Original Article

Correlation of Ki 67 proliferative index with clinical and pathological features on tissue sections of non Hodgkins lymphoma by immunostaining

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Abstract

Objectives: To assess Ki 67 proliferative index (PI) in tissue sections, with relation to grade and clinical parameters associated with non Hodgkin's lymphoma in Pakistani population.

Methods: All cases of non Hodgkin's lymphoma, diagnosed at histopathology section of Dow Diagnostic research and reference laboratory from October 2008 to June 2010, were included for this cross sectional study. Immunohistochemical study using monoclonal antibody MIB 1, Dako, Denmark against Ki 67 antigen was carried out on paraffin embedded tissue blocks of 62 patients. Ki 67 PI, with cutoff 45% was assessed in relation to specific phenotype, age, gender, site of origin and B symptoms. Mean Ki 67 PI was also calculated considering specific phenotype according to WHO classification.

Results: Out of 62 patients, Ki 67 PI >45% was noted in 39 (62.9%) cases, whereas, in 23 (37%) cases the PI was <45%. The mean Ki 67 PI was highest in Burkitt's lymphoma. Furthermore Ki 67 PI > 45% was seen in 21/25 (84%) cases with extra nodal involvement and 24/29 (82.7%) cases with B symptoms.

Conclusion: Mean Ki 67 PI can discriminate the indolent versus aggressive behaviour of disease. In the study population significant association of high Ki 67 PI > 45% was found in relation to B symptoms and site of involvement, in terms of extra nodal origin, for non Hodgkin's lymphoma. These correlations demonstrate the significant role of high Ki 67 PI, to establish the proliferative activity of tumour as prognostic index marker along with clinical parameters at the time of diagnosis.

Keywords: Ki67 Proliferative Index, Non Hodgkin's Lymphoma, Pakistan, Histopathology (JPMA 61:748; 2011).

Introduction

Non Hodgkin's Lymphoma (NHL) is a broad category consisting of several distinct types of lymphoid neoplasms, 85% of which are B cell lymphomas and 15% being T cell lymphomas. The incidence is increasing largely in Pakistan. Higher incidence of NHL >15% was reported in hospital data from Punjab and it was linked with the indiscriminate use of pesticides (organophosphates).[1] To establish the therapeutic approach, clinically NHL is categorized into two major subtypes, indolent and aggressive. Indolent group includes the small lymphocytic and the follicular cleaved cell categories, while the aggressive group which responds well against treatment, constitutes the large cell types, the Burkitt's lymphoma and the lymphoblastic lymphomas.[2] Examination of pretreatment clinical variables, like age, sex, stage, B symptoms such as drenching night sweats, unexplained weight loss, fever and severe itching, tumour bulk, site of origin and lactate dehydrogenase (LDH) with proliferative index also shows clinical importance in diagnosis, grading and determination of prognosis in terms of patient's outcome in NHL.

Proliferation is a key feature of the progression of tumours and in recent years as markers of cell proliferation are identified, it is widely estimated by the immunohistochemical assessment of the nuclear antigen Ki-67.[3] Its role as a Proliferative index (PI) marker is due to the fact that, Ki 67 is expressed during the active phases of cell cycle, which are G1, S, G2, and mitosis. Therefore it is an excellent marker for determining the growth fraction of a given cell population.[4] PI of Ki 67 is a measure of the number of dividing (proliferating) cells, positive for Ki 67 immunostaining with a total number of cells in a biopsy sample, which gives a more complete understanding of how fast a tumour is growing.[5] Experimental and clinical data indicate that tumour progression and malignancy are associated with increased angiogenesis and higher Ki-67 PI. Furthermore, higher Ki-67 PI is associated with a poor prognosis, in both solid and haematological malignancies.[6] Ki 67 relates with the disseminated disease, it is therefore helpful in the prediction of disease stage and progression. Ki-67 proliferation rate greater than 20% in extra nodal marginal zone B cell lymphoma corresponds to at least stage II lymphoma.[7]

A highly significant correlation is demonstrated between the proportion of Ki-67 positive cells and the classification into high and low-grade malignant NHL.[8] Moreover, a positive correlation exists between prognosis and proliferation rates in chronic B and T cell lymphoproliferative disorders.[9] Although the number of long-term follow-up studies is limited, Ki 67 does appear to provide early and accurate information with regard to long-term outcome and prediction of response to treatment.[10] The diagnostic criterion of Ki 67 PI approaches 100% in Burkitt's and atypical Burkitt's lymphoma.[11] Hence, due to the clinical significance of Ki 67 PI in diagnosis as well as in determination of prognosis of NHL, this study was conducted to assess Ki-67 PI in tissue sections for NHL with correlation to grade and clinical parameters in Pakistani patients, to predict the behaviour of disease at the time of presentation.

