Original Article (APMC – 601) DOI: 10.29054/APMC/19.601

Ki-67 Proliferative Index in The Non-Hodgkin's Lymphoma and Its Clinical Significance

Muhammad Mudassar, Sadia Hameed, Shazia Aslam, Rehana Majeed, Rizwan ullah Khan, Munazza Majeed

ABSTRACT

Introduction: For prognostic purposes, it is of utmost importance to know the aggressiveness of the tumor. Ki-67 Proliferative index has been used in tumors of many organ systems as a potential marker to establish biological behavior and aggressiveness of the tumor. In Non Hodgkins lymphoma, there are controversial results in the literature, where most of the studies highlighting it as an important prognostic marker, while others reported to have no correlation with clinical or pathological parameter. The objective of study is to correlate Ki-67 proliferative index in tissue sections of Non-Hodgkins lymphoma with grade of lymphoma, NHL types and clinical parameters. **Study Design:** Descriptive Retrospective Study. **Settings:** Meezan lab, Faisalabad Pakistan. **Duration:** January 2015- December 2018 (4 years). **Sample Size:** 86 cases confirmed as NHL on IHC were taken and Ki-67 was evaluated. **Sampling Technique:** Non-probability consecutive sampling. **Data Collection Procedure:** 86 Paraffin-embedded tissue blocks of Non-Hodgkins lymphoma (NHL) cases, which were confirmed on Immuno-histochemistry were taken and Ki-67 immunostain was performed. Correlation of Ki-67 Proliferative index was done with immunophenotype, age, gender and site of origin. All the collected information was entered and analyzed using SPSS version 24. Chi-square calculator for 2x2 contingency table was employed to assess the relationship of clinical parameters like age, gender, lymphoma grade and site of origin with Ki-67 PI, taking cut off value of 45%. P value of < 0.05 was taken as significant. **Results:** 26 out of 30 cases of Extra-nodal lymphomas were having Ki-67 PI greater than 45% which was statistically significant with a p-value of 0.039731 (taking P-valve < 0.05 as significant). Similarly, aggressive lymphomas were statistically significant (P-value=0.000257) in staining ≥ 45 % of Ki-67 positive staining in tumor cells. Moreover, patients with age less than 30 years were shown to have ≥ 45 % Ki-67 positivity in lymphomas (p value= 0.00458). However, gender was not related to Ki-67 positivity with insignificant results (p value=0.8201). **Conclusion:** There was significant association of high Ki-67 Proliferative Index (>45%) with the aggressiveness of the lymphoma. Extranodal origin and age <30 years of age was also associated with high Ki-67 PI. However, no relationship was established between gender and Ki-67 expression.

Keywords: Ki-67 proliferative index, Non Hodgkins lymphoma, Proliferative marker, Aggressive lymphoma, Ki-67 positivity, NHL immunohistochemistry.

Corresponding Author
Submitted for Publication: 07-05-2019 *Accepted for Publication: 19-06-2019*
DR. MUHAMMAD MUDASSAR, Associate Professor & Head, Pathology Department, Batterjee Medical College, Jeddah, Saudi Arabia
Contact / Email: +966534989769, pathology5.jed@bmc.edu.sa
Citation: Mudassar M, Hameed S, Aslam S, Majeed R, Khan R, Majeed M. Ki-67 Proliferative Index in The Non-Hodgkin's Lymphoma and Its Clinical Significance. APMC 2019;13(1):108-12.

INTRODUCTION

Immunohistochemistry (IHC) has really changed the game for histopathologist too much. It has helped not only to establish a diagnosis in many tumors of the body but also differentiate between benign and malignant tumors or aggressive or non-aggressive tumor.[1,2] For prognostic purposes, it is of utmost importance to know the aggressiveness of the tumor. Many immune markers have been reported in the literature that can help in this regard.[3] One of them is Ki-67. This particular immune marker has been used in tumors of many organ systems as a potential marker to establish biological behavior and aggressiveness of the tumor. For example, cervical tumors,[4] urothelial carcinomas,[5] bladder cancers,[6] gliomas,[7] breast cancers,[8,9] prostate cancers,[10] and many other tumors.[11,12]

Ki-67 is a non-histone nuclear antigen which is present in the proliferating cells. In the cell cycle, the increased expression was seen in all stages. However, in the G0 phase, Ki-67 expression was absent, which makes it an excellent marker of growth fraction.[13,14] It was identified and described first time by Gerdes et al[15] in the city of Kiel (hence "Ki"). It was initially recognized to bind only in fresh tissue (unfixed tissue) but after the development of newer antibodies like MIB-1, it was used even in paraffin-embedded tissues.[15] Expression of Ki-67 is usually estimated in the percentage of tumor cells being stained positively by the antibody. Nuclear staining pattern is considered as a criterion for positivity.

