Diagnostic Yield of PAX5 in Lymphoma

Hiba Rafea* , Abdul Kareem Yonis Al-Tae , Nazar Mohamed Jawhar*****
***Postgraduate Student , Department of Pathology, College of Medicicn, University of Mosul ,**
****Department of Pathology College of Medicine , University of Mosul , *****Department of Pathology,**
College of Medicine, University of Nineveh , Mosul, Iraq
Correspondence: h.r.t.m.w.t@gmail.com

(Ann Coll Med Mosul 2020; 42 (2):109-118).
Received: 20th Oct. 2020; Accepted: 6th Dec. 2020.

ABSTRACT

Background: Lymphoma is a heterogeneous group of neoplasm affecting any stage of lymphocyte development. Nowadays, WHO (2017) adapted their classification depending on their morphological, immunohistochemical appearance, and their clinical and molecular characteristics.

Objective: To determine the expression of PAX5 in Hodgkin and Non Hodgkin lymphoma and their association with clinical parameters (age & site).

Patients & Methods: During a period of 1 year starting from June 2019 to June 2020, 60 lymphoma patients were included in this prospective-retrospective case series study from medical records and histopathological results in governmental and private labs. Immunohistochemistry examination using PAX5 was performed, and data of the positive PAX5 expression were analyzed.

Results: A total of 60 patients were recruited in this study with the majority (58%) were Non Hodgkin lymphoma, dominated by diffuse large B- cell lymphoma subtype (42.8%) and (42%) were Hodgkin lymphoma where nodular sclerosis subtype formed (52%) and mainly of the nodal site in both of them. The age range of Non Hodgkin lymphoma 7-76 years (median 50 years) and 5-67 years (median 21 years) for Hodgkin lymphoma and male predominance in both of them and there is no significant association between the site and lymphoma (p value=0.0647) while a significant association with age (p value =0.037). Moreover, the immunohistochemical examination demonstrates that positive PAX5 was expressed in 77% of Non Hodgkin lymphoma and 100% of Hodgkin lymphoma. The P-value between them was 0.016, which was statistically significant ,no matter of using PAX5 or CD30 in the diagnosis of Hodgkin lymphoma,in other hand there is a significant difference between PAX5 and CD3 expression (P value=0.004), while it is not statistically significant when comparing PAX5 and CD20 (P-value =0.317) in the diagnosis of Non Hodgkin lymphoma .

Conclusion: This study demonstrates that PAX5 is one of the most sensitive and reliable immunohistochemical marker in diagnosing Hodgkin lymphoma and B-cell Non Hodgkin lymphoma.

Keywords: Hodgkin lymphoma, Immunohistochemistry, Non Hodgkin lymphoma, PAX5.

ألعائد ألتشخيصى لمعلمة باكس ٥ فى اورام الغدد اللمفاوية

هبة رافع الاطرقجي* ، عبد الكريم يونس الطائي ، نزار محمد جوهر*****
طالبة دراسات عليا ، فرع علم الامراض ، كلية الطب ، جامعة الموصل ، ****فرع علم الامراض ، كلية الطب ، جامعة الموصل ، ** فرع علم الامراض ، كلية الطب ، جامعة نينوى ، الموصل ، العراق**

الخلاصة

الخلفية: اورام الغدد الليمفاوية هو مجموعة غير متجانسة من الأورام تؤثر على أي مرحلة من مراحل نضوج الخلايا اللمفاوية، وقد تم اعتماد منظمة الصحة العالمية (WHO) (٢٠١٧) في تصنيفها في الوقت الحاضر اعتمادًا على مظهرها النسيجي والمناعي الكيميائي بالإضافة إلى خصائصها السريرية والجزيئية.

الهدف: تحديد معلمة الاورام باكس٥ في اورام الغدد الليمفاوية ونسبة انتشارها لاورام الهودجكن واللاهودجكن والترابط السريري بينهما.

المرضى والأساليب: خلال فترة سنة واحدة ابتداء من حزيران ٢٠١٩ إلى حزيران ٢٠٢٠، تم إدراج ٦٠ مريضاً من مرضى اورام الغدد الليمفاوية في دراسة رجعية ومستقبلية من السجلات الطبية والنتائج الهيستوباثولوجية في المختبرات الحكومية والخاصة. وأجري عليها فحص كيميائي نسيجي مناعي باستخدام معلمة الاورام الباكس٥.

النتائج: تم تعيين مجموعة من ٦٠ مريضاً في هذه الدراسة وكان غالبيتهم (٥٨%) ورم الغدد الليمفاوية اللاهودجكن من نوع الخلايا الكبيرة المنتشرة(٤٢.٨%) و(٤٢%) كانوا اورام الغدد الليمفاوية هودجكن من نوع التصلب العقدي (٥٢%) متركزة في العقد المفاوية لكليهما . وكانت الفئة العمرية لاورام الغدد الليمفاوية اللاهودجكن بين ٧ الى ٧٦ سنة ومعدل العمر ٥٠ سنة و ٥ الى ٦٧ سنة وبمعدل ٢١ سنة لاورام الغدد الليمفاوية نوع هودجكن مع غلبة الرجال لكليهما ولايوجد ارتباط كبير فيما يتعلق بالموقع (P=0.647) بينما هناك ارتباط مع العمر(p=0.037). علاوة على ذلك ، أظهر فحص الكيمياء المناعية والنسيجية أن باكس٥الموجب وجد على التوالي في ٧٧٪ من ليمفوما اللاهودجكين و ١٠٠٪ من اورام الغدد الليمفاوية هودجكين وقيمة P بينهما 0.016 وهي ذات دلالة إحصائية وإن لا فرق من استخدام معلمة باكس٥ وعنقود التمايز٣٠ في تشخيص اورم الغدد الليمفاوية الهودجكن في الجانب الاخر هناك فرق كبير بين باكس٥ وعنقود التمايز٣ لأن قيمة (P = 0.004) بينما ليس ذو دلالة إحصائية بين باكس ٥ وعنقود التمايز٢٠ لأن قيمة (P = 0.317) في تشخيص اورام الغدد اللمفاوية الاهودجكن .

