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Immunophenotype evaluation of Non-Hodgkin's lymphomas

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Abstract

Background: Non-Hodgkin's lymphoma (NHLs) is known as a heterogeneous group of malignant lymphoproliferative disorders. NHLs are classified into B cell and T cell types. Immunophenotypical assessment of the biopsy specimens can help diagnose NHLs.

Methods: In this study, 77 patients with B cell and T cell lymphoma were selected from Shahid Sadoghi hospital during 2010 to 2013. Immunohistochemical method was used to detect biomarkers like CD2, CD3, CD20, and CD45.

Results: In this study, 67 patients (87.01 %) had B cell lymphoma. Moreover, the most primary tissues in B cell group were lymph node and stomach, followed by bone marrow and neck. Positive co-expression of CD45 and CD20 was found in 61 patients (91.04%) with B cell lymphoma. However, 10 patients (12.98%) had T cell lymphoma, and the most primary tissue in T cell lymphoma group was the skin. Moreover, CD3 expression was seen in all patients with T cell lymphoma.

Conclusion: This study confirmed the main role of immunohistochemistry method in classifying and diagnosing NHLs. Moreover, the difference in CD marker expression and age in patients with B cell and T cell lymphoma, compared to other studies may be due to geographic area and genetic and ethnic differences.

Keywords: Non-Hodgkin's Lymphomas, CD20, CD3, CD2

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Introduction

Malignant lymphoma is a primary malignant neoplasm of lymphoid tissue (1, 2) and is characterized by one or more genetic abnormalities. It is categorized into 2 classes: Hodgkin's lymphoma and non-Hodgkin's lymphomas (NHLs). Non-Hodgkin´s lymphoma is known as a heterogeneous group of malignant lymphoproliferative disorders (1). Such factors as genetic differences, weak immune system, and consumption of some drugs after organ transplantation (3, 4) can be involved in this disease. NHLs can occur in nodal or extra nodal locations including sites such as skin, stomach, and brain, and spread in unpredictable ways (2). NHLs are classified into B cell and T cell type (two main types of lymphocytes). T cell non-Hodgkin´s lymphomas are 4 types of lymphoma that affect T cells (5). It has been demonstrated that nearly 10% to 15% of all lymphomas are diagnosed in Western countries (2). The B-cell lymphoma is a type of lymphoma that originates from B cells (6). B-cell lymphoma, which is known as gray-zone lymphoma (7), has clinical and biological overlaps for diffuse large B-cell lymphoma and classical Hodgkin lymphoma (CHL). CD20 antigen is a transmembrane phosphoprotein (2), which plays a main role in activation and differentiation of B cell (2). It is expressed by almost 95% of B cell lymphomas (8). The fact that CD20 is not expressed by some tumors indicates that CD20 is not necessary for B-cell survival (8). CD3 is a marker for T cells and natural killer cells (9). It is specific for T-cell derivation, and a minority of HL cases expresses it (10, 11). CD2 is expressed by T and natural killer (NK) cells and has been reported in T/NK cell lineage neoplasms, as well as in immature B-lymphoblastic and myeloid leukemia (12). CD45 expression as a glycoprotein was seen in all lymphohemopoietic cells. Moreover, CD45 expression is increased within B-lymphocyte ontogeny (13). Since immunohistochemical markers were used to confirm and classify NHLs, including CD20 (B

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↑What is "already known" in this topic:
Immunohistochemical markers have been used to confirm and classify NHLs (B cell and T cell types).

→What this article adds:
The most common original sites of B cell lymphoma were lymph nodes and stomach. Moreover, expression of CD markers may be related to geographic area and genetic differences.

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Table 1. Primary antibodies used for immunohistochemical staining of tumor markers in lymphomas

Antibody	Isotype	Dilution	Source
CD20	Monoclonal mouse antibody L26 Ready to use	Ready to use	Dako
CD3	Polyclonal rabbit	Ready to use	Dako
CD45	Monoclonal mouse 2B11+PD7/26	Ready to use	Dako
CD2	Monoclonal mouse antibody AB75	Ready to use	Dako

cell marker) and CD3 (T cell markers), little work has been done on NHLs, therefore, this study aimed at determining the immunophenotyping of non-Hodgkin's lymphomas in central part of Iran.

Methods

In the current study, a total of 77 patients (67 patients with B cell lymphoma and 10 with T cell lymphoma) were selected via available sampling method from Shahid Sadoghi hospital during 2010 to 2013 in central part of Iran. The hematoxylin and eosin (H & E) as histological method was used to stain and analyze tissue sections. Immunohistochemistry technique was done on specimens embedded on wax paraffin from the main tumors. Then, slides were dewaxed with xylene and rehydrated with alcohol. To block endogenous peroxidase activity, peroxide hydrogen was used in sections. For antigen retrieval, slides were transferred to citrate buffer (pH: 9) and boiled for 20 minutes in a microwave oven. After washing, non-specific binding sites were blocked by Tris Buffered Saline (TBS). Then, the section was incubated with primary antibody (Table 1), and sections were exposed to secondary antibody Horseradish peroxidase after washing. Then, sections were incubated with three 3-diamino-benzidine tetrahydrochloride (Sigma). The sections were counter-stained with hematoxylin, rinsed in tap water, and immersed in graded alcohol and xylene, and finally mounted (14, 15).

