RESEARCH ARTICLE

Distribution of Ki67 Proliferative Indices among WHO Subtypes of Non-Hodgkin's Lymphoma: Association with other Clinical Parameters

Atif Ali Hashmi*, Zubaida Fida Hussain, Naveen Faridi, Amna Khurshid

Abstract

Background: Non-hodgkin lymphoma (NHL) is a diverse group of disease encompassing divergent tumor types with contrasting clinical behaviors. We aimed to evaluate the usefulness of Ki67 index in segregating indolent from aggressive NHL and its association with clinical parameters. Materials and Methods: During a study period of 4.5 years, a total of 215 cases of lymphomas were diagnosed among of which 172 cases were NHL. Ki67 immunohistochemical staining was performed by the DAKO envision method. Average proportion of tumor cells stained was calculated to determine the proliferative index. Results: The mean age at diagnosis was 46.2 years +19.8 (3-81) with a male to female ratio of 1.5:1. Mean Ki67 index for indolent NHL included 23% for small cell, 25% for mantle cell, 28.5% for marginal zone and 34.6% for follicular lymphoma. On the other hand, mean Ki67 index for aggressive lymphomas were 66.4%, 66.9%, 80.3%, 83.3% and 94.4% for diffuse large B cell, T cell (NOS), anaplastic large cell, lymphoblastic and burkitts lymphoma respectively. No significant correlation was found between Ki67 index and other clinical parameters like age and extra nodal involvement. Conclusions: Ki67 index is a valuable IHC marker to distinguish indolent from aggressive lymphomas especially in small needle biopsies where exact typing may not be possible.

Keywords: Ki67 index - non-hodgkin lymphoma - proliferative index - Pakistan

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Introduction

Traditional grading parameters like nuclear pleomorphism and mitotic count doesn't apply to lymphoid neoplasms where classification is mainly based on cell size and morphology. Estimation of proliferative index as a marker of tumor aggressiveness has long been known. Several methods to assess proliferative fraction of tumor cells have been identified, of which flow cytometric determination of S-phase fraction gained most popularity (Pinto et al., 2003). Ki67 is a nuclear antigen that is expressed in mid G1, S, G2 and M phase of the cell cycle (Gerdes et al., 1984). The Ki67 antigen was first described by Gerdes & colleagues in the early 1980s, by use of a mouse monoclonal antibody against a nuclear antigen from a Hodgkins lymphoma (HL) derived cell line (Gerdes et al., 1983). Immunohistochemical expression of Ki67 antigen in paraffin section called Ki67 proliferative index, represents the active growth fraction of the tumor. As various studies confirmed the correlation of Ki67 index with tumor grade and clinical behavior of the tumors, it became the routine part of various tumor workup especially breast cancer and lymphoid neoplasms (Gunia et al., 2012; Niikura et al., 2012; Haroon et al, 2013).

An increased incidence and rising trend for non-hodgkins lymphoma (NHL) was observed in our population in recent years. About 429 incident cases of NHL were registered at Karachi cancer registry which is the only population based cancer registry in Pakistan. Children and adolescent in lower socioeconomic group appear to have highest susceptibility to this disease (Bhurgri et al., 2005). The cause of this increased trend is not well established. Ishtiaq et al suggested a role of Ebstein bar virus (EBV). They found 12.7% cases of NHL to express EBV-LMP by immunostaining. Interestingly 44.4% of cases of DLBCL also expressed EBV-LMP which seemed to be most prevalent NHL in our population (Ishtiaq et al., 2013).

NHL is both clinically and biologically a heterogeneous disease comprising of divergent groups of indolent and aggressive tumors. Indolent lymphomas are slow growing with relative resistance to chemotherapy and include mainly Small lymphocytic lymphoma (SLL), Marginal zone lymphoma (MZL), low grade Follicular lymphoma (FL) and Mantle cell lymphoma (MCL) whereas aggressive lymphoma category comprise of Diffuse large B cell lymphoma (DLBCL), Burkitts lymphoma (BL) and Lymphoblastic lymphoma (Swerdlow et al., 2008).

*Department of Histopathology, Liaquat National Hospital and Medical College, Karachi, Pakistan *For correspondence: doc_atif2005@yahoo.com*

Compared to indolent lymphomas, aggressive lymphomas like BL and DLBCL are associated with significantly higher expression of Ki67 index (Rabenhorst et al., 1996; Kalogeraki et al., 1997; Broyde et al., 2009).

