Ref: Ro J Med Pract. 2024;19(2)
DOI: 10.37897/RJMP.2024.2.4

Immunohistochemical study of non-Hodgkin lymphoma patients in Basrah (Single center experience)

Haider Abdul Hassan ALI[1], Jasim Mohammed AL-DIAB[2], Saad Abdulbaqi ALOMAR[2]

[1]Pathology Department, Al-Sader Teaching Hospital, Basrah, Iraq
[2]College of Medicine, University of Basrah, Iraq

ABSTRACT

Background. *Non-Hodgkin lymphoma (NHL) represents a diverse group of lymphoid tissue cancers, predominantly arising from B-cells, T-cells, or natural killer cells. The accurate diagnosis of NHL subtypes is crucial, given their varying clinical behavior and treatment responses. This study focuses on the application of immunohistochemistry (IHC) in diagnosing and classifying NHL, comparing its efficacy to traditional hematoxylin and eosin (H&E) staining methods.*

Methods. *Conducted at Al-Sadr Teaching Hospital, Basrah, this cross-sectional study involved 48 newly diagnosed NHL cases in 2015. Patients were initially examined using H&E-stained sections from lymph nodes and extranodal tissue biopsies. IHC staining, employing a range of primary antibodies, was then used to confirm diagnoses and subtype NHL cases.*

Results. *The study revealed a predominance of diffuse large B-cell lymphoma (DLBCL) at 50%. Other subtypes that were identified included mantle cell lymphoma, Burkitt lymphoma, lymphoblastic lymphoma, MALToma, peripheral T-cell lymphoma, and follicular lymphoma. The IHC method proved critical in identifying specific NHL subtypes, which were not detectable by H&E staining alone, such as mantle cell lymphomas and peripheral T-cell lymphomas. The study highlighted discrepancies with regional studies, emphasizing variations due to sample size and regional factors.*

Conclusion. *Immunohistochemical staining is an indispensable tool for accurately diagnosing and classifying NHL, complementing H&E staining. This study underscores the need for a comprehensive diagnostic approach, including a panel of antibodies, to optimize the diagnostic process and ensure accurate treatment planning.*

Keywords: non-Hodgkin lymphoma, immunohistochemistry, diagnosis, classification, hematoxylin, Eosin Staining

INTRODUCTION

Lymphoma, a complex form of cancer that arises in the lymphatic system, primarily affects lymphocytes, a type of white blood cell pivotal in the immune response [1]. Broadly classified into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), lymphomas are distinguished by unique clinical, pathological, and epidemiological features. While both types involve similar lymphoid tissues, their behavior, treatment responses, and outcomes significantly differ [2]. Lymphoma's complexity lies in its varied manifestations, ranging from indolent to highly aggressive forms, each requiring a distinct therapeutic approach [2].

Non-Hodgkin lymphoma encompasses a heterogeneous group of lymphoid tissue cancers that show greater diversity in presentation and prognosis compared to HL [2]. NHL represents about 90% of all lym-

Corresponding author:
Haider Abdul Hassan Ali
E-mail: mohammedjamal38@yahoo.com

Article History:
Received: 11 February 2024
Accepted: 25 June 2024

phomas, characterized by an array of different types arising from either B-cells, T-cells, or natural killer (NK) cells. Epidemiologically, NHL's incidence varies globally [3]. In the United States, it accounts for a significant portion of lymphoma cases, predominantly of the B-cell type [2]. Similarly, in the Kingdom of Saudi Arabia, NHL was one of the most common cancers in 2008, notably among men. This variance in incidence rates and demographic profiles underlines the need for in-depth regional research and targeted healthcare strategies [4].

The classification of NHL has evolved markedly over time. Initially guided by the Rappaport system in the 1960s [5], it transitioned to the more refined Kiel classification [6], the Revised European-American Classification of Lymphoid Neoplasms (REAL) [7], and eventually to the World Health Organization (WHO) classifications [8]. These evolving classification systems reflect a deeper understanding of NHL's complex biology, incorporating morphological, immunological, and genetic characteristics for a more accurate diagnosis and classification.

