Original Article

The Combination of GATA3 and CD79a as A Promising Supplemental Panel for the Diagnosis and Differentiation of Hodgkin Lymphomas. A Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Hodgkin lymphoma is a hematologic malignancy that shows excellent prognosis once accurately diagnosed. Its diagnosis provides a challenge to the pathologists, as it has histopathological features that overlap other lymphoid lesions. As a result confirmatory immune-stains are used to either confirm or exclude Hodgkin Lymphoma before starting therapy. Despite the well-established immune panel approved for diagnosing Hodgkin Lymphoma, still some cases are not fitting in a specific category and hence further investigations to reach more sensitive and specific markers are still required. This requirement is very important in Egypt as morbidity and mortality from Hodgkin Lymphoma is high in Africa. This study investigates the validity of GATA3 coupled with CD79a immune-histochemical markers in diagnosing Hodgkin lymphoma.

Material and Methods: Seventy three cases of Hodgkin lymphoma were included in this study: sixty Classic Hodgkin lymphoma cases, ten Nodular Lymphocytic Predominant Hodgkin Lymphoma cases confirmed by the immune staining panel and three Classic Hodgkin Lymphoma cases with atypical immune-staining pattern, excluded from statistics.

Results: GATA3 was expressed in forty-nine out of sixty Classic Hodgkin Lymphoma cases and was completely negative in all Nodular Lymphocytic Predominant Hodgkin Lymphoma cases. CD79a was expressed in all cases of Nodular Lymphocytic Predominant Hodgkin Lymphoma and in only seven cases of CHL cases. Two out of the three Classic Hodgkin Lymphoma cases with atypical immune-staining pattern were positive for GATA3 despite being completely negative for CD15. These three cases were also positive for CD79a.

Conclusions: GATA3 is a good confirmatory marker for Classic Hodgkin Lymphoma, and when coupled with CD79a forms a good panel to differentiate Nodular Lymphocytic Predominant Hodgkin Lymphoma from Classic Hodgkin Lymphoma (GATA3 negative and CD79a positive).

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Key Words: CD79a, classic hodgkin lymphoma, GATA3, hodgkin lymphoma, nodular lymphocytic predominant hodgkin lymphoma.

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INTRODUCTION

Hodgkin lymphoma (HL) is an enigmatic peculiar disease that has attracted many medical investigators, including pathologists. Whether it represents a malignant neoplasm, an infectious process, or simply inflammation was a source of controversy until recently. The cytogenetics clearly established the neoplastic nature of HL[1].

Since then, HL is defined as a malignant disease affecting mainly the lymphoid system represented in lymph nodes, and to a lesser extent; extranodal sites as spleen, liver and even bone marrow[2]. It was first recognized by Thomas Hodgkin in 1832, when he recorded seven patients suffering from lymph nodal and splenic enlargements. HL meets the usual criteria for most malignancies, including production of large tumorous masses containing malignant neoplastic cells with the potential of spreading to many sites[3].

According to many statistical analyses in recent researches, the incidence of HL is high and unfortunately is on the rise. With respect to age affection, it affects wide range of age groups including; children, late teenagers, adults and even old age people. Distribution of HL cases over age, sex, geographic territory and socioeconomic statuses has suggested multiple etiologically distinct entities for HL, rather than a single disease[4].

Since its earliest description in the first half of the 19th century, HL has proved to be a difficult form of disease to understand because of its unusual histopathological aspects, as it resembles other infectious processes[5].

Most of the cases of HL present as painless peripheral lymphadenopathy, usually the cervical group is the commonest to be affected. HL is associated with constitutional symptoms which are also called B-symptoms, in quarter of the cases. B-symptoms include

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fever, night sweats, weight loss (10% of body weight) and pruritis. Involvement of extranodal sites as spleen, liver or bone marrow is less common in HL than in Non-HL[6].

