



Case-controlled Study

Immunohistochemical expression of transcription factors PAX5, OCT2, BCL6 and transcription regulator P53 in Non-Hodgkin lymphomas: A diagnostic cross-sectional study

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ABSTRACT

Background: Non-Hodgkin lymphoma represents a heterogeneous group of tumors that constitute the seventh most common malignancy. Immunohistochemistry plays a major role in the detection of specific cell receptors. Transcription factors are a heterogeneous group of genes that play a critical role in the commitment, differentiation, and proliferation of specific cell types.

Methods: Paraffin-embedded tissue sections of non-Hodgkin lymphoma cases were selected, classified, and evaluated before staining with immunohistochemical markers (PAX5, OCT2, BCL6, and P53). Expression of the aforementioned markers was compared with histological subtypes and grades of lymphoma cases. Means of expression were also compared among histological subtypes.

Results: A total of 55 cases of NHL including 26 cases of low-grade lymphomas and 29 cases of high-grade lymphomas were included in the study. DLBCL and FL were the most common subtypes of high-grade and low-grade lymphomas respectively. Both PAX5 and OCT2 were positive in 44 cases of NHL (80%) including all cases of B-cell lymphomas. BCL6 and P53 demonstrated positive expression in 29.1% and 67.3% respectively. Interestingly, we found a significant association between the histological subtypes and the aforementioned markers (P-value<0.05).

Discussion: Expression of PAX5, OCT2, BCL6, and P53 played a major role in the diagnosis and grading of non-Hodgkin lymphomas in our study. Both PAX5 and OCT2 provided more accuracy and specificity in the diagnosis of B-cell neoplasms compared to the classical B-cell markers. BCL6 expression reflected its role in germinal center formation in normal and malignant lymphoid tissues, and expression of P53 mirrored the accumulation of gene mutations in more aggressive lymphoma subtypes.

Conclusion: In this manuscript, we aimed to present a unique study that highlights the immunohistochemical expression of all the aforementioned factors among various histological subtypes of non-Hodgkin lymphomas with disparities in histological aggressiveness, highlighting a promising diagnostic and prognostic panel for non-Hodgkin lymphomas.

1. Introduction

Non-Hodgkin lymphoma represents a heterogeneous group of tumors that constitute the seventh most common malignancy and the eighth leading cause of cancer mortality. NHL results from the malignant proliferation of either B-lymphocytes (in more than 85% of cases) or T-lymphocytes and natural killer cells (in less than 15% of cases). More than fifty histological subtypes of NHL were identified in 2016 WHO

classification of lymphoid malignancies, with DLBCL representing the more common subtype, as the classification of NHL revealed a significant enhancement in the past few decades through detailed clinical, morphological, immunohistochemical, and molecular correlations. Immunohistochemistry plays a major role in the diagnosis and management through the detection of specific cell receptors that could represent promising diagnostic markers and therapeutic targets [1–3].

On the other hand, it has been well demonstrated that NHL subtypes are associated with disparities in histological aggressiveness, speed of

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List of abbreviations

NHL	Non-Hodgkin Lymphoma
DLBCL	Diffuse Large B-Cell Lymphoma
ALCL	Anaplastic Large Cell Lymphoma
MZL	Marginal Zone Lymphoma
FL	Follicular Lymphoma
SLL	Small Lymphocytic Lymphoma
B-LBL	B-cell Lymphoblastic Lymphoma
T-LBL	T-cell Lymphoblastic Lymphoma

cell proliferation, and response to treatment. Studies identified two distinct categories: aggressive or high-grade lymphomas including diffuse large B-cell lymphoma DLBCL and lymphoblastic lymphoma, and indolent or low-grade lymphomas including follicular lymphoma FL, small lymphocytic lymphoma SLL, and marginal zone lymphoma MZL. Interestingly, although aggressive lymphomas require more intensive treatments and are associated with earlier mortality rates due to rapid proliferation, studies demonstrated that they are more likely to demonstrate a complete response to treatment. In contrast, indolent lymphomas are associated with more recurrences and incomplete remission. This highlights the crucial need to investigate the potential causes of disparities in histological aggressiveness [3–6].

Transcription factors are a heterogeneous group of genes that regulate the development of B-lymphoid cells from hematopoietic stem cells in a multi-check-point sequential process. These factors play a critical role in the initial commitment of B lineage in addition to differentiation and immunoglobulin production. Therefore, several studies raised the need to consider transcription factors in the immunohistochemical and molecular diagnostic panel of lymphoid malignancies as both potential lineage determinators and prognosis detectors.

The maturation stage of B-cells might be assessed through the expression pattern of transcription factors. Furthermore, transcription factors expression could demonstrate genetic mutations in lymphoid neoplasms, and serve as prognostic markers [7,8].

PAX-5 is a nuclear transcription factor that is encoded by a paired box domain gene on chromosome 9p13 and belongs to the PAX gene family of transcription factors. PAX5 is also known as the B-cell specific activator protein BSAP and is essential for the differentiation, development, and proliferation of B-cells. Pax5 works by activating the transcription of pre-B-cell receptor signaling pathway proteins BLNK (SLP-65), IgA (CD79a,mb1), and CD19, leading to the enhancement of B-cell differentiation. Also, it plays a major role in the formation of the pre-BCR complex and regulating IgH and IgL gene rearrangements [9–12]. Interestingly, Pax5 gene inactivation results in the dedifferentiation of mature B cells to progenitor cells in the bone marrow, which subsequently differentiate into activated T cells. Therefore, PAX5 has widely emerged as a highly sensitive and specific marker for normal and malignant B-cell detection [13].

