Original Article

Prognostic Value And Clinical Impact Of EBV And PDL1 Expression In Classical Hodgkin Lymphoma

Eman Eid Saad[1], Tawfik Ragab Elkhodary[2], Azza AbdelAziz [3], Hayam Fathy Ghazy[2], Sherif Refaat Ali[2]

1. M.Sc., Assistant lecturer of Medical Oncology, Faculty of medicine, Mansoura University, Egypt
2. MD, Professor of Medical Oncology, Faculty of medicine, Mansoura University, Egypt
3. MD, Professor of Pathology, Faculty of medicine, Mansoura University, Egypt

Email: Emygirl1988@yahoo.com

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Abstract

Background Classic Hodgkin Lymphoma (cHL) accounts for approximately 90% of Hodgkin lymphoma, while nodular lymphocyte-predominant HL (NLPHL) accounts for the remainder of cases. However, the distribution of histologic subtypes of CHL varies based on geography, socioeconomic factors, race/ethnicity, and age. Epstein-Barr virus (EBV) play a role in pathobiology of significant fraction of HL cases as EBV infected tumor cells express subset of EBV genes including LMP1, LMP2 **Aim** The aim of this study was to investigate and assess EBV and PDL 1 expression as prognostic markers in classical Hodgkin lymphoma and their relation to International Prognostic Score (IPS). **Methods** The current study was a prospective study that included patients aged ≥ 18 years with pathologically proven classical Hodgkin lymphoma. But we excluded patients received previous chemotherapy or radiotherapy, patients with advanced medical comorbidity and patients with other malignancy. Performance status was classified according to Eastern Cooperative Oncology Group (ECOG). Immunohistochemistry staining (PDL1 and EBV) was done for the obtained biopsies. **Results** PDL1 was positive in 75 patients (89.3%), while 69 (82 %) of the patients were EBV negative. Near 83% of the patients with EBV positive were presented with early-stage Hodgkin lymphoma with favorable risk, patients with neutrophils lymphocytes ratio less than 3, near 93,6 % of the patients achieved complete remission. **Conclusion** Hodgkin lymphoma is considered highly curable disease with low mortality. EBV and PDL1 should be investigated on larger number of cases to evaluate their predictive and prognostic value. Neutrophil lymphocyte ratio is an important predictive and prognostic marker for Hodgkin lymphoma which is inexpensive, easily accessible test from a CBC at diagnosis, simple, widely available, and can be easily used in clinical practice especially in resource-poor areas.

Keywords: Hodgkin Lymphomas, Nodular Sclerosis, Epstein-Barr virus, Reed Sternberg cells, Eastern Cooperative Oncology Group

Introduction

Classic Hodgkin Lymphoma (cHL) accounts for approximately 90% of Hodgkin lymphoma, while nodular lymphocyte-predominant HL (NLPHL) accounts for the remainder of cases (1). However, the distribution of histologic subtypes of CHL varies based on geography, socioeconomic factors, race/ethnicity, and age (2).
Hodgkin lymphomas (HL; formerly called Hodgkin's disease) are lymphoid neoplasms in which malignant Hodgkin/Reed-Sternberg cells are mixed with a heterogeneous population of non-neoplastic inflammatory cells (3). HL is divided into two major subgroups, based on morphology and immunophenotype (4). cHL which is further categorized according to histology; nodular sclerosis CHL (NSCHL), mixed cellularity CHL (MCCHL), lymphocyte CHL (LRCHL), lymphocyte depleted CHL (LDCHL) and nodular lymphocyte predominant HL (NLPHL).

Epstein-Barr virus (EBV) play a role in pathobiology of significant fraction of HL cases as EBV infected tumor cells express subset of EBV genes including LMP1, LMP2 (5). LMP1 produces tonic survival signal through activation of NF-KB pathway while LMP2 is an integral membrane protein that contain motifs resemble those of immunoglobulin molecules, its expression prevent apoptosis of pre B cells that fail to express Ig molecules (6). Both help tumor cells to escape programmed cell death (apoptosis)(7). The presence of EBV is generally associated with inferior prognosis in cHL, compared to patients who are EBV negative (8).

On the other hand, Reed Sternberg cells of cHL often express PDL1 and PDL2, ligand for PD1 receptors (9). Activation of PD1 leads to exhaustion and inactivation of effector T cells and is believed to have an important role in immunoevasion of Reed Sternberg cells from host immune system (10). checkpoint inhibitors targeting PD1 pathway are effective treatment in relapsed and refractory cHL, but in first line therapy efficacy of these drugs is still unknown

(11). consequently, the biological dynamicity of PD1 pathway in cHL during disease progression needs to be described (12). cHL patients with high proportions of both PD1 and PDL1 leucocytes have inferior outcome (13).

It has been found that there is association between EBV associated neoplasia and PD1 and/or PDL1 expression in some lymphomas (14). In EBV associated cHL, the presence of immunosuppressive microenvironment of EBV with high numbers of M2 macrophages and elevated expression levels of PDL1 make EBV related cHL patients more susceptible to checkpoint blockade (15).