Lymphoma diagnosis is incomplete without IHC. Since the typing, treatment, and prognosis of lymphoma completely rely on it. From morphological to treatment point of view, Hodgkin's lymphoma (HL) is totally different from Non-Hodgkins lymphoma (NHL). Similarly, among NHL, B cell lymphomas are way out of the league from T cell lymphoma. Furthermore, subtypes of B and T cell lymphoma have different immuno-phenotyping and prognosis. All of which is impossible without doing IHC.[1,16,17] Since Hodgkin's lymphoma has very distinct morphological characteristics, so in clear cut cases, it is possible to give a diagnosis without IHC. However, in NHL, Ki-67 PI has been reported as an important and independent marker for prognosis.[18,19]

In Pakistan, because of unavailability of resources and policy matters, only very few tertiary care hospitals are doing immunohistochemistry for diagnostic purposes. So, there are sparse studies locally, which have highlighted the Ki-67 prognostic value in the context of NHL.[20] Moreover, there are controversial results in the literature, where most of the studies highlighting it as an important prognostic marker[21,22] while others reported to have no correlation with clinical or pathological parameter.[23,24] In this context, the purpose of our study is to correlate Ki-67 proliferative index in tissue sections of Non-Hodgkins lymphoma with grade of lymphoma, NHL types and clinical parameters.

METHODOLOGY

Study Design: Descriptive Retrospective Study.
Settings: Meezan lab, Faisalabad-Pakistan.
Duration: January 2015- December 2018 (4 years).
Sample Technique: Non-probability Consecutive Sampling.
Sample Size: 149 cases with the morphological diagnosis of Lymphoma or lymphoproliferative disease were included. Immunohistochemistry was performed on these cases, after which 43 cases of Hodgkin's lymphoma, 16 cases of metastatic carcinoma and 4 cases with reactive lymph node or other benign diagnosis were excluded from the study. Only 86 cases confirmed as NHL on IHC were taken and Ki-67 was evaluated in these cases.

Inclusion Criteria:

- All cases of Non-Hodgkin's Lymphoma (NHL) presented during the study period and who underwent Routine IHC and Ki-67 for diagnosis and typing purposes.

Exclusion Criteria:

- After IHC, if the final diagnosis was other than NHL, they were excluded.
- If IHC was inconclusive because of technical reasons.

Data Collection Procedure: 86 Paraffin-embedded tissue blocks of Non-Hodgkins lymphoma (NHL) cases, fulfilling the inclusion and exclusion criteria were selected. These were previously categorized as different B or T cell types using a panel of antibodies including LCA, CD 20, CD 3, CD 30, and CD 15. Further antibodies like CD 10, Tdt, Cyclin D1, BCL 2 and PAX5 were used, for confirmation, if needed. All Immunohistochemistry was conducted at Fatimah memorial hospital, Lahore, Pakistan. Above categorization was done according to WHO classification of lymphoid neoplasm.[25] Ki-67 was assayed with monoclonal antibody MIB1 (Dako, Denmark). The endogenous peroxidase was quenched with methanol and 3% hydrogen peroxidase for 5 min. Target retrieval solution (Dako Denmark) with citrate buffer (ph 6.0) was placed in the pressure cooker for 2 minutes, followed by incubation with primary antibody for 30 minutes at room temperature. It was washed with Tris-buffer saline (TBS) afterward. The secondary antibody (ChemMate Dako Envision, Dako Cytomation) was applied for 30 minutes, and then washed with TBS and color developed by incubation in diaminobenzidine (DAB) for 5 minutes. Counterstaining of slides were done with hematoxylin afterward.[26] The Ki-67 PI was quantified by estimating the number of positive lymphoma cells expressing nuclear staining (Brown colored, see Figure 1) among the total number of malignant cells. All analysis and cell counts were done by two histopathologists, who were blinded with the clinical characteristics. A cut off value of 45% was used to differentiate between high versus low proliferative activity.[20,24] Correlation of Ki-67 Proliferative index was done with immunophenotype, age, gender and site of origin. Age of the patient was divided into two groups, below 30 and above 30 for correlation purposes.