While the role of Ki67 index as a prognostic and predictive factor is extensively evaluated in several studies (Erlanson et al., 1999; Hadzi-Pecova et al., 2007; Gaudio et al., 2011), its significance has not been widely studied in our population. Therefore we aimed to determine distribution of Ki67 index in various WHO subtypes of non-hodgkins lymphoma in our setup and its association with other clinical parameters like age at diagnosis and extra-nodal involvement.

Materials and Methods

This is a retrospective cross-sectional study conducted at Liaquat National Hospital, Karachi involving 215 newly diagnosed cases of lymphomas from august 2008 till December 2012 for a period of 4.5 years. Specimens include trucut/incisional and exicisional biopsies from nodal and extra-nodal sites. Cases with available clinical records were included in the study. All post-chemo and radio-therapy cases were excluded from the study. Immunohistochemical (IHC) stains using antibodies against CD 20, CD 3, CD 10, CD 5, CD 23, BCL2 and cyclinD1 were done in each case. Other markers including CD 79a, ALK, CD 43, CD 30 and CD 15 were done in selected cases. The cases were diagnosed by morphology on H and E sections and IHC profile according to WHO classification of lymphoid neoplasms by senior histopathologists.

One representative section from the tumor is selected for Ki67 immunostaining for which 4mm thick sections were deparaffinized in xylene and dehydrated. Antigen retrieval was done by boiling target DAKO Envision retrival solution (high PH 50x) for 40mins at 96-99°C. Endogenous peroxidase activity was blocked by treatment with DAKO Envision flex peroxidase blocking reagent. The slides were incubated for 20-30mins at room temperature in humidity chamber with appropriate dilutions of primary antibodies along with their positive and negative controls. The slides were then incubated with secondary antibody (Envision horse reddish peroxidase) for coupling reaction for 20-30mins at room temperature. The substrate (Diamino benzidine + Chromogen) was used to produce crisp brown color at the site of target antigen. The hematoxylin (1-2 dips) was used as a counter stain. Ki-67 immunoreactivity was recorded as continuous variables, based on the proportion of positive tumor cells (0%-100%). In limited tissue samples like trucut biopsies, all positively stained tumor cells were recorded and divided by total number of tumor cells. In exicisional biopsies, proportion of Ki67 positive cells were counted in atleast 100 high power fields and average Ki67 index was determined.

Results

The mean age at diagnosis was 46.2 years +19.8(3-81). Male to female ratio was 1.5:1. 43 cases were diagnosed as hodgkins lymphoma while 172 were that of NHL as shown in Figure 1. Total 54 (31.4%) cases of NHL were seen in >60 years of age while the most common age group was 31-60 years comprising 82 (47.7%) cases of NHL. Male to female ratio for NHL was 1.4:1. 125 cases (58%) were confined to lymph nodes while extranodal sites were involved in 90 cases (42%). DLBCL was the most common lymphoma comprising 66% of cases. Mean Ki67 index of NHL was 65.9%+20.5 (5-100). Significantly high Ki67 index values were seen in aggressive NHL like BL, DLBCL, lymphoblastic and T-cell lymphomas as compared to indolent ones (Figures 2 and 3). Highest mean Ki67 index was found in Burkitts lymphoma (94%) followed by lymphoblastic lymphoma (83%) and Anaplastic large cell lymphoma (80%) as shown in Table 1. Mean Ki67 index for indolent NHL include 23 % for small cell, 25% for mantle cell, 28.5% for marginal zone and 34.6% for follicular lymphoma. Cases of DLBCL, follicular lymphoma, marginal zone lymphoma, small cell lymphoma and T-cell lymphomas are further grouped according to defined Ki67 index cut off values as shown in Table 2. No significant correlation was found between

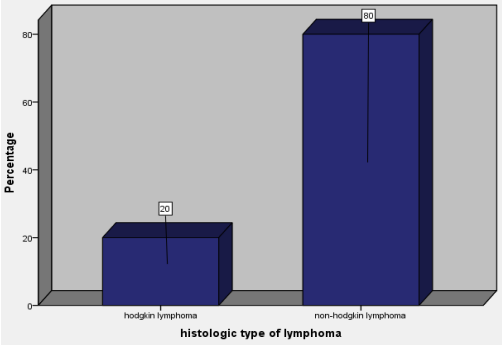

Figure 1. Frequency Distribution of Lymphoma Categories

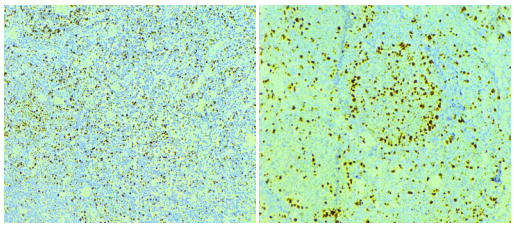

Figure 2. Ki67 Index in Indolent Lymphomas: 2a: Marginal Zone Lymphoma with 20% Mean Ki67 Index. 2b: Follicular Lymphoma with 24% Mean Ki67 Index