Several studies have attempted to use prognostic factors to identify patients at high risk for first relapse and who might benefit from more intensive initial therapy (16). The strongest predictor of outcome that can be easily applied in the clinical setting is the International Prognostic Score (IPS). IPS for patients with HL calculated based on the following seven potential unfavorable features at diagnosis; serum albumin <4 g/dL, hemoglobin <10.5 g/dL, male gender, age >45 years, stage IV disease, white blood cell count ≥ 15,000/microL, and absolute lymphocyte count <600/microL and/or <8 percent of the total white blood cell count. In this system, one point is given for each of the characteristics above (total score of 0 to 7), representing increasing degrees of risk (17).
There are other pathological features that have been evaluated as potential prognostic factors for cHL, but none is routinely applied in clinical practice (18); tumor grade (19), inflammatory infiltrate (20), cytokine responses (21), and Epstein-Barr virus (EBV) (22).

Aim

The aim of this study was to investigate and assess EBV and PDL 1 expression as prognostic markers in classical Hodgkin lymphoma and their relation to International Prognostic Score (IPS).

Patients and Methods

The current study was a prospective study targeting 84 patients with classical Hodgkin lymphoma. Patients were recruited from our outpatient medical oncology Clinic at Oncology Center Mansoura University from 2019 to 2021. We included patients aged ≥ 18 years with pathologically proven classical Hodgkin lymphoma. But we excluded patients received previous chemotherapy or radiotherapy, patients with advanced medical comorbidity and patients with other malignancy.

Methods

After being approved by the institutional research board (IRB) of the Faculty of Medicine, Mansoura University. All patients with classical Hodgkin lymphoma were subjected to history taking inform of personal history (name, age, gender, residence, marital status, occupation and special habits such as smoking), present history that included the Complaint (onset, course, duration, and associated symptoms). We asked about the symptoms suggesting other systems' affection as neurologic manifestations (weakness, sensory disturbance, sphincter disorder), GIT manifestations (mucosal ulcer, abdominal pain, diarrhea, mucus in the stool), urinary tract symptoms (dysuria and frequency), dermal manifestations (skin rash, ulcer and nodule), and cardiopulmonary symptoms (dyspnea, chest pain, cough). Past history included history of other illnesses (DM, HTN), infection and autoimmune diseases, history of prior operations, transfusion of blood or hospital admission and the family history included similar conditions and other malignancies. Performance status was classified according to Eastern Cooperative Oncology Group (ECOG) (23) into:

Performance status	Definition
0	Fully active; no performance restrictions.
1	Strenuous physical activity restricted; fully ambulatory and able to carry out light work.
2	Capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair >50% of waking hours.
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair.

Examination

The clinical examination included general examination that included body mass index (BMI), Complexion (pallor, jaundice and cyanosis), vital signs (temperature, pulse, blood pressure and respiratory rate), chest, cardiac, head, neck, abdominal, neurologic examination and lymph node examination.

Investigations

The investigations included Laboratory examination inform of complete blood picture, liver and renal functions, erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), virology testing including HBV, HCV and HIV, and bone marrow evaluation by aspirate and biopsy. The imaging procedures included PET-CT [the combination of positron emission tomography (PET) and computed tomography (CT)], CT neck, chest, abdomen and pelvis, and MRI.

We took Biopsies from suspected lesions to diagnose cHL to detect histological subtypes. Sites and type of biopsy differs according to clinical presentation. Lymph node biopsies either excisional or incisional biopsy from peripheral lymph nodes were done and that provided adequate material for pathological evaluation.

Immunohistochemistry

Immunohistochemistry staining for PD- L1 quartett (Clone QR1) rabbit monoclonal antibody (Berlin, Germany) and EBV (CS1-4) Mouse Monoclonal Antibody Sigma-Aldrich (USA) was done for the biopsies. Positive immunostaining is defined as membranous staining for PDL1 and cytoplasmic/nuclear staining for EBV. the percentage of tumor cells present in tissue sections was estimated for each case.

Evaluation of immunohistochemistry

The percentage of the total cellularity staining for rabbit anti-PD-L1, including malignant and nonmalignant cells, and the percentage of the tumor cell population staining for PD-L1 were scored independently. Staining intensity was scored as follows: 0 (no staining), 1+ (weak or equivocal staining), 2+ (moderate staining), or 3+ (strong staining). PD-L1 was considered positive if 5% or more of the tumor cell population showed 2+ or 3+ membrane staining. A case was considered to have a microenvironment positive for PD-L1 if 20% or more of the total tissue cellularity showed 2+ or 3+ membrane or cytoplasmic staining in malignant and/or nonmalignant cells (24). For EBV, positive immunostaining was defined as cytoplasmic staining in Reed Sternberg cells (25, 26).

Statistical Analysis

Data were analyzed on a personal computer running SPSS© for windows (Statistical Package for Social Scientists) version 19. A p value of < 0.05 was considered statistically significant. For descriptive statistics of qualitative variables, the frequency distribution procedure was run with calculation of the number of cases and percentages. For descriptive statistics of quantitative variables, the mean, and standard deviation or the median and range were used to describe central tendency and dispersion as appropriate. Normality of the sample distribution of each continuous variable was tested with the Kolmogorov–Smirnov test. Association between categorical variables was tested by the Chi Square Test. Fishers exact test was used if the assumptions of Chi square were violated. The independent-samples t-test was used to compare the means between two groups. Survival and progression free survival analyses were calculated by the Kaplan-Meier Product-Limit Estimator. Comparison of the survival was performed by the Log-Rank Test Exploring variables for their independent prognostic effect on survival was carried out using the multivariate stepwise Cox's proportional regression hazard model.

