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Original Article / Orijinal Araştırma

Immunohistochemical Expression of B Cell Transcription Factors in Hodgkin's Lymphoma and Their Use in Differential Diagnosis

Hodgkin Lenfomada B Hücre Transkripsiyon Faktörlerinin İmmünohistokimyasal İfadesi ve Ayırıcı Tanıda Kullanımı

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Abstract

Aim: Classical Hodgkin lymphoma is common; it is one of the lymphomas whose differential diagnosis can be difficult. It is thought that Hodgkin's cell may originate from the germinal centre. Our aim in this study was to determine the germinal centre transformation markers OCT-2, BOB.1, BCL-6, PAX-5, CD20 and MUM-1 in Classic Hodgkin Lymphoma (CHL), Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) and Diffuse Large B-cell Lymphoma (DLBCL) to evaluate the expressions of by immunohistochemical method and chromogenic in-situ hybridization (ISH) of EBV early RNAs (EBER).

Material and Method: 49 biopsies diagnosed with Hodgkin lymphoma (HL), 5 with NLPHL and 19 with DLBCL were evaluated for CD30, PAX-5, OCT-2, BOB.1, MUM-1, BCL-6, and CD20, and EBER positivity. SPSS 18 was used for statistical analysis.

Results: 73 lymphoma cases were included in the study: 61.6% males and 38.4% females. The median age of patients was 50 years. CHL (67.1%) was the most common type, and mixed cellular Hodgkin lymphoma (MCHL) was the most common subtype. There was a statistically significant difference in CD30, OCT-2, BOB.1, MUM-1, PAX-5, CD20, BCL-6, EBER expression between CHL and DLBCL cases (p<0,001). In addition, there was a statistically significant difference between CHL and NLPHL for all antibodies except BCL-6 and EBER.

Conclusion: It would be beneficial to use a panel consisting of CD30, PAX-5, OCT-2, BOB.1, MUM-1, BCL-6, and CD20, and EBER in the differential diagnosis of CHL from NLPHL and DLBCL.

Keywords: Hodgkin's Lymphoma, OCT-2, BOB.1, PAX-5, EBER

Öz

Amaç: Klasik Hodgkin lenfoma (KHL) Sık görülmek ile birlikte ayrıcı tanısı zor olabilen lenfomalardandır. Hodgkin hücresinin germinal merkezli olabileceği düşünülmektedir. Bu çalışmadaki amacımız, KHL, nodular lenfosit baskın Hodgkin lenfoma (NLBHL) ve diffuse büyük B hücreli lenfomada (DBBHL) germinal merkez transformasyon belirteçleri olan OCT-2, BOB.1, BCL-6, PAX-5, CD20 ve MUM-1'in ekspresyonlarını immünohistokimyasal yöntem ile, EBV early RNAs (EBER)'in kromojenik in-situ hibridizasyon (KISH) ile değerlendirmektir.

Gereç ve Yöntem: 49 KHL, 5'i NLBHL ve 19'u DBBHL tanılı biyopsi, CD30, PAX-5, OCT-2, BOB.1, MUM-1, BCL-6 ve CD20 ve EBER pozitifliği açısından değerlendirildi. İstatistiksel analiz için SPSS 18 kullanıldı.

Bulgular: Çalışmaya %61,6 erkek ve %38,4 kadın olmak üzere 73 lenfoma olgusu dahil edildi. Hastaların medyan yaşı 50 idi. En sık görülen lenfoma tipi KHL (%67,1), en sık görülen alt tip mikst hücreli Hodgkin lenfoma (MSHL) idi. KHL ve DBBHL olguları arasında CD30, OCT-2, BOB.1, MUM-1, PAX-5, CD20, BCL-6, EBER ekspresyonu açısından istatistiksel olarak anlamlı fark vardı (p<0,001). Ek olarak BCL-6 ve EBER haricinde diğer tüm antikorlarda KHL ve NLBHL arasında istatistiki olarak anlamlı fark mevcuttu.

Sonuç: KHL'nin NLBHL ve DBBHL'dan ayırıcı tanısında CD30, PAX-5, OCT-2, BOB.1, MUM-1, BCL-6 ve CD20 ve EBER'den oluşan bir panelin kullanılması çok faydalı olacaktır.

Anahtar Kelimeler: Hodgkin Lenfoma, OCT-2, BOB.1, PAX-5, EBER

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INTRODUCTION

Lymphoid neoplasms are clonal in origin. The World Health Organization (WHO) updated the classification of lymphoid neoplasms in 2022, and approximately 80 types of lymphoid neoplasia have been defined. Lymphomas are divided into two major groups: non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL). Hodgkin lymphomas constitute approximately 20% of all lymphomas. Hodgkin lymphoma is divided into two groups: Classic Hodgkin Lymphoma (CHL) and Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) (1-3). At the same time, CHL is divided into four subgroups: mixed cellular (MSHL), nodular sclerosing (NSHL), lymphocyte-rich type (LRHL), and lymphocyte-poor (LPHL).