Results

In this study, all samples with B cell and T cell lymphoma were selected from Shahid Sadoughi hospital in a period of 3 years. The number of patients with B cell lymphoma was 67 (87.01 %). Moreover, 35 (52%) patients with B cell lymphoma were male and 32 (48%) were female. The mean ±SD age of patients with B cell was 53.62±12.3 years. Table 2 demonstrates the classification of patients based on age range in B cell lymphoma.

As presented in Table 2, most patients were in the age group of 40 to 80 years. Also, Table 3 shows the primary tissue involvement in B cell lymphoma.

As demonstrated in Table 3, most primary tissues in B cell are lymph node and stomach, followed by bone marrow and neck.

Table 4 displays biomarkers expression in B cell lymphoma group

As presented in Table 4, CD45 and CD20 biomarker expressions were seen in 61 and 64 cases with B cell lymphoma.

Table 5 shows positive co-expression of biomarkers in B cell lymphoma patients

As Table 5 illustrates, coexpression of CD45 and CD20 biomarker was seen in 61 samples.

Figure 1 shows CD45, CD3, and CD20 positive biomarker in non-Hodgkin's lymphomas.

Figure 1 displayed the immunohistochemical assessment of biomarkers expression in non-Hodgkin's lymphomas.

Table 2. Classification of patients based on age range in B cell lymphoma

Age rang (year)	n (%)
B cell lymphoma group	
< 30	5(7.4)
30 ≤ age≤ 40	8 (11.94)
40< age≤ 50	12 (17.9)
50< age≤ 60	11 (16.41)
60< age≤ 70	11(16.41)
70< age≤ 80	12 (17.9)
80< age≤ 90	8 (11.9)

Table 3. Primary tissue involvement in B cell lymphoma group

Primary tissue	n (٪)
	B cell lymphoma
Stomach	8 (11.94 ٪)
Bone marrow	7 (10.44٪)
Mediastinal	4 (5.97٪)
Spine	3 (4.47٪)
Neck	7 (10.44٪)
Lymph nodes	8 (11.94٪)
Skin	2 (2.98٪)
Colon	1 (1.49٪)
Eye	1 (1.49 ٪)
Cervical	1 (1.49 ٪)
Parotide	3 (4.47 ٪)
Spleen	3 (4.47 ٪)
Pleural	3 (4.47 ٪)
pelvic	1 (1.49 ٪)
Lung	1 (1.49 ٪)
Ovary	2 (2.98 ٪)
Brain	1 (1.49 ٪)
Axilarry lymphoma	2 (2.98 ٪)
Heart Valve	1 (1.49 ٪)
Breast	1 (1.49 ٪)
Tongue	2 (2.98 ٪)
Unknown tissues	5 (7.46 ٪)
Total	67 (100 ٪)

Table 4. Biomarkers expression in B cell lymphoma group

Biomarker expression in B cell lymphoma	Number
CD45	
Positive	61 (91.04 ٪)
Negative	1(1.49٪)
Missed value	5 (7.4 ٪)
CD20	
Positive	64 (95.5 ٪)
Negative	1(1.49 ٪)
Missed value	2 (2.98٪)
CD3	
Positive	29 (43.2 ٪)
Negative	35(52.2٪)
Missed value	3(4.47٪)
CD2	
Positive	18 (26.86 ٪)
Negative	42(62.6 ٪)
Missed value	7(10.4٪)

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Table 5. Positive coexpression of biomarkers in B cell lymphoma patients

Positive Coexpression of biomarkers	Number	Percent
CD20, CD45	61	91.04
CD20, CD2	18	26.86
CD20, CD3	29	43.28

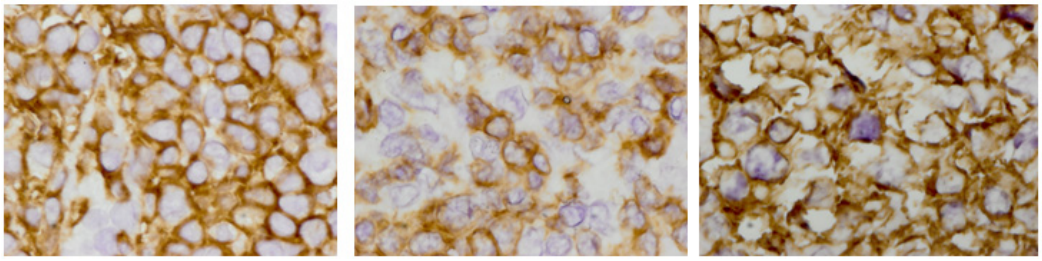

Fig. 1. A: CD45 (Diffuse positive) B: CD3 (positive) C: CD20 (positive)

Moreover, 10 patients (12.98%) had T cell lymphoma, among whom 6 were male and 4 were female. The mean ±SD age of patients with T cell lymphoma was 27.75±8.4 years. Table 6 displays the classification of patients based on age range in T cell lymphoma group.