Histopathologically, HL is distinguished from other lymphomas by the polymorphous reactive background and scattered large neoplastic cells called Hodgkin Reed Sternberg (HRS) cells. Sometimes histopathology is straight forward and enough to establish the diagnosis, however, in many cases immunohistochemistry is required for confirmation[7].

Hodgkin lymphoma includes two biologically and clinically distinct entities: Nodular Lymphocytic Predominant HL (NLPHL) and classic HL (CHL). NLPHL is considered a B-cell neoplasm, while CHL represents a neoplasm of crippled B-cells. The currently accepted nomenclature system for HL is the World Health Organization (WHO) 2016 revision for the original classification of 2008, which recognizes NLPHL to be morphologically, biologically and clinically distinct from CHL. So, HL is classified broadly into:

1. Nodular Lymphocytic Predominant Hodgkin Lymphoma (NLPHL)
2. Classical Hodgkin Lymphoma (CHL)
    a. Nodular Sclerosis (NS)
    b. Mixed Cellularity (MC)
    c. Lymphocyte-rich (LR)
    d. Lymphocyte-depleted (LD)[8].

The immunophenotype of the malignant cells, as well as the microenvironment in CHL differs significantly from that in NLPHL[9]. Where, HRS cells of CHL stain consistently positive for CD15 and CD30, occasionally positive for CD20 and negative for CD45, while lymphocyte predominant (LP) cells of NLPHL are characterized by the expression of CD20 and CD45, but they lack CD15 and CD30[10].

Despite the validity of the previous stains, there are still plenty of cases that couldn't be subtyped and remain in the grey zone. Also, CHL and NLPHL differ clinically, have different prognosis and even treatment protocols, so, differentiating these types is crucial to ensure appropriate therapy[11].

GATA3 is a transcription factor that is important in the differentiation of breast epithelium, urothelium, and subsets of T lymphocytes. It has been suggested to be useful in the diagnosis of carcinomas of mammary or urothelial origin. Its distribution in normal and neoplastic tissues is incompletely mapped. It's also a useful marker in the characterization renal and germ cell tumours, mesotheliomas, and paragangliomas[12].

Research suggests CD79a - Cluster of Differentiation 79- to substitute CD20 as a Pan B cell marker, as CD79a is a transmembrane protein[13] present on the surface of B-cells throughout their life cycle and is absent from the surface of all other healthy cells, making it a highly specific marker for B-cells neoplasms[11]. It plays both diagnostic and targeted therapeutic roles in Non Hodgkin Lymphoma (NHL)[14]. This study investigated the expression of GATA3 immunostaining in HL different subtypes.

MATERIALS AND METHODS

Materials

Seventy-three lymph node specimens were included in this study. The specimens were collected retrospectively as Formalin Fixed Paraffin Blocks (FFPB) from the archive of the Pathology Department at Medical Research Institute (MRI), Alexandria University, in the period between April 2019 and April 2020.

The diagnosis of the included HL specimens was based on standard histopathological examination and confirmed by immunostaining. All the results were reviewed before including the cases in this study. Seventy of the cases showed all the characteristic features of the final diagnosis of HL, while the rest three cases showed histopathological features of CHL yet with atypical immune-staining features and were excluded from the statistical analysis of this study.

Inclusion criteria of the cases in this study were: for all specimens of NLPHL; characteristic features by H&E, complete negativity for CD15 and CD30, and positivity for CD20 by IHC detected in the large neoplastic LP cells. For CHL; inclusion criteria were characteristic features by H&E as well as positivity for both CD15 and CD30 in HRS cells by IHC, with negativity for CD20 and CD3 in HRS cells, yet positivity of both in the reactive inflammatory background cells.

The biopsies were categorized according to availability into

NLPHL, CHL including Nodular Sclerosis (NS)-CHL, Mixed Cellularity (MC)-CHL, Lymphocytic Rich (LR)-CHL, Lymphocytic Depletion (LD)-CHL and the three CHL cases with atypical immune-staining features.