Results

In this study, 84 patients were included, 59 patients (70.2%) had PS ECOG=0, while 25 (29.7%) patients had PS ECOG=1. In this study HL is most common among young adults (20 to 39 years) with male predominance 56% compared with females 37%. Patients with history of DM were 20 (23.8%), while 11 patients (13%) had history of hypertension, 45 patients (53.6%) were smokers, 64 patients (76.1%) were overweight and obese while no patients had history of autoimmune disease (table 1). Eighty patients (95.2%) had cervical lymphadenopathy at their first presentation, 45 patients (54.7%) had B symptoms while 11 patients (13%) had initial bulky disease (Fig. 1). Nodular sclerosis was the most common pathologic variant in our study 46.43% (Fig. 2). Most cases presented with advanced stage, 38.1% were presented with stage III while 23.8 % were presented with stage IV disease (Fig. 3).

Table (1) Personal history and Risk factors related

		No	%
DM	Present	20	23.8
	Absent	64	76.1%
Hypertension	Present	11	13%
	Absent	73	87%
PS	0	59	70.2%
	1	25	29.7%
Smoking	Present	45	53.6%
	Absent	39	46.4%
Autoimmune disease	Present	0	0%
	Absent	84	100%
BMI Categories	Normal Weight	20	23.8%
	Overweight	50	59.5%
	Obese	14	16.6%
Family history of lymphoma	Present	10	12%
	Absent	74	88 %

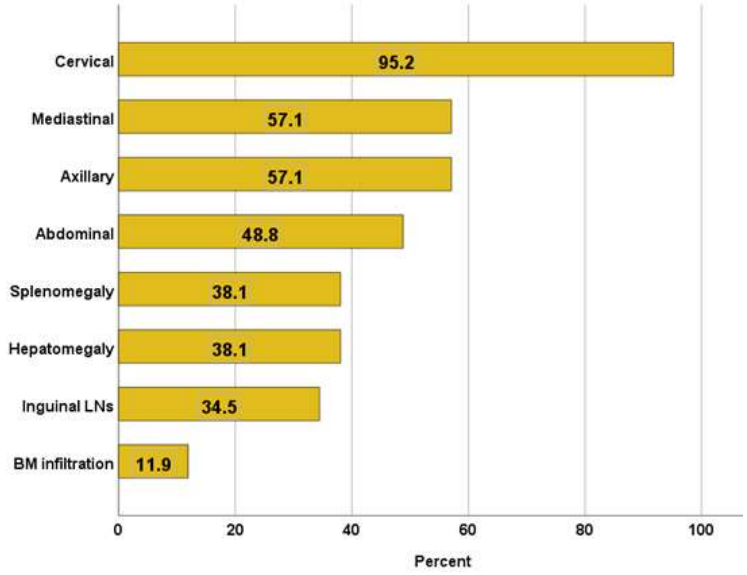
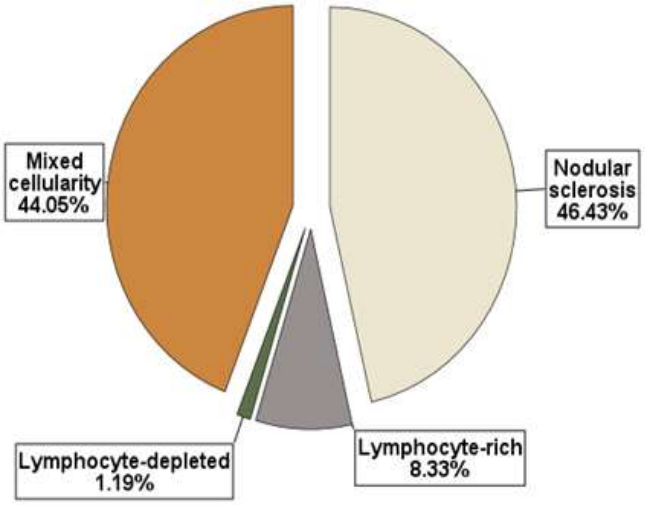
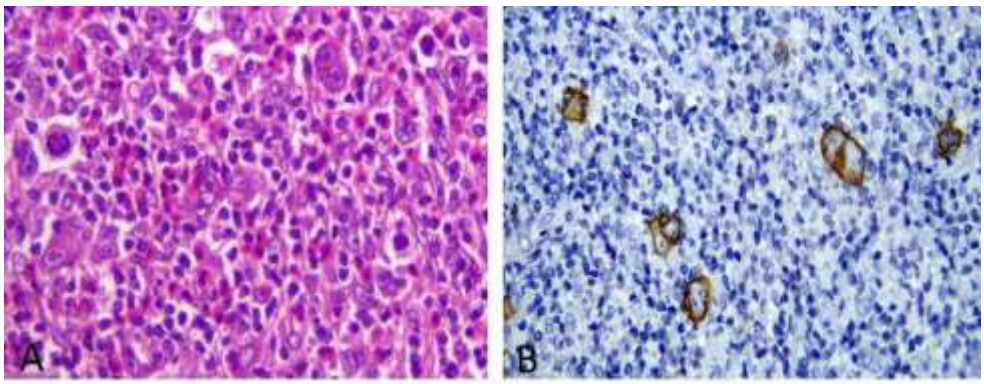
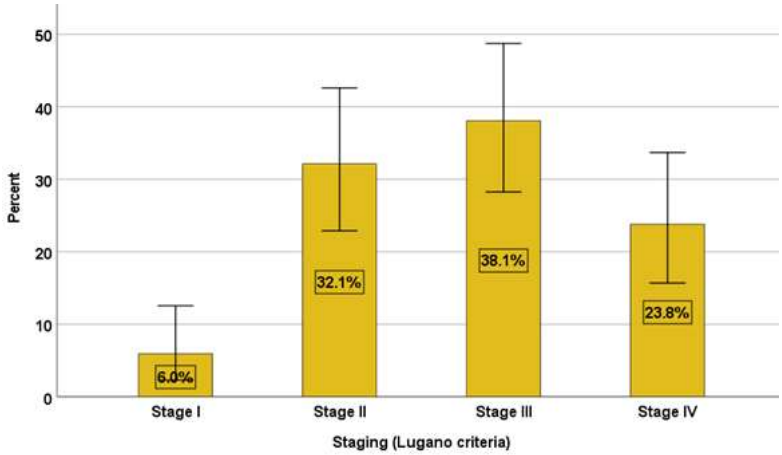


