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Cluster of Differentiation 274 Antigen Immunohistochemical Expression in Tumor and Peri-tumor Cells of Hodgkin and Non-Hodgkin Lymphoma and Clinicopathological Relation (Single-center Study)

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Abstract

BACKGROUND: Cluster of differentiation 274 (CD274) antigen has been investigated in tumors to evaluate its regulation and effect as a predictive of targeted therapy. Its expression and effect in lymphoma have raised interest recently. However, results were mixed and showed wide variations.

AIM: This study aims to explore and compare CD274 antigen immunohistochemical expression in tumor and peri-tumor cells of classic Hodgkin lymphoma (HL) and diffuse large B cells non-HL (NHL) and its relation with clinicopathological criteria.

METHODS: This work was carried out on 78 cases of lymph node excision biopsy (48 HL and 30 NHL). Prepared sections were applied for immunohistochemistry using CD274 monoclonal rabbit anti-human (programmed cell death protein 1 [PD-L1] ZR3-ASR, a Sigma Aldrich company). Assessment of CD274 antigen in tumor cells was considered positive if detected in >10% (membranous staining with cytoplasmic accentuation). Peri-tumor cells were scored as: 0, no positive cells/high-power field (HPF); 1, <10 positive cells/HPF; 2, 10–30 positive cells/HPF; 3, >30 positive cells/HPF.

RESULTS: CD274 antigen was expressed in 53.8% of total lymphoma cases with significantly more expression of CD274 antigen in HL than NHL (66.7% vs. 33.3%). Classic HL showed significantly higher expression of CD274 antigen in tumor and peri-tumor cells and significant association with elevated erythrocyte sedimentation rate and lactate dehydrogenase and male gender.

INTERPRETATION AND CONCLUSION: There is a more frequent and significant expression of CD274 antigen in classic HL than NHL cases in tumor and peri-tumor cells and a significant association with bad prognostic criteria in classic HL. High expression of CD274 antigen in classic HL proposes its potential use as a marker, especially for prognostic indication.

Introduction

Cluster of differentiation 274 (CD274) antigen is one of two ligands for programmed cell death protein 1 (PD-1), a receptor representing the second checkpoint of immune response that decreases the function of effector T-cell in tissues [1]. CD274 antigen was recognized in variable tumors, and researchers have been investigating its regulation. An example of such a regulation mechanism is interferon-gamma (IFNγ) that induces CD274 antigen surface expression on tumor cells [2].

Although CD274 antigen is not normally expressed on epithelial cells, it can be detected on the surface of many neoplastic cells, such as its expression in 20% of triple-negative breast cancer cases [2] and 20% of urothelial carcinoma [3]. Researchers pointed out that utilizing monoclonal antibodies such as CD274 antigen-targeted treatment could obstruct the pathway of CD274 antigen inhibitory effects and induce T cell activating functions, thus improving prognosis in cancer patients [4]. Furthermore, tumor expression of CD274 antigen is considered predictive of therapy response [5]. Anti-PD-1 targeted therapy succeeded in improving the prognosis and outcomes in tumors with CD274 antigen high expression by immunohistochemistry (IHC) [6].

Lymphoma is a common concern worldwide. Non-Hodgkin lymphoma (NHL) represents the fifth to ninth most frequent malignancy globally. However, the frequency of the subtypes differs according to the country [7]. In adults, diffuse large B-cell lymphoma (DLBCL) is the most frequently encountered type of NHL. Treatment with chemotherapy gives the opportunity of 60–70% overall survival for 5 years in such cases [8]. NHL has a heterogeneous nature

regarding genetics and prognosis. Sub-classification into the germinal center and non-germinal center could be helpful regarding prognostic prediction by IHC tissue microarray (TMA) application [9]. However, new markers are still needed, particularly for estimating prognosis and targeted therapy [10]. Conversely, Hodgkin lymphoma (HL) is not considered a common lymphoma according to the worldwide report of 79,990 newly registered cases (0.4% of all tumors), and 0.3% of all deaths of cancer recognized in 2018 [11].