Patients and Methods

The present immunohistochemical study was carried out on the same 62 paraffin embedded tissue blocks of NHL, which were previously categorized into B and T cell type according to WHO classification of lymphoid neoplasms,[12] by Immunohistochemistry (IHC) in our study, conducted from the period of October 2008 to June 2010, at the histopathology section of DDRRL.[13] Ki 67 PI was assessed in addition to previous work with relation to specific immunophenotype, age, gender, site of origin and occurrence of B symptoms. Age of the patients was divided into two groups according to Vose et al[14] by cutoff at 60 years. Age less than 60 years was considered as group (1) and equal to and more than 60 years was group (2). Ki-67 was assayed with monoclonal antibody MIB1 (Dako, Denmark). The endogenous peroxidase was quenched with methanol and 3% hydrogen peroxide for 5 min followed by target retrieval, which was done with target retrieval solution (Dako, Denmark) with citrate buffer (pH 6.0) in a pressure cooker for 2 minutes. The slides were incubated with the primary antibody for 30 minutes at room temperature, and washed with Tris-buffer saline (TBS). The secondary antibody (ChemMate Dako Envision, Dako Cytomation) was applied for 30 minutes. Slides were again washed with TBS and colour was developed by 5 minutes incubation in diaminobezidine (DAB) solution. Slides were counterstained with haematoxylin.[15]

Positive control (5 to 6 µm thick section of tonsil tissue) and negative control with omission of primary antibody was included with each batch. Slides were examined first separately and then consensus was made by joint review of authors on multi-head microscope. The presence of nuclear staining in terms of intensity and proportion was done, to assess PI in tissue sections. A cut-off value of 45% was used to differentiate high versus low proliferative activity.[16,17]

The data was analyzed using SPSS version 16. Age was computed in terms of mean and standard deviation, while gender and type of NHL was described in terms of frequency. Pearson chi-square (x^2) was applied to assess the relationship of clinical parameters i.e. age, gender, type of NHL, site of origin and occurrence of B symptoms with Ki 67 PI using cutoff value of 45% and the mean Ki 67 PI within the NHL groups (according to WHO classification) was tested by one

way analysis of variances (ANOVA). P value ≤ 0.05 was considered significant.

Results

The Ki 67 PI more than 45% was seen in 39 (62.9%) cases and in 23 (37%) cases the PI was less than 45%. The mean age for NHL in this series was 41.3 ± 17.5 years with median of 42.5 years and the age range was 6 to 80 years, already described previously in our study. The Ki 67 PI in the tissues from 62 patients ranged from 0 to 100%, with a mean of 38.48 ± 28.7. Follicular lymphoma and small lymphocytic lymphoma showed much lower mean than rest of the others, while Burkitt's lymphoma had highest mean. Significant association (P, ANOVA = <0.0001) was found between the high Ki 67 PI mean with aggressiveness of disease in terms of phenotypes (Table-1).

Table-1: Mean Ki 67 proliferative index (PI) in 62 patients with Non Hodgkin's lymphoma (WHO classification).

NHL type	No.of patients	Ki 67 PI (%) Mean ± SD	Minimum	Maximum
BL	2	97.5 ± 3.5	95	100
B-ALL	1	50.0	50	50
DLBCL	36	47.0 ± 25.1	0	90
ALCL	7	44.29 ± 22.2	0	70
Plasma cell type	2	15.0 ± 21.2	0	30
MCL	1	10.0	10	10
T-ALL	1	10.0	10	10
PTCL	1	10.0	10	10
FL	6	7.5 ± 7.5	0	20
SLL/CLL	5	6.2 ± 3.8	1	10
All types	62	38.4 ± 28.7	0	100

SD= Standard deviation, BL=Burkitt lymphoma, B-ALL= Acute lymphoblastic lymphoma (B cell type), DLBCL =Diffuse large B cell lymphoma, ALCL= Anaplastic large cell lymphoma, MCL= Mantle cell lymphoma, T-ALL= Acute lymphoblastic lymphoma (T cell type), PTCL=peripheral T cell lymphoma, FL= Follicular lymphoma, SLL/CLL= Small lymphocytic lymphoma/Chronic lymphocytic leukaemia.