Data Analysis: All the collected information was entered and analyzed using SPSS version 24. The qualitative variables like NHL types, anatomic site, and Ki-67 positivity were presented by calculating frequency and percentage. Chi-square calculator for 2x2 contingency table was employed to assess the relationship of clinical parameters like age, gender, lymphoma grade and site of origin with Ki-67 PI, taking cut off value of 45%. P value of < 0.05 was taken as significant.

RESULTS

Out of total 86 cases, 68(79.1%) cases were diagnosed as diffuse large B cell lymphoma with Ki-67 proliferative index of 45 % ≥ of tumor cells in total 53 cases (Table 1). All 4 cases of Burkitt lymphoma were having high Ki-67 (>95%-not shown in the table). As shown in table 2, 26 out of 30 cases of Extra-nodal lymphomas were having Ki-67 PI greater than 45% which was statistically significant with a p-value of 0.039731 (taking P-valve < 0.05 as significant). Similarly, aggressive lymphomas were statistically significant (P-value=0.000257) in staining ≥ 45 % of Ki-67 positive staining in tumor cells. Moreover, patients with age less than 30 years were shown to have ≥ 45 % Ki-67 positivity in lymphomas (p value= 0.00458). However, gender was not related to Ki-67 positivity with insignificant results (p value=0.8201). Regarding the site, all "6" nasopharyngeal and soft tissues NHL were of high Ki-67 PI. While all NHL arising from para-aortic and stomach was having Ki-67 PI less than 45% (Table 3).

Table1: Correlation of Percentage of Ki-67 positivity with NHL Types (using cutoff value 45%)

NHL types	Ki-67 < 45%	Ki-67 ≥ 45%	Total cases	%
Diffuse large B cell Lymphoma	15	53	68	79.1
Small lymphocytic lymphoma	2	0	2	2.3
B cell Lymphoblastic lymphoma	1	3	4	4.7
Burkitt lymphoma	0	4	4	4.7
Follicular lymphoma	2	0	2	2.3
T cell lymphoma	1	2	3	3.5
Mantle cell lymphoma	1	0	1	1.2
Anaplastic large cell lymphoma	0	1	1	1.2
Maltoma (Marginal zone B cell lymphoma)	1	0	1	1.2
Total cases	23	63	86	

Table 2: Ki-67 Proliferative Index correlation with clinical parameters of the site, gender, age and lymphoma grade (using a cutoff value of 45%)

Parameters		Ki-67 < 45% in tumor cells	Ki-67 ≥ 45% in tumor cells	Total	chi-square test	P-Value
Anatomic site	Nodal	19	37	56	4.2293	0.039731
	Extra-nodal	4	26	30		
Gender	Male	17	45	62	0.0517	0.82014
	Female	6	18	24		
Age (years)	1 to 30	1	22	23	8.0384	0.00458
	31 to 100	22	41	63		
Lymphoma grade	Aggressive	16	61	77	13.3626	0.000257
	Non Aggressive	7	2	9		

P-value < 0.05 is significant

Table 3: Correlation of Ki-67 positivity with different anatomic sites

Site of Specimen	Ki-67 <45%	Ki-67 >45%	No. of cases	%
Cervical Lymph node	9	24	33	38.4
Inguinal lymph node	1	3	4	4.7
Axillary lymph node	2	5	7	8.1
Supraclvicular lymph node	0	1	1	1.2
Para-aortic lymph node	3	0	3	3.5
Lymph node (site not mentioned)	4	4	8	9.3
Testis	1	5	6	7.0
Intestine	1	2	4	4.7
Bone	1	3	4	4.7
Thyroid	0	1	1	1.2
Breast	0	1	1	1.2
Soft tissue	0	6	6	7.0
Nasopharynx	0	6	6	7.0
Stomach	1	0	1	1.2
Spleen	0	1	1	1.2
Total	23	63	86	100.0