Methods

Seven 5-µm thick paraffin sections were cut from each block included in this study, then one section was stained by routine H&E stain, four sections were subjected to IHC staining for CD15, CD30, CD20 and CD3, while the other two were subjected to IHC staining for GATA3 and CD79a.

1. Routine H&E, for review of histopathological features.
2. Immunostaining for CD15, CD30, CD20 & CD3
3. Immunostaining for both GATA3 and CD79a:
    - Two 5-µm thick sections from each block are mounted on coated slides, with preservation of the archival number
    - It is carried by Dako Agilent Autostainer Link 48 slide stainer (AS480)

- Incubation with primary antibodies:
    - One section is incubated with mouse monoclonal antihuman antibody for GATA3 (L50-823, Millipore sigma, cell marque, dilution range 1:250)
    - The other section is incubated with mouse monoclonal antibody for CD79a (JCB117 clone, Dako, ready to use)
- Then chromogen -Diaminobenzidine tetrahydrochloride (DAB)- is applied to allow detection of the reaction. Rinsing excess chromogen is carried and finally, the slides are counterstained with haematoxylin, rinsed in water, dehydrated, cleared then mounted for examination.

For positive control: breast tissue from lobular carcinoma mass lesion was used -for similarity for lymphomatous cells- in case of GATA3 (Figure 1a) and a reactive lymph node, in case of CD79a (Figure 1b). While for negative control: staining was carried with omission of the primary antibody incubation, tonsil was used in case of GATA3 (Figure 1c) and appendix in case of CD79a (Figure 1d).

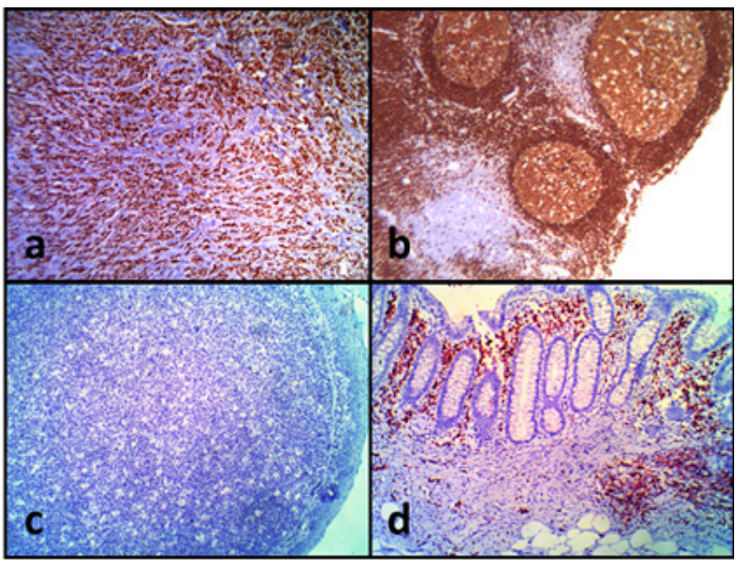

Fig. 1: Photomicrographs of sections showing: 1] positive control for the IHC stains used in the study: (a) GATA3 positivity in lobular carcinoma cells of breast (X40) and (b) CD79a positivity in B-cells in reactive LN (X40), 2] Negative control for IHC stains used in the study: (c) GATA3 negativity in tonsil (X40) and (d) CD79a negativity in epithelial lining of an appendix (X40)

Interpretation of immune staining results

a) GATA3

The positivity of GATA3 is nuclear and in this study was graded depending upon the percent of positive neoplastic cells as follows: negative (0%), moderate immunostaining (<30%), and strong (>30%)[15].

b) CD79a

The positivity of CD79a is cytoplasmic and was graded in this study depending upon the cytoplasm of HRS cells as follows: positive if more than 50% of tumour cells showed cytoplasmic staining[16].

Sensitivity as well as specificity were investigated for both markers, in addition to, the predictive values whether positive or negative and level of accuracy.

