Immunophenotypic Analysis of Non-Hodgkin's Lymphoma

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Suhail Muzaffar, Shahid Pervez, Farrukh Aijaz, Syed Abdul Aziz, Sheema H. Hasan (Department of Pathology, The Aga Khan University Hospital, Karachi.)

Abstract

One hundred and three cases of Non-Hodgkin's lymphoma were evaluated immunohistochemically using a panel of monoclonal antibodies which includes leucocyte common antigen (LCA), CD45R (Pan-B marker), L-26 (CD 20-Pan-B marker) and UCHL-1 (Pan-T marker). Of the total 63 cases (61.17%) showed a B-cell phenotype while 40(38.83%) were of T-cell origin. Most B-cell neoplasms belonged to intermediate (79.36%) or high grade (15.87%) according to the international Working Formulation (WF). Most T- cell lymphomas were of either intermediate (52.5%) or high grade (32.5%) neoplasms. Some T-cell neoplasms presented as specific clinicopathological entities like lymphomatoid granulomatosis (2 cases), mycosis fungoides (1 case) and AILD type NHL (1 case). in 27 cases the immunostaining pattern of two .Pan-B markers i.e., L26 and CD45R was compared. L26 staining was expressed in all 27 cases (100% sensitivity) while CD45R showed positive reaction in 22 cases (82% sensitivity). UCHL-1 is proved to be a sensitive and lineage specfic T-cell markerand in 67% cases the staining pattern was moderate (++) to intense (+++). The mean age for the B-cell lymphomas was 49 years and 36 years in T-cell neoplasm. Male to female ratio in both types of lymphomas was 2:1. The study indicates a high prevalence of T-cell lymphomas when comparing the data from western countries and lower to those from Japan and Caribbean countries (JPMA 47:106,1997).

Introduction

Non-Hodgkin's lymphoma (NHL) is an extremely heterogenous gmup of tumors with marked variability in morphological features, growth pattern, antigenic phenotype and biological behaviour. These neoplasms are characterized by neoplastic proliferation of cells of lymphoid tissue i.e. lymphocytes, histiocytes and their precursors. At present the Kiel classification and International Working Formulation (WF) are the most widely used lymphoma classifications worldwide. The Kiel classification, based on the immunological concepts, divides the non-Hodgkin's lymphomas into B-cell and T-celI subtypes, each again into low grade and high grade categories. This scheme has been the first to characterize many important entities, but it excludes prirnary extranodal lymphomas other than mycosis fungoides[1,2]. The WF is purely a morphologic classification in which histologic categories are placed into low, intermediate and high grade based on natural history, response to therapy and survival. In this scheme the immunological features are not taken into account[3]. Recently, International Lymphoma Study Group proposed a new classification which is designated as "Revised European-American lymphoma classification" (REAL classification). They believe that the most pmctical approach of lymphoma characterization is to define the diseases that have already been recognized with currently available morphologic, immunologic, cytogenetic and molecular biologic techniques[4].
The current approach for the diagnosis of non-Hodgkin's lymphoma is multi-disciplinary and includes routine morphologic evaluation, immunophenotypic characterization and genotypic studies. There are certain morphologic features such as follicle formation by neoplastic cells which may be taken as reliable indicator of B cell lymphornas, however, the cytologic features in the diffuse lymphomas are unreliable for predicting T-cell and B- cell phenotypes. The immunophenotypic characterization of the diffuse lymphomas is essential as there is a general agreement that T-cell lymphomas behave more aggressively than their B-cell counterparts. Furthermore post-thymic T-ceIl lymphomas are usually

associated with a different pattern of involvement, with more frequent involvement of extra nodal sites such as skin and lungs[5]. Non-Hodgkin's lymphomas arc not widely studied immunohistochemically in Pakistan except a couple of studies in Northern areas of Pakistan[6,7]. The purpose of this study is to see the phenotypic status of non-Hodgkin's lymphomas and to see the reason of aggressive behaviour of NHLs in our patients as compared to the western population.

Material and Methods

This study was carried out as part of a research project, approved by the University, to see the immunophenotypic status of non-Hodgkin's lymphoma during the year 1992-1993. A total of 199 cases were diagnosed as non-Hodgkin's lymphoma during the study period. All patients belonged to the southern part of Pakistan, especially the Sindh province. The diagnosis and grading were made on morphologic basis using the International Working Formulation for clinical usage by two pathologists independently and their consensus was achieved. Immunohistochemical studies were performed on 103 cases including both nodal and extra-nodal biopsies, but it was not possible to cany Out immunostaining on the remaining 96 cases owing to limited resources. The case selectionpmcedure was unbiased and cases were selected according to their surgical numbers as they appeared in numerical order. Forthis study we used a panel of monoclonal antibodies (MAbs) that included leucocyte common antigen (LCA), CD45R (Pan B-marker), L26 (CD 20-Pan B-marker) and UCHL-1 (Pan T-marker). In some cases kappa and lambda light chains were also used. All antibodies were obtained from Dako Inc. Denmark. Parallel positive and negative contmls were always run. Lymph node or tonsil were used as positive control while for negative control omission of primary antibody was done on test tissue.

