Synchronization of Working Formulation and WHO Classification 2008 for Nodal Non-Hodgkin's Lymphoma

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Introduction: In Pakistan, nodal Non Hodgkin s Lymphoma (NHL) cases are being reported according to Working Formulation (WF). A new and broader classification of lymphomas has been introduced by WHO classification 2008 which includes immunohistochemical markers and cytogenetics of these tumors. This study was carried out to synchronize WF and 2008 WHO classification of nodal NHL.

Materials and Methods: This study was done on 50 consecutive diagnosed cases of nodal NHL. The paraffin blocks of these cases were retrieved from laboratory. New sections were cut and immunohistochemical staining was carried out on these sections.

Results: These results showed that small lymphocytic lymphoma (SLL) according to WF have been recategorised into (WHO) SLL-T cell and SLL-B cell. Diffuse Large cell lymphoma (WF) classified into (WHO) Diffuse large B cell NOS, Peripheral T cell NOS, Anaplastic large cell lymphoma and Angioimmunoblastic lymphoma. Lymphoblastic lymphoma (WF) revealed T lymphoblastic and B lymphoblastic lymphoma types (WHO). The small cleaved, mixed and large subtypes of follicular lymphoma (WF) have been merged as follicular lymphoma(WHO). Diffuse large B cell lymphoma NOS (WHO) includes immunoblastic lymphoma (WF), diffuse mixed (WF), and some cases of diffuse large cell lymphoma (WF) in this study.

Conclusion: The classification of lymphomas has always been a debatable issue for the pathologists and oncologists. Despite of this disparity, WF classification of lymphomas is still being used in Pakistan. However, a revised unified classification would be a better step towards enhanced understanding in this field.

Key Words: Non-Hodgkin's, lymphoblastic, immunoblastic, anaplastic.

Introduction

Nodal Non-Hodgkin's Lymphoma (NHL) is a challenging diagnostic domain for pathologists. Various classifications have been devised for Non Hodgkin s lymphoma. Working Formulation (WF) has been frequently employed by Pathologists[1] as it is based on clinical features and defines prognostic groups of NHL i.e., low grade, intermediate grade and high grade. Genetic studies and immunohistochemistry is not required. This feature attracts pathologists in developing countries where facilities and resources are limited. However in WF, lymphomas are not separated into T and B cell types which are considered nowadays necessary for the treatment and prognosis. In an attempt to standardize the diagnosis of lymphoma, WHO classification 2008 has been introduced.[2] This is a revision of WHO 2001 classification which is in turn based on REAL classification.[3] The major difference of these newer classification and WF is that these systems incorporate genetic and immunohistochemical features of lymphoma. The pitfall in newer classification is that newly included disease entities are not yet supported by enough clinical information. Moreover, this classification does not give knowledge about predictable biological behavior of lymphoma. In our study, we have carried out immunohistochemical studies on diagnosed cases of nodal NHL. These lymphomas were initially classified according to WF and have now been assigned to the categories of WHO Classification 2008. This study attempts to unify WF and WHO 2008 Classifications by determining the similarities and the differences.

Materials and Methods

This study was carried out in the department of Histopathology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from December 21, 2008 to March 31, 2009. In this period, immunophenotyping of 50 diagnosed cases of nodal NHL was carried out. Age groups ranging from 4 to 74 years were included irrespective of gender. Cases with inadequate biopsy material were excluded. From laboratory record, H&E sections and paraffin blocks were retrieved. Sections of each paraffin block were cut and immunohistochemical staining was done by using the cell markers given in Table 1.

Table 1: *Immunohistochemical Markers.*

B Cell Markers	CD19, CD20, CD74, CD23, Kappa and Lambda Light Chains
T Cell Markers	CD3, CD5, CD43, CD4, CD8, CD45 RO
Others	NK Cell Markers, CD30, CD15, CD68, KI-67, BCL2, EMA & CK

To ensure the specificity of positive staining reaction, both positive and negative control slides were used. Diagnosis was made by simultaneous examination of positive and negative control slides along with immunohistochemically stained sections. For all immunostains, positive cases revealed dark brown color. Staining was graded on the percentage of stained cells as: Weak (+) [less than 25% of cells stained], Satisfactory (++) [25 to 50% cells stained], Strong (+++) [50 to 75% of the cells stained] and Very Strong (++++) [75 to 100% cells stained].

Results

This study included 50 diagnosed cases of Non-Hodgkin's lymphoma with ages ranging from 4 to 74 years (Mean ± SD: 39 ± 16.5). There were 36 (72%) males and 13 (28%) females with male to female ratio of 2.7: 1. According to WF, 16 (32%) cases were low grade, 26 (52%) were intermediate grade and 8 (16%) were high grade lymphomas. The segregation of cases according to WF is given in Table 2.