NHL is further categorized into various subtypes, each with unique characteristics. Among the most common are Diffuse Large B-cell lymphoma (DLBCL), Follicular Lymphoma, Mantle Cell Lymphoma, and Burkitt Lymphoma. These subtypes are identified based on their distinct cellular origin, genetic alterations, and clinical behavior, which significantly influence their treatment and prognosis [9].

Accurate diagnosis of NHL often necessitates biopsy methods, which involve tissue sampling for microscopic examination. The primary types include excisional or incisional biopsy, where either the entire lymph node (excisional) or a portion of a large mass (incisional) is removed [10]. Another less invasive method is Fine Needle Aspiration Cytology (FNAC), which is cost-effective and less traumatic for patients [11]. These biopsy techniques provide essential tissue samples for histopathological examination, crucial for diagnosing and classifying NHL.

Immunohistochemistry (IHC) plays a pivotal role in the diagnosis and classification of NHL. This technique involves using specific antibodies to detect antigens in cells of a tissue section, thereby providing crucial insights into the type and nature of lymphoma [12]. Historically, IHC evolved from the 1930s, with significant advancements enhancing its diagnostic capabilities [13]. It employs a variety of antibodies to identify specific antigens, enabling pathologists to differentiate between lymphoma subtypes and other similar diseases [14].

IHC is crucial in determining the lymphoma's cell of origin and its molecular characteristics, which are pivotal for accurate classification and treatment planning. For instance, in B-cell lymphomas, antibodies against CD20 or CD79a are commonly used, while T-cell lymphomas are typically tested for CD3 or CD5 markers. The staining patterns observed through IHC provide a more precise diagnosis than traditional histopathological methods alone, offering invaluable information for tailoring patient treatment strategies [14].

Immunohistochemistry (IHC) is pivotal in pathology and research, particularly for diagnosing and differentiating diseases like lymphomas, leukemias, and carcinomas. Its applications extend to identifying malignant neoplasias' origins and researching disease prognostics [15]. This study harnesses IHC's potential to accurately diagnose and subtype non-Hodgkin lymphomas (NHLs) following the latest WHO classification. A key aim is to evaluate the effectiveness of IHC staining against traditional hematoxylin and eosin methods, highlighting IHC's role in refining diagnostic accuracy and informing NHL treatment strategies.

PATIENTS AND METHODS

This cross-sectional study was executed in the pathology laboratory at Al-Sadr Teaching Hospital in Basrah over the course of 2015, specifically from January 1st to December 31st. It involved forty-eight newly diagnosed cases of non-Hodgkin lymphoma (NHL), selected based on their initial histopathological examination. These examinations utilized conventional hematoxylin and eosin-stained sections from lymph nodes and extranodal tissue biopsies. The materials employed for the preparation of paraffin-embedded tissue sections included 10% paraformaldehyde, a water bath, graded alcohol, xylene, paraffin wax, a microtome, and other standard laboratory instruments like forceps, staining holders, glass slides, and cover slides.

For the immunohistochemistry (IHC) aspect, a range of primary antibodies were utilized, targeting markers such as CD3, CD4, CD5, CD8, CD10, CD19, CD20, and others, to confirm diagnoses and subtype the NHL cases. The IHC process encompassed several stages: tissue biopsies were fixed, processed, and embedded in paraffin wax, followed by sectioning at 3-5 µm thickness and staining with hematoxylin and eosin. The IHC staining protocol included meticulous steps such as deparaffinization, rehydration, antigen activation, endogenous peroxidase blocking, incubation with primary and secondary antibodies, and detection using Diaminobenzidine (DAB). This process was complemented by counterstaining, dehydration, and slide mounting. The interpretation of the IHC slides was crucial, where the expression of markers was identified as nuclear, cytoplasmic, or membranous. The presence of proteins was indicated by a brown color due to DAB, while absence was shown in blue due to hematoxylin counterstaining.

RESULTS

Table 1 displays the age and sex distribution of patients, revealing a predominance of male patients (62.5%) across all age groups. Notably, the incidence of patients increases with age, peaking in the >60 years group (31.2%). Younger age groups (10-20 and 21-30 years) show the lowest patient counts (4.2% each). A significant gender disparity is observed in the >60 and <10 years groups, suggesting potential gender-specific factors influencing the condition.