Immunostaining procedure

Sections from paraffinblocks were cut at 5um evaluated imniunohistochemically using the panel of MAbs by peroxidase anti- peroxidase (PAP) technique. Paraffin sections were first dewaxed, hydrated through graded alcohols and taken down to water. Trypsin digestion was performed for LCA antibody. Endogenous peroxidase reaction was then blocked by immersing sections for 30 minutes in a solution of 0.3% hydrogen peroxide (H2O2) in phosphate buffer solution (PBS). Sections were then rinsed in PBS and placed on a rack in a humid atmosphere, First layer (primary) antibodies were then applied at pre-established dilutions. The preparations were incubated overnight at 40 centrigrade. Next morning sections were washed in PBS and second layer antibody, rabbit-anti-mouse globulin (1:200), was applied for 30 minutes at room temperature. The slides were then washed again in PBs and PAP complex was incubated for 30 minutes. The peroxidase development was carried out by immersing the slides in substrate solution of diaminobenzidine tetrahydrochloride dihydrate (DAB). The slides were developed in the above solution for 3-8 minutes and controlled by microscopic examination. After the development was completed the slides were rinsed well in water, counterstained with Harris hema-toxylin, differentiated in acid alcohol, dehydrated, cleared and mounted in synthetic mountant. Evaluation of immunohistochemical staining was done depending upon the intensity and the number of tumour cells. All 4 antibodies showed the distinct brown membrane staining. The sections were semi quantitatively scored showing staining as follows: weak positive (+). moderate positive (++), intense positive (+++).

Results

Immunophenotypic studies were performed on 103 cases of NHL, following International Working Formulation most of our cases fell under the category of intermediate grade i.e., 68.93%, while 22.33% cases belong to the high grade group. Among the intermediate grade, diffuse large cell and mixed cell

lymphomas were predominant subtypes i.e., 49.29% and 40.84% respectively. Cases, belonging to both nodal and extranodal sites, were evaluated immunohistochemically. The results of histologic evaluation and immunohistochemical analysis are summarized in the Table.

Table. Classification and immunohistochemical analysis of our cases of NHL with inference to International Working Formulation (Total cases=103).

Non-Hodgkin's lymphoma	No. of cases	No. of cases Reactive to Pan B Markers (L-26, CD45R)	No. of cases Reactive to Pan T-marker (UCHL-1)
Low grade			
Small lymphocytic lymphoma	1	1	-
Follicular, mixed small cleaved and large cell	1	1	-
Low grade B-cell MALT* type	1	1	-
Total number of cases	3	3	-
Intermediate grade			
Follicular, large cell	1	1	-
Diffuse, small cleaved cell	6	5	1
Diffuse, mixed small and large cell	29	19	10
Diffuse, large cell	35	25	10
Total	71	50	21
High grade			
Diffuse, large cell immunoblastic	10	6	4
Lymphoblastic	10	1	9
Small non-cleaved cell	3	3	-
Total	23	10	13
Miscellaneous			
Lymphomatoid granulomatosis	2	-	2
Mycosis fungoides	1	-	1
AIL (Angioimmunoblastic lymphadenopathy)	1	-	1
Grading not possible	2	-	2
Total No. of cases	103	63	40

*MALT: Mucosa associated lymphoid tissue

The study shows that 61.17% cases (63 cases) were of B- cell phenotype while 38.83% cases (40 cases) belonged to T-cell lineage. In the B-cell category, 79.36% (50 cases) belonged to intermediate grade while 15.87% (10 cases) were high grade lymphomas; only 3 cases (4.76%) were low grade B-cell lymphomas. Post-thymic (peripheral) T-cell neoplasms formed the significant proportion of non-Hodgkin's lymphomas in our study. Thymic (Central) lymphomas comprised of 10 cases of lymphoblastic lymphomas while 25 cases (62.50%) came under the category of post thymic lymphomas or peripheral T-cell lymphomas (PTCL). Most of these PTCL were of intermediate grade (21 cases - 52.5%) and large cell immunoblastic, high grade (4 cases - 10.0%). Two cases of lymphomatoid granulomatosis, one case each of mycosis fungoides and angioimmunoblastic (AJLD) type lymphomas were included in the T-cell neoplasm. In two cases grading was not possible owing to the scanty biopsy material.