In Egypt, lymphoma represents one of the world's highest incidence rates, with a NHL incidence rate of 5%. It ranks the fourth most frequent adults tumor, as NHL represents 76.6%, while HL is found in the rest of lymphoma cases [12]. While less incidence of HL was reported in underdeveloped regions, mortality rates are elevated in contrary to developed regions of the world [11]. Moreover, the relapse and refractory stage of the disease was experienced in 10–30% of HL treated cases [13]. Hence, there is a significant need for research to help decrease the incidence of HL and develop lines of treatment with the incorporation of more targeted therapy.

In variable lymphoma cell lines, CD274 antigen blockade showed a decline of proliferation effect. Such effect proposes a significant role for CD274 antigen expression in tumorigenesis of lymphoma [14]. There is variability in reports of CD274 antigen expression in NHL tumors with a wide range of expression from as low as 10.5% to near two-third of NHL tumor cells (61.1%) [15], [16], [17]. According to Menter *et al*., CD274 antigen was reported in 31% of primary DLBCL and 35% of 1ry B-cell lymphomas of mediastinal type. In HL; it was reported as 70% of classic HL, 54% of nodular lymphocyte-predominant HL [17]. Thus, the variability of results of CD274 antigen expression in lymphoma requires investigation. The purpose of this study is to explore and compare the immunohistochemical expression of CD274 antigen in tumor and peri-tumor cells of classic HL and NHL (DLBCL) cases and its relation with available clinicopathological data.

Materials and Methods

Material of this study

For this study, all excision biopsy cases of lymphoma submitted in the hospital were collected as paraffin-embedded blocks in a retrospective manner from January 2016 to December 2019. All cases were examined, and available data regarding age, sex, history, clinical, serological, and radiological findings were obtained from patient's data records. All cases were anonymous and handled according to legal and ethical standards.

Exclusion criteria

Core or incision biopsy and cases with insufficient clinical data or paraffin blocks with deficient material suitable for immunohistochemical staining were not included in the study. Furthermore, cases of patients who received therapy and gray zone lymphoma cases were excluded from the study.

This retrospective work was carried out on 78 cases of performed lymph node (LN) excision biopsy of the whole node or multiple nodes after the approval of an institutional ethical committee of research was obtained.

Methods

Histopathological examination

Whole sections of LN were prepared using H and E staining and submitted for histopathological examination to confirm the diagnosis of lymphoma and selection of the most cellular areas with malignant cells to be prepared for the TMA blocks after confirmation of the lymphoma type using immunophenotyping (CD 20, CD 3, CD 15, CD 30, CD5, CD23). Furthermore, 10 complete sections (5 from each type of lymphoma) were prepared to be treated with CD274 antigen IHC to compare complete section results with that of TMA.

Methodology for TMA

TMA was performed using the known manual method of punching a core from donor paraffin block, then embedded into a "recipient block". Screening of donor blocks for adequate thickness and content of tissue was performed first [18]. TMA map was designed according to each donor block core location and orientation, and it was recorded before the construction of TMA. According to this map, cores punched from donor blocks were then embedded into the corresponding hole in the recipient block. Two TMA blocks from each type of lymphoma were constructed [18]. Sections cut from the recipient TMA block have the same predesigned map orientation.

CD274 antigen IHC

Prepared sections from constructed microarray blocks, and 10 complete sections from cases of classic HL and NHL (DLBCL) for comparison (5 of each) were applied through the auto-stainer for CD274 antigen IHC using monoclonal rabbit anti-human CD274 antigen (PD-L1 ZR3-ASR, diluted in Tris buffer, PH.33.7 with 1% BSA and <0.7% sodium azide, a Sigma Aldrich company).

Interpretation of IHC

Assessment of CD274 antigen IHC results in tumor cells was performed according to Herbst *et al*. and Kwon *et al*. [5], [19] as follow:

1. The percentage of the cells displaying membranous and/or cytoplasmic staining was evaluated as no or any staining <10% of tumor cells were considered negative, and staining of more than or equal to 10% of tumor cells was considered positive. In addition, the intensity of membranous staining with cytoplasmic accentuation was reported as follows: 0; No staining, +1; mild, +2; moderate and +3; strong
2. Distribution through tumor-infiltrating cells (peri-tumor) was scored as follow: 0, no positive cells/high-power field (HPF); 1: no more than 10 positive cells/HPF; 2: 10–30 positive cells/HPF; 3: more than 30 positive cells per HPF. Then final interpretation was scored as negative or positive results.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp**).** The Kolmogorov-Smirnov test was used to verify the normality of variables distribution; comparisons between groups for categorical variables were assessed using the Chi-square test (Fisher or Monte Carlo). Student t-test was used to compare two groups for normally distributed quantitative variables. The significance of the achieved results was judged at the 5% level.