TABLE 1. *Age and sex distribution of the patients*

Age groups (years)	Sex		Total
	Male	Female	
<10	4 (8.3%)	3 (6.3%)	7 (14.6%)
10-20	2 (4.2%)	0 (0.0%)	2 (4.2%)
21-30	0 (0.0%)	2 (4.2%)	2 (4.2%)
31-40	3 (6.3%)	1 (2%)	4 (8.3%)
41-50	5 (10.4%)	2 (4.2%)	7 (14.6%)
51-60	6 (12.5%)	5 (10.4%)	11 (22.9%)
>60	10 (20.8%)	5 (10.4%)	15 (31.2%)
Total	30 (62.5%)	18 (37.5%)	48 (100%)

Table 2 presents the distribution of Non-Hodgkin Lymphoma (NHL) across various sites. Nodal involvement accounts for 48% of cases, while extranodal sites constitute 52%. The most common extranodal sites are the duodenum and skin (10.4% each), followed by the stomach and tonsils (8.3% each). Less frequent sites include the spleen, ovary(s), bone marrow, spine, cecum, and a popliteal mass (2.08% each). This data underscores the diverse nature of NHL, affecting both nodal and various extranodal sites, highlighting the need for a comprehensive diagnostic approach in NHL patients.

Table 3 reveals key immunophenotypic characteristics across lymphoma types. Diffuse large B-cell lymphomas were universally positive for CD45(LCA) and

TABLE 2. *Distribution of NHL according to sites*

Site		Number	Percentage %
Nodal		23	48
Extra nodal (n=25, 52%)			
	Duodenum	5	10.4
	Skin	5	10.4
	Stomach	4	8.3
	Tonsil(s)	4	8.3
	Spleen	2	4.2
	Ovary(s)	1	2.08
	Bone marrow	1	2.08
	Spine	1	2.08
	Cecum	1	2.08
	Popliteal mass	1	2.08
Total		48	100

CD20, negative for CD4, CD8, CD30, and cyclin D1. Mantle cell lymphomas consistently expressed CD19, CD20, CD79a, with variable CD5 and BCL2, and a mix of cyclin D1 results. Burkitt lymphomas showed uniform positivity for CD10, CD20, CD79a, and Ki67, with some cases expressing CD5, CD19, and BCL6. Lymphoblastic lymphomas (B-cell type) were positive for TdT, CD19, CD20, CD79a, BCL2, but negative for CD3, CD4, CD5, CD8, and CD23. MALToma cases all expressed CD20, CD79a, BCL2, without CD3, CD4, CD5, CD8, CD10, and BCL6. Peripheral T-cell lymphoma displayed CD3, CD4, CD8 positivity, and lacked CD10, CD19, CD20, CD79a, and TdT. Follicular lymphomas mostly expressed CD19, CD20, CD79a, BCL2, BCL6, with some variability in CD5 and CD10. The single case of small lymphocytic lymphoma showed positivity for CD5, CD20, CD79a, and negativity for several other markers.

The pie chart in Figure 1 illustrates the morphological classification of Non-Hodgkin Lymphoma (NHL) cases based on Hematoxylin and Eosin (H&E) staining. The largest segment represents NHL-unclassified type,