As presented in Table 6, most patients were younger than 30 years. Primary tissue involvement in T cell lymphoma group is shown in Table 7.

As shown in Table 7, the most primary tissue was the skin. Expression of biomarkers in patients with T cell lymphoma is displayed in Table 8.

As shown in Table 8, CD3 was seen in 100% of cases.

Discussion

The result of this study revealed that 87.01% of patients had B cell lymphoma and 12.98% had T cell lymphoma. Hamid et al. obtained the same result and reported that B cell NHLs were 87.1%, while T cell NHLs were 12.9% (2). Ammen et al., in their study, showed a high percentage of B cell NHLs and reported that 81.8% of NHLs patients had B cell and 14.2% had T cell (16). Burg et al. reported that among NHLs patients, 65% had T cell and 25% had B cell (17). Piccaluga et al. reported that in Western countries, T cell lymphomas constituted about 12% of all non-Hodgkin's lymphomas (18). OB reported that T cell NHLs incidence is higher in Far East Asia compared to Western countries (19). Another study reported that 101 of 586 Korean patients had T cell lymphoma (19). Also, a high incidence of NHLs was reported in Turkey, as 185 patients with NHL were found in Cukurova University hospital in 1988. This disease covered 13% of all malignant neoplasms diagnosed in the oncology department (20). The frequency of NHLs is 18.3% in Hong Kong, and 1.5% of the total neoplasm in Vancouver. It seems that occurrence of T cell NHLS may be due to variation of geographic frequency of NHLs (21). Moreover, geographic variation is due to increased exposure to pathologic factors such as Human T cell leukemia virus-1 and Epstein-Barr virus in Asian nations (21).

In this study, the most common original sites of NHLs (B cell lymphoma) were lymph nodes and stomach, followed by neck and bone marrow (2). Bone morrow biopsy is an integral part of staging work-up for non-Hodgkin's lymphomas (22). Hamid et al. reported that the most common site of NHLs is lymph node followed by stomach (2). Also, they reported that primary malignant lymphoma of skin constitutes nearly 1% to 3.5% of all NHLs in children (17) and that 15% to 25% of patients with NHLs

Table 6. Classification of patients based on age range in T cell lymphoma group

Age range (year) in T cell lymphoma group	Number (%)
< 30	5 (50 %)
30 ≤ age≤ 40	3 (30 %)
40< age≤ 50	1 (10 %)
50< age≤ 60	1 (10 %)
60< age≤ 70	0
70< age≤ 80	0
80< age≤ 90	0

Table 7. Primary Tissue involvement in T cell lymphoma group

Primary tissue	Frequency (%)
Stomach	0
Bone marrow	0
Mediastinal	0
Spine	0
Neck	0
Lymph nodes	0
Skin	3 (30 %)
Colon	0
Eye	0
Cervical	0
Parotide	1 (10 %)
Spleen	1 (10 %)
Pleural	0
pelvic	0
Lung	1 (10 %)
Ovary	1 (10 %)
Brain	1 (10 %)
Axilarry lymphoma	1 (10 %)
Heart Valve	0
Breast	1 (10 %)
Tongue	0
Unknown tissues	0
Total	10 (100 %)

Table 8. Expression of biomarkers in patients with T cell lymphoma

Biomarker expression	Number of patients with T cell lymphoma positive
CD3	
Positive	10 (100 %)
CD2	
Positive	7 (70 %)
Miss value	2 (20 %)
CD20	
Positive	5 (50 %)

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have mediastinal abnormalities (2).

Moreover, the result of this study showed that most patients aged 40 to 80 years. However, Ameen R. et al. found that most cases were 41 to 60 year sold (16). Hamid et al. in a study reported that high incidence of B cell NHLs was seen in the age group of 47 to 67 years and low incidence of B cell NHL was seen in age group 26 to 46 years (2). Sarpel et al. reported that mean age of men and women with NHL in Turkey was 45.5 and 41 years, respectively Moreover, they reported that histological condition of NHL demonstrated the difference between western and developing countries. There are significant differences between clinical presentation, histological condition and natural history of NHLs in terms of geographic location of the disease NHL constitutes (20).

Conclusion

This study confirmed the main role of immunohistochemistry method in classifying and diagnosing NHLs. Furthermore, the difference in CD marker expression and age in patients with B cell and T cell lymphoma, compared to other studies, may be due to geographic area and genetic and ethnic differences.

Conflict of Interests

The authors declare that they have no competing interests.

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