Statistics

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. Tests used in this study included: Chi-square test for categorical variables, to compare different groups, Fisher's Exact or Monte Carlo correction for Chi-Square when >20% of the cells have expected count less than 5, Student t-test for normally distributed quantitative variables, to compare between two studied groups, F-test (ANOVA) for normally distributed quantitative variables, to compare more than two groups, Receiver operating characteristic curve (ROC) in comparing performance between two tests, sensitivity, specificity, positive predictive and negative predictive values and accuracy.. Significance of the obtained results was judged at the 0.05 level.

All procedures performed in the current study were approved by IRB (Medical Research Institute Ethics Committee IORG#: IORG0008812) in accordance with the 1964 Helsinki declaration and its later amendments. Formal written informed consent wasn't required with the waiver by IRB (Medical Research Institute Ethics Committee IORG#: IORG0008812) (E/C.S/N. T51/2018 in 25th of August 2018)

RESULTS

Clinicopathological data

Age

It varied among studied cases in this study and it ranged from 5 to 70 years. There was no statistical significant difference in age distribution among different HL subtypes as the mean age was 33.3, 33.2, 34.2, 47.7 and 33 in NLP, NS, MC, LR and LD subtypes respectively.

Sex

There was significant difference (P<0.05) in sex distribution among HL different subtypes in this study. In NLP, MC and LR male sex predominated, while in NS and LD female sex predominated.

Site

Prevalence of the head and neck site being 74.2%. Head and neck site in this study included cervical, submental, submandibular, posterior triangle and intraparotid LNs. Other included sites in this study were axillary, inguinal and abdominal sites. There was no significant difference (p>0.05) in site distribution among different HL subtypes in this study.

Histopathological findings

Nodular Lymphocytic Predominant HL

The included ten NLPHL cases revealed the characteristic nodular pattern of growth, with scattered characteristic pathognomonic large LP cells having small pale cytoplasm and poly-lobular nuclei showing multiple basophilic small nucleoli within finely dispersed chromatin. The background was formed mainly of small mature lymphocytes. (Figure 2)

Classical HL

Twenty-four cases where of NS-CHL subtype showing fibrosis in the form of well-formed fibrotic bands and complete nodules. All the cases showed the characteristic pathognomonic lacunar cells –variant of HRS cells- showing perinuclear condensation of the cytoplasm surrounded by clear empty space. (Figures 3 A,B) Twenty-two cases where of MC-CHL subtype showing scattered HRS cells within reactive background with abundant eosinophils. (Figure 3C) Twelve cases were of LR-CHL subtype showing scattered HRS cells with predominance of small lymphocytes in the reactive background. (Figure 3D) Only two cases were LD-CHL with effaced architecture, abundant fibrosis and scattered large atypical HRS cells reminiscent to anaplastic cells.

Immune Staining data

NLPHL cases: All cases showed cytoplasmic positivity for CD20 in the large LP cells as well as scattered lymphocytes in the reactive background. CD15 and CD30 were completely negative in all cases. CD3 was negative in the large LP cells but positive in lymphocytes in the background with obvious rosetting around LP cells. (Figure 4)

CHL cases: All cases were positive for both CD15 and CD30 in the large HRS cells as well as in some scattered granulocytes. Both CD20 and CD3 were negative in the large HRS cells and showed scattered positivity in the cytoplasm of the reactive lymphocytes in the background with slight predominance of CD3. (Figure 5)

GATA3 immune-expression

Forty-nine cases included in this study showed positivity to this marker, while the rest twenty-one cases were negative. From the forty-nine positive case; twenty-six showed strong nuclear expression (15 NS-CHL, 10 MC-CHL & 1 LD-CHL) and twenty-three cases showed moderate nuclear expression (7 NS-CHL, 10 MC-CHL & 6 LR-CHL) (Figure 6). The rest twenty-one cases negative for GATA3 were the 10 NLPHL, 2 NS-CHL, 2 MC-CHL, 6 LR-CHL & 1 LD-CHL. (Figure 7)

There was high significant difference (P<0.001) in GATA3 expression among different HL subtypes in this study, as all the cases of NLPHL were negative while positivity was expressed with varying percentage in cases of NS, MC, LR and LD. (91.7%, 90.9%, 50% and 50% respectively).

