Immunohistochemical expression of PD-L1 and EBV in Hodgkin's Lymphoma

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(Ann Coll Med Mosul 2024; 46 (1):6-15).
Received: 17th Janu. 2024; Accepted: 10th Febr. 2024.

ABSTRACT

Background: Hodgkin Lymphoma (HL) constitutes 10% of lymphomas and 1% of cancers in industrialized nations, classified into classical Hodgkin Lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL). The four cHL subtypes include nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted. Etiologically, HL stems from B-cell clonal transformation, influenced by genetic predisposition and viruses like Epstein-Barr virus (EBV) and Human immunodeficiency virus (HIV). HL cells exhibit programmed death ligand 1 (PD-L1) overexpression, enabling immune evasion through interaction with PD-1 on T cells. The PD-1-PD-L1 axis is a promising therapeutic target, with ongoing scrutiny of PD-L1 identification by immunohistochemistry (IHC) in HL as a potential marker for immunotherapy efficacy.

Aim: To detect the frequency, and association between EBV & PDL-1 expression in Hodgkin lymphoma cases.To investigate the association between the expression of PD-L1 & EBV in Hodgkin lymphoma and some clinic-pathological parameters like age of patients & subtype.

Material and Methods: This is both a retrospective and prospective case series study which was conducted on 40 cases of Hodgkin Lymphoma, that were collected from private laboratories in the North of Iraq extending from November 2022 through September 2023. Reviewing of diagnosis & classification was done according to WHO of HL. PD-L1 & EBV status were evaluated immunohistochemically using intensity and percentage guided scoring for PD-L1 & cytoplasmic staining for EBV.

Results: In this study of 40 Hodgkin Lymphoma cases, the M: F was 3:2 & and nodular sclerosis subtype form the majority of cases (67.5%). PD-L1 expression occurred in (67.5%) of cases and (35%) in the tumor micro-environment. No significant associations were found with age, gender and subtype. EBV LMP-1 expression was found in (30%), with more frequency in males (83%), and associated with mixed cellularity HL subtype. PD-L1 intensity showed significant association with its cutoff in HRS cells and tumor microenvironment but not with EBV status, gender and HL subtype. PD-L1 and EBV LMP-1 expressions did not show a significant association.

Conclusion: In Hodgkin Lymphoma (HL), immunohistochemical expression of programmed death ligand 1 (PD-L1) is found in 67.5% of HL cases and 35% of the tumor micro-environment. However, there is no significant association between PD-L1 expression and the presence of EBV latent membrane protein-1 (LMP-1). Only 29.6% of patients with positive PD-L1 expression also show positive EBV LMP-1. Additionally, no significant associations were identified between PD-L1 expression and HL subtype, age, and gender. The expression of PD-L1 in the tumor micro-environment does not show a statistically significant difference when compared with HL subtypes. On the other hand, EBV LMP-1 immunohistochemical expression is significantly associated with male gender (83.3%) and certain histological subtypes but reveals no statistically significant difference concerning the age of the patients.

Keywords: Hodgkin lymphoma, PDL-1, HRS cells, EBV LMP-1.

التعبير المناعي النسيجي الكيمائي لPDL-1 وفايروس ايبشاتن بار في ورم الغدد المفاويه من نوع هودجكن

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الخلاصة

الخلفية: تشكل ورم الغدد المفاويه من نوع ليمفوما هودجكين ١٠% من الأورام اللمفاوية و١% من حالات السرطان في الدول الصناعية، وتصنف إلى ليمفوما هودجكين الكلاسيكية وسرطان الغدد الليمفاوية العقدية السائدة. تشمل الأنواع الفرعية الأربعة لـليمفوما هودجكين الكلاسيكية:التصلب العقدي، والخلوية المختلطة، والخلايا اللمفاوية الغنية، والخلايا اللمفاوية المستنفدة. من الناحية المسببة، تتبع لمفوما هودجكين من التحول النسيلي للخلايا نوع B، متأثرًا بالاستعداد الوراثي والفيروسات مثل فيروس EB وفيروس نقص المناعة البشرية. تظهر خلايا لمفوما هودجكين فرط التعبيرعن PDL-1 بواسطة الكيمياء المناعية في لمفوما هودجكين كعلامة محتملة لفعالية العلاج المناعي.

