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CLINICOPATHOLOGICAL CORRELATION OF NON-HODGKIN LYMPHOMA AN IMMUNOHISTOCHEMICAL PROFILE

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ABSTRACT

Introduction: Non-Hodgkin lymphomas (NHL) are assorted group of malignant lymphoproliferative disorder and showing distinctive patterns of behavior and responses to treatment. **Objectives**: The study aims to assess the diagnostic role of immunohistochemistry for immunophenotyping of non-Hodgkin lymphoma into B-cell and T – cell phenotypes, and to correlate them with age, sex and site of biopsy. **Methods**: A total of 40 cases of NHL were collected during two years 2012-2013.H&E, IHC were done. The markers used in this study were LCA, CD20, CD3, Ki67, CD10, CD15, CD30, EMA, Bcl2, TdT, CD5, CD34, using En Vision system. **Results:** 22 cases were males (55%) and 18 cases were females (45%), M: F ratio 1.2:1, age range 2-85. B-cell lymphomas were (87.5%), T-cell lymphomas were (7.5%), unclassified (5%). The most common type of NHL B-cell type was DLBCL (32.5%), followed by Burkitt lymphoma (12.5%), follicular lymphoma (7.5%), MZL (7.5%), B-cell lymphoma unclassified (5%). T-cell lymphomas were three, Precursor T- lymphoblastic lymphoma, T-cell lymphoma (unspecified), anaplastic large cell lymphoma. Two cases were unclassified. The major affected age group was 45-59 (37.5%). Nodal and extranodal were equally affected 50% each. The most common nodal site were cervical lymph nodes (20%). The most common extranodal site were the GIT (17.5%). **Conclusions:** We concluded that IHC is a very important investigation for all lymphomas and for immunotherapy. IHC is also important for differential diagnosis with other malignancies. B-cell lymphomas outnumber T-cell lymphomas, the most common type of B-cell NHL was diffuse large B-cell lymphoma (DLBCL), followed by Burkitt lymphoma. B-cell lymphoma most commonly nodal with cervical predominant. All T-cell lymphomas were extranodal.

KEYWORDS: Non-Hodgkin lymphoma, immunophenotyping, Yemen.

INTRODUCTION

Lymphoma is a malignancy of lymphoid tissue. The diagnosis of lymphoma is regulated according to the stratification of neoplasms depending on their precursor cell and clinical manifestations, based on the standards laid down by World Health Organization (WHO).[1] The initial diagnosis of lymphoma by light microscopy constantly remains the typical diagnostic modality.[2] The keying of lymphomas need immunophenotyping.

NHLs are malignant assorted group of lymphoid disorder and showing distinctive responses to treatment. NHL consists of many subtypes, each with distinct etiology, morphology, clinical manifestations and immunophenotyping.[3,5]

NHL is the 8th most common malignancy in men and the 11th in women. The disease accounts for 5.1% of all cancer cases and 2.7% succumbed from the disease.[4, 5]

NHL is one of the top ten most frequently diagnosed cancers in Yemen. In north Yemen out of a total number of 3782 cases registered at the National Oncology Center, NHL ranked 2nd among females 8% and 1st among males 11%.[6] Similarly, in south Yemen NHL ranked 2nd among malignant neoplasms, constituting 8.2 % out of a total of 3389 cases in Cancer Registry Center in Aden in both sexes.[7]

The pathogenesis of NHL become Probable with the aid of immunohistochemistry (IHC) and its careful application aids identification of immunophenotype in most of NHLs.[8] IHC methods have become a necessary investigation of diagnostic pathologic studies, in NHL it is used for classification of lymphoid tumors into B-cell and T-cell phenotypes and for differential diagnosis with other malignancies.