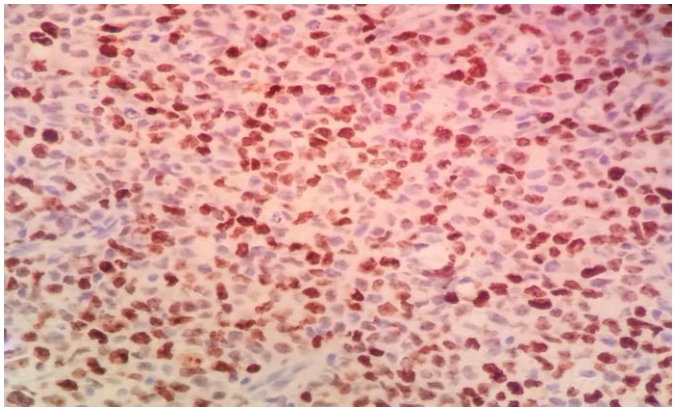


Figure 1: Ki-67 Immunohistochemical staining on diffuse large B cell lymphoma showing 80% of the tumor cells, positively staining with Ki-67 (Brown color) (magnification x 400)

DISCUSSION

Since the inception of Ki-67 by Gerdes et al,[15,27] the validity of this protein as a prognostic marker has been well studied in the literature in many tumor areas.[22,28-31] In Non-Hodgkins Lymphoma (NHL), Ki-67 has a significant relationship with the prognosis as mentioned by Xin He et al.[21] In our study, we also found that high Ki-67 PI has a statistically significant relationship with tumor aggressiveness (P-value=0.000257). This is consistent with Broyde et al[24] and Erum Naz et al.[20] We included Burkitt's, DLBCL, B cell lymphoblastic lymphoma and Anaplastic lymphoma in the aggressive category based on the literature.[32,33] However, some studies found no relationship between tumor grade and Ki-67 PI.[34] In our study, Burkitt lymphoma showed Ki-67% PI of >90 % which is reported also in other studies.[16] There were total 30 cases of extra-nodal lymphomas, 86% of which (26/30) were showing Ki-67 PI of ≥ 45%. The chi square test showed value of 4.2293 in our study with P-value of 0.039731 which was statistically significant (significant p valve <0.05). This particular evidence was also reported by Erum et al.[20] However, Mehrdad et al[18] did not find this relationship. Probably the reason for this variation is that they used a different cut off of Ki-67 % (65%). In a meta-analysis done by Xin He et al, numerous studies were reported with different cut off for Ki-67 PI ranging from 30% to 90%. Actually, they found out that the cut off was different for different sub-types of NHL.[35] This difference in cut off was also noticed by Broyde et al, where 70% cut off was found significant in DLBCL while 45% cut off was reported on Receiver Operator Characteristics (ROC) curve analysis which significantly discriminate indolent from aggressive diseases.[24] This was the particular reason to choose this cut off in our study to differentiate non aggressive from aggressive lymphomas.

Regarding Gender, our study is in accordance with all the studies, reported to date, where no significant relationship (P value=0.82014) was found with the Ki-67% positivity.[20,24,34] Most of the studies have highlighted that Overall survival (OS) is strongly associated with Ki-67 index.[24,34,35] However, in our study, the survival data was not available for us to comment on this particular variable. We also noticed that all lymphomas (6/6) arising from the nasopharynx and soft tissue sites were showing high Ki-67 PI. This feature was also revealed by Kim et al, however, their study was limited to NK/T cell lymphoma of Nasal type.[36] Regarding age, there was a significant relationship between high Ki-67 PI (>45%) and patients <30 years of age (P=0.00458). Others study are in contrary to this finding. The possible reason for this variation is the different cut off used by different studies, ranging from 50-60 years of age.[18,20,34] We used a cut off of 30 years in our study, because many studies internationally[37,38] and nationally[39] have used this cut off point while studying demographics of different tumors of the body.