الاستنتاج : تبرهن من هذه الدراسة على أن الباكس٥ هو احد العلامات الكيميائية المناعية المناسبة الأكثر حساسية وموثوقية في تشخيص اورام الهودجكن واللاهودجكن لاورام الغدد اللمفاوية نوع بي .

الكلمات المفتاحية : اورام هودجكن , الفحص الكيميائي النسيجي المناعي, اورام اللاهودجكن , باكس٥.

INTRODUCTION

Lymphoma is a heterogeneous group of malignant tumors of lymphoid cells at different maturation stages, which could be nodal or extranodal. The World Health organization's (WHO) classification system recognizes more than 90 different subtypes, and it classifies lymphoma into Hodgkin (HL) and Non Hodgkin lymphoma (NHL). [1]

Lymphoid neoplasms occupy the sixth leading cause of cancer related death in the United States in 2016 as there are 136,960 new cases diagnosed annually. [2]

The category of HL encompasses classical Hodgkin lymphoma (CHL) and less commonly nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), and the diagnosis of them depends on the presence of neoplastic Hodgkin Reed-Sternberg (HRS) cells. [3] and lymphocyte predominant (LP) cells within the reactive inflammatory background respectively. [4] Both of these types arise from germinal center B-cell. [5]

NHL comprises of different neoplasm of the immune system, Eighty-five to ninety percent of them are derived from B- cell, while 10-15% are derived from T cell or NK cell. [6]

Immunohistochemical (IHC) markers are crucial for recognition of the B-cell lineage to help in the diagnosis and classification of lymphoma [7] due to overlapping morphology and antigenic variation. [8] Accurate lineage assignment has contributed to our fundamental understanding of lymphoma and advances in therapeutic and disease monitoring strategies.

IHC detection of PAX5 protein, also known as B-cell specific activation protein (BSAP), in lymphoid neoplasm using polyclonal and monoclonal antibodies was used as a well-identified marker for recognizing the B-cell lineage cells. [9,10]

The BSAP protein is encoded by the PAX5 gene [11], which is located in 9p13 chromosome [12]. It consists of a family of 9 members (PAX1-PAX9) arranged into four groups (Ⅰ, Ⅱ, Ⅲ, and Ⅳ) according to their structural similarity. It has a significant role in regulating cell migration, proliferation, and differentiation through organogenesis. [13]

PAX5 protein is a subgroup Ⅱ member of highly conserved paired box (PAX) family, and the only member that involved in the hematopoietic system [13] that is important for cellular commitment, immunoglobulin rearrangement, pre BCR (B cell receptor) signaling, and survival of mature B- cell. [14] in addition to functional maintenance of B- cell during late B lymphopoiesis. [15]

PAX5 is expressed primarily in pro-, pre- and mature B-cells but not in plasma cells. [16]

Aim of the Study

To find the frequency of PAX5 expression in lymphoma (HL &NHL) and its association with clinicopathological parameters (age and site).

MATERIAL AND METHODS

This study was reviewed and approved by the Medical Research Ethnics Committee (MREC), College of Medicine, University of Mosul. In this case series study, extending over one year from June 2019 through June 2020, all cases diagnosed

as lymphoma at governmental hospitals and those referred from private laboratories in Nineveh province in the North of Iraq were enrolled in this current study.

The study includes 60 cases of lymphoma. All histopathological reports were reviewed regarding clinicopathological data (age, gender, and site). For each case, the author reviewed the hematoxylin and eosin-stained sections. The tumor was diagnosed and subtyped according to the WHO classification system 2017. [1] One section from each case was selected for the immunohistochemical study.

Immunohistochemistry:

Each case was studied for PAX5 expression. Formalin-fixed paraffin-embedded block of cases were obtained. Section of 5 microns thick were deparaffinized in xylene and rehydrated. The immunohistochemical study was performed according to manufacturer instructions using the IgG rabbit monoclonal antibody (Clone: EP156 PR064-3ml RTU, Pathnsitu company, India), and PathnSitu PolyExcel detection system with DAB chromogen. Positive and negative control slides were involved in each run of staining. Negative controls were prepared by replacing the primary antibody with Tris-buffered saline; positive control slides were obtained from tonsil.

For the evaluation of PAX5 expression, histopathological features were correlated with cell specific immunohistochemical staining pattern and are compared with the control slides. Nuclear brown color staining in more than 10% of neoplastic cells is regarded as positive, [17] and the expression of PAX5 in normal B- cell is more strong than HRS, which is faint. [18]

Apart from PAX5, which was done in 60 patients, other IHC markers reports were obtained from several cases of the same patients in our study. The results of immunohistochemical markers CD30, CD3, CD20 previously used to confirm the diagnosis and subtyping of lymphoma were studied and determined as positive or negative to obtain the association between them and PAX5 expression.

The immunohistochemical markers and clinicopathological variables were analyzed using the Statistical Package for Social Science version 23 SPSS and association between PAX5 expression and variable categories, and other markers were evaluated using the Fisher Exact test and McNemar Chi-square test when indicated, and the probability level accepted for significance was $P < 0.05$.