Diffuse large B cell lymphoma (DLBCL) is the most common subtype of NHL in the west and accounts for approximately 60% of patients with B cell lymphomas in the East.[9] Although World Health Organization (WHO) define these tumors as single disease entity, the variety of clinical presentations and pathologic, genetic characteristics strongly suggest that these neoplasms denote a dissimilar group of tumors.[10]

The incidence of subtypes of malignant lymphomas is different according to geographic regions paralleled with western countries; regions of Asia have stated greater frequency of T-cell lymphoma and lesser frequency of follicular lymphoma and Hodgkin lymphoma.[11,12]

CD20 is a cell surface antigen expressed precisely on most human B cells.[13] Because CD20 is also expressed on more than 90% of B-cell lymphomas, CD20 has become a good molecular goal for monoclonal antibody therapy[14,15] CD20 is the most broadly used Pan B-cell marker, is expressed from the native B cell upto last stages of B-cell development just preceding to plasmacytic differentiation.[16]

The aim of this study is to assess the diagnostic role of immunohistochemistry for immunophenotyping of Non-Hodgkin lymphoma on formalin fixed paraffin embedded sections according to the WHO classifications,[17] and correlate them to age, sex, site of biopsy and to compare our results with other geographic regions.

PATIENTS AND METHODS

A retrospective study of 40 cases diagnosed as lymphoma in their biopsy specimens during two years 2012-2013 from a private laboratory in Sana'a, one case was excluded because it did not fit the criteria. The data of age, sex, site of biopsy were collected from request forms and histopathology and immunohistochemistry data were collected from laboratory records, all slides were reviewed to know the phenotype of lymphoma cases into B-cell and T-cell according to WHO classifications using immunohistochemistry. Data processing was done using the Statistical Package for Social Sciences SPSS 19, Chi-square test was applied with P-value >0.05 considered significant.

All the biopsies were fixed in 10% neutral buffered formalin and processed for paraffin embedding, sectioning and staining with H&E. IHC was done on 3 µm thick section of representative tumor areas. Of all cases, histological slides were deparaffinized in xylene, different alcohol series, endogenous peroxidase blockage for 20 min with 3% H_2O_2 methanol, followed by antigen retrieval in pressure cooker (citrate buffer PH 6.0). The sections were placed in phosphate buffer saline (PBS) (PH.7.4). Overnight incubation with primary antibodies at 4◦C, rinsing in PBS 3x5min. Secondary antibody labelled polymers with horseradish peroxidase. Color was developed with Diaminobenzidin (DAB) + Buffer substrate for liquid DAB. Positive and negative control slides were used.

The markers available were LCA, CD20, CD3, CD10, Ki 67, CD15, CD30, EMA, Bcl2, TdT, CD5, CD34, with optimal dilutions using En vision system. For differential diagnosis, also these markers were also used CK, Vimentin, Actin, CD99, CD117, Synaptophysin, S100, CEA and PSA.

RESULTS

A total of 40 cases of NHL were collected retrospectively, 22 cases were males (55%) and 18 cases were females (45%) M:F ratio was 1.2:1 age range was 2-85 years mean age 43.28. B-cell lymphomas 35 cases, (87.5%) T-cell lymphomas were three cases, (7.5%) and unclassified two (5%). The B-cell lymphomas were DLBCL 55%, Follicular lymphomas (7.5%), Extranodal marginal zone B-cell lymphoma (MALT) (7.5%), Burkitt lymphoma (12.5%), B- cell lymphomas (unclassified) were (5%).

T cell lymphomas were (7.5%), precursor T- cell lymphoblastic lymphoma, T cell lymphoma-unspecified, anaplastic large cell lymphoma, two cases were unclassified. (Table 1) The age group was bimodal, early age ≤ 29 (30%) and late age 45-59 years (37.5%).

Table 1: Distribution of patients according to subtypes of NHL and sex.