Moreover, certain tumors are more aggressive below 30 years of age, for example Burkitt's lymphoma is more common below 30 years of age and is by far highly aggressive with Ki-67 PI approaching 100%.[16]

In our study, 68/86 (79.1 %) cases were of diffuse large B cell lymphoma (Table 3). This is consistent with Erum et al and Broyde et.[20,24] On contrary, a study conducted at Uganda[40] shows Burkitt's lymphoma as the top most type. Follicular lymphoma is 2nd most common tumor in the study of Broyde et al[24] while in Anaplastic large cell lymphoma is the 2nd most common lymphoma in the study conducted by Erum Naz.[20] In our study, Burkitt's lymphoma and B-cell lymphoblastic lymphoma share the 2nd spot (Table 1). These variations can be attributed due to geographical and racial differences. All SLL/CLL cases were showing low Ki-67 PI, which is seen in all studies published so far. Many studies which have been stated so far, discussed the Nodal and Extra-nodal statistics, but individual sites were not correlated with Ki-67 PI. However, in our study we noticed that most of the biopsies were taken from cervical lymph node (33/86, 38.4%). Out of these, 24 out of 33 were showing Ki-67 PI ≥ 45%. It was followed by axillary lymph nodes (4.7%). Among extra-nodal lymphomas, most common site was shared by testis, soft tissues and nasopharynx, which were 6 each in number (see Table3)

LIMITATION OF STUDY

The main limiting factor in our study was the unavailability of follow up of the patients, which hindered to comment on the survival data and thereby, limitation of correlation with the survival analysis. Secondly, the results can be more conclusive, if the sample size would be further increased. Lastly dearth of clinical data was also a limiting factor. So, the need of the hour is to have future studies which will counteract these deficiencies and limiting factors.

CONCLUSION

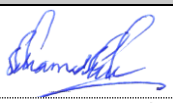
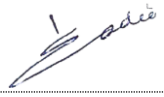
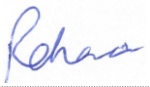
There was significant association of high Ki-67 Proliferative Index (>45%) with the aggressiveness of the lymphoma. Extranodal origin and age <30 years of age was also associated with high Ki-67 PI. However, no relationship was established between gender and Ki-67 expression.

REFERENCES

1. Zhang X, Aguilera N. New immunohistochemistry for B-cell lymphoma and Hodgkin lymphoma. Arch Pathol Lab Med. 2014;138(12):1666–72.
2. Disanto MG, Ambrosio MR, Rocca BJ, Ibrahim HAH, Leoncini L, Naresh KN. Optimal minimal panels of immunohistochemistry for diagnosis of B-Cell lymphoma for application in countries with limited resources and for triaging cases before referral to specialist centers. Am J Clin Pathol. 2016;145(5):687–95.
3. Guy N, Michel K, Abdon M, François T, Etienne O, Bienvenu L, et al. Expression of Ki-67 and Prognosis of Breast Invasive Carcinoma in Congolese Women. Researchgate.net. 2018;3(1):1–9.
4. Piri R, Ghaffari A, Gholami N, Azami-Aghdash S, PourAli-Akbar Y, Saleh P, et al. Ki-67/MIB-1 as a prognostic marker in cervical cancer - a systematic review with meta-analysis. Asian Pacific J Cancer Prev. 2015;16(16):6997–7002.