RESULTS

Among 60 cases included in this study, 25 (42%) cases were diagnosed as HL, whereas 35 cases (58%) diagnosed as NHL

As shown in Table (1). The age of HL patients ranged from 5-67 years (mean 26 ± 14.73) years (median 21 years) peaked at the teenage group (36%). Patient's age in NHL ranged from 7-76 years (mean 46 ± 18.72) (median 50 years) peaked at the fifth decade (31.5%), with male to female ratio of 1.2: 1

There was a statistically significant association between PAX5 and age (P value=0.037).

Table (2) showing the histopathological subtyping of lymphoma which revealed nodular sclerosis (figures 1&2) was the commonest HL subtypes 13/25 (52%) of cases, followed by mixed cellularity 11/12 (44%) then a single case of lymphocyte predominant HL whereas diffuse large B-cell lymphoma (figures 3&4) dominated NHL cases 15/35, forming (42.8%) of NHL cases followed by 3 cases (8.6%) of each small lymphocytic lymphoma, lymphoblastic lymphoma (figures 5&6), follicular lymphoma (figures 7&8), marginal zone lymphoma (figures 9&10), then 2 cases (5.7%) of each Burkitt lymphoma (figures 11&12), lymphoplasmacytic lymphoma, peripheral T-cell lymphoma and single cases (2.85%) of mantle cell lymphoma and large unclassified cases.

PAX5 was expressed in all 25 cases of HL, whereas it showed positivity in 27 out of 35 (77%) and negative in 8 cases (23%) of NHL with a significant statistical association between them (P=0.016) as shown in table (3).

Most of the NHL were of B- cell lineage 30/35 (85.7%), and they were much more than T- cell lineage 4/35 (11.4%), while one case (2.9%) was not classified under a specific cell type and was reported as unclassifiable NHL. PAX5 has been observed in 27/30 cases (90%) of B-cell NHL while it was negative in all T-cell lymphoma 4/4 (100%) and the single unclassifiable case.

Regarding the location, 82% of NHL were nodal, and 18% were extranodal .while all HL cases were nodal, and it has been shown the association of PAX5 expression in relation to the site does not show statistically significant value (P=0.647) as shown in table (4).

CD 30 expression results were available in 14 cases of HL, and all of them have expressed PAX5, as shown in table (5).

In NHL of a paired match group, CD3 results were also available in 22 cases of NHL (18 were negative and 4 were positive). PAX5 was expressed in only 17 cases of them (77.3%), and the P-value between them was 0.004, which is statistically significant as shown in table (6). These

cases consisting of 1/4 (25%) case of PAX5 positive and CD3 positive whereas the other 3/4 (75%) cases showing PAX5 negative and CD3 positive while there is 2/18 (11%) showing simultaneous negativity and 16/18 (89%) showing positive PAX5 and negative CD3 as shown in Table (6)

PAX5 was found to be positive in 20/26 (77 %) in NHL in paired match cases, and the positivity for CD20 was found to be 21/26 (81%), and the P-value between them was 0.317, which is statistically not significant as shown in table (7).

Table (1): Age distribution of lymphoma patients.

Age groups (years)	Hodgkin lymphoma		Non-Hodgkin lymphoma	
	No.	%	No.	%
<10	1	4.0	2	5.7
10-19	9	36.0	3	8.6
20-29	8	32.0	0	0.0
30-39	2	8.0	4	11.4
40-49	2	8.0	6	17.1
50-59	2	8.0	11	31.5
≥ 60	1	4.0	9	25.7
Total	25	100.0	35	100.0

Table (2): Frequencies of different lymphoma types in the current study.

Final Diagnosis	No.	Percentage (%)
Hodgkin Lymphoma		
A. Classical HL		
Nodular sclerosis	13	52.0
Mixed cellularity	11	44.0
Nodular lymphocyte predominant	1	4.0
Total	25	100.0
Non-Hodgkin Lymphoma		
Diffuse large B-cell lymphoma	15	42.8
Follicular lymphoma	3	8.6
Small lymphocytic lymphoma	3	8.6
Marginal zone lymphoma	3	8.6
Lymphoblastic lymphoma	3	8.6
Burkitt lymphoma	2	5.7
Lymphoplasmacytic lymphoma	2	5.7
Mantle cell lymphoma	1	2.85
Peripheral T-cell lymphoma	2	5.7
Large unclassified	1	2.85
Total	35	100.0

Table (3): PAX5 expression in lymphoma in the current study

Types of lymphoma	PAX5 Positive		PAX5 Negative		P-value*
	No	%	No	%	
Hodgkin lymphoma (n=25)	25	100.0	0	0.0	0.016
Non Hodgkin lymphoma (n=35)	27	77.0	8	23.0	

* Fisher Exact test was used.

Table (4): PAX5 expression in relation to the site of lymphoma

Site	PAX5 Positive		PAX5 Negative		P-value*
	No	%	No	%	
Nodal (n=49)	42	85.7	7	14.3	0.647
Extranodal (n=11)	10	90.9	1	9.1	

* Chi-square test was used.

Table (5): Associations between PAX5 & CD30 in 14 cases of HL

IHC	Positive cases		Negative cases		P-value*
	No.	%	No.	%	
PAX5 (n=14)	14	100.0	0	0.0	-----
CD30 (n=14)	14	100.0	0	0.0	

Table (6) Test analysis results for 22 cases PAX5 & CD3 in a paired group NHL

IHC		CD3						P-value*
		+ ve		- ve		Total		
		No.	%	No.	%	No.	%	
PAX5	+ve	1	25.0	16	89.0	17	77.3	0.004
	-ve	3	75.0	2	11.0	5	22.7	
Total		4	100	18	100	22	100.0	

* McNemar Chi-square test was used.

Table (7): Association between PAX5 & CD20 in 26 cases of NHL

IHC	Positive cases		Negative cases		P-value*
	No.	%	No.	%	
PAX5 (n=26)	20	77.0	6	23.0	0.317
CD20 (n=26)	21	81.0	5	19.0	

* McNemar Chi-square test was used.