Type of lymphoma	Sex				Total		p-value
	Male		Female				
	No	%	No	%	No	%	
B-cell lymphoma							
Diffuse large B cell lymphoma	13	32.5	9	22.5	22	55	
Follicular lymphoma	2	5	1	2.5	3	7.5	
Extranodal marginal zone B cell lymphoma (MALT)	1	2.5	2	5	3	7.5	0.001
Burkitt Lymphoma	2	5	3	7.5	5	12.5	
B-cell lymphoma (unclassified)	2	5	-	-	2	5	
Subtotal	20	50	15	37.5	35	87.5	
T- cell lymphoma							
Precursor T- cell lymphoblastic lymphoma /leukemia	1	2.5	-	-	1	2.5	
T cell lymphoma- unspecified	-	-	1	2.5	1	2.5	0.148
Anaplastic large cell lymphoma	1	2.5	-	-	1	2.5	
Subtotal	2	5	1	2.5	3	7.5	
Unclassified	-	-	2	5	2	5	-
Total	22	55	18	45	40	100	

DLBCL were distributed in all age groups with the highest peak between 45-59. Burkitt lymphomas all were in younger age group (Table 2).

Table 2: Distribution of patients by subtypes of NHL and age.

Lymphoma subtypes	Age group (years)							
	< 15	15-29	30-44	45-59	60-74	≥ 75	Total	%
Diffuse large B cell lymphoma	1	5	2	8	3	3	22	55
Follicular lymphoma	-	-	-	1	1	1	3	7.5
Extranodal marginal zone B cell lymphoma (MALT)	-	-	1	2	-	-	3	7.5
Burkitt Lymphoma	5	-	-	-	-	-	5	12.5
B-cell lymphoma (unclassified)	-	-	-	1	1	-	2	5
Precursor T- cell lymphoblastic lymphoma /leukemia	-	-	-	1	-	-	1	2.5
T cell lymphoma- unspecified	-	-	-	1	-	-	1	2.5
Anaplastic large cell lymphoma	-	1	-	-	-	-	1	2.5
Unclassified	-	-	1	1	-	-	2	5
Total	6 15%	6 15%	4 10%	15 37.5%	5 12.5%	4 10%	40	100

According to the specific anatomical location of NHL, the nodal location were (50%), with cervical predominant. The extranodal locations were (50%), with GIT predominant. (Table 3).

Table 3: The anatomic distribution of specific types of NHL.

Site	Specific anatomic Location	B-cell phenotype					T-cell phenotype				No %
		DLBCL	FL	MZL	BL	B-cell Lymphoma Unclassified	PTLL	T-cell Lymphoma unspcified	ALCL	Lymphoma Unclassified	
Nodal	Cervical LN	5	1	-	1	1	-	-	-	-	8 (20)
	Submandibular LN	3	-	-	-	-	-	-	-	1	4 (10)
	Abdominal LN	2	-	-	1	-	-	-	-	-	3 (7.5)
	Axillary LN	1	1	-	-	-	-	-	-	-	2 (5)
	Inguinal LN	2	1	-	-	-	-	-	-	-	3 (7.5)
	Subtotal	13	3	0	2	1	0	0	0	1	20 (50)
Extranodal	Head&neck	3	-	1	-	-	-	-	-	1	5 12.5)
	GIT	3	-	2	2	-	-	-	-	-	7 (17.5)
	Retroperitoneum	1	-	-	1	-	-	-	-	-	2 (5)
	Spine	1	-	-	-	-	1	-	-	-	2 (5)
	Soft tissue	-	-	-	-	-	-	1	-	-	1 (2.5)
	Chest	-	-	-	-	1	-	-	-	-	1 (2.5)
	Mediastinum	-	-	-	-	-	-	-	1	-	1 (2.5)
	Breast	1	-	-	-	-	-	-	-	-	1(2.5)
	Subtotal	9	0	3	3	1	1	1	1	1	20 (50)
Frequency Of different Types of NHL(%)		22(55)	3 (7.5)	3 (7.5)	5 (12.5)	2 (5)	1 (2.5)	1 (2.5)	1 (2.5)	2 (5)	40 (100)

DLBCL= diffuse Large B-cell lymphoma, FL= follicular lymphoma, MZL= marginal zone lymphoma, BL= Burkitt lymphoma, PTLL= precursor T-lymphoblastic lymphoma ALCL=anaplastic large cell lymphoma

According to typing of the NHL based on IHC markers. (Table 4) In DLBCL, one case was F+ve for CD 15, two cases show CD30 +ve (anaplastic variant), in one case the Ki67 +ve in few cells only, and one shows T-cell rich. Follicular lymphomas showed follicular pattern, Bcl 2 was mandatory for differential diagnosis with follicular hyperplasia.