Octamer-binding protein 2 (OCT2) is a member of the POU domain family of DNA binding proteins and is widely known as a B-cell-restricted transcription factor. OCT2 is encoded by the gene POU2F2 and works by binding to an octamer DNA motif which is a conserved DNA sequence located on the promoters and enhancers of immunoglobulin encoding genes. OCT2 is essential for post-natal survival and maturation of B cells and plays a major role in immunoglobulin production and germinal center formation. Due to its high expression in immature and mature B cells, it's well-recognized as a sensitive marker similar to Pax5 for detecting B-cell lineage in controversial cases [14–16].

BCL6 is a transcription repressor that is encoded by the BCL6 gene located on chromosome 3q27. BCL6 plays a major role in the formation of germinal centers in normal and malignant lymphoid tissue mainly

DLBCL and BL. It's considered a proto-oncogene that interacts with corepressors to regulate gene expression through inducing heterochromatin formation and epigenetic remodeling. Due to the repression of genes involved in cell cycle arrest and apoptosis mainly P53, BCL6 represents a major transcription repressor [17,18].

P53 is a transcriptional regulator that is encoded by the P53 gene on chromosome 17p and plays a critical role in suppressing malignant transformation through inducing apoptosis and cell cycle arrest. Mutations of the P53 gene are considered one of the most important molecular events in promoting malignant transformation and are found in 25–30% of NHL. Also, P53 expression is frequently detected in high-grade NHL [19–22].

Herein, we aim to investigate the immunohistochemical expression of the aforementioned markers in several cases of indolent and aggressive non-Hodgkin lymphomas.

2. Methods

2.1. Cases selection

This is a retrospective cross-sectional study that was performed on paraffin-embedded archival blocks in the department of pathology at Tishreen University. Ethical approval was obtained from the Research Ethics Committee of Tishreen University in accordance with the Declaration of Helsinki. The research was reported in line with the STROCSS criteria [23]. Paraffin-embedded blocks from fifty-five cases of non-Hodgkin lymphomas were retrieved from the archive of the pathology department. Demographic data of the cases including age, gender, and site were recorded. The cases were previously immunostained with the classical markers of non-Hodgkin lymphoma including CD45, CD20, CD19, CD79a, CD3, CD5, CD23, CD10, CD15, CD30, ALK-1, BCL2, TDT, MPO, PAS, and Cyclin D1 to diagnose and subtype the lymphomas according to the WHO classification of lymphoid malignancies.

After reviewing the morphological and immunohistochemical characters of the selected cases by two pathologists (ZA and AD), one representative section for each case was selected to perform the study markers (PAX5- OCT2- P53- BCL6). Appropriate tissue sections from tonsils and reactive lymph nodes were used as positive controls, whereas processed sections without primary antibodies were used as negative controls.

2.2. Immunohistochemistry

4 µm sections were cut and attached to charged slides. The sections were then deparaffinized in xylene and rehydrated in three gradually decreased grades of alcohol followed by water. After that, the slides were put in EDTA solution in a microwave oven for 15 min for antigen retrieval. After endogenous peroxidase blockage, the sections were stained with the primary antibodies (PAX5: Clone RBT-Pax5, OCT2: Clone EP115, BCL6: Clone RBT-BCL6, P53: Clone DO7- Company BioSB) and incubated for 45 min. Later, slides were rinsed with wash buffer for 5 min and incubated with the secondary antibody (Mouse poly-detector HRP LABEL, BIOSP) for 45 min. After that, the sections were incubated for 10 min with poly-detector chromogen before rinsing and counterstaining with hematoxylin and eosin. Representative immunohistochemical images are demonstrated in Figs. 1-4. The intensity was considered either positive or negative, and expression was considered positive if more than 10% of the cells demonstrated positive nuclear staining.

2.3. Statistical analysis

This is a retrospective cross-sectional study. SPSS Program (version 26) was used for the statistical analysis. Independent Samples T-Test and One-way Anova were used to compare parametric variables whereas