Although there is still controversial information about the origin of the Hodgkin cell, it is emphasized that it originates from the germinal centre in the lymph node but has undergone some changes and lost its B cell characteristics. In addition, the marker on the Hodgkin cell is CD30. Hodgkin cells show CD20 expression in NLPHL. Hodgkin cells have quite different morphologies. Therefore, it can be confused with many lymphomas. Sometimes it can be quite challenging to distinguish between non-Hodgkin and classic Hodgkin lymphomas from the other group. In this case, some cell antigen expressions are used; Germinal centre transformation markers are (1, 2). These markers are OCT-2 and BOB.1 (4), BCL-6, PAX5 (5), and MUM-1 (6), and these markers are upregulated on the lymphocyte in the germinal centre during the plasma cell differentiation stage of the lymphocyte. These markers are expressed by cells forming the germinal center. Hodgkin cells also express some of these markers. But they are usually either weakly or partially expressed. This feature can be used in the differential diagnosis (7-10).

Epstein-Barr virus (EBV) is an oncogenic virus from the herpes virus family. It has been proven to be associated with lymphoid malignancies in children and adults. There are geographical differences between lymphoma types and EBV positivity (7, 8).

In our study, we wanted to look at the expression of germinal center transformation markers (OCT-2, BOB.1(2), BCL-6, PAX5 (3), and MUM-1) and EBV positivity in HL, NLPHL, and and diffuse large B-cell lymphoma (DLBCL) and the differential diagnosis of HL from the other two groups.

MATERIAL AND METHOD

This retrospective study included 54 biopsies diagnosed with HL and 19 diagnosed with DLBCL between 2016 and 2021. All cases were diagnosed from the lymph node. The age, gender, and clinical information of the patients were obtained from the hospital information system. Hematoxylin-eosin (HE) stained preparations prepared by embedding in paraffin after 10% formaldehyde fixation from the tissues were examined. CD30, PAX-5, OCT-2, BOB.1, MUM-1, BCL-6, and CD20 immunohistochemical (IHC) studies and EBER staining of all biopsies were performed.

The Method Used in the Immunohistochemical Studies and Chromogenic In-situ Hybridization

Four micron-thick sections from tissues in appropriate paraffin blocks for each antibody were taken on poly-L-lysine coated slides. The antigen retrieval technique was used in IHC studies; the avidin-biotin-peroxidase complex method was applied. Antibodies were stained on a Leica band max automated immunohistochemical staining device. A Bond Polymer Refine Detection kit (Leica, DS9800) was used for each antibody. The necessary staining procedure was performed according to the datasheet of each antibody, and appropriate positive and negative controls were used for each antibody. The characteristics of the primary antibodies used in the immunohistochemical study are listed in **Table 1**. After covering them with a coverslip ultra-mount, the prepared samples were examined under an Olympus BX51 model microscope. EBV early RNAs (EBER) and chromogenic in-situ hybridisation (ISH) method were used for EBV. 4 µm thick sections were taken from the paraffin-embedded tissue. Chromogenic ISH with Leica brand EBV RNA probe and ISH kit on the Leica band max device were performed automatically according to the manufacturer's recommendations using standard procedure. Examined under the Olympus BX51 model microscope.

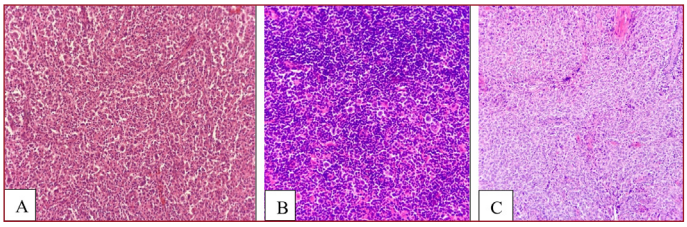
Table 1: The characteristics of the antibodies used in the immunohistochemical study

Primary Antibody	Clone	Dilution Rate	Incubation Time	Antigen Revealing	Company
CD30	JCM182	1:100	20 minute	ER2	Novocastra
Pax-5	Polycylonal	1:80	40 minute	ER2	Thermo
OCT-2	ZM90	1:50	30 minute	ER2	Zeta
BOB.1	TG14	1:20	30 minute	ER2	Novocastra
MUM-1	EAU32	1:200	20 minute	ER1	Novocastra
BCL-6	LN22	1:60	40 minute	ER2	Leica
CD20	L26	1:200	40 minute	ER2	Leica