Table-2: Ki 67 proliferative index (PI) in relation to subtypes of NHL, using cutoff value of 45%.

Cell type	Subtypes of NHL	Ki 67 PI < 45% (n= 23)	Ki 67 PI ≥45% (n=39)	N= 62 (%)
B cell	DLBCL	14	32	36 (58.1)
(n=53)	FL	6	0	6 (9.7)
	SLL/CLL	5	0	5 (8.1)
	Plasma cell type	2	0	2 (3.2)
	BL	0	2	2 (3.2)
	B-ALL	0	1	1 (1.6)
	MCL	1	0	1 (1.6)
T cell	ALCL	3	4	7 (11.3)
(n=9)	T-ALL	1	0	1 (1.6)
	PTCL	1	0	1 (1.6)

DLBCL =Diffuse large B cell lymphoma, SLL/CLL= Small lymphocytic lymphoma/Chronic lymphocytic leukemia, ALCL= Anaplastic large cell lymphoma, T-ALL= Acute lymphoblastic lymphoma (T cell type), B-ALL= Acute lymphoblastic lymphoma (B cell type), B.L = Burkitt's lymphoma, F.L = Follicular lymphoma, MCL = Mantle cell lymphoma.

Table-3: Ki 67 proliferative index (PI) in relation to clinical parameters, using cutoff value of 45%.

		Ki 67 PI < 45% (N)	Ki 67 PI >45% (N)	Total	P value (x^2 test)
Age	<60 years (gp. 1)	20	29	49	0.50
	≥60 years (gp. 2)	3	10	13	
Gender	Male	14	28	42	0.374
	Female	9	11	20	
Anatomic Site	Nodal	19	18	37	0.005*
	Extranodal	4	21*	25	
B symptoms	Present	5	24*	29	0.002*
	Absent	18	15	33	

P value ≤ 0.05 is significant
x^2 test = Chi square test
< = less than, > = more than.

Amongst total of 62 cases, 53 (85.4%) were of B cell phenotype and 9 (14.5%) cases were of T cell type. Ki 67 PI with cutoff value of 45% was assessed with relation to cell lineage (either B or T cell origin) and subtypes of NHL, depicted in Table-2. Total 35/53 (66.0%) cases of B cell phenotype showed the PI more than 45%, however 18/53 (33.9%) cases had figures less than 45%. Regarding T cell phenotype 5/9 (55.5%) cases had PI less than 45% as compared to 4/9 (44.4%) cases with PI more than 45%. There was no significant association of Ki 67 PI with cutoff value of 45% in relation to cell lineage (P, x2 = 0.622) and specific subtypes of NHL (P, x2 = 0.075). Table 3 exhibits the Ki 67 PI in relation to clinical parameters. Patient's age and gender showed no significant association with Ki 67 PI at cutoff value of 45% while the correlation of Ki 67 PI more than 45% with extra nodal origin was highly significant (P, x2 = 0.005). Total of 37/62 (59.6%) patients with nodal origin, 18/37 (48.6%) were assessed as having PI more than 45%, however 19/37 (51.3%) had PI less than 45%. Out of 25/62 (40.3%) patients with extra nodal involvement the Ki 67 PI was more than 45% in 21/25 (84%) cases while only 4/25 (16%) cases showed the PI less than 45%. B symptoms were present in 29/62 (46.7%) patients, in which 24/29 (82.7%) cases had Ki 67 PI more than 45% while only 5/29 (17.2%) cases with B symptoms had Ki 67 PI less than 45%. Whereas 33/62 (53.2%) cases showed absence of B symptoms at the time of diagnosis, in which 15/33 (45.4%) patients had Ki 67 PI more than 45% as compared to 18/33 (54.5%) patients with Ki 67 PI less 45%. The Ki 67 PI more than 45% also showed significant association (P, x2 = 0.002) with presence of B symptoms.