Figure (1) Presentations of the studied cases.





Figure(2) Pathology of the studied cases. A)Hodgkin's lymphoma mixed cellularity type(H&E X400). B)CD30 positivity in Reed Sternberg cells (IHC,DAB X400).



Figure(3) Staging of the studied cases.

Regarding laboratory studies, 42 patients (50%) were presented with anemia, while 74 patients (88.1%) had elevated LDH and 37 patients (44%) had neutrophils/lymphocytes ratio more than 3 (Table 2). Regarding prognostic factors, early stage (NCCN, EORTC and GHSG) had no difference as regard favorable or unfavorable risk, while 16 (30 %) patients had IPS =1 (Table 3). PDL1 was positive in 75 patients (89.3%), while 69 (82 %) of the patients were EBV negative (Fig. 4)(Table 4).

Table (2) laboratory studies related parameters.

		No	%
N/L Ratio	<3	47	56.0%
	≥ 3	37	44.0%
ESR	<30	40	47.6%
	30-50	17	20.2%
	>50	27	32.1%
Leukocytosis (> 15x1000/µL)		15	17.9%
Lymphocytopenia (Lymphocytes < 8%)		9	10.7%
Anemia		42	50.0%
Thrombocytopenia (Platelets < 100 x 1000/µL)		3	3.6%
Serum albumin (gm/dL)	< 4	15	17.9%
	≥ 4	69	82.1%
Elevated LDH		74	88.1%
Renal Impairment		1	1.2%
Abnormal Liver Transaminases		11	13.1%
Hyperbilirubinemia		4	4.8%
HCV Positive		4	4.8%
HBV Positive		2	2.4%
HIV Positive		1	1.2%

Table (3) Prognostic factors related parameters.

		No	%
Early stage NCCN risk categories	Favorable risk	11	34.4%
	Unfavorable risk	21	65.6%
Early EORTC	Favorable risk	11	34.4%
	Unfavorable risk	21	65.6%
Early stage GHSG risk categories	Favorable risk	11	34.3%
	Unfavorable risk	21	65.6%
Advanced stage IPS risk categories	0	5	9.4%
	1	16	30.2%
	2	14	26.4%
	3	9	17.0%
	4	7	13.2%
	5	2	3.8%

Table (4) PDL1 and EBV staining.

PD-L1	**Negative**	9	10.7%
	Positive	75	89.3%
EBV	**Negative**	69	82.1%
	Positive	15	17.9%

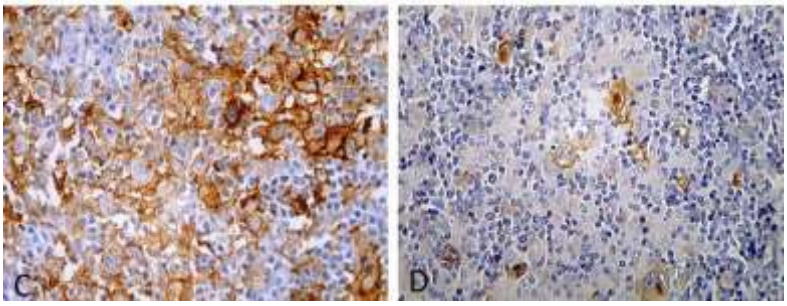


Figure (4) PDL1 and EBV staining. C)PDL1 membranous staining in Reed Sternberg cells and mature lymphocytes (IHC,DAB X400). D)Cytoplasmic/nuclear staining for EBV in Reed Sternberg cells (IHC,DAB X400).

Regarding the relation of PDL1 expression to clinicopathologic features, near 88% of the patients with negative PDL1 had high ESR more than 30 with P value =0.02 (Table 5). Regarding relation of EBV expression to clinicopathologic features, near 83% of the patients with EBV positive were presented with early-stage Hodgkin lymphoma with favorable risk with P value=0.003 (Table 6).

Table (5) Relation of PDL1 expression to clinicopathologic features.