أهداف الدراسة: الكشف عن التكرار و معرفة العلاقة بين تعبير فيروس EB و PDL-1 في حالات ليمفوما هودجكين.

التحقق من العلاقة بين التعبيرعن PDL-1 وفيروس EB في الخلايا السرطانية والعديد من العوامل المرضية السريرية مثل العمر والنوع النسيجي الفرعي .

طرق البحث: هذه دراسة أجريت بأثررجعي ومستقبلي على ٤٠ حالة من حالات سرطان الغدد الليمفاوية ليمفوما هودجكين والتي تم جمعها من مختبرات خاصة في شمال العراق على مدى ١١ شهرًا تمتد من شهرتشرين الثاني (نوفمبر) ٢٠٢٢ حتى ايلول ٢٠٢٣.

تم تأكيد التشخيص وتصنيف الأورام وفقًا لنظام تصنيف منظمة الصحة العالمية. PDL-1 وفيروس EB أجريت عليها الصبغات النسيجية المناعية الكيميائية بأستخدام كلاً من النسبة المئوية وشدة التصبغPDL-1 والتصبغ الغشائي لفيروس EB.

النتائج: شاركت في الدراسة الحالية أربعون حالة, وجدت ان نسبة الذكور الى الأناث هي ٣:٢ و شكلت حالات التصلب العقدي الأغلبية بنسبة (٦٧.٥٪) . تم اكتشاف تعبير مناعي كيميائي مناعي إيجابي لـ PDL-1 في (٦٧.٥%) من بين الحالات المدروسة والتعبير عنه ايضا في البيئة الدقيقة للورم والتي شكلت (٣٥%) في الدراسة، لم يكشف عن اي دلالة احصائية مع العمر والجنس والنوع الفرعي.

وجد فيروس EB بين الحالات المدروسة بنسبة (٣٠.٠%) مع انتشاره بشكل اكبر بين الذكور بنسبة (٨٣%) و بالاشتراك مع النوع النسيجي الفرعي للمفوما هودجكين.

شدة التعبير الكيميائي المناعي لبروتين PDL-1 أظهر وجود ارتباط بين قطع بروتين PDL-1 في خلايا السرطانية لهودجكين وبيئة الورم. و لم يتم الحصول على ارتباط فيما يتعلق بحالة فيروس EB مع الجنس، والنوع النسيجي الفرعي لسرطان الغدد الليمفاوية هودجكين.

مقارنة التعبير المناعي النسيجي الكيميائي لبروتين PDL-1 مع تعبير فيروس EB لم تظهر ارتباطا احصائيا مهما.

الاستنتاج: وجد أن بروتين PDL-1 يتم التعبيرعنه كيميائيًا مناعيًا في ٦٧.٥% من حالات ليمفوما هودجكين و في البيئة الدقيقة للورم وبنسبة ٣٥.٠% من الحالات ولم يتم اكتشاف ارتباط ملحوظ مع النوع النسيجي الفرعي لسرطان الغدد الليمفاوية هودجكين والعمر والجنس.

لم يتم الكشف عن وجود علاقة ذات دلالة إحصائية بين تعبير بروتين PDL-1 وتعبير فيروس EB. لكن فقط ٢٩.٦% من المرضى الذين لديهم تعبير إيجابي لبروتين PDL-1 أظهروا إيجابية فيروس EB, بالإضافة إلى ذلك لم يتم تحديد أي ارتباطات مهمة بين تعبير PDL-1 والنوع الفرعي للمفوما هودجكين والعمر والجنس. لا يُظهر تعبير PDL-1 في البيئة الدقيقة للورم فرقًا ذو دلالة إحصائية عند مقارنته بأنواع لمفوما هودجكين الفرعية. من ناحية أخرى، يرتبط التعبير المناعي الكيميائي لفيروس EB بشكل كبير بجنس الذكور (٨٣.٣٪) وبعض الأنواع الفرعية النسيجية ولكنه لا يكشف عن أي فروق ذات دلالة إحصائية فيما يتعلق بعمر المرضى.

