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Immunophenotypic characterization of malignant lymphoma in Iraqi patients using immunohistochemical CD-marker study

Hameda Abd Al-Mahdi Ghazi[1], Rajaa Ali Moheiseen Al-Taee[2], and Hayder Abdul-Amir Makki Al-Hindy*[3]

1,2 Hammurabi Medical Collage. Babylon University/Iraq
3 (Medical Physiology). College of Pharmacy. University of Babylon/Iraq
Corresponding Author
Corresponding Author: phar.hayder.abdul@uobabylon.edu.iq.

ABSTRACT

Background: In diseases affecting lymph nodes (LN), this highly organized morphological structure of the node is disturbed in a way or another. The lymphoid follicles may become larger, similar to what has been found in case of follicular hyperplasia. On the other hand, the sinuses may enlarge in cases of sinus hyperplasia as a response to certain types of inflammatory disorders. The LN architecture may be dramatically destroyed due to metastatic malignant disorder or due malignant neoplasia affecting primarily the LN itself, as is the case in malignant lymphoma.

Aim of the study: The poverty of local studies justified the planning and conduction of the current study to evaluate the prevalence of different types of malignant lymphoma in Al-Diwaniyah city, Middle Euphrates zone of Iraq

Materials and methods: This study conducted at the teaching labs of Al-Diwaniyah hospital and a number of private laboratories. At the end of the study we were able to retrieve 27 paraffin block of patients documented to have malignant lymphoma, either Hodgkin or Non-Hodgkin lymphoma. The study started on June 2018 and ended at June 2019.

Results: The current study included 27 lymphoma patients; 17 Hodgkin and 10 non-Hodgkin cases. The overall mean patients' age was 48.09 ±12.7 years, that of Hodgkin lymphoma was 46.03 ±7.81 and that of non-Hodgkin lymphoma was 49.81 ±13.41 years. The study included 13 females and 14 males. Hodgkin lymphoma included 7 females and 10 males; while non-Hodgkin lymphoma included 6 females and 4 males. The study revealed no significant variation in the mean ages. Likewise, no sex-variation in the distribution of Hodgkin/non-Hodgkin groups ($p > 0.05$).

Conclusion: The use of conventional "hematoxylin/eosin-stain" allows the identification of malignant lymphoma. However, for proper characterization, immunophenotyping is necessary.

Keywords: Immunohistochemistry, Hodgkin lymphoma, Non-Hodgkin lymphoma, Iraq

Correspondence:
Hayder Abdul-Amir Makki Al-Hindy
(Medical Physiology). College of Pharmacy. University of Babylon/Iraq
Corresponding Author

*Corresponding author: Hayder Abdul-Amir Makki Al-Hindy email-address: phar.hayder.abdul@uobabylon.edu.iq.

INTRODUCTION

The lymph-nodes are a large number of specialized tissues situated along the pathway of lymphatic vessels. Typically, every LN is composed of several lobules that are bounded by sinuses containing lymph and enclosed within a capsule [1]. However, pathologists prefer to describe LNs referring to cortex, para-cortex, and medulla, but these are lobular-structures that have variation in cellular composition [2]. The base of each lobule is directed toward the hilus and a base that is bulbous in appearance and separated from the capsule by sub-capsular sinus. The afferent lymphatic vessel enters the sub-capsular sinus whereas the efferent lymphatic vessels leave the node at the site of entrance of LN arterial supply at the region of the hilus [3].

In diseases affecting LNs, this highly organized morphological structure of the node is disturbed in a way or another. The lymphoid follicles may become larger, similar to what has been occurred in follicular hyperplasia [4, 5]. On the other hand, the sinuses may enlarge in cases of sinus hyperplasia as a response to certain types of inflammatory disorders [5, 6]. The LN architecture may be dramatically destroyed due to metastasis [7, 8] or because of the malignant neoplasia affect the LNs itself initially [9, 10]. In general, malignant neoplasms originating from lymphatic tissues had categorized into two major classes, Hodgkin lymphoma (HL) [11] and Non-Hodgkin lymphoma (NHL) [12].

