities ,out of them 13/25(52%) patients had Ki-67 PI less than 45%. 69% cases of low risk group have Ki-67 proliferation less than 45% and 89% cases of high risk group have Ki-67 PI more than 45%

Amongst total of 103 cases, 94 (91.3%) were of B cell type and 9 (8.7%) cases were of T –cell type. Regarding the frequency of NHL, DLBCL (47.5%) had highest frequency, followed by CLL/SLL (15.5%) and Plasma cell neoplasm (11.6%).

Highest mean Ki-67 index is present in Burkitt's lymphoma (94.3%) followed by B-Cell lymphoblastic lymphoma (74.2%) and DLBCL (58%). Mean Ki-67 index include 30.6 for Plasma cell neoplasm,26.0 for CLL/SLL, 28.75 for Mantle cell, 25 for MALT lymphoma and 45 for follicular lymphoma. Significant association was found between high level of mean Ki-67 PI with aggressiveness of the disease subtypes.

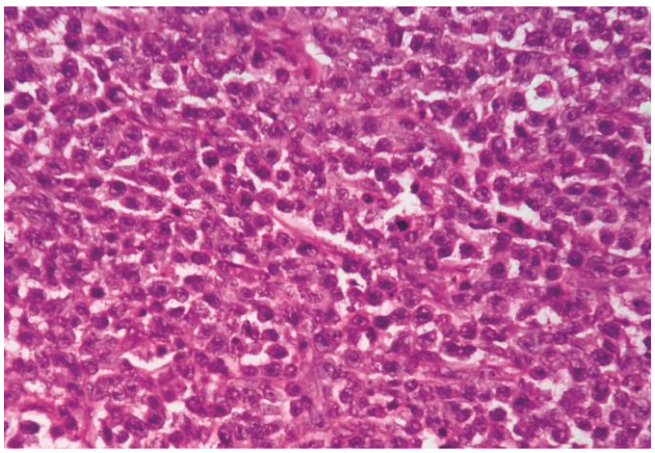

Fig 1: DLBCL show diffuse sheets of lymphoma cells. Nuclei are large, round, indented having vesicular chromatin and prominent nucleoli. (H&E, 400X)

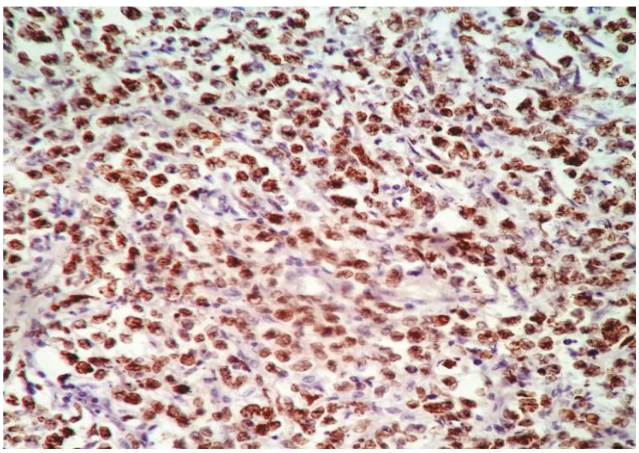

Fig 2: DLBCL show positivity for MIB score-60%

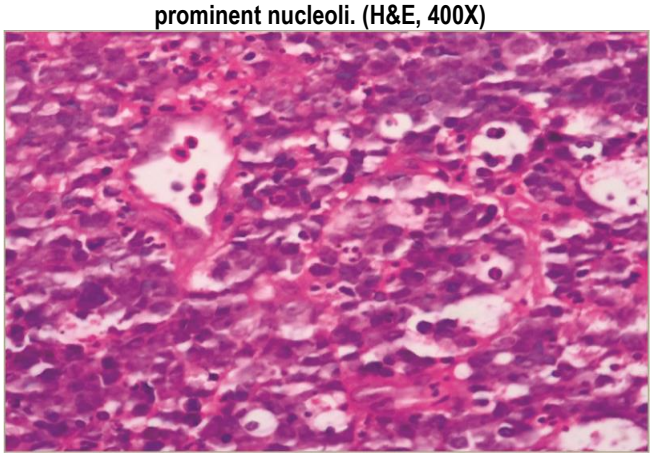

Fig 3: Burkitt lymphoma show medium sized neoplastic cells with "squaring off " of nuclear membrane with typical "starry-sky " appearance (H&E, 400X)