الكلمات المفتاحية : ورم الغدد اللمفاوية نوع هودجكين , PDL-1 , خلايا السرطانية لهودجكين, فيروس EB.

INTRODUCTION

Hodgkin Lymphoma (HL) comprises 10% of lymphomas and 1% of cancers in industrial countries [1], with an annual incidence of 2–3 per 100,000 in Europe and the USA. In Iraq, HL ranks tenth among cancers, varying from 0.8 to 2.78 per 100,000 in different regions [2], The WHO classifies HL into classical and nodular lymphocyte-predominant types, with nodular sclerosis cHL being the most common subtype [3]. Diagnosis relies on histological examination revealing Hodgkin Reed-Sternberg cells expressing CD30 and CD15 and lacking CD45 [4].

HRS cells, originating from germinal center B cells, acquire key characteristics, such as MYC, NF-B, and JAK/STAT pathway activity [4], enabling their survival and escape from programmed cell death during malignant transformation. The precise mechanisms underlying this transition remain incompletely understood [5].

The etiology of HL is primarily linked to genetic predisposition [6] and environmental factors [7]. Epstein-Barr virus (EBV) [8] is implicated in certain cases, providing survival signals.

The immune system plays a dual role in cancer development through immunoediting [9], with the

elimination phase damaging cancer cells, followed by an escape phase where tumors express T-cell checkpoint regulators like CTLA-4, PD-1, and PD-L1 [10].

In nodular-sclerosis HL, chromosome 9p24.1 amplification leads to PD-L1 overexpression [11], indicating genetic predisposition to PD-1 blockade sensitivity [12]. Immune checkpoint drugs targeting the PD-1 pathway, such as Nivolumab, have shown promising outcomes in treating HL patients with 9p24.1 amplification [13].

This approach, focusing on the microenvironment, represents a significant advancement in cancer treatment, particularly in high-risk cases [14]. In relapsed or resistant cHL, anti-PD-1 monotherapy has demonstrated response rates of 50-80%, expanding the indications for immune checkpoint inhibitors either alone or in combination and representing a substantial breakthrough in cancer treatment [15].

Epstein-Barr virus (EBV) is associated with a third of cHL cases in affluent nations, with a higher prevalence in underdeveloped areas [16].

In EBV-associated Hodgkin Reed-Sternberg (HRS) cells express viral transcripts and proteins, following a latency II pattern. Although the exact role of EBV in cHL pathophysiology is not fully understood, Latency II antigens are believed to rescue germinal center B cells from apoptosis, leading to their transformation into HRS cells [16].

Additionally, juvenile cHL cases with EBV exhibit immune profiles suggesting the presence of regulatory mechanisms hindering antitumoral reactions in the cHL microenvironment [17].

AIMS OF THE STUDY

To detect the frequency and association between EBV & PDL-1 expression in HL cases.

To investigate the association between the expression of PD-L1 & EBV in HL and some clinic-pathological parameters including age, & subtype.

MATERIAL AND METHODS

A retrospective and prospective case series study was conducted on 40 HL cases collected from private laboratories in North Iraq extending from November 2022 through September 2023. Cases were immunohistochemically confirmed, reviewed and subtyped according to the WHO classification 2016 [18].

Immunohistochemical stains for PDL-1 and EBV LMP-1 were performed on Formalin-Fixed Paraffin-Embedded (FFPE) tissues at Private laboratory in north of Iraq.