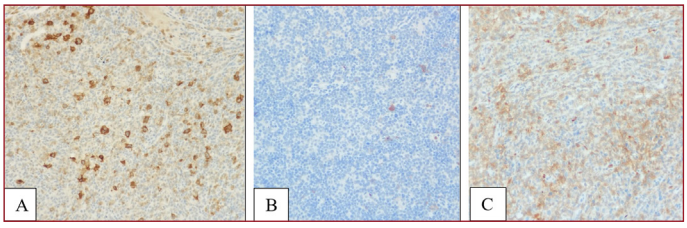
ER1 : Citrat Buffer, pH:6; ER2 : EDTA Buffer, pH:9

2.2 Immunohistochemical and Chromogenic In-situ Hybridization Evaluation

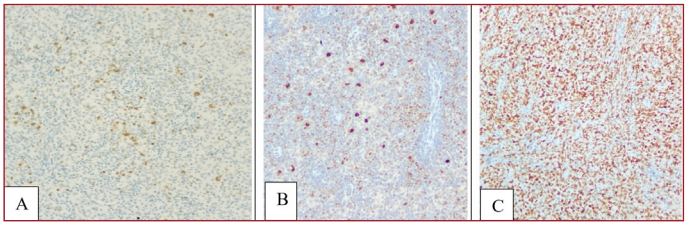
The severity and diffusiveness of expression were taken into account when evaluating IHC studies with CD30, PAX5, OCT-2, BOB.1, MUM-1, BCL-6 and CD20. The germinal center in the tissue is used as the internal positive control and the external negative control tissue. Absence of staining was scored as 0, weak and focal staining as 1, and strong and diffuse staining as 2. EBER was recorded as positive and negative (11). **Table 1** presents the characteristics of the antibodies used in the immunohistochemical study. The images of the results of the immunohistochemical studies of the lymphoma groups included in the study are in **Figure 1-7**. Positive staining with EBER in CHL (**Figure 8**).



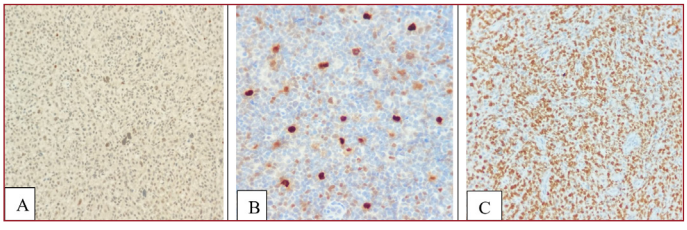
Figüre 1. In classical Hodgkin lymphoma, mixed cellular and nodular lymphocyte predominant Hodgkin lymphoma, there is an infiltration consisting of hodgkin and reed-sternberg cells in the nonneoplastic cellular background, while mass-forming lymphoma cells are present in diffuse large B-cell lymphoma (A, B, C , respectively x200 HE)



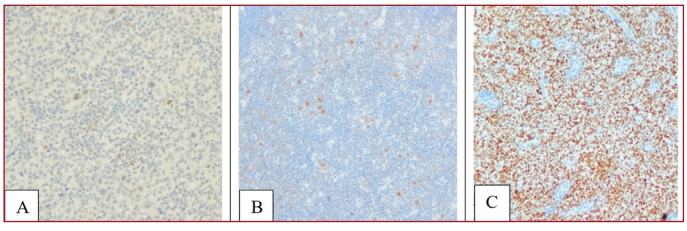
Figüre 2. In classical Hodgkin lymphoma, membranous-golgi zone CD30 antibody expression is present, whereas expression is not observed in nodular lymphocyte predominant Hodgkin lymphoma. Cytoplasmic CD30 antibody expression is observed in diffuse large B-cell lymphoma (A, B, C , respectively x200)



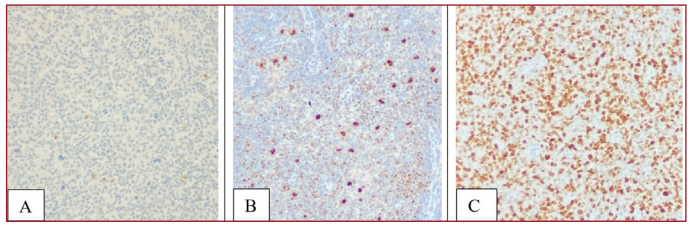
Figüre 3. Strong expression by BCL-6 antibody in all lymphoma groups (x200).