5. Krabbe LM, Bagrodia A, Lotan Y, Gayed BA, Darwish OM, Youssef RF, et al. Prospective analysis of Ki-67 as an independent predictor of oncologic outcomes in patients with high grade upper tract urothelial carcinoma. J Urol. 2014;191(1):28–34.
6. Ding W, Gou Y, Sun C, Xia G, Wang H, Chen Z, et al. Ki-67 is an independent indicator in non-muscle invasive bladder cancer (NMIBC); Combination of EORTC risk scores and Ki-67 expression could improve the risk stratification of NMIBC. Urol Oncol. 2014;32(1):13-9.
7. Chen W-J, He D-S, Tang R-X, Ren F-H, Chen G. Ki-67 is a valuable prognostic factor in gliomas: evidence from a systematic review and meta-analysis. Asian Pac J Cancer Prev. 2015;16(2):411–20.
8. Kim K Il, Lee KH, Kim TR, Chun YS, Lee TH, Park HK. Ki-67 as a predictor of response to neoadjuvant chemotherapy in breast cancer patients. J Breast Cancer. 2014;17(1):40–6.
9. Chen X, He C, Han D, Zhou M, Wang Q, Tian J, et al. The predictive value of Ki-67 before neoadjuvant chemotherapy for breast cancer: A systematic review and meta-analysis. Future Oncol. 2017;13(9):843-57.
10. Spratt DE. Ki-67 Remains Solely a Prognostic Biomarker in Localized Prostate Cancer. Int J Radiat Oncol Biol Phys. 2018;101(3):513–5.
11. Sugimoto S, Hotta K, Shimoda T, Imai K, Yamaguchi Y, Nakajima T, et al. The Ki-67 labeling index and lymphatic/venous permeation predict the metastatic potential of rectal neuroendocrine tumors. Surg Endosc. 2016;30(10):4239-48.
12. He QY, Jin F, Li YY, Wu WL, Long JH, Luo XL, et al. Prognostic significance of downregulated BMAL1 and upregulated Ki-67 proteins in nasopharyngeal carcinoma. Chronobiol Int. 2018;35(3):348-57.
13. Scholzen T, Gerdes J. The Ki-67 protein: From the known and the unknown. J Cell Physiol. 2000;182(3):311-22.
14. Oka S, Uramoto H, Shimokawa H, Iwanami T, Tanaka F. The expression of Ki-67, but not proliferating cell nuclear antigen, predicts poor disease-free survival in patients with adenocarcinoma of the lung. Anticancer Res. 2011;31(12):4277–82.
15. Scholzen T, Gerlach C, Cattoretti G. An insider's view on how Ki-67, the bright beacon of cell proliferation, became very popular. A tribute to Johannes Gerdes (1950-2016). Histopathology. 2018;73(2):191–6.
16. O'Malley DP, Auerbach A, Weiss LM. Practical applications in immunohistochemistry: Evaluation of diffuse large B-cell lymphoma and related large B-cell lymphomas. Arch Pathol Lab Med. 2015;139(9):1094–107.
17. Agarwal R, Lade S, Liew D, Rogers TM, Byrne D, Feleppa F, et al. Role of Immunohistochemistry in the era of genetic testing in MYC-positive aggressive B-cell lymphomas: A study of 209 cases. J Clin Pathol. 2016;69(3):266–70.
18. Payandeh M, Sadeghi M, Sadeghi E. The Ki-67 index in Non-Hodgkin's Lymphoma: Role and Prognostic Significance. Am J Cancer Prev. 2015;3(5):100–2.
19. Li ZM, Huang JJ, Xia Y, Zhu YJ, Zhao W, Wei WX, et al. High Ki-67 expression in diffuse large B-cell lymphoma patients with non-germinal center subtype indicates limited survival benefit from R-CHOP therapy. Eur J Haematol. 2012;88(6):510–7.
20. Naz E, Mirza T, Aziz S, Ali A, Danish F. Correlation of ki 67 proliferative index with clinical and pathological features on tissue