Results

In this study, we investigated a total of 78 cases of lymphoma (48 cases of classic HL and 30 cases of NHL DLBCL). All patient records of the studied lymphoma groups are summarized in (Table 1). The 10 complete sections IHC results were the same as obtained by TMA. As shown in Table 1, 53.8% of all studied lymphoma cases showed positive CD274 antigen expression. There is a significant difference when comparing CD274 antigen-positive and negative cases in classic HL and NHL (DLBCL) as two-third of HL cases expressed CD274 antigen. At the same time, one-third of NHL showed positivity ($p = 0.004$). In addition, there was a significant difference when comparing different intensities of CD274 antigen expression in HL and NHL patients ($p < 0.001$). Strong intensity of CD274 antigen expression was detected in 30.8% of all studied cases and was more common in HL cases (50%). Almost half of the peri-tumor microenvironment cells (48.7%) showed positive CD274 antigen expression with significantly more frequent expression in HL cases ($p = 0.002$) (Figures 1 and 2). When comparing positive and negative CD274 antigen cases in relation to different clinical parameters, only serological findings showed significant difference ($p < 0.001$), with combined elevated erythrocyte sedimentation rate (ESR) and lactate dehydrogenase (LDH) being found in two-third of positive CD274 antigen cases (Table 2).

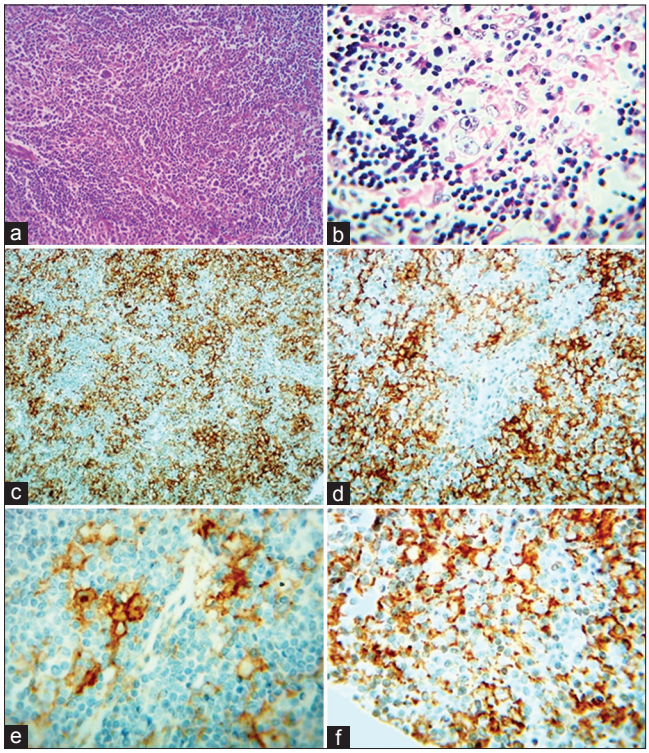

Figure 1: Hodgkin lymphoma: (a) hematoxylin and eosin (H and E) of Hodgkin lymphoma with scattered RS cells (×100). (b) H and E Hodgkin lymphoma (×400). (c) Cluster of differentiation 274 (CD274) antigen-positive RS cells of moderate-intensity (×100). (d) CD274 antigen moderate and strong intensity (×200). (e) CD274 antigen strong intensity RS cells (×400). (f) CD274 antigen strong intensity peri-tumor cells (×400)