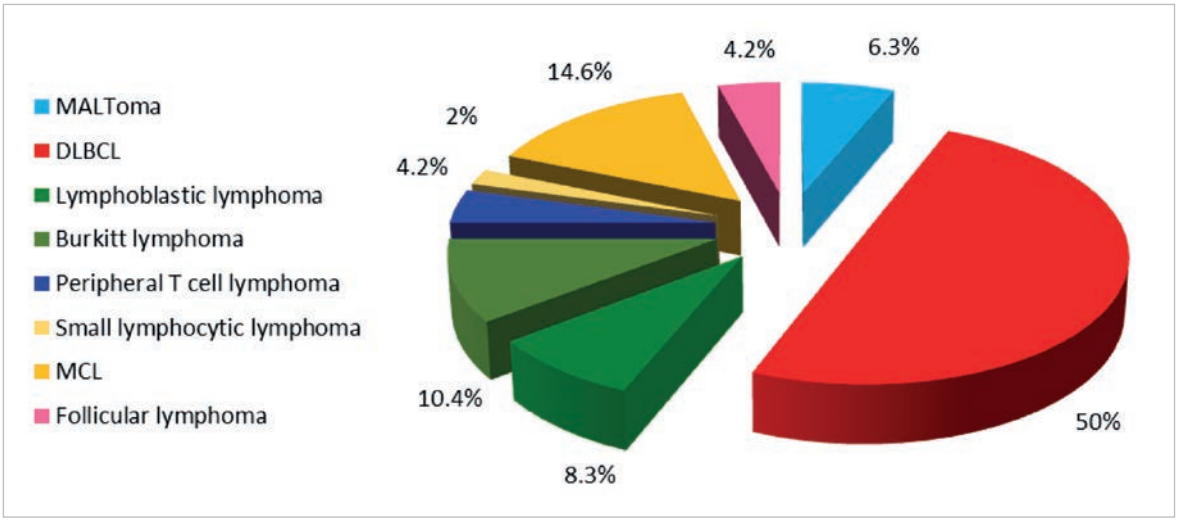

FIGURE 1. *Morphological classification of NHLs based on H&E stains*

TABLE 3. *Classification of NHL according to the positivity and negativity to immunohistochemical stains*

	DLBCL (50%)	MCL (14.6%)	Burkitt lymphoma (10.4%)	Lymphoblastic lymphoma (8.3%)	MALToma (6.3%)	Follicular lymphoma (4.2%)	Peripheral T-cell lymphoma (4.2%)	Small lymphocytic lymphoma (2.0%)
CYCLYNDI1								
BCL2								
BCL6								
CD3								
CD4								
CD5								
CD8								
CD10								
CD20								
CD19								
CD23								
CD30								
CD34								
CD45								
CD68								
CD79a								
CD138								
CK								
KI67								
ALK								
EMA								
MUM1								
TdT								

Positive in all cases
Positive in some cases
Negative in all cases
Marker not done