PD-L1		**Negative**		**Positive**	
		No	**%**	**No**	**%**
Age (median cut-off)	**<35**	5	55.6%	37	49.3%
	≥35	4	44.4%	38	50.7%
Gender	**Female**	3	33.3%	34	45.3%
	Male	6	66.7%	41	54.7%
B-Symptoms	**Absent**	3	37.5%	34	45.9%
	present	5	62.5%	40	54.1%
Cervical LNs	**Absent**	1	11.1%	3	4.0%
	Present	8	88.9%	72	96.0%
Axillary LNs	**Absent**	3	33.3%	33	44.0%
	Present	6	66.7%	42	56.0%
Inguinal LNs	**Absent**	6	66.7%	49	65.3%
	Present	3	33.3%	26	34.7%
Mediastinal LNs	**Absent**	2	22.2%	34	45.3%
	Present	7	77.8%	41	54.7%
Abdominal LNs	**Absent**	6	66.7%	37	49.3%
	Present	3	33.3%	38	50.7%
Splenomegaly	**Absent**	6	66.7%	46	61.3%
	Present	3	33.3%	29	38.7%
Hepatomegaly	**Absent**	7	77.8%	45	60.0%
	Present	2	22.2%	30	40.0%
BM infiltration	**Absent**	9	100.0%	65	86.7%
	Present	0	0.0%	10	13.3%
ESR***	***<30***	***1***	***11.1%***	***39***	***52.0%***
	≥30	***8***	***88.9%***	***36***	***48.0%***
Leucocytosis	**≤ 15x1000/µL**	7	77.8%	62	82.7%
	> 15x1000/µL	2	22.2%	13	17.3%
Lymphocytopenia	**Lymphocytes ≥8%**	9	100.0%	66	88.0%
	Lymphocytes <8%	0	0.0%	9	12.0%
Anaemia	**No anaemia**	4	44.4%	38	50.7%
	Anaemia	5	55.6%	37	49.3%
Thrombocytopenia	**Platelets ≥100 x1000/µL**	9	100.0%	72	96.0%
	Platelets < 100 x 1000/µL	0	0.0%	3	4.0%

Serum albumin (gm/dL)	**< 4**	1	11.1%	14	18.7%
	≥ 4	8	88.9%	61	81.3%
Normal LDH	**Normal**	1	11.1%	9	12.0%
Elevated LDH		8	88.9%	66	88.0%
Renal Impairment	**Normal kidney function**	9	100.0%	74	98.7%
	Renal Impairment	0	0.0%	1	1.3%
Liver Transaminases	**Normal liver transaminases**	8	88.9%	65	86.7%
	Abnormal liver transaminases	1	11.1%	10	13.3%
Hyperbilirubinemia	**Normal bilirubin**	9	100.0%	71	94.7%
	Hyperbilirubinemia	0	0.0%	4	5.3%
HCV	**Negative**	9	100.0%	71	94.7%
	Positive	0	0.0%	4	5.3%
HBV	**Negative**	9	100.0%	73	97.3%
	Positive	0	0.0%	2	2.7%
Pathology	**Nodular sclerosis**	5	55.6%	34	50.7%
	Mixed cellularity	4	44.4%	33	49.3%
Staging (Lugano criteria)	**Stage I-II**	5	55.6%	27	36.0%
	Stage III-IV	4	44.4%	48	64.0%
Bulky disease	**No Bulky disease**	9	100.0%	64	85.3%
	Bulky disease	0	0.0%	11	14.7%
Early stage NCCN risk categories	**Favourable risk**	1	20.0%	10	37%
	Unfavourable risk	4	80.0%	17	63%
Response	**CR**	6	66.7%	65	86.7%
	Refractory	3	33.3%	10	13.3%
N/L Ratio	**<3**	3	33.3%	44	58.7%
	≥ 3	6	66.7%	31	41.3%
EBV	**Negative**	8	88.9%	61	81.3%
	Positive	1	11.1%	14	18.7%

**** significant difference at p=0.02**

Table (6) Relation of EBV expression to clinicopathologic features.

EBV		**Negative**		**Positive**	
		No	%	No	%
Age (median cut-off)	**<35**	35	50.7%	7	46.7%
	≥35	34	49.3%	8	53.3%
Gender	**Female**	31	44.9%	6	40.0%
	Male	38	55.1%	9	60.0%
B-Symptoms	**Absent**	29	43.3%	8	53.3%
	Present	38	56.7%	7	46.7%
Cervical LNs	**Absent**	2	2.9%	2	13.3%
	Present	67	97.1%	13	86.7%
Axillary LNs	**Absent**	30	43.5%	6	40.0%
	Present	39	56.5%	9	60.0%
Inguinal LNs	**Absent**	46	66.7%	9	60.0%
	Present	23	33.3%	6	40.0%
Mediastinal LNs	**Absent**	26	37.7%	10	66.7%
	Present	43	62.3%	5	33.3%
Abdominal LNs	**Absent**	34	49.3%	9	60.0%
	Present	35	50.7%	6	40.0%
Splenomegaly	**Absent**	43	62.3%	9	60.0%
	Present	26	37.7%	6	40.0%
Hepatomegaly	**Absent**	41	59.4%	11	73.3%
	Present	28	40.6%	4	26.7%
BM infiltration	**Absent**	60	87.0%	14	93.3%
	Present	9	13.0%	1	6.7%
ESR	**<30**	32	46.4%	8	53.3%
	≥30	37	53.6%	7	46.7%
Leucocytosis	**≤ 15x1000/µL**	55	79.7%	14	93.3%
	> 15x1000/µL	14	20.3%	1	6.7%
Lymphocytopenia	**Lymphocytes ≥8%**	62	89.9%	13	86.7%
	Lymphocytes <8%	7	10.1%	2	13.3%
Anemia	**No anemia**	34	49.3%	8	53.3%