Sensitivity of GATA3 in diagnosing CHL was 78; as it had the capability of identifying forty-seven cases of CHL out of the sixty cases included. Its specificity was 100; as none of the included ten NLPHL cases expressed this marker and so this possibly signifies the capability of excluding NLPHL. The positive predictive value was also 100, as the probability to detect CHL was 47 against none of the NLPHL. Its negative predictive value was 43.48. Its accuracy in diagnosing CHL was 81.43, as GATA3 expression was in agreement of CHL subtype in forty-seven biopsies out of the seventy biopsies of HL included in this study. There was a high statistical significant association between GATA3 expression as a diagnostic marker and the CHL subtype of HL. (p <0.001)

CD79a immune expression

Seventeen cases were positive for CD79a (the 10 NLPHL, 2 NS-CHL, 3 MC-CHL & 2 LR-CHL) while the rest fifty-three cases were negative for CD79a (22 NS-CHL, 19 MC-CHL, 10 LR-CHL & 2 LD-CHL) (Figure 8)

There was significant difference in CD79a expression among different HL subtypes (P<0.001). All NLPHL cases were positive for CD79a, while positivity for this marker in the other types was as follows: 8.3%, 13.6%, 16.7% and 0% in NS, MC, LR and LD subtypes respectively.

Sensitivity of CD79a in diagnosing NLPHL was 100; as all the ten biopsies of NLPHL included in this study were positive for CD79a. Its specificity was 88.3l as fifty-three biopsies of CHL were negative to this marker out of the sixty included CHL biopsies. Its positive predictive value was 58.82, as its probability to detect and diagnose

NLPHL was 100% against only 11.7% of the CHL biopsies. Its negative predictive value was 100, as its probability to exclude NLPHL was 100% as none of the included cases of NLPHL expressed this marker against 88.3% of CHL not expressing this marker. Its accuracy in diagnosing NLPHL was 90, as CD79a expression was in agreement with NLPHL subtype in the included ten biopsies out of the seventy HL biopsies.

There was a high statistical significant association between CD79a expression as a diagnostic marker and the NLPHL subtype of HL. (p <0.001)

Summary of relationships between GATA3 expression and different parameters in this study (Table 1)

The three CHL cases with atypical immune-staining features

The morphological features of the three cases were highly reminiscent to CHL. The lymph nodes were effaced by a neoplastic growth separated by thick fibrous tissue strands in vague attempts at nodule formation. The neoplastic growth was formed of abundant large neoplastic cells HRS like as well as large cells with convoluted nuclei against a background of reactive lymphocytes with some eosinophils and histiocytes. (Figure 9)

CD15 results came against predicted in the three cases, as it was completely negative. CD30 was positive in the large neoplastic cells as well as in small granulocytes in the reactive background. CD20 was positive in the large neoplastic cells and few reactive cells in the background. CD3 was diffusely positive in the small reactive lymphocytes in the background, but negative in the large neoplastic cells. (Figure 10)

Two of the three cases showed moderate positivity for GATA3. (Figures 11 a,b) The other case was completely negative for GATA3.The three cases showed cytoplasmic positivity for CD79a in more than 50% of the large neoplastic cells as well as in scattered small reactive lymphocytes in the background. (Figures 11 c,d)