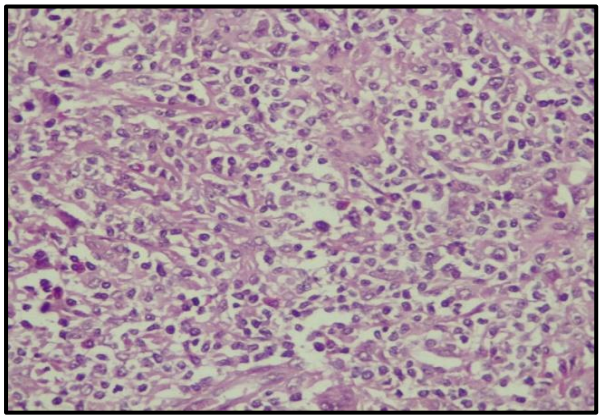

Figure (1): HL-Nodular sclerosis (H&E x 400)

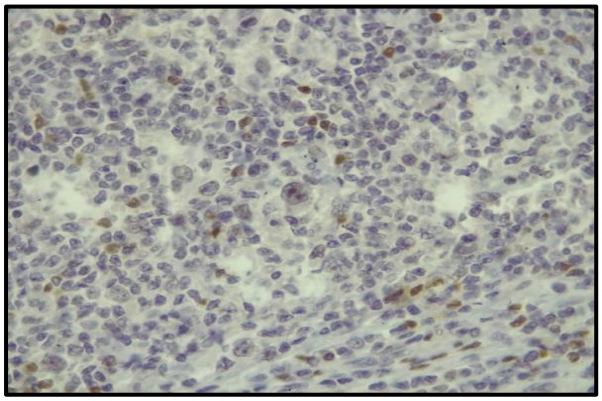

Figure (2): HL-Nodular sclerosis (PAX5positive x400)

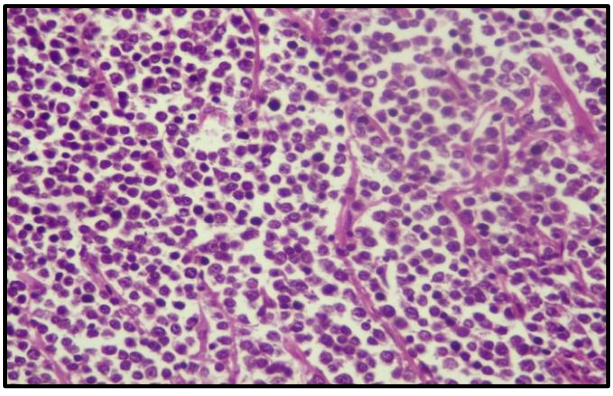

Figure (3): Diffuse large B cell lymphoma (H&Ex400)

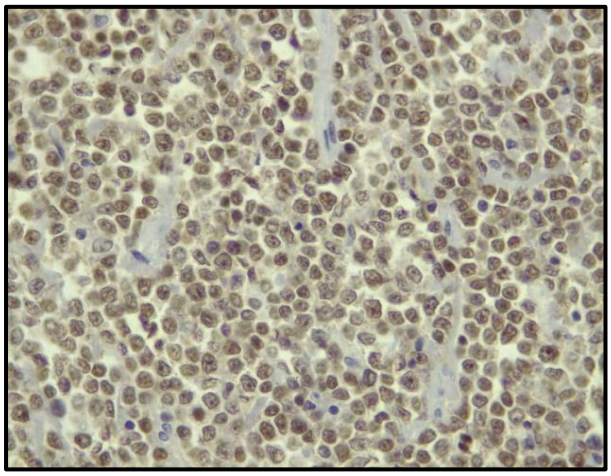

Figure (4): Diffuse large B cell lymphpma (PAX5 positive x400)

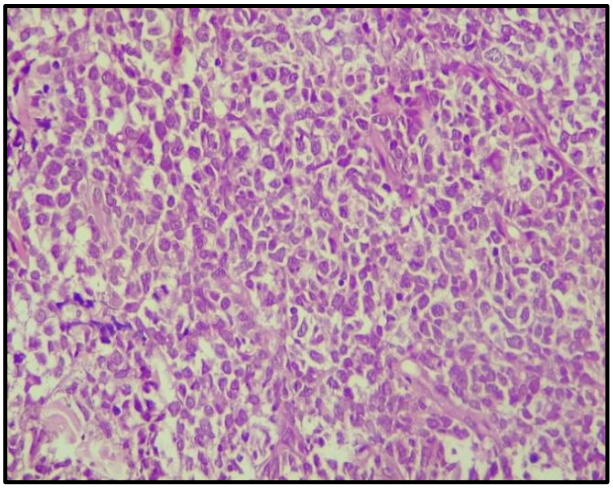

Figure (5): Lymphoblastic Lymphoma (H&E x400)

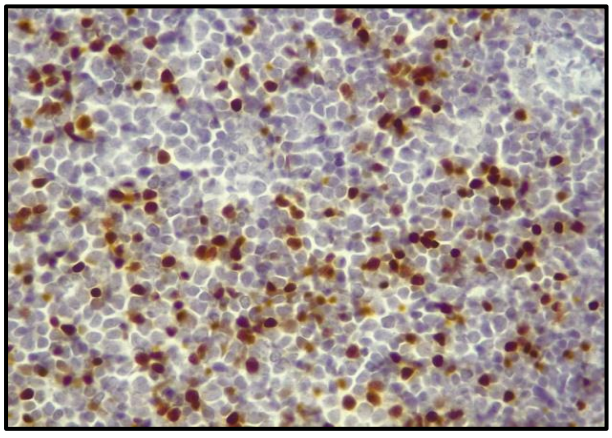

Figure (6): Lymphoblastic Lymphoma (PAX5 positive x400)