	Anemia	35	50.7%	7	46.7%
Thrombocytopenia	**Platelets ≥100 x1000/µL**	66	95.7%	15	100.0%
	Platelets < 100 x 1000/µL	3	4.3%	0	0.0%
Serum albumin (gm/dL)	**< 4**	13	18.8%	2	13.3%
	≥ 4	56	81.2%	13	86.7%
Elevated LDH	**Normal**	7	10.1%	3	20.0%
	Elevated LDH	62	89.9%	12	80.0%
Renal Impairment	**Normal kidney function**	68	98.6%	15	100.0%
	Renal Impairment	1	1.4%	0	0.0%
Liver Transaminases	**Normal liver transaminases**	60	87.0%	13	86.7%
	Abnormal liver transaminases	9	13.0%	2	13.3%
Hyperbilirubinemia	**Normal bilirubin**	67	97.1%	13	86.7%
	Hyperbilirubinemia	2	2.9%	2	13.3%
HCV	**Negative**	65	94.2%	15	100.0%
	Positive	4	5.8%	0	0.0%
HBV	**Negative**	67	97.1%	15	100.0%
	Positive	2	2.9%	0	0.0%
Pathology	**Nodular sclerosis**	34	54.8%	5	35.7%
	Mixed cellularity	28	45.2%	9	64.3%
Staging (Lugano criteria)	**Stage I-II**	26	37.7%	6	40.0%
	Stage III-IV	43	62.3%	9	60.0%
Bulky disease	**No Bulky disease**	59	85.5%	14	93.3%
	Bulky disease	10	14.5%	1	6.7%
Early stage NCCN risk categories **	**Favorable risk**	**6**	**23.1%**	**5**	**83.3%**
	Unfavorable risk	**20**	**76.9%**	**1**	**16.7%**
Response	**CR**	56	81.2%	15	100.0%
	Refractory	13	18.8%	0	0.0%
N/L Ratio	**<3**	39	56.5%	8	53.3%
	≥ 3	30	43.5%	7	46.7%

**** Significant difference at p=0.003**

Regarding Relation of neutrophils lymphocytes ratio to clinicopathologic features, patients with neutrophils lymphocytes ratio less than 3, near 93,6 % of the patients achieved complete remission (CR) with p value =0.004 (Table 7).

Table (7) Relation of neutrophils lymphocytes ratio to clinicopathologic features.

N/L Ratio		**<3**		**≥ 3**	
		No	%	No	%
Age (median cut-off)	**<35**	20	42.6%	22	59.5%
	≥35	27	57.4%	15	40.5%
Gender	**Female**	19	40.4%	18	48.6%
	Male	28	59.6%	19	51.4%
B-Symptoms	**Absent**	24	51.1%	13	37.1%
	B-Symptoms	23	48.9%	22	62.9%
Cervical LNs	**Absent**	1	2.1%	3	8.1%
	Present	46	97.9%	34	91.9%
Axillary LNs	**Absent**	20	42.6%	16	43.2%
	Present	27	57.4%	21	56.8%
Inguinal LNs	**Absent**	32	68.1%	23	62.2%
	Present	15	31.9%	14	37.8%
Mediastinal LNs	**Absent**	19	40.4%	17	45.9%
	Present	28	59.6%	20	54.1%
Abdominal LNs	**Absent**	27	57.4%	16	43.2%
	Present	20	42.6%	21	56.8%
Splenomegaly	**Absent**	30	63.8%	22	59.5%
	Present	17	36.2%	15	40.5%
Hepatomegaly	**Absent**	32	68.1%	20	54.1%
	Present	15	31.9%	17	45.9%
BM infiltration	**Absent**	41	87.2%	33	89.2%
	Present	6	12.8%	4	10.8%
ESR	**<30**	26	55.3%	14	37.8%
	≥30	21	44.7%	23	62.2%
Leucocytosis	**≤ 15x1000/µL**	45	95.7%	24	64.9%