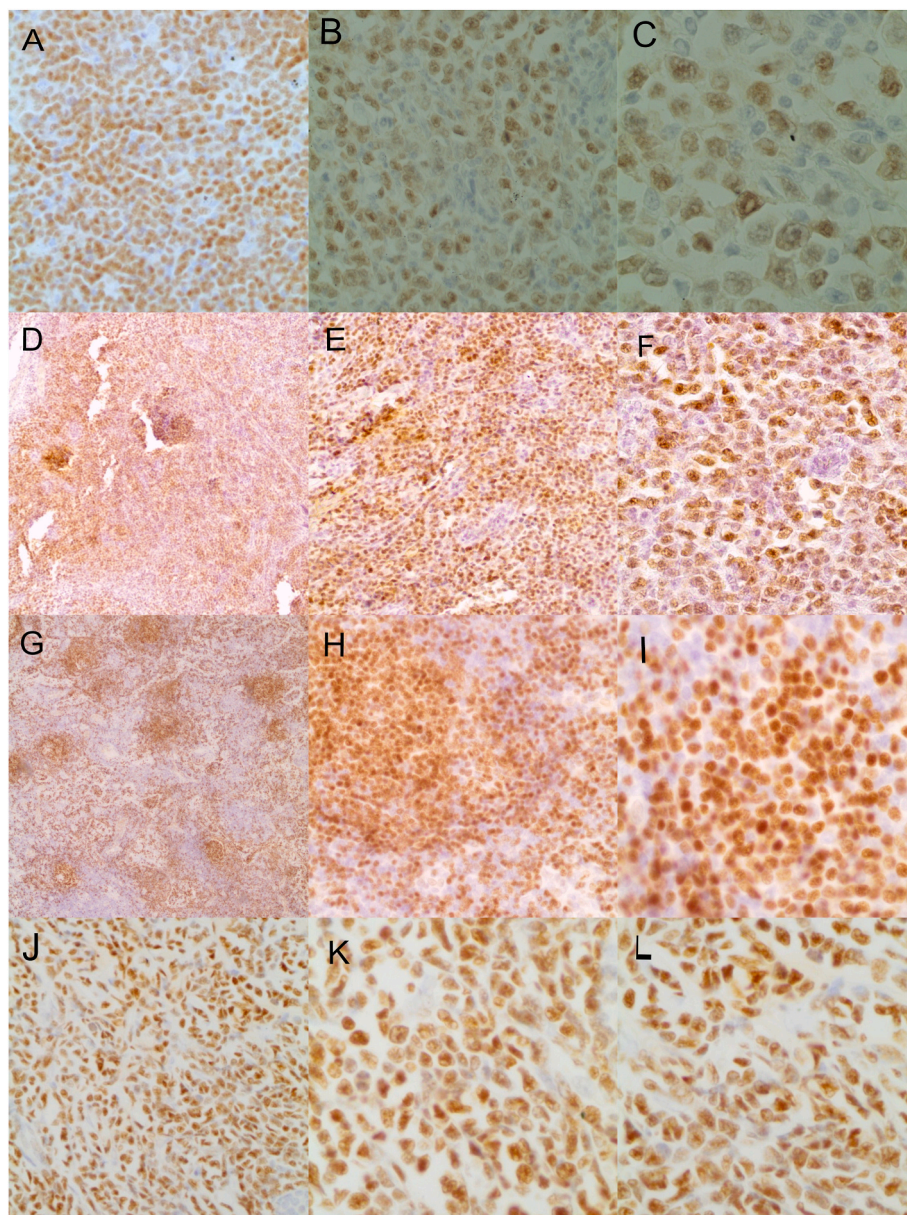


Fig. 1. Immunohistochemical expression of PAX5 in cases of NHL: (A,B,C: expression in DLBCL, D,E,F: expression in MZL, G,H,I: expression in FL, J,K,L: expression in B-LBL).

Fisher's exact test was used to compare nonparametric variables. *P-value* <0.05 was considered significant.

3. Results

A total of fifty-five cases of non-Hodgkin lymphomas were selected in the study. The age of the patients at presentation ranged from 6 to 85 years, with a mean age of 46.29. Male to female ratio was 2:1 (36 males and 19 females). Regarding the primary site of the lymphomas: 38 cases were nodal (69.1%), whereas 17 cases (30.9%) were primarily diagnosed in extra-nodal sites (Table 1).

All the cases were histologically diagnosed by two pathologists (AD and ZA), and confirmed by a third pathologist (YE) according to the WHO classification of lymphoid malignancies. All cases were previously stained with the classical markers of non-Hodgkin lymphomas to subtype the lymphomas. The cases included 29 cases of high-grade lymphomas (52.7%) and 26 cases of low-grade lymphomas (47.3%). The selected cases were diagnosed as follows: 16 cases of diffuse large B-cell

lymphoma (DLBCL 29.1%), 9 cases of anaplastic large cell lymphoma (ALCL 16.4%), 10 cases of follicular lymphoma (FL 18.2%), 9 cases of small lymphocytic lymphoma (SLL 16.4%), 7 cases of marginal zone lymphoma (MZL 12.7%), 2 cases of T-cell lymphoblastic lymphoma (T-LBL 3.6%), and 2 cases of B-cell lymphoblastic lymphoma (B-LBL 3.6%) characterized by the malignant proliferation of lymphoblasts in lymph nodes with positive expression of TDT, CD19, and CD79a.

3.1. Immunohistochemical results

Fifty-five cases were stained with the study immunohistochemical markers (PAX5, OCT2, BCL6, P53). The intensity was considered either positive or negative, and expression was considered positive if more than 10% of the cells demonstrated positive nuclear staining according to a previous study [31]. All cases were reviewed by two pathologists (ZA and AD) and confirmed by a third consultant pathologist (YE). All the positive cases demonstrated nuclear staining for the markers (Pax5, OCT2, BCL6, P53)