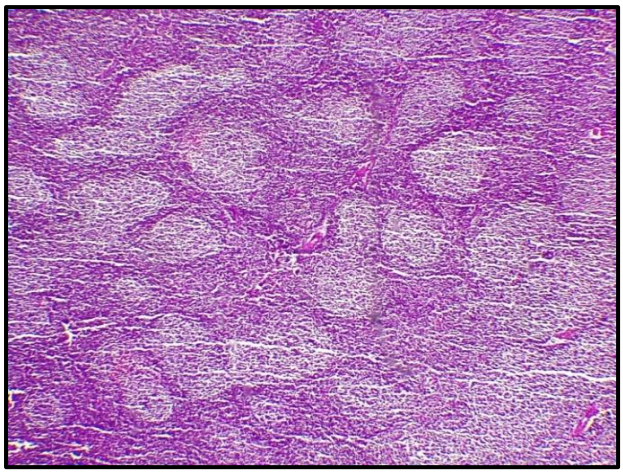

Figure (7): Follicular lymphoma (H&E x40)

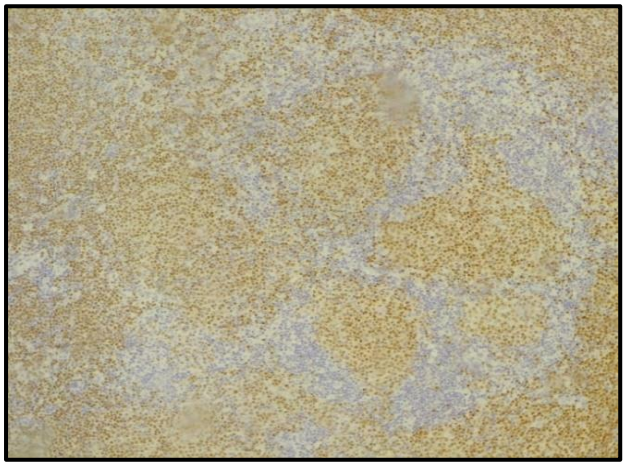

Figure (8): Follicular lymphoma (PAX5 positive x40)

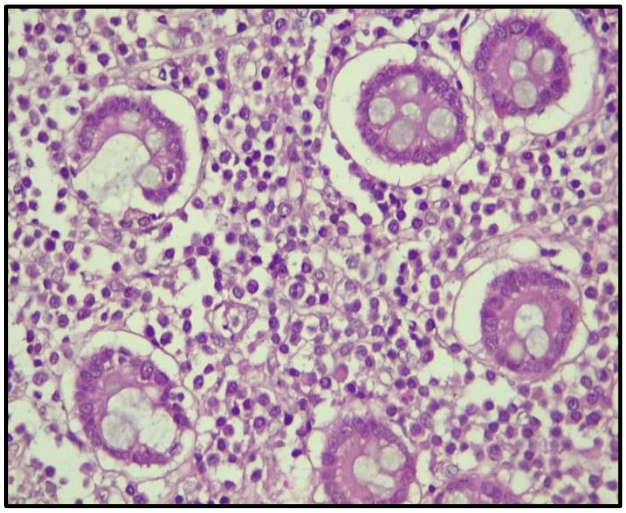

Figure (9): Marginal zone lymphoma (H&E x400)

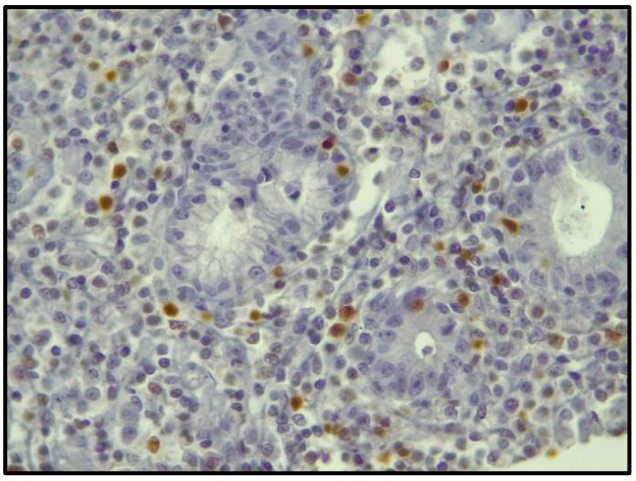

Figure (10): Marginal zone lymphoma (PAX5 positive x400)

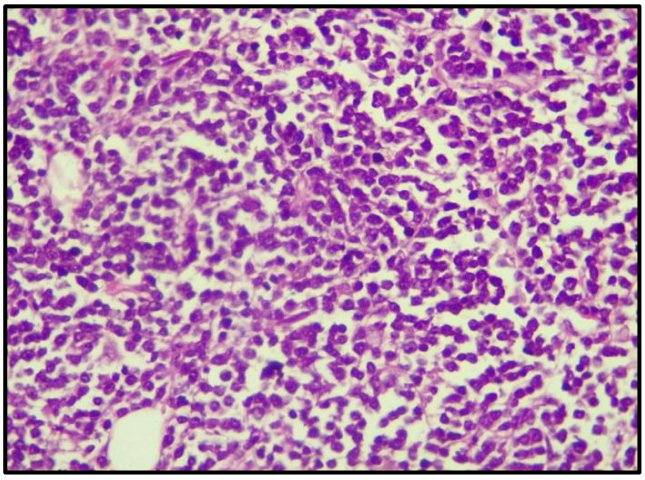

Figure (11): Burkitt lymphoma (H&E x400)