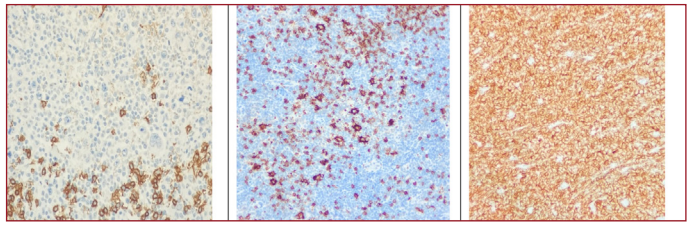
Figüre 4. While weak nuclear expression is detected with PAX-5 antibody in classical Hodgkin lymphoma (A), strong nuclear expression is observed in nodular lymphocyte predominant Hodgkin lymphoma (B) and diffuse large b-cell lymphoma (C) (X200).



Figüre 5. While OCT-2 antibody has weak and sparse nuclear expression in classical Hodgkin lymphoma (A), strong nuclear expression is observed in nodular lymphocyte predominant Hodgkin lymphoma (B) and diffuse large b-cell lymphoma (C) (X200).



Figüre 6. There was no reaction with BOB-1 antibody in classical Hodgkin lymphoma (A), while strong nuclear expression was present in nodular lymphocyte predominant Hodgkin lymphoma (B) and diffuse large b-cell lymphoma (C) (X200).



Figüre 7. There was no reaction with CD20 antibody in classical Hodgkin lymphoma (A), while strong cytoplasmic expression was present in nodular lymphocyte predominant Hodgkin lymphoma (B) and diffuse large b-cell lymphoma (C) (X200).

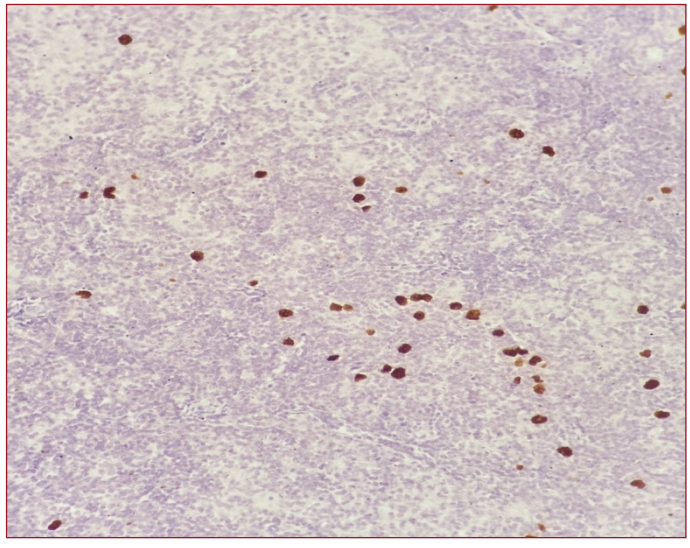


Figure 8. Nuclear positivity of EBER (CISH) in classical Hodgkin lymphoma (x200).

Ethics Committee Approval

The study was approved by the Clinical Research Ethics Committee number: 2021/408. dated 06.08.2021.

Statistical analysis

The immunohistochemical studies and the EBER results of the lymphomas included in the study were divided into three groups, and statistics were made. Three main groups are divided into Classical Hodgkin Lymphoma, Nodular Lymphocyte Predominant Hodgkin Lymphoma, and Diffuse Large B Cell Lymphoma. SPSS 18 was used for statistical analysis. Continuous data were expressed as median, minimum, and maximum values. Categorical variables were defined as percentage frequency. The

distribution characteristics of continuous data were evaluated with the Shapiro-Wilk test, and it was found that it did not fit the normal distribution. The Kruskal Wallis H test was used to compare the ordinal and continuous variables with more than two groups. Mann Whitney U test with Bonferroni correction was used in post hoc analyses. In statistical analysis, the level of significance was accepted as $p<0.05$.

RESULTS

Of the 73 lymphoma cases included in the study, 61.6% were male, and 38.4% were female. The median age of patients was 50, the youngest was 18, and the largest was 83. The most common lymphoma type included in the study was CHL (67.1%), and the most common subtype of CHL was MCHL (57.2%) (**Table 2**).

Table 2. Distribution of Lymphoma Types

Types of lymphoma	N	%
Classical Hodgkin Lymphoma	49	67.1
Nodular Lymphocyte Predominant Hodgkin Lymphoma	5	6.8
Diffuse Large B-cell Lymphoma	19	26.1
Classical Hodgkin Lymphoma Subtypes	N	%
Mixed Cellular	28	57.2
Nodular Sclerosing	16	32.6
Lymphocyte Rich	5	10.2

When lymphoma types were compared according to their age, it was observed that there was a statistically significant difference between the ages (p=0.001). In addition, as a result of in-group comparisons, the age of the diffuse large B-cell lymphoma group was found to be statistically significantly higher than the other disease groups ($p<0.001$) (**Table 3**).