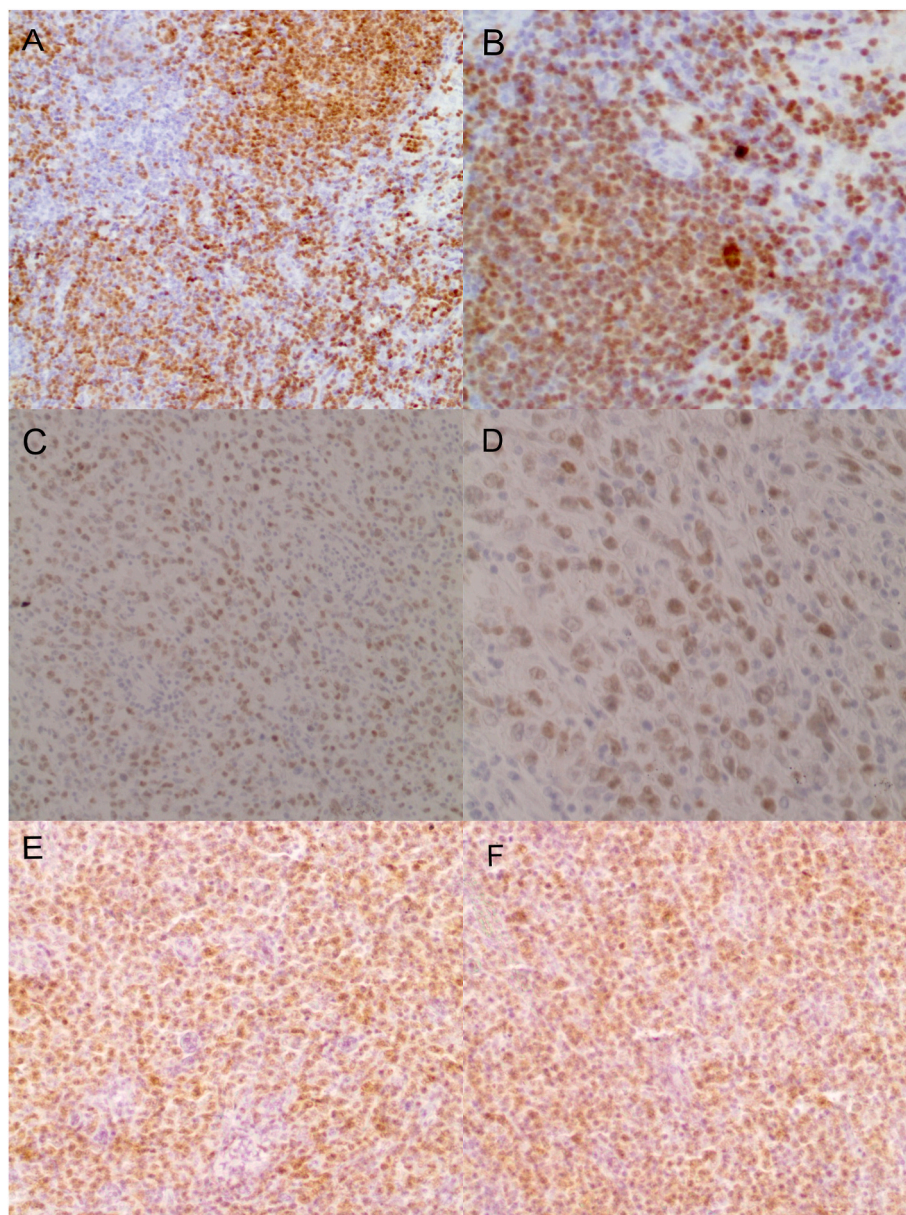


Fig. 2. Immunohistochemical expression of OCT2 in cases of NHL: (A,B: expression in FL, C,D: expression in DLBCL, E,F: expression in MZL).

For Pax5: Forty-four cases of NHLs (80%) demonstrated diffuse positive expression. All the positive cases were B-cell non-Hodgkin lymphomas, whereas 11 cases of T-cell NHLs (20%) including ALCL and T-LBL were negative. For positive cases, positive expression ranged from 40 to 95% with a mean value of 77.61%. 32 cases (58.2%) demonstrated positive expression in more than 75% of cells, whereas 10 cases (18.2%) revealed positive expression in 50–75% of cells.

For OCT2: Similar to Pax5, all cases of B-cell NHLs ($n = 44$, 80%) revealed diffuse positive expression of OCT2, whereas 11 cases of T-cell NHLs (20%) including ALCL and T-LBL were negative. For positive cases, the mean of expression was 64.43% and frequency ranged from 25 to 90%. 17 cases (30.9%) demonstrated positivity in more than 75% of cells, whereas 22 cases (40%) demonstrated positive expression in (50–75%) of cells.

BCL6 and P53 were positive in 16 cases (29,1%) and 37 cases (67.3%) respectively. The frequency of BCL6 expression ranged from 20 to 70% (Mean value 45.93%) and was mainly concentrated in follicular centers, and for P53, expression ranged from 10 to 90% (Mean value 29.1%) in a scattered pattern.

3.2. Statistical correlation

Both Pax5 and OCT2 were positive in all cases of DLBCL, FL, SLL, MZL, and B-LBL with a total of 44 positive cases (80%) for both stains, whereas they were negative in all cases of ALCL and T-LBL ($n = 11$, 20% of cases) (P -value: $0.000 < 0.05$). Upon comparing with histological grading, positive expression was found in 18 cases of high-grade lymphomas (62.06%) and 100% of low-grade lymphomas. (P -value: 0.00). Both stains were strictly expressed in B-cell lymphomas. PAX5 demonstrated stronger expression (Mean Value of positive cases 77.61%) than OCT2 (Mean Value of positive cases 64.43%).

Regarding BCL6: We found that BCL6 was positive in 6/16 cases of DLBCL, 4/9 cases of ALCL, and 6/10 cases of FL. There was no significant association between BCL6 and histological grading (P -value: 0.929), whereas there was a significant association between BCL6 and diagnosis (P -value: 0.005). Positive expression was highly demonstrated in follicular centers.