Burkitt lymphomas most commonly found in children, and show high proliferation index. Two cases were B - cell lymphomas (unclassified), were +ve for LCA and CD20 only.

Precursor T –cell lymphoblastic lymphoma / leukemia, TdT was mandatory for diagnosis, whereas Ki67 50%, T – cell lymphoma (unspecified) showed focal positivity for CD3.

Anaplastic large cell lymphoma was CD3, LCA, CD30 + ve in most of the cells. Unclassified cases were only positive for LCA, B-cell and T-cell markers were negative.

Table 4: Typing of the subtypes of NHL based on IHC markers.

Lymphoma subtypes	LCA	CD20	CD10	CD3	CD15	CD30	Ki67	EMA	TdT	Bcl2
Diffuse large B cell lymphoma	+	+	+	-	-	-	80%	-		
Follicular lymphoma	+	+		-			40-60%			+
Extranodal marginal zone B cell lymphoma (MALT)	+	+		-			20-30%			
Burkitt Lymphoma	+	+	+	-			80-90%			
B-cell lymphoma (unclassified)	+	+		-						
Precursor T- cell lymphoblastic lymphoma /leukemia	+	-	-	+			50%		+	
T cell lymphoma- unspecified	F+	-		F+						
Anaplastic large cell lymphoma	+	-		+		+		-		
Unclassified lymphoma	+	-		-						

DISCUSSION

NHLs are assorted group of malignant lymphoproliferative disorders. A total of 40 cases of non – Hodgkin lymphoma, The male to female ratio was 1.2:1. This finding was in consistent with a previous study conducted by Enonomophos T et al.[18] whom they found M:F ratio of 1.2:1, other study done by Hamid KH et al[19] in which the M:F ratio was 1.6:1, This male preponderance has been reported in other studies.

Age range was 2-85 years. In the study of salwa HS[20] from Sudan, she noted that the age of cases was ranged 0 – 90 years.

According to the immunophenotypic profile, Sharma M et al.[3] Found that B – cell type was (89.3%), T- cell type was (10.7%). Yaqo RT et al.[21] in Iraq found (91%) B – cell and (9%) were T – cell, which is similar to our study.

The most common subtype of NHL was DLBCL. Sharma M et al.[3] decided that DLBCL constituted 46.8% of all NHL.

DLBCL constituted in different Asian countries, Iran 37.8%, Korea 43.2%, Tiwan 47.2% / 39%, India 34%, China 35.1%, Thiland 50.5%, Japan 33% / 33%, Asia 41.2%, UK 41.3%, USA 20%.[22] our results was more or less similar to the results of Thiland but higher than all other countries. This is because of the small sample that we were having.

Burkitt lymphoma was the second most common type of NHL. Burkitt lymphoma amounted in Egypt 6.9 %, Pakistan 1%, Kuwait 4%, India 2%, India 4.1%.[23] In one study no Burkitt lymphoma cases were seen.[3] our result was higher than all the other studies.

In the study of sharma M et al.[3] follicular lymphoma accounted 4.3% of all NHL. There was a significant variation in the proportion of follicular lymphoma which accounted for 15% in India and 11% in Kuwait, but 3%, 5.2% in Pakistan and Egypt.[23] All of these results were significantly lower than those in western series. Howell et al.[24] reported 8% FL in the Gastrointestinal NHL cases. Our result was in agreement with the result of Howell et al.

In the study of sharma M et al.[3] MZL accounted for 2.1% of all NHL, Naresh et al.[23] reported 8.2% MZL. Our results were in agreement with Naresh et al. But higher than sharma et al.

In our current study, the age group was bimodal, One study done by Sharma M et al.[3] the incidence of NHL including both sexes was seen in age group 31-40 (21.3%) followed by age group 61-70 (19%). Another study decided by Hingorjo MR et al.[25] showed bimodal age group, the first peak occurring in the age range 12-13 years, the second peak between (52-62) years. Our late age group was similar to the study of Hingorjo MR et al. which was one decade earlier than the west.