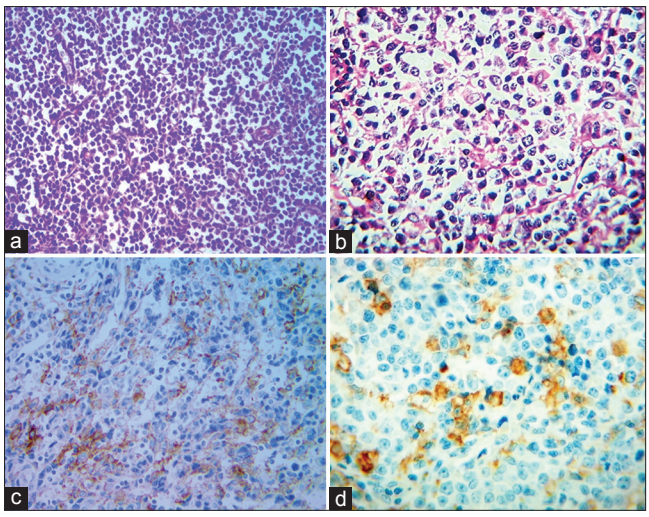

Figure 2: Non-Hodgkin lymphoma: (a) hematoxylin and eosin (H and E) of Non-Hodgkin lymphoma (×200). (b) H and E of Non-Hodgkin lymphoma (×400). (c) Cluster of differentiation 274 (CD274) antigen-positive tumor cells of mild and moderate intensity (×200). (d) CD274 antigen-positive tumor cells of moderate-intensity (×400)

Table 1: Comparison between the two lymphoma groups according to different parameters

Clinicopathological parameters	Total (n = 78)	HL classic (n = 48)	NHL DLBCL (n = 30)	Test of sig.	p
Age (years)					
Median (Min.–Max.)	45 (17–66)	28.5 (17–65)	55 (48–66)	t = 9.820*	<0.001*
Mean ± SD	42.6 ± 15.8	34 ± 13.8	56.4 ± 6		
Sex (%)					
Male	46 (59)	40 (83.3)	6 (20)	χ^2 = 30.607*	<0.001*
Female	32 (41)	8 (16.7)	24 (80)		
Site (%)					
Cervical LN	42 (53.8)	34 (70.8)	8 (26.7)	χ^2 = 14.498*	0.001*
Axillary LN	26 (33.3)	10 (20.8)	16 (53.3)		
Inguinal LN	10 (12.8)	4 (8.3)	6 (20)		
Clinical symptoms (%)					
Swelling	44 (56.4)	20 (41.7)	24 (80)	χ^2 = 11.033*	0.001*
Fever and swelling	34 (43.6)	28 (58.3)	6 (20)		
Serological data received (%)					
No serological findings	30 (38.5)	6 (12.5)	24 (80)	χ^2 = 40.582*	<0.001*
Elevated ESR	12 (15.4)	8 (16.7)	4 (13.3)		
Elevated LDH	2 (2.6)	2 (4.2)	0 (0)		
Elevated ESR and elevated LDH	34 (43.6)	32 (66.7)	2 (6.7)		
Radiological findings received (%)					
Localized lymphadenopathy	66 (84.6)	40 (83.3)	26 (86.7)	χ^2 = 0.158	0.758
Generalized lymphadenopathy	12 (15.4)	8 (16.7)	4 (13.3)		
CD274 antigen IHC results					
Negative	36 (46.2)	16 (33.3)	20 (66.7)	χ^2 = 8.254*	0.004*
Positive	42 (53.8)	32 (66.7)	10 (33.3)		
Intensity of staining (%)					
Negative	36 (46.2)	16 (33.3)	20 (66.7)	χ^2 = 26.809*	<0.001*
Mild	6 (7.7)	2 (4.2)	4 (13.3)		
Moderate	12 (15.4)	6 (12.5)	6 (20)		
Strong	24 (30.8)	24 (50)	0 (0)		
Peri-tumor microenvironment (%)					
Negative	40 (51.3)	18 (37.5)	22 (73.3)	χ^2 = 9.488*	0.002*
Positive	38 (48.7)	30 (62.5)	8 (26.7)		

χ^2: Chi-square test, t: Student t-test, p: p value for comparison between the two studied groups, *: Statistically significant at p ≤ 0.05. HL: Hodgkin lymphoma, NHL: Non-Hodgkin lymphoma, DLBCL: Diffuse large B-cell lymphoma, LN: Lymph node, ESR: Erythrocyte sedimentation rate, LDH: Lactate dehydrogenase, IHC: Immunohistochemistry, CD274: Cluster of differentiation 274.