Table 3: Comparison of the types of lymphoma and the mean age of patients

Group	Median	25-75	p
Classical Hodgkin Lymphoma, Mixed Cellular	43.5	43.50-58.25	0.001*
Diffuse large B-cell lymphoma	68.0	68.00-78.00	
Classical Hodgkin Lymphoma, Nodular Sclerosing	52.5	52.50-61.75	
Nodular Lymphocyte Predominant Hodgkin Lymphoma	44.0	44.00-55.00	
Classical Hodgkin Lymphoma, Lymphocyte Rich	29.0	29,00-42,00	

Kruskal Wallis H Test, *p<0,05

Table 4 presents the results of immunohistochemical staining and EBER for CD20, CD30, OCT.1, OCT.2, BOB.1, BCL-6, PAX-5 and MUM.1 in CHL, NLPHL, DLBCL. The results of the same immunohistochemical staining and EBER in CHL subtypes are shown in **Table 5**. Finally, the comparison of immunohistochemical staining results between lymphoma groups is presented in **Table 6**.

OCT-2

28.6% of CHL cases showed weakly positive, 8.2% positive, and 63.3% negative reactions with OCT-2. In CHL subtypes, independent of the degree of positivity, the highest OCT-2 expression was seen in 60 % in LRHL, and the lowest OCT-2 expression was 18.8% in NSHL. OCT-2 expression was positive in 80% and negative in 20% of NLPHL cases. OCT-2 expression was positive in 94.7% and weakly positive in 5.3% of DLBCL cases. A statistically significant difference was found between CHL, NLPHL (p=0.006), and DLBCL cases regarding OCT-2 expression ($p<0.001$). No statistically significant difference was found between NLPHL and DLBCL cases following OCT-2 expression (p=0.226).

Table 4: An immunohistochemical study and EBER results based on lymphoma types

	Classical Hodgkin Lymphoma		Nodular Lymphocyte Predominant Hodgkin Lymphoma		Diffuse Large B-cell Lymphoma		Total	
	n	%	n	%	n	%		
Oct-2								
0 (Negative)	31	63.3	1	20.0	0	0.0	32	43.8
1 (Weakly Positive)	14	28.6	0	0.0	1	5.3	15	20.5
2 (Strong Positive)	4	8.2	4	80.0	18	94.7	26	35.6
BOB.1								
0 (Negative)	46	93.9	0	0.0	1	5.3	47	64.4
1 (Weakly Positive)	3	6.1	2	40.0	4	21.1	9	12.3
2 (Strong Positive)	0	0.0	3	60.0	14	73.7	17	23.3
Pax-5								
0 (Negative)	6	12.2	0	0.0	0	0.0	6	8.2
1 (Weakly Positive)	43	87.8	1	20.0	1	5.3	45	61.6
2 (Strong Positive)	0	0.0	4	80.0	18	94.7	22	30.1
BCL-6								
0 (Negative)	34	69.4	2	40.0	0	0.0	36	49.3
1 (Weakly Positive)	13	26.5	2	40.0	3	15.8	18	24.7
2 (Strong Positive)	2	4.1	1	20.0	16	84.2	19	26.0
MUM-1								
0 (Negative)	6	12.2	4	80.0	4	21.1	14	19.2
1 (Weakly Positive)	8	16.3	1	20.0	10	52.6	19	26.0
2 (Strong Positive)	35	71.4	0	0.0	5	26.3	40	54.8
CD20								
0 (Negative)	36	73.5	1	20.0	0	0.0	37	50.7
1 (Weakly Positive)	10	20.4	1	20.0	0	0.0	11	15.1
2 (Strong Positive)	3	6.1	3	60.0	19	100.0	25	34.2
CD30								
0 (Negative)	0	0.0	5	100.0	13	68.4	18	24.7
1 (Weakly Positive)	2	4.1	0	0.0	3	15.8	5	6.8
2 (Strong Positive)	47	95.9	0	0.0	3	15.8	50	68.5
EBER								
Negative	28	57.1	5	100.0	19	100.0	52	71.2
Positive	21	42.9	0	0	0	0.0	21	28.8
Total	49	100.0	5	100.0	19	100.0	73	100.0

EBER: EBV early RNAs

Table 5: Results of İmmunohistochemical Studies in Classical Hodgkin Lymphoma Subgroups