For P53: Our study found revealed that P53 was positive in 20/29 cases of high-grade lymphomas and 17/26 cases of low-grade

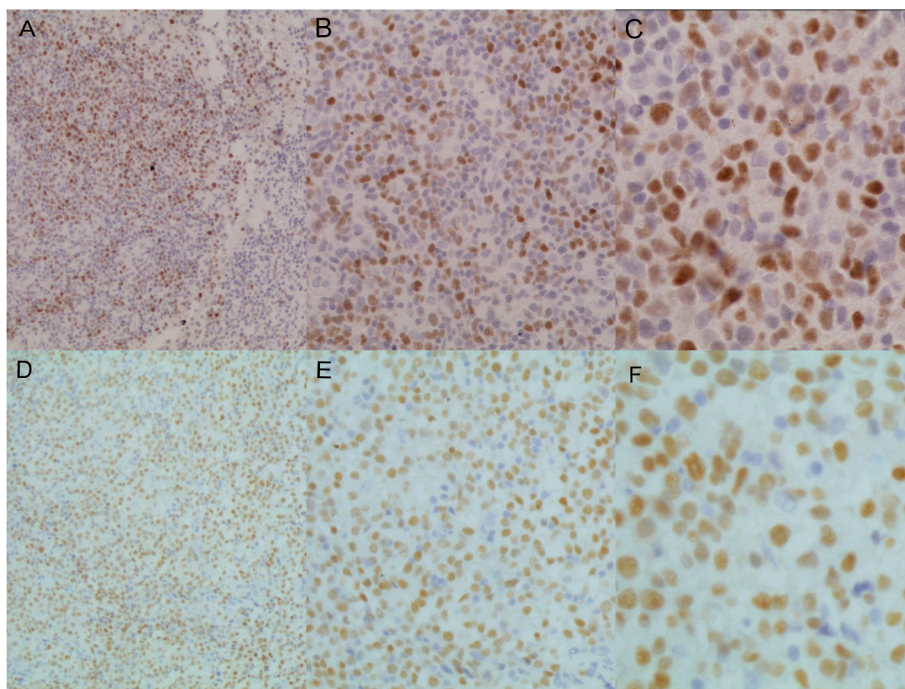


Fig. 3. Immunohistochemical expression of BCL6 in cases of FL and DLBCL.

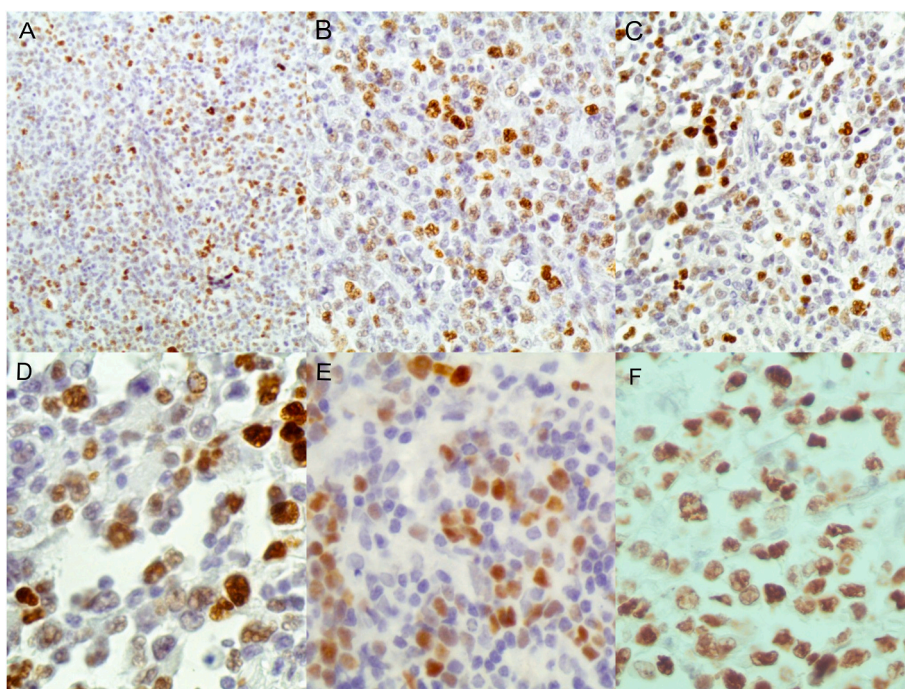


Fig. 4. Immunohistochemical expression of P53.

lymphomas in a scattered pattern. There was a significant association between P53 expression and diagnosis (P -value: 0.033). There was a significant association between P53 and grade (P -value: 0.016). Fig. 5 demonstrates the frequency of positive and negative cases among histological diagnoses.

We also aimed to compare the percentage of positive cells among diagnoses (Table 2). For Pax5: the strongest expression was found in MZL (Mean 83.57%) followed by FL and SLL (Means 80.5% and 80% respectively). Regarding OCT2: the highest positive expression was detected in FL.

BCL6 was negative in all cases of SLL, MZL, and LBL, whereas the highest positive expression was found in follicular lymphoma (Mean 35%). And finally, the highest expression of P53 was detected in cases of B-cell lymphoblastic lymphoma and DLBCL, whereas the lowest expression was demonstrated in FL (Mean 10%).