Table 2: Relation between CD274 antigen IHC and different parameters in total lymphoma cases (n = 78)

Clinical data	CD274 antigen IHC		Test of Sig.	p
	Negative (n = 36)	Positive (n = 42)		
Age (years)				
Median (Min.–Max.)	49 (19–66)	33 (17–64)	t = 1.483	0.142
Mean ± SD	45.4 ± 13.9	40.2 ± 17.1		
Sex (%)				
Male	22 (61.1)	24 (57.1)	χ^2 = 0.126	0.722
Female	14 (38.9)	18 (42.9)		
Clinical symptoms (%)				
Swelling	22 (61.1)	22 (52.4)	χ^2 = 0.601	0.438
Fever and swelling	14 (38.9)	20 (47.6)		
Serological data received (%)				
No serological findings	22 (61.1)	8 (19)	χ^2 = 23.977*	<0.001*
Elevated ESR	8 (22.2)	4 (9.5)		
Elevated LDH	0 (0)	2 (4.8)		
Elevated ESR and elevated LDH	6 (16.7)	28 (66.7		

χ^2: Chi-square test, t: Student t-test, p: p value for the association between CD274 antigen staining result and different parameters, *: Statistically significant at p ≤ 0.05. ESR: Erythrocyte sedimentation rate, LDH: Lactate dehydrogenase, IHC: Immunohistochemistry, CD274: Cluster of differentiation 274.

When comparing positive and negative CD274 antigen IHC results in different parameters found in cases of classic HL, gender and serological findings showed significant difference ($p = 0.039$ and $p < 0.001$, respectively), with combined elevated ESR and LDH being found in 87.5% of positive CD274 antigen HL cases (Table 3). While, when comparing positive and negative CD274 antigen staining results in different parameters of the NHL (DLBCL) group, only age showed a significant difference ($p < 0.001$) (Table 3).

Discussion

Lymphoma represents a heterogeneous neoplastic group with various manifestations, predictions, and treatment responses. Possibly more than any other type of malignancy, lymphoma diagnosis and prognosis implicate incorporating clinical presentations, histologic criteria, immunophenotyping, and even molecular and cytogenetic findings. A precise lymphoma diagnosis and identification of new markers typically permit appropriate patient categorization and treatment [20]. Thus, many researchers have been working to recognize biomarkers of a checkpoint blockade immune response, to categorize patients who are most probably benefiting from such immune therapy. Recently, CD274 antigen has been studied in different tumors to predict targeted immunotherapy and prognostic criteria. However, CD274 antigen expression and effect in lymphoma showed wide variations in results [5], [6], [15], [16], [17].

This study detected a significant difference between HL and NHL cases, as NHL patients were two decades older than HL patients. Furthermore, there was a significant difference regarding gender with male predominance in HL patients. Cervical LN was the most affected site, especially in HL patients, while axillary LN was affected more in NHL cases ($p = 0.001$). A significant difference was detected

Table 3: Relation between CD274 antigen IHC and different parameters in classic Hodgkin Lymphoma group (n = 48) and Non-Hodgkin lymphoma group (n = 30)

Clinical data	CD274 antigen IHC in classic Hodgkin Lymphoma n = 48		Test of Sig.	p
	Negative (n = 16)	Positive (n = 32)		
Age (years)				
Median (Min.–Max.)	28.5 (19–65)	29 (17–63)	t = 0.336	0.738
Mean ± SD.	35 ± 14.2	33.6 ± 13.8		
Sex (%)				
Male	16 (100)	24 (75)	χ^2 = 4.800*	0.039*
Female	0 (0)	8 (25)		
Clinical symptoms (%)				
Swelling	6 (37.5)	14 (43.8)	χ^2 = 0.171	0.679
Fever and swelling	10 (62.5)	18 (56.3)		
Serological data received (%)				
No serological findings	6 (37.5)	0 (0)	χ^2 = 24.083*	<0.001*
Elevated ESR	6 (37.5)	2 (6.3)		
Elevated LDH	0 (0)	2 (6.3)		
Elevated ESR and elevated LDH	4 (25)	28 (87.5)		
Clinical data	CD274 antigen IHC in non-Hodgkin Lymphoma n = 30		Test of Sig.	p
	Negative (n = 20)	Positive (n = 10)		
Age (years)				
Median (Min.–Max.)	53 (48–66)	63 (56–64)	t = 4.247*	<0.001*
Mean ± SD.	53.8 ± 5.3	61.6 ± 3.2		
Sex (%)				
Male	6 (30)	0 (0)	χ^2 = 3.750	0.074
Female	14 (70)	10 (100)		
Clinical symptoms (%)				
Swelling	16 (80)	8 (80)	χ^2 = 0.000	1.000
Fever and swelling	4 (20)	2 (20)		
Serological data received (%)				
No serological findings	16 (80)	8 (80)	χ^2 = 1.312	0.629
Elevated ESR	2 (10)	2 (20)		
Elevated LDH	0 (0)	0 (0)		
Elevated ESR and elevated LDH	2 (10)	0 (0)		