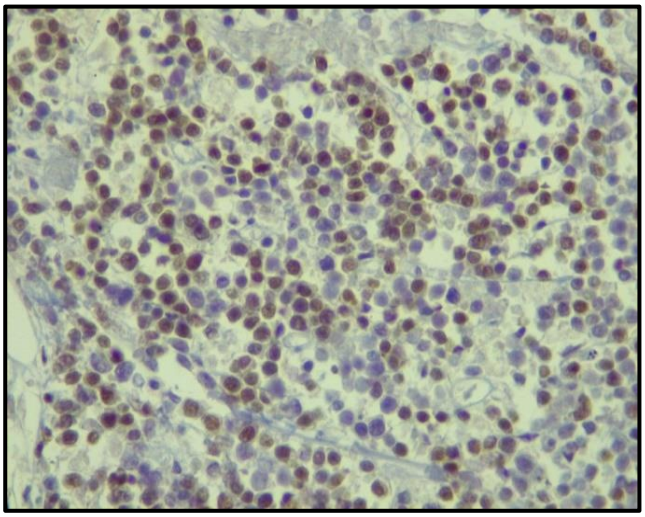

Figure (12): Burkitt lymphoma (PAX5 positive x400)

DISCUSSION

Lymphoma is a group of lymphoid neoplasm with different genetic alterations, immunophenotypes, and clinical features, The prevalence of lymphoma is less in developing countries than in developed countries. [19] Although the overall incidence is increased worldwide. [20]

HL is more prevalent cancer in the young age group [21], as seen in the North of Iraq [22] in Saudi Arabia [23] in Pakistan [24], which is in agreement with the result of this study. The high family number and early exposure to viral infection may be all attributed to the high incidence of HL in childhood [25] . However, other studies in this locality showed another peak incidence in advanced age [26] also in Kuwait [27] in China [28] and Western and developed countries [25] . In this series, a bimodal series was not apparent. The absence of age-related bimodality might contribute to the rising incidence of nodular sclerosis subtype which is more associated with unimodal distribution [29] or small sample size. Hence a furthermore extensive study is recommended.

Regarding NHL was found to be more prevalent among patients above 50 years. [23] and the rate of increasing NHL cases with age has been reduced. [30] This is in accordance with the result of this study and other studies in Saudi Arabia [23] and Turkey [30] but lesser than seen in Pakistan. [24]

We found a statistically significant association between the age of patients and PAX5 expression (P-value =0.037).

The incidence of lymphoma was higher in males than in females. This has been clearly shown in the current study and in concordance with other studies too. [23,31,32] Occupational exposure of males contributes to a high-risk factor in males more than females. [33]

In the present study, the recorded NHL cases are more than HL, which is consistent with the results of other studies conducted in the north and south of Iraq [31,34], and another study in the USA. [35]

The most frequent histological subtype of HL in the current study was nodular sclerosis (NS) (52%), which is similar to another study from Erbil city in the north of Iraq. [22] but it is different from a study in Misan city in the south of Iraq where the mixed cellularity (MC) is the highest frequency [31], also in Pakistan [24], Indonesia [36], and China [28]. This changing trend might be linked to environmental and host factors and reduced risk of EBV exposure in early childhood that is associated with MC rather than NS. [37]

Diffuse large B-cell lymphoma (DLBC) has also been reported to be the most common subtypes of NHL (42%). This finding is similar to other series from North and South of Iraq but in different proportions (52.2%) (73.3%), respectively [31,34] in addition to most studies worldwide. However, it varies considerably from region to region (33.6%) in Saudi Arabia [23] , (55.7%) in Bahrain [38] , (78.7%) in Turkey [30] , (30%) in Pakistan [24] , (25%) in US [2]. These differences might belong to different geographical regions, genetic, lifestyle, and environmental factors. [39]

All the reported cases of HL in the current study show PAX5 positivity (100%). A similar finding was noticed by other studies [40-44] and very low frequency noticed in a study done in North America [45] in Indonesia [36] and in Europe [46]. This wide range of detection may be due to the properties of different antibodies, subjectivity in interpretation, [47] also tissue fixation procedure, protocol variation in the technique of incubation antigen retrieval, as well as the number of cases studied .

Most of the studies noticed that PAX5 is expressed in B- cell lineage NHL in a different higher proportion [14,41,44] which is similar to this work, that could be attributed to different antibodies used, interpretation results, and differences in population groups.

NHL presents a wider systemic extranodal distribution than HL, and this has been observed in the current study and other studies [35,41,44] although extranodal HL can occur in few cases. [28,48]

No significant difference was noted between PAX5 expression and site in this study as well as other studies. [40,44]

CONCLUSION

PAX5 is a most sensitive and reliable B cell marker in the diagnosis of HL and NHL. It is expressed in 100% of HL cases and 77% of NHL, and there is a significant difference between PAX5 immunoreactivity with HL and NHL. There is no significant difference in using PAX5 or CD30 in HL, and there is a significant negative association between PAX5 and CD3 while it is no significant association between PAX5 and CD20 in NHL

HL was found to be more prevalent among younger age groups while NHL in older people and predominance of male gender in both of them. NS was the most common subtypes of HL, while DLBC lymphoma was the major subtypes of NHL. Lastly, there is mainly nodal involvement in lymphoma cases, and there is no difference of PAX5 immunostaining in relation to the site, but it is significant with age.

Acknowledgment

The authors are grateful to Dr. Hatem Abd-AlMajeed Al-Nuaimi a supervisor of Arab board pathology-Mosul center for his assistance in immunohistochemistry and to all members of the Department of Pathology, College of Medicine, University of Mosul for their kind cooperation.