PDL-1 status was assessed using the PDL-1 IHC 22C3PharmDx Kit (Dako, SK006) in an automated staining process. FFPE tissue, cut into 4-micron thickness, underwent deparaffinization, re-hydration, and epitope retrieval using Dako target retrieval solution and a water bath-based Dako PT Link tank. This process aimed to enhance staining intensity by unmasking antigens with a single primary antibody.PD-L1 status in HL was scored based on both percentage and intensity of staining in Hodgkin Reed-Sternberg (HRS) cells [19]. A 5% PD-L1 staining cutoff for HRS cells was used. Staining intensity was categorized as negative (0), weak (+1), moderate (+2), or strong (+3). Patients with moderate and strong staining in at least 5% of HRS cells were deemed "positive for PD-L1." Tumor microenvironment staining (>20%) was considered separately from HRS cell scoring.

EBV LMP-1 status was assessed using the Bio SB EBV detection kit, which includes a ready-to-use anti-mouse monoclonal antibody (IgG-1, clone CS1-4) targeting latent membrane protein-1 (LMP-1). Positive interpretation involved observing cytoplasmic staining in Hodgkin cells.

Positive & negative control slides were included in each run of staining for both markers.

RESULTS

The study included 40 cases of classical Hodgkin Lymphoma (HL), with a mean age of 31.4 ± 17.46 years and M:F =3:2. as demonstrated in tables (1,2). Nodular sclerosis was the most common histological subtype (67.5%) as demonstrated in table (2). Age distribution analysis revealed a statistically significant association with the histological subtype (P=0.046), with predominance of mixed cellularity in early decades, whereas nodular sclerosis showing a bimodal age distribution as demonstrated in table (1).

It is noteworthy that in this study, the subtypes of Hodgkin Lymphoma characterized by lymphocyte-rich and lymphocyte-depleted compositions were not identified.

Positive PD-L1 immunohistochemistry (IHC) expression was observed in 67.5% and 35% in Hodgkin Reed-Sternberg (HRS) cells and the tumor microenvironment (TME), respectively, as depicted in figures (1,2), photomicrograph (1,2,3). with no significant association with either age or gender as demonstrated in table (3) and (4). EBV LMP-1 expression was found in 30% as shown in figure (3) photomicrograph (4,5), showing a significant association with gender (p=0.050) and histological subtype (p=0.032) as demonstrated in table (5). A comparison between PD-L1 and EBV LMP-1 expression revealed no significant association (p=1.000) as demonstrated in table (6).

DISCUSSION

Immune checkpoint inhibitors, particularly anti-PD-1/PD-L1 antibodies, represent a significant advancement in cancer treatment, gaining approval for various cancers [20]. In cases of relapsed or refractory classical Hl with 9p24.1 mutation, anti-PD-1 monoclonal antibodies like pembrolizumab and nivolumab have shown impressive response rates, leading to accelerated approval [21].

The prevalence of HL differs between developing and developed countries, with variations in age distribution [22]. The study reports a mean age of (31.4 +-17.46) with a predominant occurrence of cases in the 2nd and 3rd decades, aligning with findings in other studies [19,23,24]. Variations in age distribution across studies may be influenced by factors such as ethnic, geographic, and socioeconomic diversity [25]. This study reveals a higher occurrence in male patients (60%) with M: F = 3:2, consistent with earlier study conducted in Iraq [26]. In contrast, some studies in Iraq found an equal gender ratio than those conducted by (Saeed MS, Majeed AH) [23,24]. Regarding HL subtypes, there has been a change in the trend compared to earlier Iraqi studies, with nodular sclerosis HL forming the majority of cases (67.5%) [26,27]. This is consistent with findings in the North of Iraq and some neighboring Eastern Mediterranean countries as Saudi Arabia, Jordan and Turkey [28-30] resembling patterns in developed western countries [31]. The study also revealed a bimodal age distribution for nodular sclerosis (HL), with the first peak in the 2nd and 3rd decades and another in the 6th and 7th decades, while mixed cellularity HL was most commonly encountered early in life. These results differ from earlier study reporting predominance of mixed cellularity in Iraq [24].