	Classical Hodgkin Lymphoma, Mixed Cellular		Classical Hodgkin Lymphoma, Nodular Sclerosing		Classical Hodgkin Lymphoma Lymphocyte Rich	
	n	%	n	%	n	%
Oct-2						
0 (Negatif)	16	57.1	13	81.3	2	40.0
1 (Weakly Positive)	9	32.1	2	12.5	3	60.0
2 (Strong Positive)	3	10.7	1	6.3	0	0.0
BOB.1						
0 (Negatif)	26	92.9	15	93.8	5	100.0
1 (Weakly Positive)	2	7.1	1	6.3	0	0.0
2 (Strong Positive)	0	0.0	0	0.0	0	0.0
Pax-5						
0 (Negatif)	2	7.1	3	18.8	1	20.0
1 (Weakly Positive)	26	92.9	13	81.3	4	80.0
2 (Strong Positive)	0	0.0	0	0.0	0	0.0
BCL-6						
0 (Negatif)	16	57.1	14	87.5	4	80.0
1 (Weakly Positive)	10	35.7	2	12.5	1	20.0
2 (Strong Positive)	2	7.1	0	0.0	0	0.0
MUM-1						
0 (Negatif)	2	7.1	3	18.8	1	20.0
1 (Weakly Positive)	5	17.9	1	6.3	2	40.0
2 (Strong Positive)	21	75.0	12	75.0	2	40.0
CD20						
0 (Negatif)	23	82.1	12	75.0	1	20.0
1 (Weakly Positive)	3	10.7	4	25.0	3	60.0
2 (Strong Positive)	2	7.1	0	0.0	1	20.0
CD30						
0 (Negatif)	0	0.0	0	0.0	0	0.0
1 (Weakly Positive)	0	0.0	2	12.5	0	0.0
2 (Strong Positive)	28	100	14	87.5	5	100.0
EBER						
Negative	16	57.1	9	56.3	3	60.0
Positive	12	42.9	7	43.8	2	40.0
Total	28	100.0	16	100.0	5	100.0

Table 6. An analysis of the statistical results between lymphoma types and antibodies

ANTIBODY	CHL/ NLPHL/ DLBCL1	CHL/ NLPHL2	CHL/ DLBCL2	NLPHL / DLBCL2
		P Value		
Oct-2	<0.001*	0.006#	<0.001#	0.226
BOB.1	<0.001*	<0.001#	<0.001#	0.622
Pax-5	<0.001*	<0.001#	<0.001#	0.229
BCL-6	<0.001*	0.143	<0.001#	0.003#
MUM-1	<0.001*	<0.001#	0.002#	0.019
CD20	<0.001*	0.004#	<0.001#	0.005#
CD30	<0.001*	<0.001#	<0.001#	0.299
EBER	0.001*	0.064	0.001#	0.160

1 Kruskal Wallis H Test.*p<0.05, 2 Mann Whitney U test was used as posthoc analysis. Bonferroni With the correction. statistical significance was taken as #p<0.017. CHL: Classical Hodgkin Lymphoma ; NLPHL: Nodular Lymphocyte Predominant Hodgkin Lymphoma ; DLBCL: Diffuse Large B-cell Lymphoma.

BOB.1

Of the CHL cases, 93.9% were negative with BOB.1, and 6.1% had weak expression. Strong expression was not observed in any case. The highest expression of BOB.1 in CHL subtypes was seen in MCHL at 7.1 %. There was no statistically significant difference between CHL subtypes regarding BOB.1 expression (p=0.837).

BOB.1 showed positive expression in 60% of NLPHL cases and weakly positive expression in 40%. On the other hand, it showed positive expression in 73.7% of DLBCL cases, weakly positive expression in 21.1%, and negative expression in 5.3%.

Regardless of the degree of staining, lower BOB.1 expression was detected in CHL cases compared to NLPHL and DLBCL cases. This difference was also statistically significant (p<0.001). On the other hand, no statistically significant difference between NLPHL and DLBCL cases regarding BOB.1 expression (p=0.622) was found.

PAX-5

87.8% of (CHL cases showed weak positive and 12.2% negative expression with PAX-5. No strong expression was detected. The highest weak positive expression of CHL subtypes was in MCHL, 92.9%.

Strong positive expression in 80% of NLPHL cases and weak positive expression in 20%. Strong positive expression was observed in 94.7% of DLBCL cases, and weakly positive expression was observed in 5.3% of DLBCL cases.

A statistically significant difference was found between CHL, NLPHL, and DLBCL cases regarding PAX-5 expression (p<0.001). However, there was no statistically significant difference in PAX-5 expression between NLPHL and DLBCL cases (p=0.229).

BCL-6

CHL cases showed weak positive, 69.4% negative, and 4.1% strong positive reactions with BCL-6. Regardless of the degree of staining, the highest BCL-6 expression was seen in MCHL, 42.8 %, and the lowest BCL-6 expression was 12.5% in NSHL, in CHL subtypes.

BCL-6 showed positive expression in 20%, weakly positive in 40%, and negative in 40% of NLPHL cases. It showed positive expression in 84.2% of DLBCL cases and weakly positive expression in 15.8%.