REFERENCE

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Revised 4t. 2017. Lyon, France: IARC; 189–465 p.
2. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR. US lymphoid malignancy statistics by World Health Organization subtypes. CA Cancer J Clin. 2016; 66(6):443–59.
3. Agostinelli C, Pileri S. Pathobiology of Hodgkin Lymphoma. Mediterr J Hematol Infect Dis. 2014. 6(1):1–12.
4. Hartmann S, Eichenauer DA. Nodular lymphocyte predominant Hodgkin lymphoma: pathology, clinical course and relation to T-cell/histiocyte rich large B-cell lymphoma. Pathology. 2020; 52(1):142–53.
5. Re D, Küppers R, Diehl V. Molecular pathogenesis of Hodgkin's lymphoma. J Clin Oncol. 2005; 23(26):6379–86.
6. Armitage JO, Gascoyne RD, Lunning MA, Cavalli F. Non-Hodgkin lymphoma. Lancet [Internet]. 2017.390(10091):298–310. Available from: http://dx.doi.org/10.1016/S0140-6736(16)32407-2
7. Disanto MG, Ambrosio MR, Rocca BJ, Ibrahim HAH, Leoncini L, Naresh KN. Optimal minimal panels of immunohistochemistry for diagnosis of B-Cell lymphoma for application in countries with limited resources and for triaging cases before referral to specialist centers. Am J Clin Pathol. 2016. 145(5):687–95.
8. Gill KZ. Mantle cell lymphoma mimicking chronic lymphocytic leukemia/small lymphocytic lymphoma on flow cytometry. J Hematop [Internet]. 2020.13(1):25–31. Available from: https://doi.org/10.1007/s12308-020-00384-x
9. Krenacs L, Himmelmann AW, Quintanilla-Martinez L, Fest T, Riva A, Wellmann A, et al. Transcription factor B-cell-specific activator protein (BSAP) is differentially expressed in B cells and in subsets of B-cell lymphomas. Blood. 1998.92(4):1308–16.
10. Torlakovic E, Torlakovic G, Nguyen PL, Brunning RD, Delabie J. The value of anti-Pax-5 immunostaining in routinely fixed and paraffin-embedded sections: A novel pan pre-B and B-cell marker. Am J Surg Pathol. 2002.26(10):1343–50.
11. Adams B, Dorfler P, Aguzzi A, Kozmik Z, Urbanek P, Maurer-Fogy I, et al. Pax-5 encodes the transcription factor BSAP and is expressed in B lymphocytes , the developing CNS , and adult testis. Genes Dev. 1992. 6(9):1589–607.
12. Falini B, Mason DY. Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: Clinical value of their detection by immunocytochemistry. Blood. 2002; 99(2):409–26.
13. Lang D, Powell SK, Plummer RS, Young KP, Ruggeri BA. PAX genes: Roles in development, pathophysiology, and cancer. Biochem Pharmacol. 2007; 73(1):1–14.
14. Mhawech-Fauceglia P, Saxena R, Zhang S, Terracciano L, Sauter G, Chadhuri A, et al. Pax-5 immunoexpression in various types of benign and malignant tumours: A high-throughput tissue microarray analysis. J Clin Pathol. 2007. 60(6):709–14.
15. Nutt SL, Eberhard D, Horcher M, Rolink AG, Busslinger M. Pax5 determines the identity of B cells from the beginning to the end of B-lymphopoiesis. Int Rev Immunol [Internet]. 2001. Jan 1;20(1):65–82. Available from: https://doi.org/10.3109/08830180109056723
16. McCune RC, Syrbu SI, Vasef MA. Expression profiling of transcription factors Pax-5, Oct-1, Oct-2, BOB.1, and PU.1 in Hodgkin's and non-Hodgkin's lymphomas: a comparative study using high throughput tissue microarrays. Mod Pathol an Off J United States Can Acad Pathol Inc. Jul 2006;19(7):1010–8.
17. Chu PG, Loera S, Huang Q, Weiss LM. Lineage Determination of CD20 – B-Cell Neoplasms An Immunohistochemical Study. Am J Clin Pathol. 2006; 126:534–44.
18. O'Malley DP, Dogan A, Fedoriw Y, Medeiros LJ, Ok CY, Salama ME. American Registry of Pathology Expert Opinions: Immunohistochemical evaluation of classic Hodgkin lymphoma. Ann Diagn Pathol. 2019. 39:105–10.
19. Huh J. Epidemiologic overview of malignant lymphoma. Korean J Hematol. 2012. 47(2):92–104.
20. Borgohain M, Krishnatreya K, Weingken CK, Das JK. Diagnostic Utility of Immunohistochemistry in Lymphoma Section. Int J Contemporary Med Res. 2017. 4(12):6–9.
21. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin. 2014. 64(2):83–103.
22. Mohammedzaki LB, Hasan KM, Polus RK, Yassin AK. Clinicopathological,