Globally, PD-L1 expression in HL varies widely, ranging from 20% to 100%. In the current study 67% of HL cases exhibit positive PD-L1 expression, which aligns with findings from some studies [32,33]. However, other studies have reported both either lower or higher percentages of PD-L1 expression [34-36]. The variation in these results may be attributed to differences in PD-L1 antibody clones, evaluation criteria, as well as genetic and geographic diversity of the studied populations [32,33,34]. Assessment of HL microenvironment in this study revealed PD-L1 expression in 35% of cases. Similar results were found in studies conducted in Turkey [19,34]. However, other studies reported higher percentages of PD-L1 expression in the tumor microenvironment [13]. These variations in results may be attributed to differences in immunological and genetic responses to Hodgkin Reed-Sternberg cells in the tumor microenvironment [37].

In the current study, the association of PD-L1 expression in both Hodgkin Reed-Sternberg (HRS) cells and the tumor microenvironment with age, gender and subtype, was not found to be significant. This agrees with similar findings in other studies [19,33,34]. Despite the lack of statistical significance, it's noteworthy that in the current study, PD-L1 expression was more common in individuals less than 40 years old (88.9%), males (59.3%), and the nodular sclerosis subtype (70.4%). This pattern is somewhat consistent with results from other studies, such as [33], which also observed a higher prevalence of PD-L1 expression in individuals less than 45 years old (58.5%), males (56.1%), and nodular sclerosis subtype (70.7%).

Concerning EBV LMP-1 expression in HL, only 30% of cases reveal LMP-1 expression which is approximately to [23] in Iraq with the mixed cellularity subtype being the most likely to be associated with EBV LMP-1 status.

Genetic factors may contribute to a predisposition for EBV-associated HL [38]. Some studies propose using circulating EBV-DNA levels as a biomarker to monitor therapy responses. There's potential for targeting EBV as a therapeutic intervention in HL. This reflects ongoing efforts to understand and develop targeted treatments for EBV-associated cancers, including HL [38].

EBV LMP-1 expression in HL shows age-related variability, with peaks in the 2nd decade (41.7%) and another in older age groups (<50 years) (33.4%). Studies from Iraq and other countries, including developed and developing nations, reported similar age-related trends [39,40] This variation may be linked to the bimodal age incidence of cases, especially in nodular sclerosis classical HL. EBV LMP-1 expression in HL exhibits a male predilection, possibly attributed to differences in immune response between genders & social factors. This study, along with others, highlights a significant statistical association (p=0.050) between EBV LMP-1 expression and gender [23,29,39,40]. EBV LMP-1 expression in HL is significantly associated with histological subtype. Mixed cellularity HL exhibits a higher frequency (58.3%) compared to nodular sclerosis (41.7%), in agreement with findings from various studies [19,41]. However, discrepancies, such as a Pakistani study [42] reporting higher EBV LMP-1 expression in nodular sclerosis, emphasize the complexity of these associations. The current study reveals that 29.6% of HL cases exhibit concurrent expression of PD-L1 and EBV LMP-1 in HRS cells. In contrast, some studies reported a higher co-expression percentage [19,33], denoting to possible other contributing factors in such relationship between these markers.

Large-scale multicenter studies and variations in detection methods further contribute to the nuanced understanding of PD-L1 and EBV LMP-1 associations in HL [43].

Research exploring the relationship between EBV LMP-1 and PD-L1 in HL suggests that while some publications highlight a connection based on gene locus aberrations, protein expression studies have not consistently confirmed such associations. In cases negative for EBV LMP-1, alternative mechanisms may activate PD-L1. There were two studies support the notion that EBV LMP-1 status may not reliably predict PD-L1 expression in Hodgkin lymphoma [9,34].

CONCLUSION

1. PD-L1 expression was found in 67.5% of HL, with no association with histological subtype, patients' age and gender.
2. EBV LMP-1 was detected in 30% of HL cases, males exhibited a higher level of expression (83.3%) compared to females, with a statistically significant association. However, expressed not significantly associated with patients' age and histological subtypes.
3. No significant correlation between was discerned between PD-L1 and EBV LMP-1 expression.

Conflict of Interest

Disclosure: The authors declare that they have no conflict of interest.