Discussion

The importance of Ki 67 as prognostic and diagnostic marker in NHL is documented in literature and it is observed in different studies that the significance of Ki 67 with clinical parameters remains highly informative. Mihaljevic et al[18] tested relation of Ki-67 in patients of NHL with survival and observed that low PI was associated with the longest survival

(median about 36 months) while it was only 12.9 months with the high PI. According to Grogan et al,[19] Ki 67 is an independent factor to predict outcome and PI more than 60% (on cutoff value of 60%), was found to be a strong predictor of poor survival. Broyde et al[16] used the cut-off value of 45% to differentiate indolent from aggressive disease and also assessed the mean Ki 67 PI within the lymphoma subgroups according to WHO classification, which yielded significant association ($P < 0.001$). Our study is accordance with Broyede et al[15] because we also found significant difference of mean Ki 67 PI (P, ANOVA = <0.0001) 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 in this study 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. We found PI of more than 45% in 62.9% of cases, which shows overall high PI in our series. It is quite alarming on looking at the importance of high PI with shorter survival[18] and poor outcome[19] and could be evaluated in our setup in long term follow up.

When we analyzed the relation of Ki 67 using cutoff value of 45% with specific phenotype, age, gender, site of origin and occurrence of B symptoms, we found that there is no significant association of Ki 67 with specific phenotype and gender. However, site of involvement and occurrence of B symptoms showed significant results with high Ki 67 PI (more than 45%), which is quite eye opening because of the relation of B symptoms with poor responders to chemotherapy[20,21] and poor survival.[22] Szczuraszek et al[23] tested the correlation of Ki-67 expression with clinical parameters of the patients and their survival. It was seen that survival of the patients was significantly shorter ($P = 0.03$) with higher Ki-67 indices but no significant correlation could be detected between Ki-67 antigen expression and clinical or pathological parameters of the patients like type of lymphoma age and gender. This is partly in accordance with our study where specific phenotype and gender showed no statistical significance. Moreover, we did not find any significant association of high PI (using cutoff value of 45%) regarding cell lineages (P, $x2 = 0.622$) and specific subtypes of NHL (P, $x2 = 0.075$). Though this is in contrast with Melo et al,[9] showed significantly higher proportions of Ki-67 positive cells in T cell (11.2%) than in B cell (2.9%) disorders ($P < 0.001$). Whereas Tominaga et al[24] studied 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. Within the B cell phenotype the percentage was higher in diffuse lymphomas than follicular lymphomas, which is conflicting with the greater expression profile in higher grades.

Regarding NHL, localized disease is considered to be a good prognostic indicator for both event free survival (EFS) and overall survival (OS).[25] Extra nodal involvement shows the aggressive nature of disease and our findings suggest high Ki 67 PI > 45% in the patients with extra nodal involvement with significant association (P, $x2 = 0.005$). Similar to our series this was also indicated by Llanos et al[26] with high Ki-67 expression in extra nodal involvement of more than one area ($P = 0.03$). On the contrary no significant association was seen in literature regarding high Ki 67 PI having cutoff at 60% with occurrence of B symptoms,[18] whereas in our study it was seen that high PI (more than 45%) was significantly associated with occurrence of B symptoms. It is worth noting that in our series we have some ranges of dissimilarities of clinical parameters with Ki 67 PI. Due to the dearth of clinical data in relation to histological and immunochemical correlations, the exact picture of patient's outcome and survival is still unclear, which should be resolved in terms of predictive value of Ki 67 expression in tissue sections. Therefore, in future studies, there is a dire need to relate the laboratory investigations with clinical parameters to identify the exact picture in a large number of clinicopathological studies for more conclusive observations.

Conclusion

Significant association of Ki 67 PI > 45% with occurrence of B symptoms and extra nodal origin, is seen in NHL. Regarding diagnostic point of view, the assessment of mean Ki 67 PI is quite helpful, to differentiate indolent versus aggressive behaviour of disease. Using cutoff value of 45%, other parameters like age, gender and specific phenotype showed no significant association and in dissimilarity with international data but highly informative in our setup as little information is available in this regard, which needs further analysis, as it has prognostic as well as therapeutic implications.

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