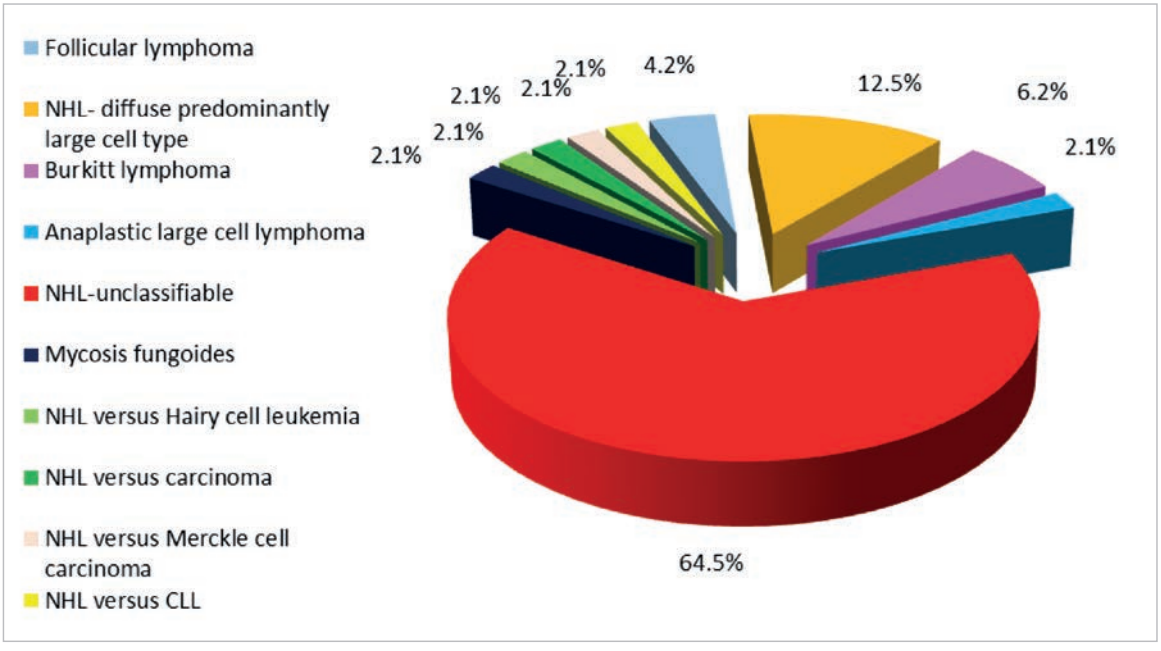

FIGURE 2. *Immunophenotyping of NHLs based on IHC stains4*

which constitutes a significant majority at 64.5%. The second most prevalent classification is NHL-diffuse predominantly large cell type at 12.5%. Other classifications such as Anaplastic large cell lymphoma, Mycosis fungoides, NHL versus Hairy cell leukemia, NHL versus carcinoma, NHL versus Merkel cell carcinoma, NHL versus Chronic Lymphocytic Leukemia (CLL), and Burkitt lymphoma each account for smaller fractions ranging from 2.1% to 6.2%. The diversity in the morphological subtypes emphasizes the heterogeneity of NHL.

Figure 2 illustrates the distribution of Non-Hodgkin Lymphoma (NHL) subtypes based on IHC staining. Diffuse large B-cell lymphoma (DLBCL) is the predominant subtype, accounting for 50% of the cases.

Mantle cell lymphoma (MCL) follows at 14.6%, with other subtypes like Follicular lymphoma and Small lymphocytic lymphoma making up smaller proportions.

DISCUSSION

In this study, the most common type of non-Hodgkin lymphomas identified was the diverse group, representing 66.6% of the cases. This was more frequent than intermediate grade (12.5%), high grade (8.3%), and low grade (4.2%) non-Hodgkin lymphomas. These results differ from those of Isikdogan et al. (2004), who reported that intermediate grade non-Hodgkin lymphoma was the most common at 69.8%, followed by low grade (14.4%) and high grade (8.7%), with 6.7% classified as miscellaneous [16]. Castella et al. (2001) found in the United Arab Emirates that intermediate grade non-Hodgkin lymphomas comprised 59%, high grade 34%, and low grade 7% [17].

Despite the expertise of pathologists and the use of well-prepared hematoxylin and eosin-stained sections, the morphological classification of non-Hodgkin lymphoma is often inadequate. This results in many cases being labeled as miscellaneous or undetermined, underscoring the importance of incorporating immunohistochemistry for more accurate diagnoses, which is crucial for patient treatment plans.

The purpose of this research was to underscore the significance of immunohistochemistry in diagnosing and classifying non-Hodgkin lymphomas. Once a morphological diagnosis is established, antigen expression is analyzed through monoclonal antibodies, a critical step in current diagnostic and classification processes for non-Hodgkin lymphoma. This study compared diagnoses from hematoxylin and eosin-stained sections with those from immunohistochemical stained sections.

Immunohistochemical staining was conducted on all 48 cases in the study, revealing that 95.8% showed B-cell differentiation and 4.2% were T-cell differentiation. This aligns with Al-Allawi et al. (2011), who found a similar distribution of B-cell and T-cell types in non-Hodgkin lymphoma in northern Iraq [18]. Notably, diffuse large B-cell lymphoma (DLBCL) was identified as the most frequent type (50%), corroborating findings from Al-Allawi et al. and other studies from the United Arab Emirates and Egypt [17,19].

Through immunohistochemistry, two cases initially diagnosed as carcinoma were reclassified as DLBCL. Additionally, 22 cases previously deemed unclassifiable or of other types were also identified as DLBCL.

Mantle cell lymphomas, not detectable by hematoxylin and eosin staining alone, were identified in 14.6% of cases using immunohistochemical stains. This differs from percentages reported in southern Iran [20] and Western Europe [21]. These mantle cell lymphomas tested positive for specific markers and negative for others, with cyclin D1 positive in five cases, highlighting its strong expression in mantle cell lymphomas, a feature not typical in other B-type non-Hodgkin lymphomas [22].

Burkitt lymphoma was identified in 10.4% of cases, aligning with reports from northern Iraq (18) and the United Arab Emirates [17]. However, lymphoblastic lymphoma, which constituted 4.8% in Al-Allawi et al.'s study, represented 8.3% in this research, all identified through immunohistochemical analysis [18].