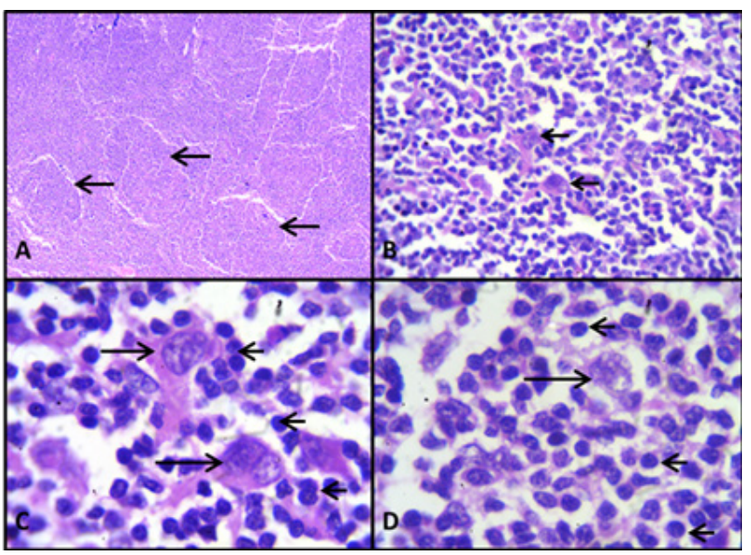

Fig. 2: Photomicrographs of sections from lymph nodes from NLPHL cases showing (A) the characteristic nodular pattern of growth (arrows) (H&E, X40), (B) scattered LP cells (arrows) in a reactive background formed mainly of lymphocytes (H&E, X400), (C) Closer view of previous case showing two LP cells with irregular folded nuclear membrane in the upper cell and multilobation of the lower one (long arrows), surrounded by mature reactive lymphocytes (short arrows) (H&E, X1000), (D) a LP cell with lobulated nucleus (long arrow) within a reactive background formed mainly of lymphocytes (short arrows). (H&E, X1000)

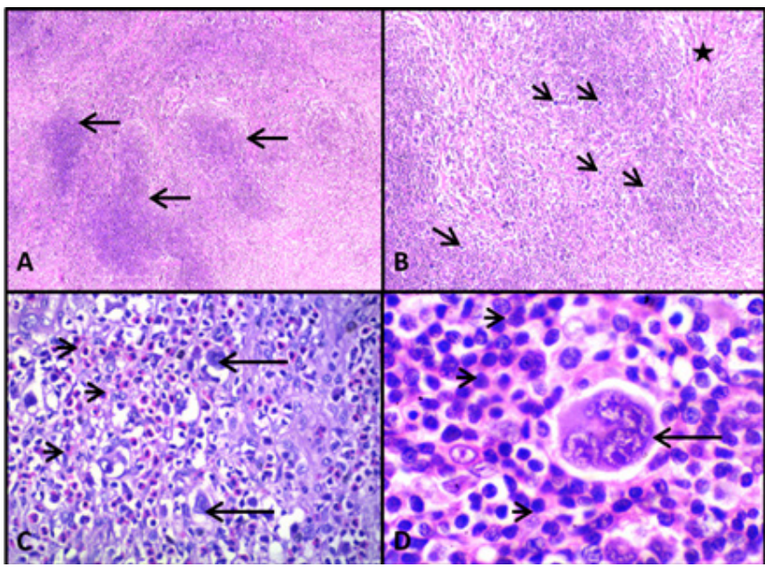

Fig. 3: Photomicrographs of sections from lymph nodes from CHL cases: (A) Well-formed fibrotic nodules (arrows) from a case of NS-CHL. (X40) (B) Closer view showing multiple lacunar cells with characteristic perinuclear halo (arrows) with reactive background and fibrotic bands (star). (X100) (C) MC-CHL case showing scattered HRS cells (long arrows) within reactive background rich in eosinophils (short arrows) (X400) (D) a large multinucleated HRS cell (long arrow) within reactive background formed mainly of lymphocytes (short arrows) from a case of LR-CHL. (X1000) (H&E)