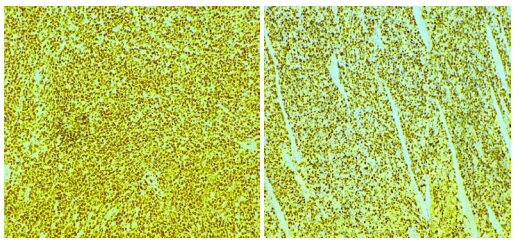

Figure 3. Ki67 Index in Aggressive Lymphomas: 3a: Burkitts Lymphoma with 95% Mean Ki67 Index. 3b: Diffuse Large B Cell Lymphoma with 80% Mean Ki67 Index

Table 1. Frequency, Age, Ki67 Index Distribution and Extranodal Involvement in Non-Hodgkins Lymphoma

Non-hodgkins lymphoma subtype	Frequency(%)	Mean age in years(+SD)	Mean Ki67 index in %(+SD)	Extranodal involvement n(%)
Diffuse large B-cell lymphoma	114(66.3%)	54.2(+15.5)	66.4(+14.6)	63(55.3%)
Follicular lymphoma	8 (4.7%)	53.8(+9.1)	34.6(+21.3%)	1(12.5%)
Mantle cell lymphoma	1 (0.6%)	80	25	1
Marginal zone lymphoma	4 (2.3%)	55.5(+28.2)	28.5(+3.7)	2(50%)
Small cell lymphoma	3 (1.7%)	59.3(+9.8)	23.0(+2.7)	0 (0%)
Lymphoblastic lymphoma	13 (7.6%)	32.2(+19.9)	83.3(+9.0)	4(30.8%)
Burkitts lymphoma	8 (4.7%)	20.6(+19.9)	94.4(+8.0)	5(62.5%)
T cell lymphoma, NOS	13 (7.6%)	32.7(+20.8)	66.9(+24.1)	7(62.5%)
Anaplastic large cell lymphoma	7 (4.1%)	39.0(+13.1)	80.3(+8.2)	2(28.6%)
Plasmacytoma	1 (0.6%)	53	35	1
Total	172			

Table 2. Ki67 Categorization in Specific Non-Hodgkins Lymphoma Subtypes

Non-hodgkins lymphoma subtype	Ki67 index category	Frequency (%)
Diffuse large B-cell lymphoma	<70%	72(63.2%)
	>70%	42(36.8%)
Follicular lymphoma	<40%	6(75%)
	>40%	2(25%)
Marginal zone lymphoma	<20%	0(0%)
	>20%	4(100%)
Small cell lymphoma	<20%	1(33.3%)
	>20%	2(66.7%)
T-cell lymphoma, NOS	<80%	11(84.6%)
	>80%	2(15.4%)

Ki67 index and clinical parameters like age (p-value 0.06), B-symptoms (p-value 0.23) and extranodal site of involvement (p-value 0.15).

Discussion

The WHO classification of hematopoietic tumors is based on morphologic, immunophenotypic, genetic, and clinical features to define distinct subtypes. The WHO classification does not take into account the aggressiveness of the tumors largely due to significant variability in specific types. However, several clinical trials have separated histologic subtypes according to the usual clinical behavior and biologic aggressiveness. They roughly segregated NHL into three groups based on proliferative index namely, indolent, aggressive and highly aggressive categories, however cutoff values for these categories are ill defined.

Several researchers tried to establish usefulness of Ki67 index in distinguishing indolent and aggressive lymphomas. Broyde et al evaluated Ki67 index in 319 newly diagnosed cases of NHL. There was a statistically significant increase in mean Ki67 index from 26.6% for indolent lymphomas to 67.2% for aggressive lymphomas to 97.6 % for very aggressive lymphomas. They established a ki67 index of 45% to differentiate indolent from aggressive lymphomas (Broyde et al., 2009).