Table 2: *Grouping Lymphomas According to Working Formulation.*

LOW GARDE:	**16**	**32%**
Small Lymphocytic	11	22%
Follicular, Small Cleaved	3	6%
Follicular Mixed	**2**	4%
INTERMEDIATE GRADE:	**26**	**52%**
Follicular, Large Cell	1	2%
Diffuse, Small Cleaved	0	0%
Diffuse, Mixed	5	10%
Diffuse, Large	20	40%
HIGH GRADE:	**8**	**16%**
Immunoblastic	**3**	6%
Lymphoblastic	5	10%

After immunophenotyping, 36 (72%) lymphomas were of B cell type and 14 (28%) were T cell type. The correlation of WF and WHO 2008 Classification for these cases is given in Table 3.

Table 3: *Correlation between Working Formulation and WHO Classification 2008*.

Working Formulation		**WHO Classification**	
Small Lymphocytic Lymphoma	11	SLL (B Type)	10 (90.9%)
		SLL (T Type)	1 (9.1%)
Follicular Lymphoma • Small Cleaved Lymphoma (3) • Mixed Lymphoma (3) • Large Cell Lymphoma (4)	6	Follicular Lymphoma	6 (100%)
Diffuse Mixed Lymphoma	5	Diffuse Large B Cell Lymphoma – NOS	5 (100%)
Diffuse Large Cell Lymphoma	20	Diffuse Large B Cell Lymphoma – NOS	11 (55%)
		Peripheral T Cell Lymphoma – NOS	5(25%)
		Anaplastic Large T Cell Lymphoma	3 (15%)
		Angioimmunoblastic T Cell Lymphoma	1 (5%)
Lymphoblastic Lymphoma	5	T Lymphoblastic Lymphoma	4 (80%)
		B Lymphoblastic Lymphoma	1 (20%)
Immunoblastic Lymphoma	3	Diffuse Large B cell Lymphoma – NOS	3 (100%)

Discussion

This study included 50 cases of nodal NHL of patients with ages ranging from 4 to 74 years (Mean ± SD: 39±16.5). This study revealed 32% low grade, 52% intermediate grade and 16% high grade lymphomas according to WF. After immunohistochemical staining, 36 cases (72%) were of B cell type. This is comparable to the findings of Khan MA and Khan MS where B Cell lymphomas were found 85.5% and 72.5% respectively in their studies.[4,5] Diffuse large B cell lymphoma was diagnosed in 19 cases (38%) and it was the most common type of B cell lymphoma in present series. This can be correlated to the study by Rittaluga S et al which observed that 32% of lymphomas were of diffuse large B cell type.[6] The follicular lymphoma was seen in 6 cases (12%) in this series. This prevalence is lower when compared with United States where follicular lymphoma comprises 30% of all lymphomas.[7] 11 cases of small lymphocytic lymphoma (SLL) after immunostaining revealed 10 cases (90.9%) of SLL-B and 1 case of SLL-T. 90-95% SLL are of B cell type and this fact is similar to findings in our study. T cell type was seen in 14 out of 50 cases (28%) of NHL. This finding is comparable to Western countries where 30% of NHL cases are of T cell origin.[8] Peripheral T cell lymphoma constitutes 10-20% of nodal NHL in Western countries[9] whereas 5 cases (25%) of peripheral T cell lymphoma NOS were diagnosed in our study. There were 3 cases (15%) of Ki-1 lymphoma and all these

cases were of T cell origin. This finding is similar to established data that all cases of Ki-1 lymphoma are T cell or rarely null cell type on immunophenotyping.[10] Among 5 cases of lymphoblastic lymphoma 4 cases (80%) on immunophenotyping turned T cell and 1 case (20) B cell type. This can be correlated with reported incidence (66-75%) of T cell lymphoblastic lymphoma.[11] When compared to WF, we find that WHO classification 2008 of lymphomas is more extensive. Along with other parameters, WHO classification 2008 also takes into account the individuality of tumor types based on genetic and immunohistochemical studies. Therefore, it is helpful for the clinicians to offer tailor made diagnosis and treatment options to the patients. However, due to more elaborate grouping, pathologists do not get adequate experience of the various sub types. Also complete panel of immunohistochemical markers is not widely available especially in developing countries.

Conclusion

It is suggested that a simpler approach in diagnosis of nodal NHL may be adopted considering the difficulties of the pathologists who have the responsibility of rendering the diagnosis.

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