4. Discussion

This is a unique study that investigates the immunohistochemical expression of Pax5, OCT2, and BCL6 together with P53 in various

Table 1
Distribution of Primary sites of cases.

Primary Site of Lymphoma		Frequency	Percent
Nodal	Cervical LN	14	25.5
	Axillary LN	10	18.2
	Inguinal LN	10	18.2
	Mediastinal LN	1	1.8
	Periaortic LN	1	1.8
	Retroperitoneal LN	2	3.6
Extra-nodal	Gastric Mucosa	7	12.7
	Spleen	5	9.1
	Testicle	2	3.6
	Mandibular mass	1	1.8
	Breast	1	1.8
	Skin	1	1.8
	Total	55	100.0

subtypes of low-grade and high-grade non-Hodgkin lymphoma. In our study, we aimed to evaluate the promising role of the aforementioned markers in innovating a new diagnostic and classification panel for non-Hodgkin lymphomas.

The most common subtype of NHL in our study was DLBCL (29.1%), whereas FL was the most common type of low-grade NHL and the second most common type of all NHL cases (18.2%), similar to previous studies highlighting that DLBCL and FL represent the most common subtypes of high-grade and low-grade non-Hodgkin lymphomas in general. The male to female ratio was 2:1 which is supported by previous studies that highlight a male predominance in lymphoid malignancies [24,25].

PAX5 is a nuclear transcription factor that is highly essential for the proliferation and differentiation of B cells, and it's considered a B-cell lineage marker. Pax5 possesses dual opposite molecular functions regarding B cell development. On one hand, it's considered a transcription factor activator of B-lineage required genes by inducing the active chromatin at target loci. On the other hand, it works as a transcription repressor of other B-cell inappropriate genes [26,27]. In our study, all cases of B-cell NHL demonstrated positive expression of PAX5, whereas it was negative in all cases of T-cell lymphomas (ALCL and T-LBL). Before this study, the diagnosis of B-cell lymphomas at our institution was built based on the classical B-cell markers (CD19, CD20, and CD79a). Nevertheless, it's now well-known that the aforementioned

markers lack specificity and sensitivity compared to PAX5. Several studies demonstrated that CD20 is expressed in mature B cells and late precursor B cells, whereas it's negative in B-cell lymphomas treated with rituximab. Also, CD79a demonstrated positive expression in cases of T-cell lymphoblastic lymphoma, and plasma cell neoplasms. And regarding CD19, despite its high specificity for B-cell lineage, it's mostly recommended to be analyzed by flow cytometry with fresh cells [28–30].

On the other hand, the strong nuclear expression of PAX5 in all developmental stages of B-cells makes it the most reliable and sensitive immunohistochemical marker in the differential diagnosis of B-cell NHLs and CHLs. And the strong expression of PAX5 in pre-B cells could provide more details regarding earlier stages of B-cell development. In our study, PAX5 was positive in 100% of B-cell NHL cases. In a study by Johri et al., PAX5 was positive in 80% of B-cell NHLs. Also, Khan et al. and Dong et al. found positive expression of PAX5 in approximately all cases of B-cell NHL cases. PAX5 is also extremely useful in excluding plasma cell neoplasms, T-cell lymphomas including ALCL, and epithelial and mesenchymal tumors that might demonstrate positive expression for other B-cell markers [28,29,31]. All the aforementioned factors supported the major role of PAX5 in cell lineage diagnosis in our study.

Furthermore, we aimed to compare the expression rates among histological subtypes. In our study, MZL demonstrated the strongest expression of PAX5 (Mean 83.57%) followed by FL and SLL (Means 80.5% and 80.0% respectively). All the aforementioned subtypes are considered low-grade lymphomas. These results could be explained by the high concentration of PAX5-positive B cells in mantle zone cells and follicles in normal lymphoid tissues, and therefore, the strongest expression of PAX5 is mostly detected in lymphomas derived from mantle zone and germinal center cells in earlier stages of malignant transformation [31,32]. Furthermore, PAX5 demonstrated diffuse expression in DLBCL, FL, SLL, and B-LBL, whereas it demonstrated a higher frequency in marginal zone cells in cases of MZL. In addition, our study demonstrated that PAX5 could represent a promising prognostic marker as it was more expressed in low-grade lymphomas compared to the more aggressive lymphomas.

Another transcription factor was included in our study for the detection of B-cell lymphomas. Oct2 or Octamer-binding protein 2 is a B-cell transcription factor that is restricted to and detected in all