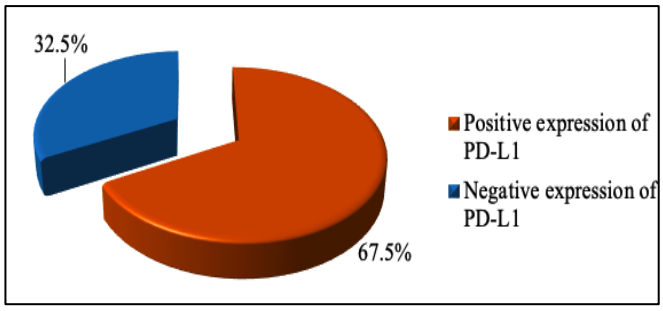


Figure (1): PD-L1 IHC expression in HRS cells.

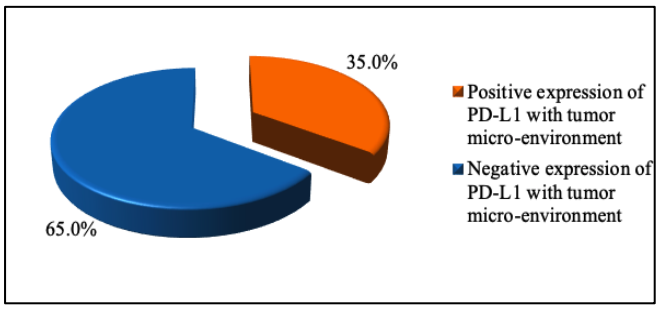


Figure (2): The expression of PD-L1 IHC with tumor micro-environment of HL.

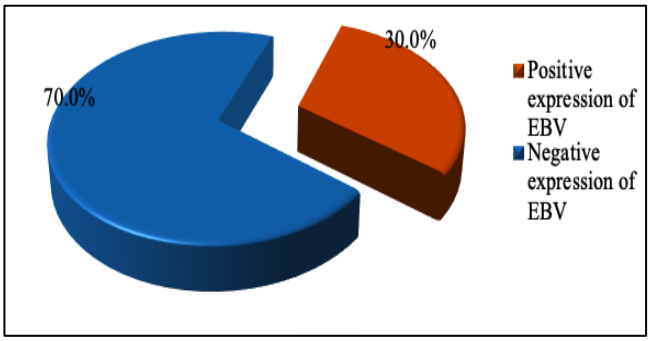
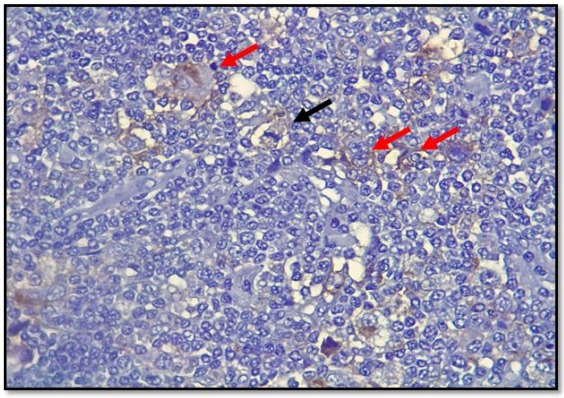
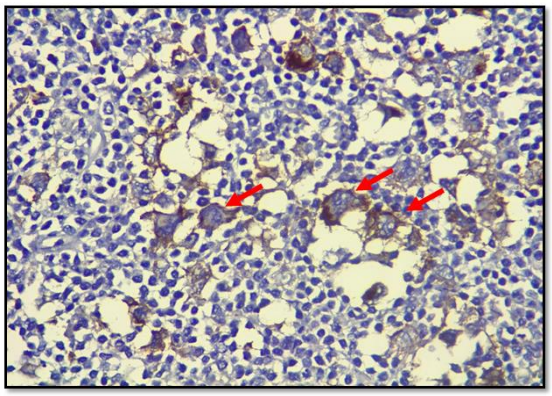


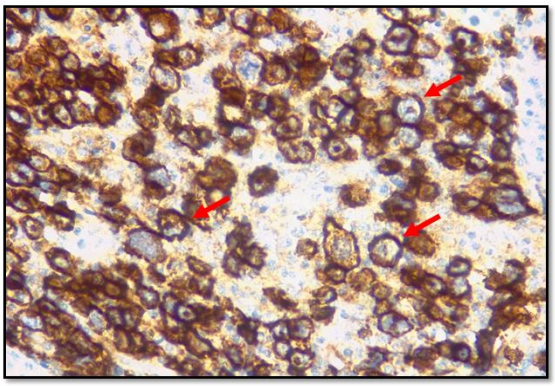
Figure (3): The EBV expression in HL.