MALTomas were identified in 6.3% of cases, peripheral T-cell lymphoma in 4.2%, and follicular lymphoma in another 4.2%. These findings show variations when compared with other regional studies, likely due to differences in sample size and regional factors.

In this study, immunohistochemical analysis led to the diagnosis of peripheral T-cell lymphoma in two instances (4.2%). Initially, these cases were identified as unspecified non-Hodgkin lymphoma and mycosis fungoides through morphological examination. The incidence of peripheral T-cell lymphoma noted by Al-Allawi et al. (2011) in northern Iraq was a lower 1.5% [18], while Castella et al. (2001) recorded it as 2% in the United Arab Emirates [17]. In contrast, Chen et al. (2010) reported a higher prevalence, with peripheral T-cell lymphomas accounting for 10.6% of non-Hodgkin lymphomas in Taiwan [23]. Such discrepancies may arise from differences in sample size, genetics, and environmental influences.

In our study, follicular lymphoma was identified in two cases (4.2%), both morphologically and subsequently confirmed via immunohistochemistry. Comparatively, Al-Allawi et al. (2011) reported follicular lymphoma constituting 2.9% of non-Hodgkin lymphomas in northern Iraq [18], whereas Ameen et al. (2010) observed a higher proportion of 15.5% in Kuwaiti Arabs [24]. These variations could be attributed to the differences in sample sizes.

Furthermore, the study identified one case (2% of the total) as small lymphocytic lymphoma through im-

munohistochemical analysis. This contrasts with the findings of Al-Allawi et al. (2011), who noted that small lymphocytic lymphoma made up 5.9% of non-Hodgkin lymphomas in northern Iraq [18].