In B – cell lymphomas, the nodal to extranodal ratio was 1.46:1, while all the cases of T – cell lymphomas were extranodal. This finding was decided with Andrew M et al.[26] whom he stated that the most common site for T cell NHL was the extranodal site.

Regarding nodal presentation, one study done by Erum Naz et al,[27] found that cervical presentation was (32.3%) followed by (14.5%) Inguinal lymph node. Sarpel et al.[28] in Turky stated that more than half of cases (54%) were nodal. Ameen R et al.[29] noticed that 54% of the cases were extranodal presentation. Our current study was intermediate among these studies.

We concluded that IHC is a very important investigation for all lymphomas which is important for immunotherapy and for differential diagnosis with other malignancies.

B-cell lymphomas outnumber T-cell lymphomas, the most common type of B-cell NHL was DLBCL, followed by Burkitt lymphoma. B-cell lymphoma most commonly nodal with cervical predominant. All T-cell lymphomas were extranodal.

REFERENCES

1. Soldini D, Campo E, New insights into the diagnosis of lymphomas Ann Oncol, Sep, 23, 2012; 10: x83-x88.
2. Ott G, Ziepert M, Klapper W, Horn H, Szczepanowski M, Bernd HW, Thorns C, Feller AC. et al. Immunoblastic morphology but not The immunohistochemical GCB/non GCB classifier predicts outcome in diffuse large B-cell lymphoma in the RICOVER-60 trial of the DSHNHL. Blood, 2010; 116: 4916-4925.
3. Non- Hodgkin lymphoma, A histopathologic and prognostic evaluation. http://www. Biooncology.com / research education / b cell / down loads/ GA 10000083900_NHL_Primer. Pdf.
4. Boffetta P. Epidemiology of adult Non-Hodgkin lymphoma. Ann Oncol, 2011; 22(4): 27-31.
5. Sharma M, Mannan R, Madhukar M, Immunohistochemical Analysis of Non-Hodgkin lymphoma Spectrum according to WHO/ REAL classification. Journal of clinical and diagnostic Research. 2014 Jan; Vol-8(1): 46-49.
6. AL-Nabhi A, Algharati AM, Abdulhamid G, Shamlan A, AL-Nehmi A, Pattern of Cancer in Yemen: First results from the National Oncology Center, Sana'a 2007, ejpmr, 2017; 4(1): 149-154.
7. Basaleem HO, Bawazir AA, Challenges and Prospects of Cancer Registry In the context of Non-Communicable Disease Surveillance in Yemen: The Example of Aden Cancer Regestry. AHOJ, Jan-Feb 2017; 1(1): 9-22.
8. Rao Sl. Role of immunohistochemistry in lymphoma. Indian J Med Paediatr Oncol, 2010; 31(4): 145-47.
9. Chen Y, Han T, Iqbal J, Irons R, Chan WC, Zhu X, Fu K, Diffuse Large B-Cell Lymphoma in Chinese Patients, Immunophenotypic and Cytogenetic Analyses of 124 Cases Am J Clin Pathol, 2010; 133: 305-313.
10. ALizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, et al. Distinct types of diffuse large B-cell identified by gene expression profiling. Nature, 2000; 403: 503-11.
11. Kim JM, Ko YH, Lee SS, Huh J, Kang CS, Kim CW, Kang YK, Go JH, Kim MK, Kim WS et al. WHO classification of malignant lymphomas in Korea: Report of the Third nationwide study. The Korean Juornal of pathology, 2011; 45: 254-260.
12. Yoon SO, Suh C, Lee DH, Chi HS, Park CJ, Jang SS, Shin HR, Park BH, Huh J, *et al.* Distribution of lymphoid neoplasms in the Republic of Korea: analysis of 5318 cases according to the World Health Organization classification. Am J Hematol, 2010; 85: 760-4.