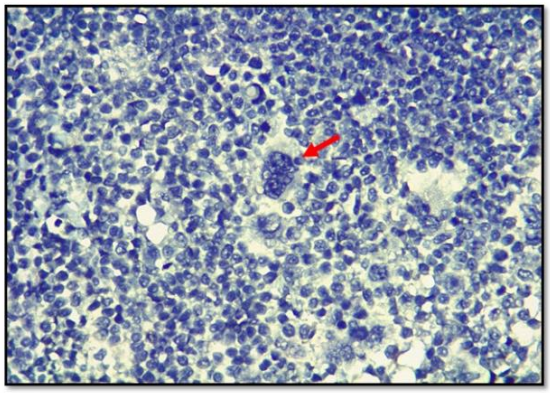
Photomicrograph 1: HL with multiple mononuclear and lacunar Hodgkin cells, showing positive membranous brown DAB staining, intensity (1+), (red arrows), one HRS cell shows mitoses (black arrow) (PDL-1 IHC staining X400).



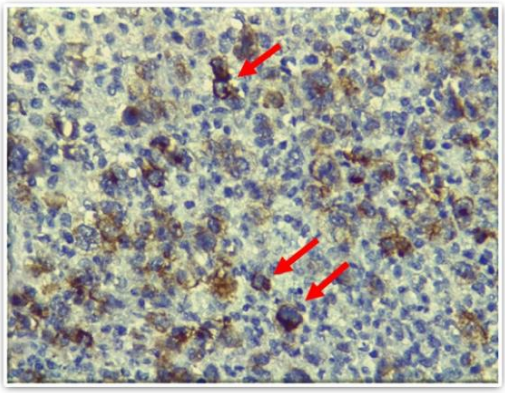
Photomicrograph 2: HL with multiple mononuclear and lacunar Hodgkin cells, showing positive membranous brown DAB staining, intensity (2+), (red arrows) (PDL-1 IHC staining X400).



Photomicrograph 3: HL with many Hodgkin cells, showing prominent positive membranous brown DAB staining, intensity (3+), (red arrows) (PDL-1 IHC staining X400).



Photomicrograph 4: HL with central multinucleate Hodgkin cells, showing negative EBV LMP-1 staining, (red arrow) (EBV IHC staining X400).



Photomicrograph 5: Hodgkin lymphoma with multiple mononuclear and lacunar Hodgkin cells, showing positive EBV, (red arrows). (PDL-1 IHC staining X400).

Table (1): Age distribution in histological subtypes of classical HL.

Age groups/years	Number (%)	Age groups / years	Types		*P*-value*
			MC No. (%)	NS No. (%)	
≤ 10	1(2.5 %)	≤ 10(n =1)	0(0.0)	1(3.7)	0.046
11-20	12(30 %)	11-20 (n=12)	6(46.1)	6(22.2)	
21-30	12(30 %)	21-30 (n=12)	2(15.4)	10(37. 1)	
31-40	6(15%)	31-40 (n=6)	2(15.4)	4(14.8)	
41-50	1(2.5 %)	41-50 (n=1)	0(0.0)	1(3.7)	
51-60	4(10%)	51-60 (n=4)	2(15.4)	2(7.4)	
≥ 61	4(10%)	≥ 61 (n=4)	1(7.7)	3(11.1)	
Total	**40(100%)**	**Total**	**13(100%)**	**27(100%)**	

Table (2): Gender distribution in histological subtypes.