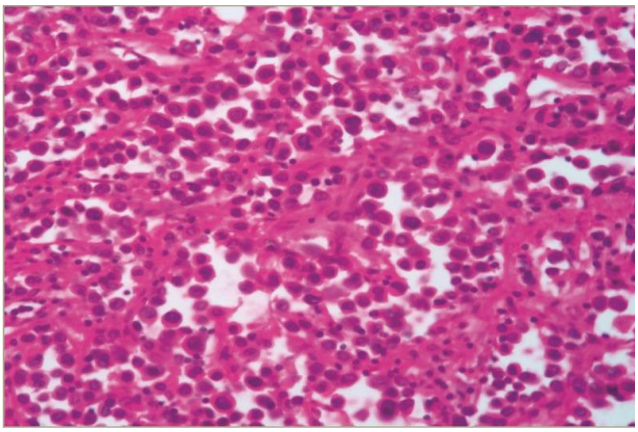

Fig 4: ALCL showing large neoplastic cells with round or horse shoe shape nuclei having amphophillic cytoplasm. (H&E,400x)

DISCUSSION

The WHO classification of hematopoietic tumors is based on morphologic, immunophenotypic, genetic and clinical features. Ki-67 PI is used as prognostic and diagnostic marker in NHL. The significance of Ki-67 with various clinical parameters is highly informative. Szczuraszek[10] et al examined the correlation of Ki-67 expression with clinical parameters of the patients and their survival that patients of high Ki-67 PI have shorter survival (p=0.03) but no significant correlation could be detected among Ki-67 expression and clinical or pathological parameters of the patients like type of NHL, age and gender. This is partly in accordance with our study. Llanos et.al.[11] suggest that Extra-nodal non-hodgkin's lymphoma of more than one site show the aggressive nature of the disease and our finding have no significant association with high Ki-67 PI. De Melo[5] et al. showed significantly higher proportions of Ki-67 positive cells in T cell (11.2%) than in B cell (2.9%) lymphoma (P < 0.001) in contrast to a study performed by Tominaga[12] et al. that the percentage of Ki-67 positive (Ki-67+) cells was lower, in T cell lymphomas than in B cell lymphomas, although the prognosis of T cell lymphomas is considered worse than that of B cell lymphomas. Our study is accordance with Broyedeet al.[13] because we also found valuable difference of mean Ki-67 PI within the NHL subgroups but using the cutoff value 45% of Ki 67 PI, we did not see any significant association of Ki-67 PI in subtypes of NHL. Hence the significance of mean Ki-67 PI to differentiate indolent versus aggressive subgroups rather cutoff value of Ki-67, demonstrated the diagnostic value of Ki-67 PI to be followed in categorization of NHL, as it is often difficult to grade NHL morphologically alone. Erum Naz[14] suggests high Ki-67 PI have significant association in relation to anatomical site and presence of B symptoms. He X, Chen Z[15] found that high Ki-67 PI is negatively correlated with overall survival and disease free survival. However no significant association was seen between Ki-67 and LDH level, presence of B symptoms, Ann Arbor stage, extra-nodal involvement and performance status. Atif Ali[16], Sanna Abdallah[17]studied that mean Ki-67 PI was less than 45% for indolent NHL and more than 45% for aggressive lymphoma. El-Esawy BH[18] found significant correlation between high Ki-67 PI and presence of B symptoms and involvement of extra-nodal site in NHL. Our study is in accordance to Erum Naz[14], El-Esawy BH[18].

CONCLUSION

Significant association of Ki-67 PI >45% with presence of B symptoms and increase LDH level is seen in NHL. KI-67 PI cut-off value 45% is quite helpful to differentiate indolent versus aggressive nature of disease. Various clinical parameters age, gender, anatomical site and performance status do not show significant association in relation to Ki-67 PI cut-off value 45%. Due to the lack of clinical data in relation to histological and immunochemical correlations, the exact picture of patient's outcome and survival is still unclear. Therefore, in future studies,

there is a need to relate the laboratory investigations with clinical parameters to identify the exact picture in a large number of clinico-pathological studies for more conclusive observations.

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