Considering the BCL-6 expressions regardless of the staining degree, the lowest expression was found in CHL cases, and the highest was found in DLBCL cases. A statistically significant difference was found between CHL and DLBCL cases in BCL-6 expression (p<0.001). No statistically significant difference between CHL and NLPHL cases was found in BCL-6 expression (p=0.143).

MUM1

71.4% of CHL cases had a positive reaction with MUM1, 16.4% had a weak positive reaction, and 12.2% had a negative response. Regardless of the staining degree, the

highest MUM1 expression was seen in MCHL, 92.8%, and the lowest MUM1 expression was 80% in LRHL, in CHL subtypes. Weakly positive MUM1 expression was detected in 20% of NLPHL cases and negative in 80%. No negative reaction was observed in any of the cases. It showed positive expression in 26.3% of DLBCL cases, weakly positive expression in 52.6% and negative expression in 21.1%.

The highest MUM1 expression was seen in CHL cases, and the lowest MUM1 expression was seen in DLBCL cases. A statistically significant difference was found between CHL and DLBCL and between CHL and NLPHL cases in MUM1 expression ($p=0.002$, $p<0.001$). There was no statistically significant difference in MUM1 expression between NLPHL and DLBCL cases ($p=0.019$).

CD20

73.5% of CHL cases showed negative expression, 20.4% weakly positive, and 6.1% positive expression reaction with CD20. Regardless of the degree of staining, the highest CD20 expression in CHL subtypes was 80% in LZHL.

Strong and weakly positive CD20 expression was detected in 80% of NLPHL cases. CD20 expression was seen in all DLBCL cases.

The highest MUM1 expression was seen in CHL cases, and the lowest MUM1 expression was seen in DLBCL cases. A statistically significant difference was found between CHL and DLBCL and between CHL and NLPHL cases in CD20 expression ($p<0.001$). There was no statistically significant difference in CD20 expression between NLPHL and DLBCL cases ($p=0.140$).

CD30

CD30 expression was present in all CHL cases. Expression was not observed in any of the NLPHL cases. A varying degree of positive words was observed in 31.6% of DLBCL cases.

A statistically significant difference was found between CHL and DLBCL and between CHL and NLPHL cases in CD20 expression ($p<0.001$). There was no statistically significant difference in CD45 expression between NLPHL and DLBCL cases ($p=0.299$).

EBER

EBER was positive in 42.9% of CHL cases. The highest EBER positivity in CHL subtypes was NSHL, with 43.8%. EBER was negative in all NLPHL and DLBCL cases.

A statistically significant difference was found between CHL and DLBCL cases regarding EBER expression ($p=0.001$). There was no statistically significant difference in EBER expression between NLPHL and DLBCL cases ($p=0.160$).

DISCUSSION

CHL is one of the lymphomas of which nearly 100 types have been defined so far; the origin of the Hodgkin cell, a malignant cell, is still controversial. It is stated that it originates from the germinal center but has lost some of its characteristics (1-3).

CHL subtype MSHL accounted for the majority of cases in our study, with 28 (57.2%) cases. Contrary to our study, the most common subtype was found to be NSHL in some studies, 52.5% (12) and 75% (13), respectively. Similar to our study another research have reported MSHL (50%) as the most prevalent subtype of CHL (5).

PAX-5 is the gene family that encodes the nuclear transcription factor; it regulates B cells' development, differentiation, and migration (4). PAX-5 CHL generally shows weak expression in Reed- Sternberg cells (5). In our study, we found 87.8% of CHL cases to have weak positive reactions with PAX-5 and 12.2% to negative reactions; we did not observe strong positivity in any of the cases. In the study, which included 60 CHLs previously performed in the literature, a weak reaction was obtained with PAX-5 in all cases, but no strong response was received. A moderate reaction was obtained in a patient with NLPHL, and a strong reaction was found in 26 cases, except for one DLBCL (5, 14).

Furthermore, our study did not find strong positivity for PAX-5 in CHL cases. In contrast, in our study, negative reactions were obtained in 12.2% of CHL cases, positive expression was found in 80% of NLPHL cases and weak positive expression in 20%. Our study found a strong reaction in almost all cases, similar to DLBCL cases.

An interaction between transcription factors and the octamer regions of the immunoglobulin promoter is required for immunoglobulin gene expression during the development of B cells. Two of these transcription factors, BOB.1 and OCT-2, belong to the POU family of transcription factors (15). Surprisingly, OCT-2 expressed in B cells can be expressed in Hodgkin cells in CHL together with the co-transcription factor BOB.1 (16-18).