χ^2: Chi-square test, t: Student t-test, p: p value, *: Statistically significant at p ≤ 0.05. ESR: Erythrocyte sedimentation rate, LDH: Lactate dehydrogenase, IHC: Immunohistochemistry, CD274: Cluster of differentiation 274.

between the clinical presentation of HL and NHL since fever and swelling were more frequent in HL while swelling only was more common in NHL. Serological findings were detected more significantly in HL cases, with the combined elevation of both ESR and LDH being the most common finding. When comparing significant variants using univariate and multivariate logistic regression analysis affecting studied classic HL and DLBCL cases; age, male gender, clinical symptoms (fever and swelling), and CD274 antigen showed significant difference.

Hodgkin lymphoma is a neoplasm that is frequently seen at a young age with two peaks of incidence (third decade and older than 55) [21]. It was previously reported that there is more male incidence in HL, especially in urban areas of Egypt [22]. Furthermore, the report of Zhou *et al*. showed the increased incidence of HL in males and the variation of HL incidence distribution according to age, gender, and geographical distribution [11].

Signaling of PD-1/CD274 leads to negative regulation of T cell-induced immune reactions to reduce the response of effector T cell. It causes peripheral T cell tolerance (defend tissues against immune-induced damage). Besides binding to PD-1, the interaction of CD274 with CD80, CD86 creates inhibitory signals on T cells and diminishes antitumor immune response [23], [24]. CD274 antigen is variably over-expressed in different types of tumors and is suggested to play a role in suppressing local responses against the tumor cells, including lymphoma [25]. The expression of CD274 on tumor cells is related to the progression of the tumor and bad prognosis [14]. Expression of CD274 is constitutively elevated in some oncogenic cells by signaling through abnormal activation of the PI3K-AKT signaling or genetic changes and amplification that is found in HL, which is self-regulated and not related to tumor micro-environment signaling. On the contrary, CD274 expression can be induced as part of adaptive resistance of the immune system as a reaction to active signals of antitumor immune reaction. Many cytokines can produce or sustain the expression of CD274, with IFNγ being the most effective [23].

PD-1/CD274 antigen pathway blockade by anti-PD-1/CD274 antigen antibodies has been studied for their potential antitumor immune-therapeutic role [26]. Such blockade of the PD-1/CD274 pathway using antibodies inhibitors interrupts the PD-1 axis hence converses the suppression of T cells, revitalizing the drained cells of immunity in the tumor micro-environment and eradicating malignant cells. Thus, this strategy of treatment stabilizes immunity defects against the tumor, which has reached a 10–40% success in clinical response [23], [24]. For example, Atezolizumab (MPDL3280) IgG1 monoclonal antibody eradicates cellular cytotoxicity related to antibodies to avoid exhaustion of T cells expressing CD274. Furthermore, it interrupts the interaction of CD274, precisely on the surface of neoplastic cells and tumor micro-environment immune cells. Hence, it improves the CD8+ T cells level by prompting cytokine increase of IFNγ, IL-18, and CXCL11 and decreasing the immune inhibition signals in the tumor's micro-environment [23].

More than half of all studied lymphoma cases (53.8%) showed positive CD274 antigen expression. However, there was a significant difference when comparing CD274 antigen-positive, and negative cases in classic HL and NHL (DLBCL) as two-third of HL cases expressed CD274 antigen (66.7%), while, one-third of NHL showed positivity (33.3%) (p = 0.004).