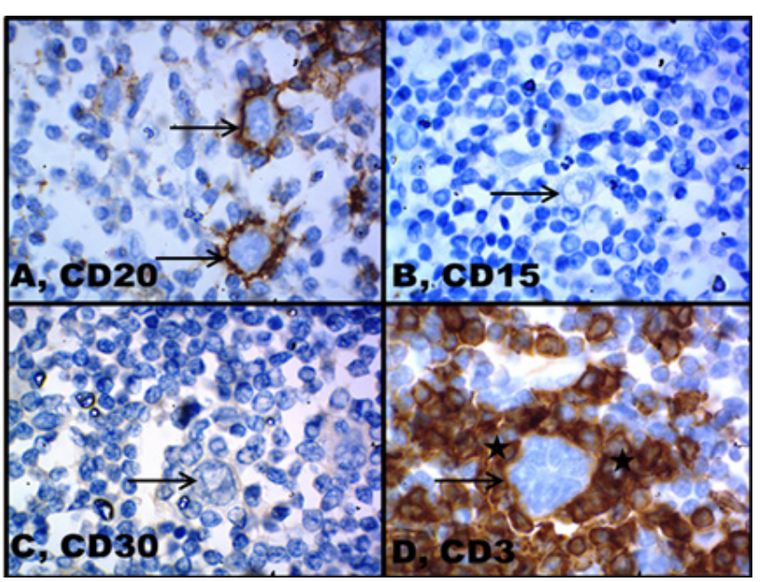

Fig. 4: Photomicrographs of sections from lymph nodes showing immunostaining features of NLPHL. (A) CD20 cytoplasmic positivity in two LP cells (arrows). (B) & (C) showing negativity for CD15 and CD30 in the large neoplastic cells (arrows) respectively. (D) Abundance of CD3 positive reactive T-lymphocytes (stars) surrounding a multilobed LP cell (arrow). (IHC, X1000)

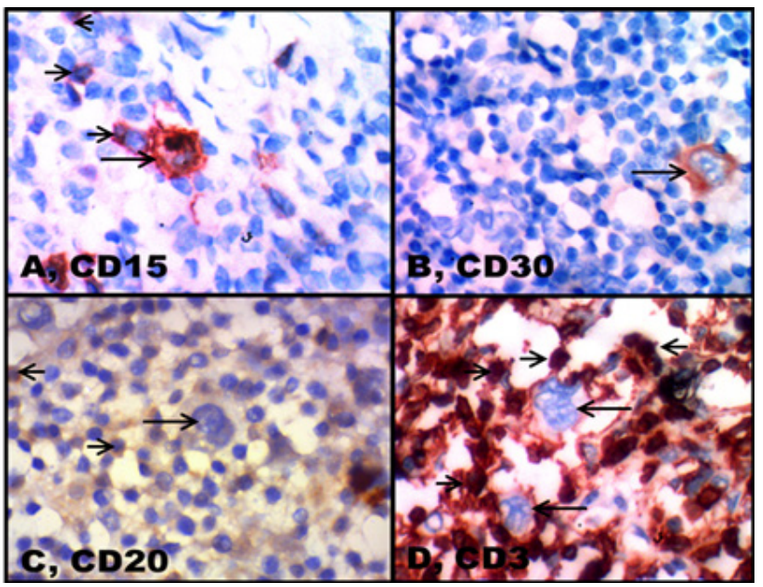

Fig. 5: Photomicrographs of sections from lymph nodes showing immunostaining features of CHL. for (A) CD15: cytoplasmic positivity in a HRS cell with paranuclear accentuation (long arrow) and few positive granulocytes (short arrows). (B) CD30: cytoplasmic positivity in a HRS cell (arrow). (C) CD20: negativity in a HRS cell (long arrow) with scattered cytoplasmic positivity in the reactive B-lymphocytes (short arrows). (D) CD3: positive reactive T-lymphocytes (short arrows) surrounding two negative HRS cells (long arrows). (X1000)