	> 15x1000/µL	2	4.3%	13	35.1%
Lymphocytopenia	**Lymphocytes ≥8%**	41	87.2%	34	91.9%
	Lymphocytes <8%	6	12.8%	3	8.1%
Anemia**	**No anemia**	**28**	**59.6%**	**14**	**37.8%**
	Anemia	**19**	**40.4%**	**23**	**62.2%**
Thrombocytopenia	**Platelets ≥100 x1000/µL**	45	95.7%	36	97.3%
	Platelets < 100 x 1000/µL	2	4.3%	1	2.7%
Serum albumin (gm/dL)	**< 4**	9	19.1%	6	16.2%
	≥ 4	38	80.9%	31	83.8%
Elevated LDH	**Normal**	4	8.5%	6	16.2%
	Elevated LDH	43	91.5%	31	83.8%
Renal Impairment	**Normal kidney function**	46	97.9%	37	100.0%
	Renal Impairment	1	2.1%	0	0.0%
Liver Transaminases	**Normal liver transaminases**	41	87.2%	32	86.5%
	Abnormal liver transaminases	6	12.8%	5	13.5%
Hyperbilirubinemia	**Normal bilirubin**	46	97.9%	34	91.9%
	Hyperbilirubinemia	1	2.1%	3	8.1%
HCV	**Negative**	46	97.9%	34	91.9%
	Positive	1	2.1%	3	8.1%
HBV	**Negative**	45	95.7%	37	100.0%
	Positive	2	4.3%	0	0.0%
Pathology	**Nodular sclerosis**	20	47.6%	19	55.9%
	Mixed cellularity	22	52.4%	15	44.1%
Staging (Lugano criteria)	**Stage I-II**	20	42.6%	12	32.4%
	Stage III-IV	27	57.4%	25	67.6%
Bulky disease	**No Bulky disease**	40	85.1%	33	89.2%
	Bulky disease	7	14.9%	4	10.8%
Early stage NCCN risk categories	**Favorable risk**	8	40.0%	2	18.2%
	Unfavorable risk	12	60.0%	9	81.8%
Response**	**CR**	**44**	**93.6%**	**27**	**73.0%**
	Refractory	**3**	**6.4%**	**10**	**27.0%**

**** Significant difference (p= 0.04 and 0.009 respectively)**

Regarding event free survival, EFS was defined from the date of diagnosis to the date of relapse or progression, unplanned treatment, or death from any cause. The median EFS was 40 months (95% CI not reached) in patients with positive PD-L1 expression vs. 26 months (95% CI 4-48 months) in cases with negative PD-L1 expression, the difference was not statistically significant (p = 0.13) (Fig. 5). The median EFS was not reached in EBV positive cases vs. 27 months (95% CI 15-39 months) in cases with negative EBV expression, the difference was of borderline significance (p = 0.057)(Fig. 6). At the end of this study 80 patient were still alive.

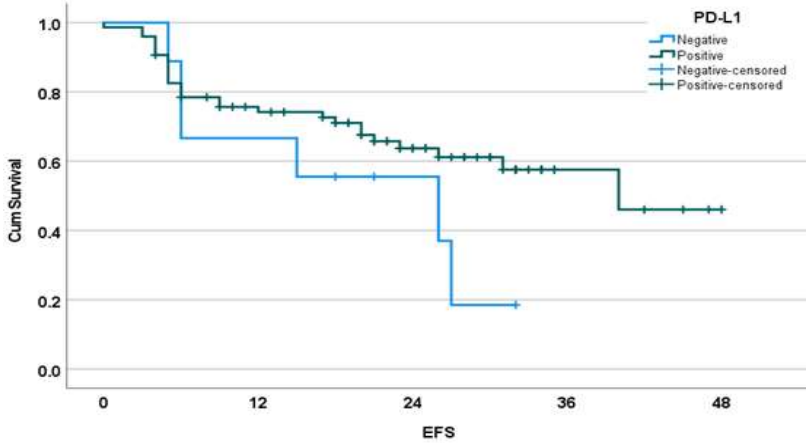


Figure (5) Relation between PDL1 expression and EFS.

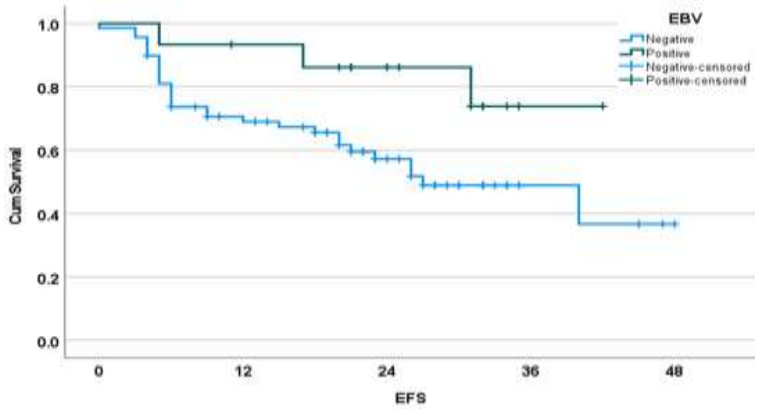


Figure (6) Relation between EBV expression and EFS.

Discussion

cHL accounts for approximately 90% of Hodgkin lymphoma, while nodular lymphocyte-predominant HL (NLPHL) accounts for 10% of cases. (27) In the United States, Europe, and other economically developed countries, HL accounts for about 10 percent of all lymphomas (the other 10 percent accounts for non-Hodgkin lymphomas), 0.5 percent of all other cancers, and 0.2 percent of all cancer deaths. (28)
In this study HL is most common among young adults (20 to 39 years) with male predominance 56% compared with females 37% and this was supported by **Khan et al.** (29)

In this study 45 patients (53.6%) were smokers while 64 patients (76.1%) were overweight and obese , this show that smoking and obesity are associated with increased risk of HL and this was agreed by **Hjalgrim and Jarrett** in their study (30). on the other hand no patients in our study had history of autoimmune disease unsupported with some data showing that there is a relation between HL and autoimmune disease but it is unclear if this is related to these disorders or related to the immunosuppressive agents used to treat them. (31)

In this study most of cases presented by lymphadenopathy, 80 patients (95.2%) had cervical lymphadenopathy at their first presentation while 57.1% of patients had mediastinal LN, 45 patients (54.7%) had B symptoms and 11.9% of patients presented with bone marrow infiltration, this was supported by **LaCasce et al.,** (32) and **Shanbhag and Ambinder** (1).