These results were in concordance with other reports showing more frequent expression of CD274 antigen in HL than NHL. CD274 antigen was expressed in 31% of DLBCL compared to 70% of classic HL in the research done by Menter and coworkers [17]. According to another research, 82% of HL expressed CD274 antigen, while only 10% of DLBCL showed CD274 antigen expression [27]. The variation in DLBCL expression was mostly related to the variation in sample size, cutoff point and analyzed cells. The cutoff applied in different studies, showed a range from 5% to 30%, and the cell compartment that was analyzed varied in different reports (tumor vs. non-tumor cells) [28], [29], [30]. In previous studies, the use of more than 30% of tumor cells as a cutoff point resulted in 10.5–11% of DLBCL tumor cells' expression of CD274 antigen [15], [16]. While, when more than 10% of tumor cells were considered positive, 41–61% of DLBCL had CD274 antigen positivity [19], [31]. More than 5% positivity was used as a cutoff point in other studies [17], [29]. This discrepancy in detecting CD274 antigen may indicate the need for agreement and consensus on settling a definite cutoff in studying the expression of CD274 antigen in lymphoma, and we support the application of 10% as a cutoff point when considering positivity as an appropriate protocol of assessment.

In this study, there was a significant difference when comparing different intensities of CD274 antigen expression in classic HL and NHL (DLBCL) patients ($p < 0.001$). The strong intensity was common in classic HL cases (50%). This result conforms to the finding of strong positivity in classic HL cases compared with other studied types by Vranic *et al*. [28].

Almost half of the peri-tumor microenvironment cells (48.7%) showed positive CD274 antigen expression with significantly more frequent expression in classic HL cases ($p = 0.002$). In comparison, slightly more than a quarter of peri-tumor cells showed positivity for CD274 antigen in NHL (26.7%). The overall immunosuppressive micro-environment occurring with tumorigenesis explains the CD274 antigen detection on non-malignant infiltrating macrophages and lymphocytes besides malignant cells [19], [31]. This peri-tumoral expression finding supported other authors' reports of classic HL cells recruitment and induction of a CD274 antigen-positive myeloid environment, mainly macrophages [30]. Furthermore, other studies detected that micro-environment CD274 antigen-positive cells in DLBCL ranged from 14% to 27% [6], [15], [16], [29], [30], [31]. Moreover, high expression of CD274 antigen in NHL tumor-infiltrating lymphocytes was evident in previous studies [31]. Expression of CD274 antigen in both tumor and peri-tumor cells contributes to immune response suppression in the local environment. It is proposed that CD274 antigen-positive non-tumor cells are significant in immune therapy [32].

In this work, when comparing positive and negative CD274 antigen cases in relation to different clinical parameters, only serological findings showed significant difference ($p < 0.001$), while the same comparison in cases of classic HL showed a significant difference in male gender in addition to serological findings ($p = 0.039$ and $p < 0.001$ respectively). Furthermore, when comparing positive and negative CD274 antigen staining results in different parameters of the NHL (DLBCL) group, only age showed a significant difference ($p < 0.001$). These results indicate the association between CD274 antigen expression and serological data (combined elevated ESR and LDH) in lymphoma with particular significance in classic HL. The elevated ESR is one of the known bad prognostic factors in early-stage HL, while the male gender is considered one of the established bad prognostic factors in the advanced stage of HL [33] that reflects a bad prognostic indication of CD274 antigen expression in classic HL cases.

There are some limitations to the current work since this was a retrospective study conducted in a single center with a sample size of 78 cases (48 cases of classic HL and 30 cases of NHL DLBCL) and limited access to clinical data. Hence, further prospective research with a larger number of cases and inclusion of other clinicopathological criteria, for example survival, size of the tumor, and EBV status, is required to confirm the prognostic value of CD274 antigen immunohistochemical expression in such cases.

Conclusion

Since CD274 antigen was expressed in 53.8% of all studied lymphoma cases with a significantly more frequent expression of CD274 antigen in classic HL (66.7%) compared to NHL (DLBCL) (33.3%) and more significant strong intensity of tumor cells and more expression in peri-tumor cells of HL studied cases, this immunohistochemical marker can be applied in classic HL. CD274 reflects a bad prognostic indication in classic HL due to significant association with elevated ESR and LDH and male gender. Therefore, the high expression of CD274 antigen in classic HL proposes its potential use as a marker, especially for prognostic indication.

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