The NHLs are not a single group but represent a heterogenous category of lymphoid malignancies with various causes, morphological appearances, clinical presentations, biological behavior, and response to treatment. It comprises many subgroups with variations in epidemiology, LN architecture, and immunophenotypic features [13]. The disease is estimated to be responsible for 5.1 % of all malignant neoplasms and 2.7 % of cancer-related mortality [14]. The NHLs are more common in developed countries, and their incidence is relatively high in the United States and Europe [15]. From a historical perspective, there were several classifications although the final one had gained wide acceptance among clinicians and pathologists globally, which was the WHO classification that published in 2001 [13]. According to the cell of origin, NHL may be considered as (B or T), and natural killer (NK) cells [16]. The use of immunohistochemistry permitted better categorization and understanding of NHL, specifically with immunophenotyping [17]. Several markers are used to categorize subtypes of NHLs, such as common leukocyte antigen (CLA), T-cell biomarkers (CD 3 and 5), B-cell markers (CD 20 and 79a) and other markers (CD138, CD10, CD15, CD23), etc. [17]. No one of these markers is specific for a precise lymphoma'-subtype, but many biomarkers form a panel for the suggestion of the final diagnosis [13].

The lack of local studies justified the planning and conduction of the current work to evaluate the prevalence of different types of malignant lymphoma in Al-Diwaniyah city, Middle-Euphrates zone of Iraq.

Materials and Methods

This study had conducted in laboratories of Al-Diwaniyah hospital and some private laboratories. At the end of the study, we were able to retrieve 27 paraffin blocks of patients documented to have malignant lymphoma, either Hodgkin or Non-Hodgkin lymphoma. The study started in June 2018 and ended in June 2019.

From each paraffin block, two thin-sections (around 4-6 micrometer in diameter) had prepared. The first section had used to perform hematoxylin and eosin stain to confirm the diagnosis and study the characteristic features of available subtypes. The second section was stained with immunohistochemistry for CD3 as a T-cell marker or CD20 as a B-cell marker.

The sections made were thin (5µm) and were put on positively charged slides. The Deparaffinization step had done then, by using a xylene bath, three times 5 minutes for each. The rehydration step had performed by descending ethanol concentrations baths 95%, 90%, and 75 %, 5 minutes for each, then followed by a distilling water bath for 5 minutes. The antigen retrieval step was carried out at microwave with EDTA buffer (pH of 8) for 20 minutes. Endogenous peroxidase was inhibited by running tissue through 6 % oxygenated water for 5 minutes. Finally, incubated with a primary antibody for 60-min at 37 ºC had been made after washing with PBS solution for 5 minutes. "The primary antibodies used were CD3 (DAKOCytomation, Denmark) and CD20 (DAKOCytomation, Denmark) in 1:50 dilution". The tissues had then wash-away with ''PBS/Tween'' to be incubated by EnVision HPR detection system for thirty-min at 37 ºC. At that point, the tissues had washed by water, followed by signal visualization using 3-3' diaminobenzidine DAB. Hematoxylin was then used for nuclei counterstaining. The dehydration step had then performed with ascending ethanol concentration baths followed by clearing-step and mounting using Canada balsam and covered by a coverslip.

The patients' age, gender, and clinical features had retrieved from available histopathology reports. Data were collected and transformed into Microsoft office Excel 2007 for statistical description. The variables had described as range, mean/SD, sum, and/or percentage. Independent *t*-test had applied to study in the ages' variation crosswise lymphoma groups. Meanwhile, *Chi*-square had conducted to investigate gender variation. A *p-value of* ≤ 0.05 had accepted as significant.

RESULTS

The current study included 27 cases: 17 HL and 10 NHLs. The overall mean ages were 48.09 ±12.7 years, that of HL was 46.03 ±7.81, and that of NHL was 49.81 ±13.41 years. The study included 13 females and 14 males. Patients with HL included 7-females and 10-males; while NHL included 6-females and 4-males (table-1.) The mean patients' ages and their gender-distribution between the HL and NHL groups were parallel (p>0.05).

Table 1: The frequency distribution of patients with malignant lymphoma according to age and gender

Characteristic	Total	Hodgkin lymphoma	Non- Hodgkin lymphoma	*p*-value
Number of cases	27	17	10	----
Age (years)				
Mean ±SD	48.09 ±12.7	46.03 ±7.8	49.81 ±13.4	> 0.05 (I)
Range	17-59	17-51	23-59	
Gender				
Male, *n* (%)	14	10	4	> 0.05 (C)
Female, *n* (%)	13	7	6	

SD: standard-deviation, ***n*****:** number**, I:** independent *t*-test; **C**: *Chi*-square test