Regarding the pattern of presentation of lymphomas, 38 B-cell lymphomas presented as nodal involvement while in 23 the biopsy sites were extranodal. In 2 cases the site of biopsy was not mentioned. In comparison, 32 cases of T-cell lymphomas presented as lymph node enlargement, while in 8 cases, the site of biopsy was extra-nodal. In some of these cases the primary nodal or extranodal location was clinically confirmed, while in many cases it was not possible to establish the primary site of lymphoma This was because a number of cases were sent to our laboratory from other medical centers in the city and various other parts of the province, so that clinical assessment and follow-up was not available in all cases. L26 (CD20) was the main MAb used as B- cell marker while in 27 cases CD45R, the other pan-B antibody was also used to compare the sensitivity. L26 staining was expressed in all 27 cases (100% sensitivity) while CD45R showed positive reaction in 22 cases (82% sensitivity).

The intensity of the staining was moderate (++) to intense (+++) in most of the cases with L26, while CD45R showed weak (+) to moderate staining pattern and in many cases the staining pattern was focal. UCHL-1 (CD45RO) is a sensitive and lineage specific markerand inmajority ofourcases (67%), the stainingpattern was moderate (++) to intense (+-H-). The age range in B-cell lymphomas was 8 to 80 years (mean 49 years). The age range in T-cell lymphomas was 7 to 71 years (mean 36 years). The male gender was dominant in both types with male to female ratio of 2:1.

Discussion

During the last several years with the advances in the immunophenotyping techniques particularly for the reason that a number of reagents (antibodies) now work on formalin fixed, routinely processed paraffin sections, non-Hodgkin's lymphomas have been classified according to their lineages as eitherB- cell, T-cell orhistiocytic. Studies from USA indicate that vast majority of non-Hodgkin's lyinphomas (80 to 85%) are of B-cell origin, the remainder are T-cell tumors. Tumors of histiocytes or macrophage are quite uncommon[8]. Malignant lymphomas of T-celI origin have been divided into thymic (central) and post-thymic (peripheral) types based on the inununophenotypic features. Post-thymic lymphomas bearing a mature T-cell phenotype have been grouped together as peripheral T-cell lymphoma (PTCL) which show significant geographic, clinical, histopathological and prognostic diversity, This type of lymphoma is less common in the western countries than in Japan. In south west Japan T-cell lymphoma accounts for upto 70% of cases primarily because adult T-cell leukemia/lymphoma associated with HTLV-l is endemic in this part[9]. In a review article Winberg[10] observed that PTCLs are uncommon in the United States, but occur with greater frequency in Japan and in Caribbean countries. In various series from United States, PTCL lymphoma constitute 15 to 30% of diffuse non- Hodgkin's lymphoma and they are most frequently placed in the international WF as diffuse mixed cell and large cell categories[11-13].

In our study of 103 cases studied immunohistochemically, 63 (61.17%) showed B-cell phenotype and 40 cases (3 8.83%) were ofT- cell origin. The results of this study show that T-cell lymphoma has a high prevalence and occupy a position in between the western and Asian patterns of the disease. The iinmunophenotypic status of NHL has not been extensively studied in Pakistani population and there are only a couple of studies perfonned to address this aspect of lymphoma. In a study carried on 100 cases of N}{Ls, most (83) cases were of B-cell phenotype, while T-cell lymphomas were uncommon (4 cases). True histiocytic lymphomas constituted a significant sub-group in this study and 8% cases belonged to this category[7]. In another study, immunophenotypic analysis was performed in 40 randomly selected cases of diffuse large cell lymphoma. Of these, 87.5% cases were positive for B- cell marker while 5% were Ki-1 lymphomas and 2.5% were of histiocytic origin. No diffuse large cell lymphoma of T-cell lineage was identified[8]. These two studies indicate the phenotypic status of NHL in the northern parts of Pakistan. Our study included those cases which belong to the southern parts of Pakistan particularly the Sindh province and shows a high prevalence of T-cell lymphomas in this part, which is in sharp contrast with those studies published from northern parts of Pakistan. This indicates a significant geographical difference in relation to inimunophenotypic status of NHL in this country. The importance of categorizing NHLs according to their cell lineage is well established and is an integral component of new REAL classification. According to this classification immunohistochemical characterization of all lymphomas is mandatory because morphology of the neoplasm cannot always predict its biological behaviour. T-cell lymphomas show significant geographic, clinical and prognostic differences from B-cell lymphomas[14].

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