immunohistochemical charachtaristic and the outcome of Hodghkin lymphoma patients in Erbil city, Iraq. Iraqi J Hematol. 2019; 8:14–20.
23. Albasri AM, El-Siddig AA, Shah A, Alhujaily AS, Hussainy AS, Alhujaily AS. Pattern of Lymph Node Pathology in Western Saudi Arabia. Asian Pacific J Cancer Prev. 2014;15(11):4677–81.
24. Shabbir S, Ahmed KN, Marri M, Jan MH, Jamali MS, Khanum S, et al. Epidemiological features of Lymphoma in Pakistan. Pure Appl Biol. 2019. 8(1):977–94.
25. Punnett A, Tsang RW, Hodgson DC. Hodgkin Lymphoma Across the Age Spectrum : Epidemiology, Therapy, and Late Effects. Radiat Oncol [Internet]. 2010. 20(1):30–44. Available from:http://dx.doi.org/10.1016/j.semradonc.2009.09.006
26. Fadhil MS, Al-Nueimy WM, Lazim AF. Hodgkin's lymphoma. An immunohistochemical profile in northern Iraq. Saudi Med J. May 2014.;35(5):448–53.
27. Al-shemmari SH, Al-humood S, Ameen R. Hodgkin's Disease : Kuwait Experience. Med Princ Pract. 2004; 13(4):201–5.
28. Shi Y, Li X. [Clinicopathologic features and association with Epstein-Barr virus infection in 235 cases of Hodgkin lymphoma from northern China]. Zhonghua bing li xue za zhi = Chinese J Pathol [Internet]. 2015. 44(2):84—89. Available from:http://europepmc.org/abstract/MED/25916637
29. Zhou L, Deng Y, Li N, Zheng Y, Tian T, Zhai Z, et al. Global, regional, and national burden of Hodgkin lymphoma from 1990 to 2017: estimates from the 2017 Global Burden of Disease study. J Hematol Oncol [Internet]. 2019.12(1):107. Available from: https://doi.org/10.1186/s13045-019-0799-1
30. Yildirim R, Sincan G. The clinicopathologic features and response to treatment of patients with Nonhodgkin Lymphoma : A single-center experiment in Turkey. Pak JMed Sci. 2019; 35(1):82–5.
31. Saadoon H, Alhilfi Q, Mansib O, Kassib K, Jihad H, Jihad I, et al. Patterns of lymphoma in Misan city , Iraq : A retrospective observational study. F1000 Research. 2019; 8(1733):8–13.
32. Khader YS, Sharkas GF, Arkoub KH, Alfaqih MA, Nimri OF, Khader AM. The Epidemiology and Trend of Cancer in Jordan , 2000 – 2013. J Cancer Epidemiol. 2018; 2018:1–7.
33. Jafari-delouei N, Naimi-tabiei M, Fazel A, Ashaari M, Hatami E. IRANIAN Descriptive Epidemiology of Lymphoma in Northern Iran : Results from the Golestan Registry 2004 – 2013. Arch Iran Med. 2020; 23(3):150–4.
34. 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. 2011; 48(4):446–51.
35. Urquhart A, Berg R. Hodgkin's and non-Hodgkin's 'lymphoma of the head and neck. Laryngoscope. 2001; 111(9):1565–9.
36. Ranuhardy D, Suzanna E, Sari RM, Hadisantoso DW, Andalucia R, Abdillah A. CD30, CD15, CD50, and PAX5 Expressions as Diagnostic Markers for Hodgkin Lymphoma (HL) and Systemic Anaplastic Large Cell Lymphoma (sALCL). Acta Med Indones. 2018; 50(2):104–9.
37. Al-Diab AI, Siddiqui N, Sogiawalla FF, Fawzy EM. The changing trends of adult Hodgkin's disease in Saudi Arabia. Saudi Med J. 2003;24(6):617–22.
38. Aljufairi EA, George SM, Alshaikh SA, Radhi AA, Mohamed RM. Spectrum of lymphoma in Bahrain. A retrospective analysis according to the World Health Organization classification. Saudi Med J. 2018; 39(7):736–9.
39. Perry AM, Diebold J, Nathwani BN, Maclennan KA, Müller-Hermelink HK, Bast M, et al. Non-hodgkin lymphoma in the developing world: Review of 4539 cases from the international Non-Hodgkin Lymphoma Classification Project. Haematologica. 2016; 101(10):1244–50.
40. Khan MR, Ahmad A, Kayani N, Minhas K. Expression of PAX-5 in B cell Hodgkin and Non Hodgkin Lymphoma. Asian Pacific J Cancer Prev. 2018; 19(12):3463–6.
41. Johri N, Patne SCU, Tewari M, Kumar M. Diagnostic utility of PAX5 in Hodgkin and non-Hodgkin lymphoma: A study from Northern India. J Clin Diagnostic Res. 2016; 10(6):4–7.
42. Morgan EA, Pozdnyakova O, Nascimento AF, Hirsch MS. PAX8 and PAX5 are differentially expressed in B-cell and T-cell lymphomas. Histopathology. 2013; 62(3):406–13.
43. Nguyen TDT, Frater JL, Klein J, Chen L, Bartlett NL, Foyil K V., et al. Expression of TIA1 and PAX5 in Classical Hodgkin Lymphoma at Initial Diagnosis May Predict Clinical Outcome. Appl Immunohistochem Mol Morphol. 2016; 24(6):383–91.
44. Madanagopaal LR, Subashchandrabose P, Sivanandam S. Analysis of PAX5 expression of Hodgkin's and non-Hodgkin's lymphomas : in comparison with routine panel. Arch Cytol Histopathol Research. 2016; 1(1):4–13.
45. Palma-Lara I, Sánchez-Aldana AE, Jiménez-Hernández E, Martínez-Villegas O, Núñez-Enríquez JC, Mejía-Aranguré JM, et al. Variable Expression of Notch1 and Pax5 in Classical Hodgkin Lymphoma and Infection with Epstein–Barr in Pediatric Patients. Microorganisms. 2020;8(6):958.

46. Adams H, Liebisch P, Schmid P, Dirnhofer S, Tzankov A. Diagnostic Utility of the B-cell Lineage Markers CD20, CD79a, PAX5, and CD19 in Paraffin-embedded Tissues From Lymphoid Neoplasms. Appl Immunohistochem Mol Morphol. 2009; 17(2):96–101.
47. Feldman AL, Dogan A. Diagnostic uses of Pax5 immunohistochemistry. Adv Anat Pathol. 2007;14(5):323–34.
48. Zapater E, Bagán J V., Carbonell F, Basterra J. Malignant lymphoma of the head and neck. 2010; Oral Dis. 16(2):119–28.