The cases of HL included 7 cases of mixed cellularity subtype and 10 cases of nodular sclerosis subtype. The typical feature of HL is the existence of a ''Reed-Sternberg (RS) cell'' that is a large cell with a large nucleus and two mirror-image nucleoli with abundant cytoplasm, as shown in figure 1 and 2. The other distinguishing feature of HL is the existence of a mixed cell population of reactive white blood cells included the lymphocytes. The distinguishing feature of the nodular sclerosis subtype is the presence of bands of fibrosis that divided the substance of the LN into well-demarcated nodules that have the typical Hodgkin cell and mixed cell population of white blood cells, as shown in figure 3 and 4. Staining with B-cell marker (CD20) revealed negativity in all cases of HL as shown in figures 2 and 4.

On the other hand, all cases of NHL were of the B-cell diffuse subtype. This subtype has characterized by the effaced structure of LN with lost follicular architecture and replacement of LN substance by a diffuse population of monomorphic large mononuclear B-cells, as shown in figures (5 and 6). Immunohistochemistry was negative for CD3 (T-cell marker), but it was positive for CD-20 (B-cell marker), which exhibited diffuse cytoplasmic staining pattern, as shown in figure-6.

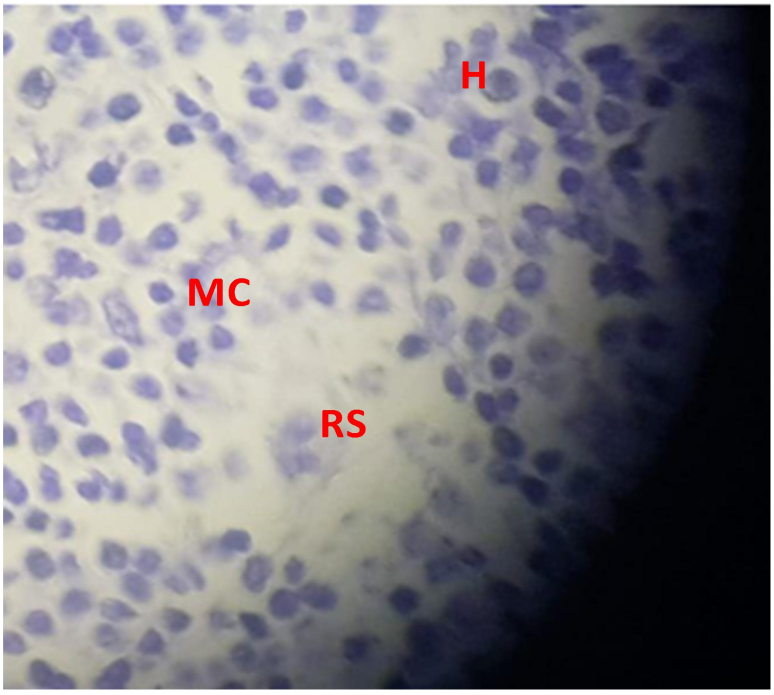

Figure 1: Section through cervical LN with mixed cellularity Hodgkin lymphoma. The sectioned had stained with H and E stain. The typical features were the presence of ''Reed-Sternberg cell (RS)'', Hodgkin cells (H), and a mixed cellular population of lymphocytes. 20 X