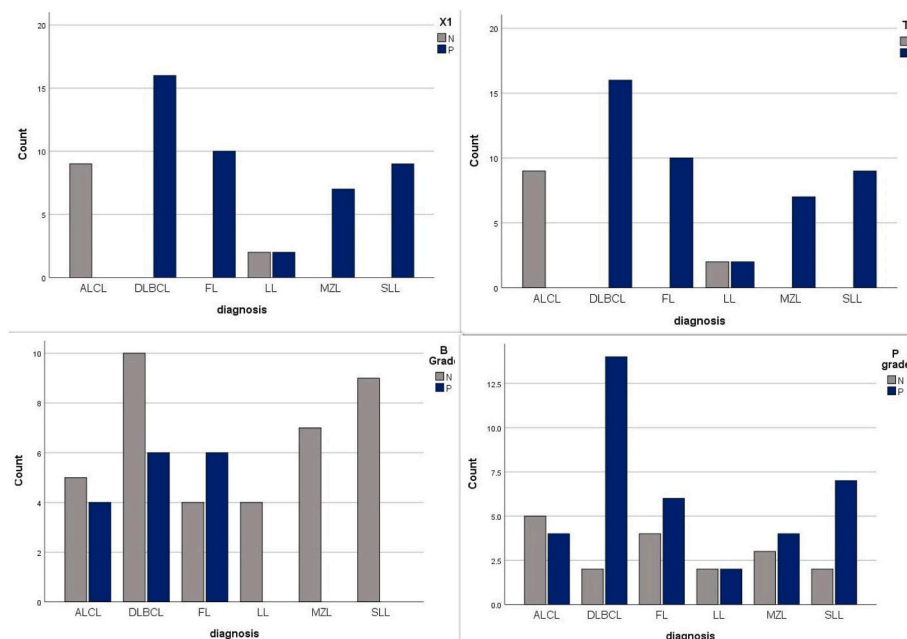


Fig. 5. Charts demonstrating positive and negative cases among diagnoses (A: PAX5 distribution among diagnoses, B: OCT2 distribution among diagnoses, C: BCL6 distribution among diagnoses, D: P53 distribution among diagnoses).

Table 2
Means of expression among histological subtypes.

diagnosis		PAX5	OCT2	BCL6	P53
ALCL	Mean	0.0000%	0.0000%	18.3333%	14.1667%
	Std. Deviation	0.0000%	0.0000%	22.63846%	15.94261%
	Minimum	0.00%	0.00%	0.00%	0.00%
	Maximum	0.00%	0.00%	50.00%	40.00%
DLBCL	Mean	71.2500%	60.9375%	13.4375%	37.6923%
	Std. Deviation	16.17611%	20.59278%	19.97655%	26.50593%
	Minimum	40.00%	25.00%	0.00%	0.00%
	Maximum	90.00%	90.00%	60.00%	90.00%
FL	Mean	80.5000%	70.0000%	35.5000%	10.0000%
	Std. Deviation	11.41393%	16.32993%	31.48633%	4.08248%
	Minimum	55.00%	30.00%	0.00%	5.00%
	Maximum	95.00%	85.00%	70.00%	15.00%
B-LBL	Mean	75.0000%	67.5000%	0.0000%	45.0000%
	Std. Deviation	7.07107%	3.53553%	0.00000%	0.00000%
	Minimum	70.00%	65.00%	0.00%	45.00%
	Maximum	80.00%	70.00%	0.00%	45.00%
MZL	Mean	83.5714%	66.4286%	0.0000%	12.5000%
	Std. Deviation	8.01784%	10.69045%	0.00000%	13.32291%
	Minimum	70.00%	50.00%	0.00%	0.00%
	Maximum	95.00%	80.00%	0.00%	35.00%
SLL	Mean	80.0000%	60.5556%	0.0000%	18.0000%
	Std. Deviation	6.61438%	7.68295%	0.00000%	2.73861%
	Minimum	70.00%	50.00%	0.00%	15.00%
	Maximum	90.00%	70.00%	0.00%	20.00%
T-LBL	Mean	0.0000%	0.0000%	0.0000%	0.0000%
	Std. Deviation	0.00000%	0.00000%	0.00000%	0.00000%
	Minimum	0.00%	0.00%	0.00%	0.00%
	Maximum	0.00%	0.00%	0.00%	0.00%

developmental stages of B cells. OCT2 is essential for post-natal survival and maturation of B cells and plays a major role in immunoglobulin production and germinal center formation. Due to its high expression in immature and mature B cells, it's well-recognized as a highly sensitive marker similar to Pax5 for detecting B-cell lineage in controversial cases [33].

Yin et al. suggested using OCT2 as a definite B-cell lineage identifier in cases that reveal morphological characteristics of B-cell lymphomas with negative expression of the conventional markers CD20, and CD79a. The study highlighted a possible role of OCT2 in replacing molecular techniques in controversial cases including ALK-positive large B-cell lymphoma (ALK + LBCL), plasmablastic lymphoma (PBL), primary effusion lymphoma (PEL), the solid variant of PEL, HHV8-positive large B-cell lymphoma (HHV8 + LBCL), and B-cell lymphomas treated with Rituximab that demonstrate negative expression of the classical B-cell markers which might lead to a delayed inaccurate diagnosis [34]. In our study, OCT2 demonstrated strong nuclear expression in all cases of B-cell non-Hodgkin lymphomas whereas it was negative in ALCL and T-LBL cases. The highest expression was detected in FL and B-LBL in a diffuse pattern (Means 70% and 67.5% respectively).

Upon comparing both PAX5 and OCT2 with histological grading, positive expression was found in 18 cases of high-grade lymphomas (62.06%) and 100% of low-grade lymphomas. (*P-value*: 0.00). Both stains were strictly expressed in B-cell lymphomas. PAX5 demonstrated slightly stronger expression (Mean Value of positive cases 77.61%) than OCT2 (Mean Value of positive cases 64.43%). This limited difference could be possibly related to the OCT2 clone that was used in our study as it was also demonstrated in positive control tissues and reviewed by three expert pathologists. Nevertheless, due to the limited number of cases in our study and the inability to perform further molecular and ancillary techniques due to economic restrictions, investigation of the aforementioned difference requires further studies.