We evaluated mean Ki67 index in different subsets of NHL and segregated them into high and low risk categories using cut off values defined by various researchers according to prognostic and survival analysis. The mean Ki67 index for FL in our studied patient population was 34.6 %. Martin et al defined a cut off Ki67 index of 40% in FL and found a significant difference in overall survival between high and low ki67 index using the same (Martin et al., 1995). We found 25% of cases of FL falling in high risk category using a cut off value of 40%. Petit et. al assessed proliferation index in 90 indolent lymphoplasmacytic and MZL. They found mean Ki67 index of 20% being associated with low overall survival (Petit et al., 2005). In our series all cases of MZL and 66.7% cases of SCL were above 20% ki67 index.

Gene expression profiling has identified proliferation signature as a powerful predictor of outcome in MCL (Rosenwald et al., 2003). Similar results were found on IHC estimation of Ki67 index on paraffin sections. Schaffel et. al defined a cut off value of 30% in determining progression free survival and overall survival in patients with MCL (Schaffel et al., 2010).

DLBCL was the most common subtype of NHL in our study comprising of 66% of cases. Ki67 index is an important prognostic and predictive parameter in DLBCL in determining response to chemotherapy. In addition to ki67 index, other IHC and molecular markers associated with dismal prognosis in DLBCL include MYC and Bcl2 expression (Akay et al., 2014; Bellas et al., 2014; Cook et al., 2014). Significant disparity was seen in establishing cut off values of Ki67 index in DLBCL as these tumors show a wide range of expression of proliferation related genes. Jerkman et al included 185 cases of DLBCL in their study. Ki67 expression was found to be low (<60%) in 116 tumors (63%), moderate (60-90%) in 59 (32%) and high (>90%) in 10 (5%) cases. These values correlated with performance status. A value of <60% was associated with failure free survival (Jerkeman et al., 2004). Broyde et. al defined a ki67 cut off value of 70% in distinguishing tumors with favorable and poor prognosis (Broyde et al., 2009). Using the same cut off value we found 36.8% of cases of DLBCL to be on the higher side.

13 cases in our study were that of T-cell lymphoma, NOS. Went et al analyzed 148 cases of T-cell/NK cell lymphoma, NOS. They found ki67 index >80% in 11 % of patients. Ki67 index had prognostic significance on univariate analysis (Went et al., 2006). We found 15.4% of cases of T-cell lymphoma to have ki67 index above 80%.

Aggressive lymphomas respond better to chemotherapy as more number of cells are in cell cycle which can be targeted. As a strong correlation between lymphoma grade and Ki67 index is well established, Ki67 became an integral part of NHL workup. Many researchers evaluated the diagnostic and prognostic significance of Ki67 in NHL.

Ki67 index has also been studied and correlated with response to newly established chemotherapy regimens (Li et al., 2013; Salek et al., 2014).

In a meta-analysis involving 27 studies (3902 patients), high Ki67 index was found to be negatively correlated with overall survival and disease free survival. However no significant association was seen between Ki67 index and clinicopathologic parameters like LDH levels, B-symptoms, tumor stage, extranodal involvement and performance status (He et al., 2014).

Several clinical parameters were studied as prognostic and predictive factors in NHL including serum beta 2 microglobulin, absolute lymphocyte count (ALC), serum immunoglobulin free light chains, vitamin D levels and serum lactate dehydrogenase (LDH) (Jung et al., 2014; Vaidya et al., 2014; Wu et al., 2014). A few investigators also established a positive correlation between Ki67 index and clinical parameters like B symptoms, LDH levels and stage (Li et al., 2011). In a retrospective analysis of 227 cases of DLBCL, an unfavorable prognostic outcome was found to be associated with Elevated LDH and β2-M levels, positive B symptoms, Ann Arbor stage III/IV, and primary nodal lymphoma (Zhou et al., 2013). However most of the studies failed to establish a significant positive correlation as seen in our study. Gene expression profiling and molecular studies uncovered several up-regulated and down-regulated pathways in NHL including overexpression of Rb binding protein 5, DKFZP586J1624 protein, protein kinase inhibitor gamma, zinc finger protein 3, choline ethanolamine phospho-transferase CEPT1, protein phosphatase, and histone deacetylase-3, however their utility in molecular diagnostics is yet to be established (Zekri et al., 2013; Zhand et al., 2013)

A few investigators also analyzed the usefulness of Ki67 index in FNAC specimens. In a study involving 86 FNAC specimens of NHL, 38% cutoff value was established to discriminate indolent from aggressive lymphomas (Ali et al., 2010).

In conclusion, Ki67 index is a valuable IHC marker to distinguish between indolent and aggressive lymphomas especially in small needle biopsies where exact typing may not be possible.

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