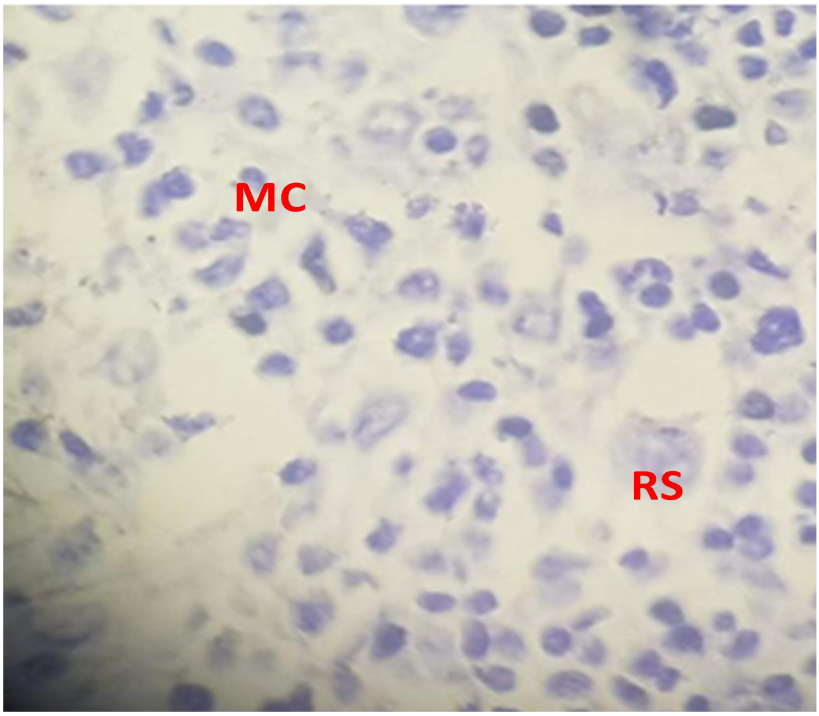

Figure 2: Section through cervical LN with mixed cellularity Hodgkin lymphoma. The sectioned was stained with B-cell immune marker but was negative. The typical features were the presence of Reed-Sternberg (RS) cells and a mixed cellular population of lymphocytes. 20 X

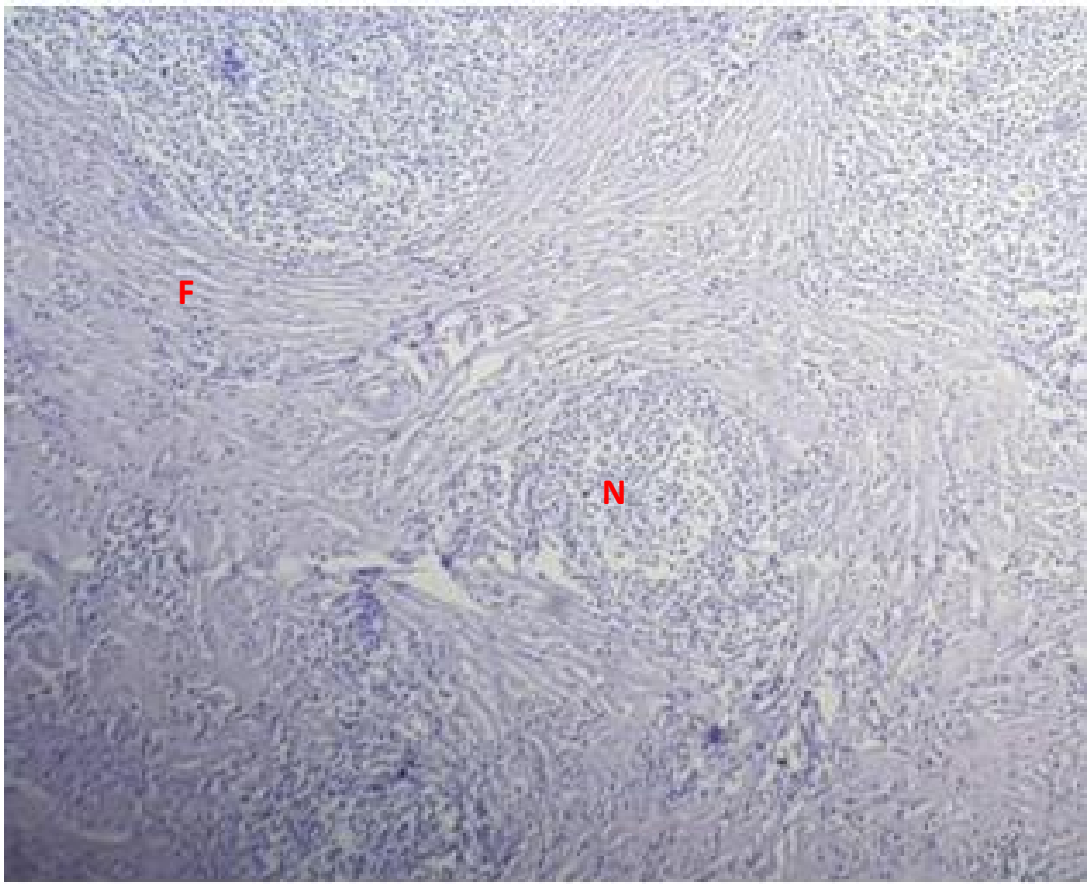

Figure 3: Section through cervical LN with nodular sclerosis Hodgkin lymphoma. This section had stained H and E stain. The typical features were the presence of fibrous bands (F) and nodules with a mixed cellular population of lymphocytes (N). 4 X