sections of non hodgkins lymphoma by immunostaining. J Pak Med Assoc. 2011;61(8):748–52.
21. He X, Chen Z, Fu T, Jin X, Yu T, Liang Y, et al. Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies in lymphoma subtypes: Evidence from a systematic meta-analysis. BMC Cancer. 2014;14(1):153.
22. Hoster E, Rosenwald A, Berger F, Bernd HW, Hartmann S, Loddenkemper C, et al. Prognostic value of Ki-67 index, cytology, and growth pattern in mantle-cell lymphoma: Results from randomized trials of the european mantle cell lymphoma network. J Clin Oncol. 2016;34(12):1386–94.
23. Payandeh M, Sadeghi M, Sadeghi E. The Ki-67 index in Non-Hodgkin's Lymphoma: Role and Prognostic Significance. Am J Cancer Prev. 2015;3(5):100–2.
24. Broyde A, Boycov O, Strenov Y, Okon E, Shpilberg O, Bairey O. Role and prognostic significance of the Ki-67 index in non-Hodgkin's lymphoma. Am J Hematol. 2009;84(6):338–43.
25. Quintanilla-Martinez L. The 2016 updated WHO classification of lymphoid neoplasias. Hematol Oncol. 2017;35:37–45.
26. Kim S, Kim B, Choi C, et al. Ki-67 expression is predictive of prognosis in patients with stage I/II extranodal NK/T-cell lymphoma, nasal type. Ann Oncol. 2007;18(8):1382–7.
27. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer. 1983;31(1):13–20.
28. Watanabe S, Suzuki T, Kondo Y, Naoe A, Uga N, Yasui T, et al. Evaluation of Ki-67 as prognostic factor for pediatric neuroblastoma and the possibility of molecular-targeted drugs with vascular endothelial growth factor and platelet-derived growth factor receptor. Minerva Pediatr. 2019;32(15):1115-25.
29. Arima N, Nishimura R, Osako T, Okumura Y, Nakano M, Fujisue M, et al. Ki-67 index value and progesterone receptor status can predict prognosis and suitable treatment in node-negative breast cancer patients with estrogen receptor-positive and HER2-negative tumors. Oncol Lett. 2019;17(1):616–22.
30. Thomas K, Voros B, Patel DCD, Boudreaux JP, Thiagarajan R, Woltering E, et al. The role of Ki-67 in determining optimal chemotherapy in high grade neuroendocrine tumors. J Clin Oncol. 2018;36(15):4100–4100.
31. Cho U, Kim HE, Oh WJ, Yeo MK, Song BJ, Lee A. The long-Term prognostic performance of ki-67 in primary operable breast cancer and evaluation of its optimal cutoff value. Appl Immunohistochem Mol Morphol. 2016;24(3):159–66.
32. Slack GW, Hsi ED. Diffuse Aggressive B-Cell Lymphomas. Hematop A Vol Ser Found Diagnostic Pathol. 2017;37(12):271-305.
33. Hapgood G, Savage KJ. The biology and management of systemic anaplastic large cell lymphoma. Blood. 2015;126(1):17-25.
34. Szczuraszek K, Mazur G, Jeleń M, Dzięgiel P, Surowiak P, Zabel M. Prognostic significance of Ki-67 antigen expression in non-Hodgkin's lymphomas. Anticancer Res. 2008;28(2):1113–8.
35. He X, Chen Z, Fu T, Jin X, Yu T, Liang Y, et al. Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies in lymphoma subtypes: evidence from a systematic meta-analysis. BMC Cancer. 2014;14:153
36. Kim S, Kim B, Choi C, Choi J, Kim I, Lee Y-H, et al. Ki-67 expression is predictive of prognosis in patients with stage I/II extranodal NK/T-cell lymphoma, nasal type. Ann Oncol. 2007;18(8):1382–7.
37. Goldie SJ, Kim JJ, Wright TC. Cost-Effectiveness of Human Papillomavirus DNA Testing for Cervical Cancer Screening in Women Aged 30 Years or More. Obstet Gynecol. 2004;103(4):619–31.
38. Van Laar M, McKinney PA, Parslow RC, Glaser A, Kinsey SE, Lewis IJ, et al. Cancer incidence among the south Asian and non-south Asian population under 30 years of age in Yorkshire, UK. Br J Cancer. 2010;103(9):1448–52.
39. Akbar A, Bhatti ABH, Khattak S, Syed AA, Kazmi AS, Jamshed A. Outcome of rectal cancer in patients aged 30 years or less in the Pakistani population. Asian Pac J Cancer Prev. 2014;15(15):6339-42.
40. Tumwine LK, Agostinelli C, Campidelli C, Othieno E, Wabinga H, Righi S, et al. Immunohistochemical and other prognostic factors in B cell non Hodgkin lymphoma patients, Kampala, Uganda. BMC Clin Patho. 2009;9(1):11.

AUTHORSHIP AND CONTRIBUTION DECLARATION

AUTHORS	Contribution to The Paper	Signatures
Dr. Muhammad Mudassar Associate Professor & Head, Pathology Department Batterjee Medical College, Jeddah, Saudi Arabia	Manuscript writing, Data Analysis, Result Compilation, Discussion, Reference Writing	
Dr. Sadia Hameed Professor & Head, Pathology Department University Medical and Dental College, Faisalabad	Data Collection, Technical Review, Discussion, Histopathology Review	
Dr. Shazia Aslam Associate Professor, Pathology Department University Medical and Dental College, Faisalabad	Technical Review, Data Collection	
Dr. Rehana Majeed Senior Registrar, Gynecology Department Jinnah Hospital, Lahore	Data Analysis, Article Review	
Dr. Rizwan ullah Khan Assistant Consultant, Pathology, King Faisal Specialist Hospital, Buraidah, Al-Qaseem, Saudi Arabia	Result Analysis, Histopathology Slides Review, Photomicrographs	
Dr. Munazza Majeed General Practitioner, Qasar al-Rayed hospital, Riyadh, Saudi Arabia	Discussion, Introduction Writing, Proof Reading