Gender	Number (%)	Histological subtype	Number (%)
Males	24(60%)	Nodular Sclerosing	27 (67.5%)
Females	16(40%)	Mixed cellularity	13 (32.5%)
Total	**40(100%)**	**Total**	**40 (100%)**

Table (3): PD-L1 IHC expression in HRS cells in relation to age groups, gender and histological subtype of HL cases.

Clinico-pathological Parameter		**PD-L1 IHC expression in HRS**		***P*-value***
		(+ve) No. (%)	**(-ve) No. (%)**	
Age group/ years	≤ 10	1(3.7%)	0(0.0%)	0.172
	11-20	11(40.7 %)	1(7.7%)	
	21-30	8(29.6%)	4(30.7 %)	
	31-40	4(14.9%)	2(15.4 %)	
	41-50	0(0.0%)	1(7.7%)	
	51-60	1(3.7%)	3(23.1 %)	
	≥ 61	2(7.4%)	2(15.4 %)	
	Total	27(100 %)	13(100 %)	
Gender	Males	16(59.3 %)	8(61.5 %)	0.89
	Females	11(40.7 %)	5(38.5 %)	
	Total	**27(100 %)**	**13(100 %)**	
Histological subtype	Nodular sclerosis	19(70.4 %)	8(61.5 %)	0.416
	Mixed cellularity	8(29.6%)	5(38.5 %)	
	Total	**27(100 %)**	**13(100 %)**	

Table (4): PD-L1 IHC expression in tumor micro-environment in relation to age groups, gender and histological subtype of HL cases.

Clinico-pathological Parameter		**PD-L1 IHC expression in tumor micro-environment**		***P*-value ***
		(+ve) No. (%)	**(-ve) No. (%)**	
Age group/ years	≤ 10	1 (7.1%)	0 (0.0%)	0.525
	11-20	5 (35.7%)	7 (27.0%)	
	21-30	4 (28.6%)	8 (30.8%)	
	31-40	2 (14.4%)	4 (15.4%)	
	41-50	0 (0.0%)	1 (3.8%)	
	51-60	1 (7.1%)	3 (11.5%)	
	≥ 61	1 (7.1%)	3 (11.5%)	
	Total	14 (100%)	26 (100%)	
Gender	Males	11 (78.6%)	13 (50.0%)	0.079
	Females	3 (21.4%)	13 (50.0%)	
	Total	14 (100%)	26 (100%)	
Histological subtype	Nodular sclerosis	8 (57.1%)	19 (73.1%)	0.480
	Mixed cellularity	6 (42.9%)	7 (26.9%)	
	Total	14 (100%)	26 (100%)	

Table (5): EBV LMP-1 IHC expression in HRS in relation to age groups, gender and histological subtype.

Clinico-pathological Parameter		EBV IHC expression		*P*-value*
		(+ve) No. (%)	(-ve) No. (%)	
Age group/ years	≤ 10	0(0.0%)	1(3.6%)	0.156
	11-20	5(41.7%)	7(25.0%)	
	21-30	1(8.3%)	11(39.3%)	
	31-40	2(16.7%)	4(14.3%)	
	41-50	0(0.0%)	1(3.6%)	
	51-60	2(16.7%)	2(7.1%)	
	≥ 61	2(16.7%)	2(7.1%)	
	Total	12(100%)	28(100%)	
Gender	Males	10(83.3%)	14(50.0%)	0.050
	Females	2(16.7%)	14(50.0%)	
	Total	12(100%)	28(100%)	
Histological subtype	Nodular sclerosis	5(41.7%)	22(78.6%)	0.032
	Mixed cellularity	7(58.3%)	6(21.4%)	
	Total	12(100%)	28(100%)	

Table (6): Comparison of PD-L1 IHC expression with EBV expression.

EBV	PD-L1 IHC expression		p-value *
	+ve No. (%)	-ve No. (%)	
+ve	8(29.6%)	4(30.8%)	1.000
-ve	19(70.4%)	9(69.2%)	
Total	27(100%)	13(100%)	

Disclosure

The authors declare that they have no conflict of interest.

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