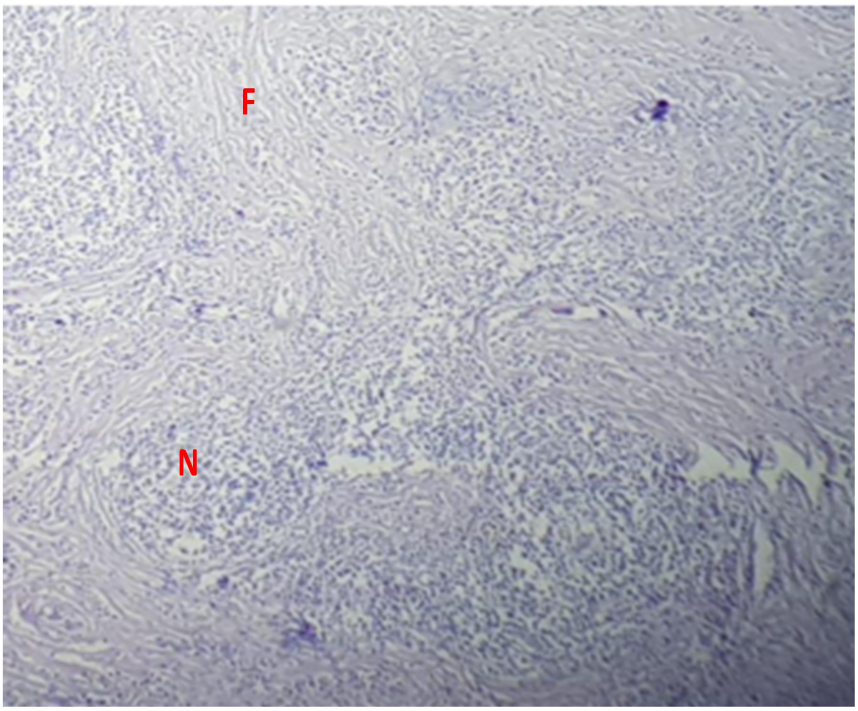

Figure 4: Section through cervical LN of a patient with nodular sclerosis Hodgkin lymphoma. This section had stained with B-cell immune marker, but it was negative. The typical features were the presence of fibrous bands (F) and nodules with a mixed cellular population of lymphocytes (N). 4X

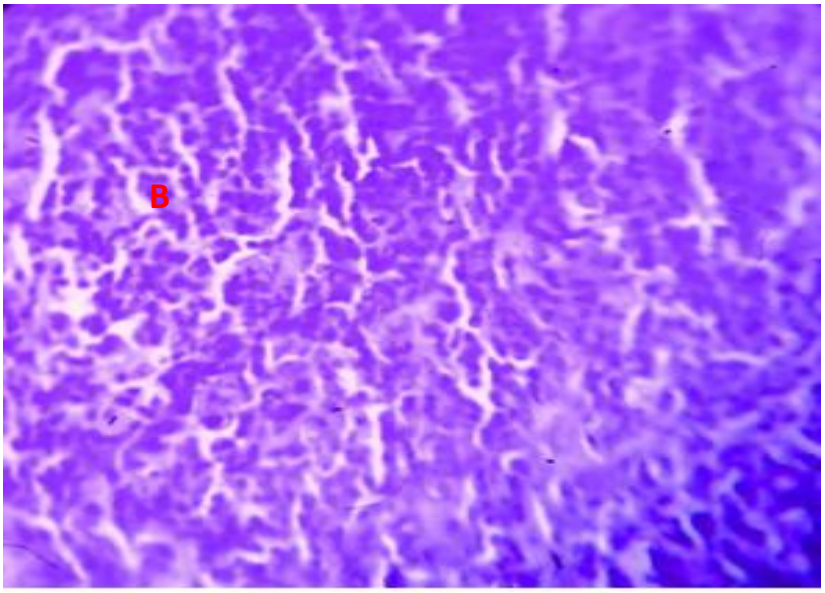

Figure 5: Section of the lymph node with diffuse large B-cell NHL. This section had stained with H and E stain. The typical features included effaced LN architecture with loss of follicular architecture besides replacement of LN substance by a diffuse population of monomorphic large mononuclear B-cells (B). 10 X