BOB.1 has been studied very little in the context of CHL, and little recent literature has been published about it. A previous study conducted with 57 CHL cases found a weak positive reaction in 28% (16/57 patients) (18). Our study had a few cases of BOB.1 (6.1%), but the staining pattern was the same.

In the same study, a strong positive reaction was obtained with BOB.1 in all DLBCL and NLPHL cases: 73.7% (14) of 19 DLBCL cases were strongly positive, 21.1% (4) weakly positive, 5.3% (1) showed a negative reaction. A strong positive reaction was observed in 60% (5) of NLPHL cases and a weak positive reaction in 40% (2).

In various studies, OCT-2 expression in CHL ranges from 0% (19) to 33% (18). In our study, 28.6% of CHL cases showed a weak positive reaction, 8.2% a strong reaction, and 63.3% a negative reaction with OCT-2. The expression pattern was like in previous studies (18). In NLPHL, OCT-2 showed 100% strong expression in almost all previous studies (4). Most cases, except for one case, showed a strong positive reaction. The OCT-2 expression in DLBCL was found to be over 90% in our study, similar to other studies (7, 18, 20).

B-cell lymphoma 6 protein (BCL-6) is a follicular helper T cell (TFH) related marker and is involved in the transformation of B cells in the germinal center (21). BCL-6 expression is particularly expressed in T-cell lymphomas of follicular origin. It is also expressed in many B-cell lymphomas, including DLBCL (22). It is expressed at rates ranging from 83-100% in NLPHL (23, 24). In this study, we found 60% expression in NLPDHL. Studies with Bcl-6 in CHL are very limited. Generally, no expression was detected or very few and weak positivity was detected (25). Although expressions were low in CHL, our study found higher positivity than previous studies.

Multiple myeloma-1/interferon regulatory factor-4 (MUM1/IRF-4), a lymphocyte-specific member of the IRF family, is expressed in the final stage of B cell differentiation in the germinal center (26). MUM-1 expression is present in almost all CHL, regardless of type (27). This study detected positive expression in 87.8% of our CHL cases, regardless of the staining pattern. The lowest expression of CHL subtypes was NSHL, and it was negative in 18.8%. We detected weak positive expression with MUM-1 in only one of our NLPHL cases, while the others were negative. Our results were similar to previous studies (26, 27). MUM-1 positive expression was 78.9% in our DLBCL cases, and in one of the earlier studies, 92% was found in 92 cases (28).

CD30 is expressed in various inflammatory conditions and malignancies and is described as a transmembrane glycoprotein receptor (120kd) of the tumours necrosis factor receptor superfamily 8 (TNFRSF8) (29). CD30 expression is reported between 90-100% in CHL (30, 31). In our study, all our CHL cases showed CD30 positive expression. CD30 expression in de nova DLBCL varies between 10-25% (29). This study shows CD30 expression is slightly higher in DLBCL, 31.6%. Similar to the literature (32), in our study, CD30 expression was not seen in NLPHL.

CD20, a pan- B cell marker, can be expressed in Hodgkin's cells and Reed Sternberg cells in CHL. These rates generally vary between 19-28% regardless of the subgroup (18, 30). Results similar to previous studies were obtained in our study. As reported in the previous study, it was found to be positive in nearly all cases of DLBCL and NLPHL CD20 (7, 18). All cases of DLBCL in our study showed strong expression. Three of our five NLPHL cases showed a strong positive reaction. One case resulted in a negative reaction. CD20 expression may be negative or weakly positive, depending on the location of NLPHL, especially in the mediastinal area.

Epstein-Barr virus (EBV) is a herpes virus that causes lymphoid neoplasms. The prevalence of EBV positivity in HL varies between countries (33). HL positivity was detected in 45.4% of cases in Turkey, most frequently in the NSHL subtype and none in the NLPHL subtype (34). The results of our study are in agreement with those of this study. There is a low level of EBER positivity in DLBCL (35). All cases of DLBCL in our study were negative.

Our immunohistochemical study revealed significant differences between CHL and the other two types of lymphoma, except for BCL-6, regardless of the staining intensity. Based on previous publications, only the frequency of antibodies has been reported (18), and no statistical comparison has yet been conducted.

CONCLUSION

Regardless of their type, lymphomas are challenging to diagnose neoplasms. For their treatment, they should be typified. In addition to morphology, immunophenotyping by the immunohistochemical method should be a must. Aberrant expressions or loss of expression seen in lymphomas make diagnosing difficult, necessitating reliable immunohistochemical markers. As a result of our study, we demonstrated that CD30 positivity, BOB.1 negativity, PAX-5 weak positivity, MUM-1 strong positivity, and EBER positivity could be used to diagnose CHL effectively.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee Sayı (2021/408), Date 06.08.2021

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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