BCL6 is a nuclear transcriptional repressor of apoptotic and cell cycle arrest genes including P53. BCL6 protein is highly expressed in germinal center B-cells and their counterparts in lymphoid malignancies mainly FL and DLBCL. Few studies also demonstrated lower expression in several cases of ALCL [35,36]. In our study, BCL6 was positive in 16 cases of NHLs (28.1%) categorized into 6/16 cases of DLBCL (37.5% of DLBCL), and 4/9 (44.4%) cases of ALCL, and 6/10 (60%) cases of FL mainly in follicular centers. Whereas all cases of SLL, MZL, and lymphoblastic lymphomas demonstrated negative expression.

Furthermore, BCL6 is the most prominent somatic alteration in DLBCL. It's required for the regulation and development of germinal centers and is associated with a better prognosis in the GCB-like subtype, whereas in the post-GCB subtype it demonstrated distinct translocations that correlate with a worse prognosis [37]. Furthermore, in FL, t(14;18) (q32;q21) represents the most common translocation, whereas BCL6 mutations reflect a worse prognosis and a more aggressive course. On the other hand, MZL is associated with t(11;18)(q21;q21), 6q23 deletion, and NOTCH2 mutations [3].

In our study, there was no significant association between BCL6 and histological grading (*P-value*: 0.929), whereas there was a significant association between BCL6 and diagnosis (*P-value*: 0.005), and the highest positive expression was found in follicular lymphoma (Mean 35%). In control tissues, BCL6 expression was restricted to germinal centers, similar to studies demonstrating the distribution of BCL6 positive cells in normal and malignant tissue [35,38]. On the molecular level, BCL6 mutations in FL reflect a tendency to transform into a high-grade lymphoma. Therefore, the high expression of BCL6 in FL could demonstrate a worse prognosis in our study.

P53 is a transcriptional regulator that plays a critical role in inducing apoptosis and cell cycle arrest. Mutations of the P53 gene reflected by P53 immunohistochemical expression are crucial for promoting malignant transformation and are found in 25–30% of NHL. In a study by Hussein et al., high P53 expression was detected in approximately 25–30% of high-grade lymphomas, whereas lower expression correlated with a lower grade. Furthermore, additional studies demonstrated that low expression of P53 was detected in low-grade lymphomas whereas higher overexpression was detected in more aggressive subtypes including DLBCL and BL, and reflected a worse prognosis [39–41]. In our study, P53 revealed a scattered diffuse pattern of positivity in 37 cases of NHLs (67.3%) including 20/29 cases of high-grade lymphomas and 17/26 cases of low-grade lymphomas. And similar to previous studies, there was a significant association between P53 expression and histological grade (*P-value*: 0.016). Also, a significant association was found between P53 and diagnosis (*P-value*: 0.033 < 0.05). The positive expression of the monoclonal antibody of P53 in our study reflected a mutational P53 gene in high-grade neoplasms. The highest expression of P53 was detected in cases of B-cell lymphoblastic lymphoma (Mean 45%), whereas the lowest expression was demonstrated in FL (Mean 10%).

Limitations of the study included the absence of molecular techniques due to economic restrictions. Nevertheless, although the molecular study would have provided more accuracy regarding the diagnosis, we managed to challenge these circumstances through utilizing a wide panel of immunohistochemical markers in our study in addition to the confirmation and detailed reviewing of morphological and immunohistochemical features by three expert pathologists. Furthermore, we managed to compare the immunohistochemical markers between low-grade and high-grade NHL subtypes, highlighting the importance of histopathological and immunohistochemical correlation.

5. Conclusion

In conclusion, immunohistochemical detection of PAX5, OCT2, BCL6, and P53 played a major role in the diagnosis, classification, and grading of non-Hodgkin lymphomas in our study. Both PAX5 and OCT2 provided more accuracy and specificity in the diagnosis of B-cell neoplasms

compared to the classical B-cell markers. Immunohistochemical staining of BCL6 reflected its role in germinal center formation in normal and malignant lymphoid tissues, and expression of P53 mirrored the accumulation of gene mutations in more aggressive lymphoma subtypes. In this manuscript, we aimed to present a unique study that highlights the immunohistochemical expression of all the aforementioned factors among various histological subtypes of non-Hodgkin lymphomas, highlighting a promising diagnostic and prognostic panel for non-Hodgkin lymphoma subtypes in the absence of molecular techniques.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Conflicts of interest

None declared.

Sources of funding

None declared. The research didn't receive any funding.

Ethical Approval

Ethical Approval was obtained from the Research Ethics Committee of Tishreen University in accordance with the Declaration of Helsinki.

Consent

Not Applicable.

Author contribution

SI: Drafted the manuscript and participated in the pathologic and immunohistochemical examination. YE: Reviewed the cases, confirmed the histopathological examination and immunohistochemical results, and participated in drafting the article. AD: Performed immunohistochemical examination and participated in the histopathological examination and in drafting the manuscript. ZA: performed histopathological and immunohistochemical examination and participated in drafting the manuscript. All authors have read and approved the final manuscript.

Registration of research studies

Registration of research studies: The research is a cross-sectional study on archival paraffin blocks that are stored at the department of pathology at Tishreen University for research purposes and does not involve human participants or direct interventions on humans. The protocol was approved and registered by the Research Ethics Committee at Tishreen University.

Guarantor

Dr. Zuheir Alshehabi.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2022.103786>.

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