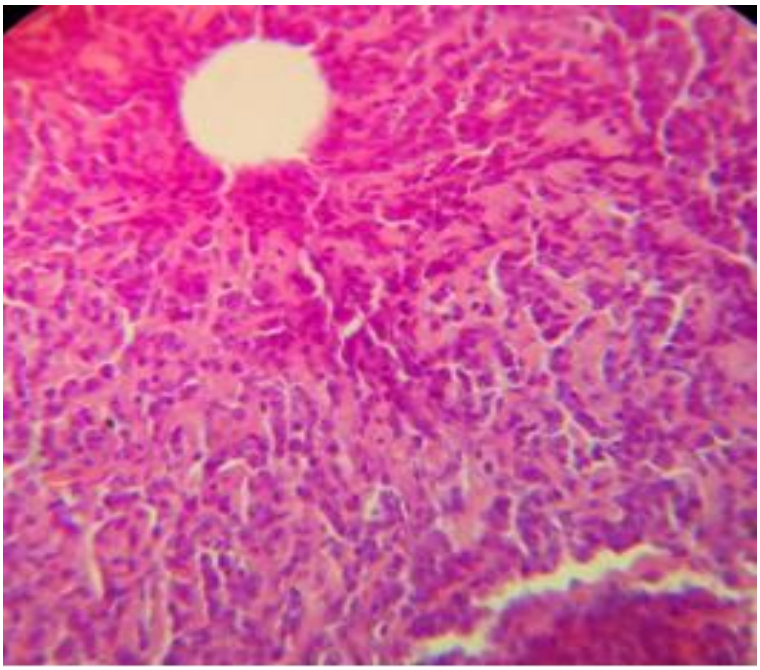

Figure 6: Section of LN with diffuse large B-cell NHL. This section had stained with B-cell marker (CD20) immunohistochemistry. There diffuse brown color intracytoplasmic positivity. The typical features included effaced LN architecture with loss of follicular architecture besides replacement of LN substance by a diffuse population of monomorphic large mononuclear B-cells. 10 X

DISCUSSION

The histological characterization of changes affecting LNs affected by malignancy depends on how these changes alter the normal LN architecture. In general, malignancy affecting LN can be primarily arising from cells inside LN itself or secondary propagating to LN from a nearby organ or a distant tissue. The primary malignant disease affecting LN is known as malignant lymphoma and had divided into HL and NHL. It is well-known that HL had characterized by the presence of the malignant RS cells and reactive mixed cell population of white blood cells. On the other hand, HL is principally characterized by effaced LN architecture with the replacement of LN substance by a malignant population of T or B lymphocytes or others.

In this study, the authors tried to evaluate the prevalence of HL and NHL subtypes in Al-Diwaniyah city. It had found that most cases of Hodgkin lymphoma were of nodular sclerosis subtype, and the rest were mixed cellularity subtypes. The recent classification of HL classified the disease into 5-subtypes that includes lymphocyte-predominant, nodular-sclerosis, mixed-cellularity, lymphocyte-rich, and lymphocyte-depleted subtypes.

On the other hand, all cases of NHL were diffuse large B-cell lymphoma. This subtype represents the most-frequently encountered subtype in Iraq, as reported by several authors. The use of B-cell (CD20) and T-cell (CD3) permitted the accurate characterization of diffuse large B-cell lymphoma in the current work.

The immunohistochemistry had applied in lymphoid tissues in three circumstances. To understand the phenotype of the abnormal population detected by H and E stain, or to characterize further the abnormal-cell population seen by flow cytometry, and finally to investigate normal tissue (based on H and E) to see if subtle abnormal cells are present [16]. Researchers had observed that the Non-Hodgkin lymphoma is more common in men than in women [13, 18]. This finding was not the case in the current study, probably due to the small sample size. However, it is worth mentioning that the statistical significance had not obtained, and the disease can be regarded as equally distributed over genders.

It has shown that non-Hodgkin lymphoma is a disease of the elderly [13]. In the current study, we observed that patients with NHL were older than patients with HL, but with no statistical significance. Diffuse large B-cell lymphoma is an aggressive form and accounts for approximately 30-50 % of cases worldwide [19]; this is probably the explanation for all our patients to be of that variety.

B-cell and T-cell markers were not beneficial in HL, but they can differentiate subtypes of NHL in the current work.

CONCLUSION

The use of conventional ''hematoxylin/eosin-stain'' allows the identification of malignant lymphoma. However, for proper characterization, immunophenotyping is necessary.

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