CONCLUSION

Non-Hodgkin lymphoma diagnosis primarily hinges on hematoxylin and eosin-stained sections, with immunohistochemical staining providing crucial supplementary information. The initial differential diagnosis guides marker selection, optimizing the diagnostic process in terms of time and resources. However, the potential for errors in immunophenotyping necessitates cautious interpretation, and since no marker is uniquely indicative of lymphoma, a panel of antibodies is used to accurately determine antigen expression.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Willard-Mack CL. Normal Structure, Function, and Histology of Lymph Nodes. *Toxicol Pathol.* 2006;34(5):409-24. http://doi.org/10.1080/01926230600867727
2. Zelenetz AD, Abramson JS, Advani RH, Andreadis CB, Byrd JC, Czuczman MS, et al. Non-Hodgkin's Lymphomas. *J Natl Compr Canc Netw.* 2010;8:288-334. http://doi.org/10.6004/jnccn.2014.0125
3. Shankland KR, Armitage JO, Hancock BW. Non-Hodgkin lymphoma. *Lancet.* 2012; 380(9844):848-57. http://doi.org/10.1016/S0140-6736(12)60605-9
4. Saudi Cancer Registry. Cancer Incidence Report in Saudi Arabia. Riyadh: Ministry of Health; 2008.
5. Rappaport H. Tumor of hematopoietic system. In Atlas of tumor pathology, series 3, fascicle 8, Washington, DC, 1966, Armed Forced Institute of Pathology.
6. Krüger GR, Grisar T, Lennert K, Schwarze EW, Brittinger G. Histopathological correlation of the Kiel with the original Rappaport classification of malignant non-hodgkin lymphomas. *Blut.* 1981; 43(3): 167-81.
7. Cousar JB, Macon WR, McCurley TL, Swerdlow SH. Hematopoietic and Lymphatic Systems. In: Millis S E, Carter D, Greenson JK, Reuter VE, Stoler MH. Sternberg's Diagnostic Surgical Pathology, Fifth Edition. 2010; p. 681-734.
8. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (eds): World Health Organization classification of tumors of hematopoietic and lymphoid tissues, ed. 4. Lyon: IARC Press, 2008; p. 1-439.
9. Jaffe ES, Harris NL, Stein H, Vardiman JW (eds): World Health Organization classification of tumors. Pathology and genetics of tumor of hematopoietic and lymphoid tissues. IARC Press. Lyon. 2001. 352: p. 185.
10. Jaffe ES, Banks PM, Nathwani B, Said J, Swerdlow SH. Recommendations for the reporting of lymphoid neoplasms: A report from the Association of Directors of Anatomic and Surgical Pathology. *Mod Pathol.* 2004;17:131-135. http://10.1038/sj.modpathol.3800028
11. Casimiro Onofre AS, Pomjanski N, Buckstegge B, Böcking A. Immunocytochemical typing of primary tumors on fine-needle aspiration cytologies of lymph nodes. *Diagn Cytopathol.* 2008;36(4):207-15. http://doi.org/10.1002/dc.20781
12. Brandtzaeg P. The increasing power of immunohistochemistry and immunocytochemistry. *J Immunol Methods.* 1998;216(1-2):49-67. http://doi.org/10.1016/s0022-1759(98)00070-2
13. Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. *Exp Biol Med.* 1941;47(2):200–2.
14. Higgins RA, Blankenship JE, Kinney MC. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. *Arch Pathol Lab Med.* 2008;132(3):441-61. http://doi.org/10.5858/2008-132-441-AOIITD
15. Matos LLD, Trufelli DC, MGLD, Silva MADS. Immunohistochemistry as an Important Tool in Biomarkers Detection and Clinical Practice. *Biomarker Insights.* 2010;5:9-20. http://doi.org/10.4137/bmi.s2185
16. Isikdogan A, Ayyildiz O, Buyukcelik A, Arslan A, Tiftik N, Buyubayram H, et al. Non-Hodgkin's lymphoma in southeast Turkey: Clinicopathologic features of 490 cases. *Ann Hematol.* 2004;83:265-9. http://doi.org/10.1007/s00277-003-0812-0
17. Castella A, Joshi S, Raaschou T, Mason N. Pattern of Malignant Lymphoma in the United Arab Emirates- A Histopathologic and Immunologic Study in 208 Native Patients. *Acta Oncologica.* 2001;40(5):660-4. http://doi.org/10.1080/028418601750444231
18. Al-Allawi NA, Hughson MD, Sulayvani FK, Yaqo RT. Malignant lymphoma in northern Iraq: A retrospective analysis of 270 cases according to the World Health Organization classification. Hematological Malignancies. *Indian J Cancer.* 2011; 48(4):446-51. http://doi.org/10.4103/0019-509X.92276
19. Naresh KN, Advani S, Adde M, Aziz Z, Banavali S, Bhatia K, et al. Report of an International Network of Cancer Treatment and Research workshop on non-Hodgkin's lymphoma in developing countries. *Blood Cells Mol Dis.* 2004;33(3):330-7. http://doi.org/10.1016/j.bcmd.2004.08.001
20. Monabati A, Safaei A, Noori S, Mokhtari M, Vahedi A. Subtype distribution of lymphomas in South of Iran, analysis of 1085 cases based on World Health Organization classification. 2016;95(4):613-8.
21. Dreyling M, Geisler C, Hermine O, Kluin-Nelemans HC, Le Gouill S, Rule S, et al; ESMO Guidelines Working Group. Newly diagnosed and relapsed mantle cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2014;25(3):83-92. http://doi.org/10.1093/annonc/mdu264
22. Yatabe Y, Suzuki R, Tobinai K, Matsuno Y, Ichinohasama R, Okamoto M, et al. Significance of cyclin D1 overexpression for the diagnosis of mantle cell lymphoma: a clinicopathologic comparison of cyclin D1-positive MCL and cyclin D1-negative MCL-like B-cell lymphoma. *Blood.* 2000;95(7):2253-61.
23. Chen W-L, Tsai W-C, Chao T-Y, Sheu L-F, Chou J-M, Kao W-Y, et al. The clinicopathological analysis of 303 cases with malignant lymphoma classified according to the World Health Organization classification system in a single institute of Taiwan. *Ann Hematol.* 2010;89(6):553-62. http://doi.org/10.1007/s00277-009-0870-z
24. Ameen R, Sajnani KP, Albassami A, Refaat S. Frequencies of non-Hodgkin's lymphoma subtypes in Kuwait: comparisons between different ethnic groups. *Ann Hematol.* 2010;89(2):179-84. http://doi.org/10.1007/s00277-009-0801-z