In this study nodular sclerosis was the most common pathologic variant accounts for 46.43% while mixed cellularity found in 44.05%, lymphocyte rich found in 8,33% and lymphocyte depleted variant was the least common pathologic variant accounts for 1.19%. These data was agreed in previous studies. (33, 34)

In this study IPS score was evaluated in all cases and one factor IPS was noticed in 30.2% of patients, this was supported by an analysis of 740 patients with cHL in which one factor IPS score represent the highest percentage which was 26% of patients (35). Early stage stratification into favorable or unfavorable risk according to NCCN, EORTC and GHSG was 32.3% for favorable risk and 67.7% for unfavorable risk for the three models and this was agreed by **Klimm et al.,** (36) in which the three models classified similar percentages of patients as having unfavorable prognosis early stage cHL (56, 55, and 57 percent, respectively).

In this study PDL1 expression was detected in 75 patients (89.3%), EBV positive staining was detected in 15 patients (17.9%) with 14 patients (93.3%) was PDL1 positive. This data was matched with that cHL is reported to have the highest incidence (82%) of PD-L1 expression among other types of lymphoma, and this expression has been correlated with the presence of Epstein-Barr virus (EBV) in malignant cells. The gene products of EBV, such as latent membrane protein-1, have been shown to upregulate PD-L1 expression in a number of patients with cHL according to **Ozturk et al.** (37) Many phase II ongoing trials are evaluating immunotherapy as 1st line therapy for patients with classic Hodgkin lymphoma as nivolumab with AVD for early stage disease (38, 39) with high remission rates and excellent 12m progression free survival and nivolumab with brentuximab Vedotin for elderly patients with comorbidities. (40)

Prognostic significance of PD-L1 expression had been evaluated in different types of solid tumors and limited number of cases with lymphoma. It has been shown that expression of PD-L1 in some solid tumors predicts poor outcome (41).In classical HL, the PD-1/PD-L1 signaling axis showed to have an inferior clinical outcome through the suppression of antitumor immunity. (42)

PD-L1 expression has been analyzed by different methods; using flow cytometry, immunohistochemistry and fluorescein in situ hybridization in cell lines and lymphoma specimens. (43) As no standardized method for detection, differences in the specificity and sensitivity of the commercial antibodies used for immunohistochemical staining of

PD-L1 and lack of concordance between expert pathologists, this explains the differences between studies and indirectly the prognostic value of the PD-L1.

In our study we didn't find an important difference for these cases, as we found that 65 patients PDL1 positive cases (86.7%) achieved CR after 1st line of treatment. The median EFS was 40 months (95% CI not reached) in patients with positive PD-L1 expression vs 26 months (95% CI 4-48 months) in cases with negative PD-L1 expression, the difference was not statistically significant (p = 0.13).

EBV has been linked to the pathogenesis of HL, however the absolute risk of developing HL after EBV is very small. (44) In our study, EBV positive staining was detected in 15 patients (17.9%), 9 patients (60%) were presented with advanced stage (stage III-IV), mixed cellularity pathology was found in 9 patients(63.4%) while nodular sclerosis was found in 5 patients, this matched with prevalence of EBV according to histological subtypes. (45) Although EBV is being associated with inferior prognosis in cHL, compared to patients who are EBV negative. (46)

In our study all patients with EBV positive staining achieved CR after 1st line of treatment. The median EFS was not reached in EBV positive cases vs. 27 months (95% CI 15-39 months) in cases with negative EBV expression, the difference was of borderline significance (p = 0.057), this is explained by difference in the prevalence of EBV detection in malignant HL cells according to geography and socioeconomic status being the highest in Europe, North America and tropical areas (47) and that differs from our country.

As regard neutrophils/lymphocytes ratio in our study, patients with N/L ratio less than 3, near 93.6 % of the patients achieved CR with p value =0.04, the ROC analysis demonstrated that at cut-off 3, the ratio has a sensitivity and specificity of 65% and 70% respectively for predicting relapsed/refractory disease, AUC 0.65, p=0.025 .The median EFS was not reached in cases with N/L ratio < 3 vs. 21 months (95% CI 4-38 months) in cases with N/L ratio ≥ 3, p< 0.001, this data was strongly supported by **Dogan and Demircioglu** (48) and **Stefaniuk et al.,** (49) that confirms importance of NLR as a predictive and prognostic factor. Also, among patients with NLR<3 anemia was not detected in 28 patients (59,6) compared with those with NLR>3 in which anemia was detected in 23 patients (62.2%) with P value=0.04, this is matched with being anemia a poor prognostic feature. (48)

Conclusion

Hodgkin lymphoma is considered highly curable disease with low mortality. EBV and PDL1 should be investigated on larger number of cases to evaluate their predictive and prognostic value. Neutrophil lymphocyte ratio is an important predictive and prognostic marker for Hodgkin lymphoma which is inexpensive, easily accessible test from a CBC at diagnosis, simple, widely available, and can be easily used in clinical practice especially in resource-poor areas.

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