13. Tedder TF, Engel P. CD20: a regulator of cell-cycle progression of B lymphocytes. Immunol Today, 1994; 15: 450–4.
14. Lim SH, Beers SA, French RR, Johnson PW, Glennie MJ, Cragg MS. Anti-CD20 monoclonal antibodies: historical and future perspectives. Haematologica, 2010; 95: 135–43.
15. Tokunaga T, Tomita A, Sugimoto K, Shimada K, Iriyma C, Hirose T, Shirahata-Adachi M. et al De novo, diffuse large B-cell lymphoma with CD20 imunohistochemistry-positive and flow cytometry-negative phenotype: Molecular mechanisms and correlation with rituximab sensitivity Cancer Sci., 2014; 105: 35–43.
16. Chen CC, Raikow RB, Sonmez-Alan E, Swerdlow SH. Classification of small B cell lymphoid neoplasms using a paraffin section immunohistochemical panel. Appl Immunohistochem Mol Morphol. 2000; 8:1- 11(Am J Clin Pathol, 2002; 117: 636-643.
17. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, eds. WHO Classif ication: Pathology and Genetics of Tumors of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: International Agency for Research on Cancer, 2008.
18. Economopoulos T, Papageorgiou S, Dimopoulos MA. Non-Hodgkin's Lymphoid Lymphoma in Greece according to the WHO classification of neoplasm. Acta Haematology, 2005; 113(2): 97-103. PubMed | Google Scholar.
19. Hamid. K H, Mirgani B, Mohamed Y, Salih M M, Eldumal A H. & Immunophenotyping of Non-Hodgkin's lymphomas in Sudan. The Pan African Medical Journal, 2014; 18: 82. 3732.
20. Salwa HS, NHL in Soba University hospital 1979-1989, Master degree. 1992. Sudan. Soba University.
21. Yaqo RT, Hughson MD, Sulayvani FK, Al-Allawi NA. Malignant lymphoma in northern Iraq: a retrospective analysis of 270 cases according to the World Health Organization classification. Indian J Cancer, Oct- Dec, 2011; 48(4): 446-51.
22. Mozaheb Z, Epidemiology of Lymphoid Malignancy in Asia, www.intechopen.com.
23. Naresh, K.N. Advani S, Adde M. Aziz Z, Banavali S, Bhatia K, Belqaumi A, Ezzat A, Khalid N,Norton A, et al. Report of an International Network of Cancer Treatment and Research workshop on non-Hodgkin's lymphoma in developing countries, Blood Cells, Molecules and Diseases, 2004; 33: 330–337.
24. Howell JM, Auer-Grzesiak I, Zhang J, Andrews CN, Stewart D, Urbanski SJ. Increasing incidence rates, distribution and histological characteristics of primary gastrointestinal non-Hodgkin lymphoma in a North American population.Can J Gastroenterol, 2012; 26(7): 452–6. [PMC free article] [PubMed]
25. Hingorjo M R, Syed S. PRESENTATION, STAGING AND DIAGNOSIS OF YMPHOMA: J Ayub Med Coll Abbottabad, 2008; 20(4): 100-104.
26. Evens A M, Querfeld C, Rosen S T. T-cell non-Hogdkin's lymphoma. Cancer treatment and research, 2006; 131: 161-220. PubMed | Google Scholar.

27. Naz E, Mirza T, Aziz S, Danish F, Siddiqui S T, Ali, A Frequency and clinicopathologic correlation of different types of Non Hodgkin's lymphoma according to WHO classification. J Pak Med Assoc. 61; No.3 2 011.260-263.
28. Sarpel SC, Paydas S, Tuncer I, Varinli S, Koksal M, Akoglu T. Non-Hodgkin's lymphomas in Turkey. Cancer, 1988; 62(8): 1653-7. PubMed | Google Scholar.
29. Ameen R, Sajnani KP, Albassami A, Refaat S. Frequencies of non-Hodgkin's lymphoma subtypes in Kuwait: comparisons between different ethnic groups. Ann hematology, 2010; 89(2): 179-84. Google Scholar.