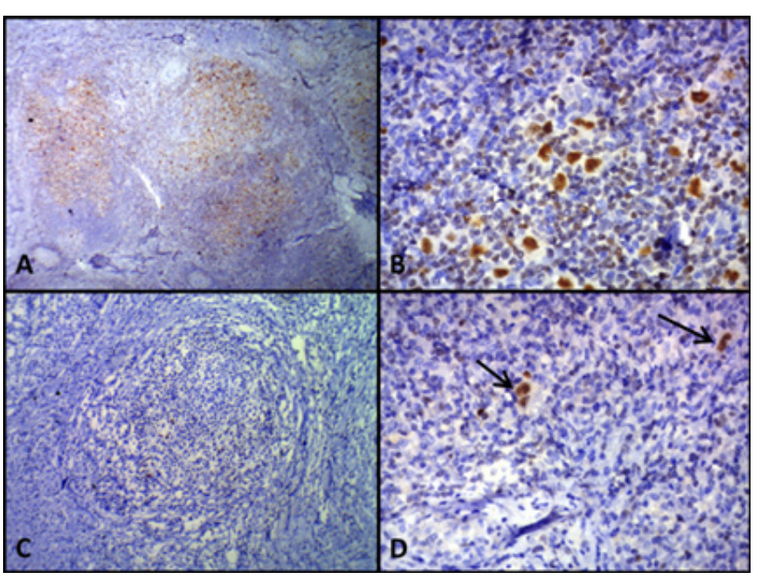

Fig. 6: Photomicrographs of sections from lymph nodes showing GATA3 immune staining in CHL cases: (A & B) strong expression (>30% of HRS cells) in NS-CHL (brown dots represent positive nuclei) (A,X40 & B X400), (C) Moderate expression (<30% of HRS cells) from a nodule of NS-CHL (X100) and (D) MC-CHL case showing scattered nuclear expression in HRS cells (arrows) (X400)

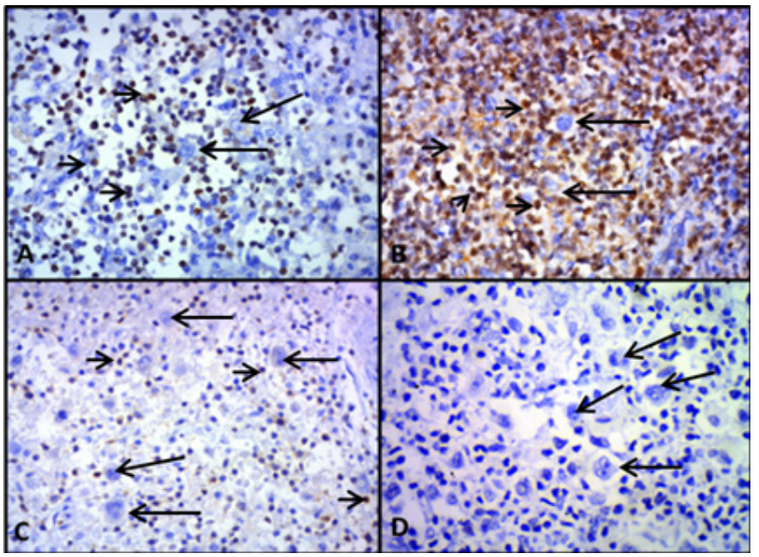

Fig. 7: Photomicrographs of sections from lymph nodes showing GATA3 negative immune expression in HL cases: (A) NLPHL case showing scattered LP cells not expressing GATA3 (long arrows) with scattered positivity in the nuclei of lymphocytes (short arrows) in the reactive background. (X400), (B) NS-CHL case showing scattered lacunar cells negative for GATA3 (long arrows) with abundance of GATA3 positive reactive lymphocytes (short arrows) (X400), (C) MC-CHL case showing abundant HRS cells not expressing GATA3 (long arrows) with scattered positivity in reactive lymphocytes (short arrows) (X400) and (D) LD-CHL showing abundant atypical HRS cells negative for GATA3 (long arrows). (X400).

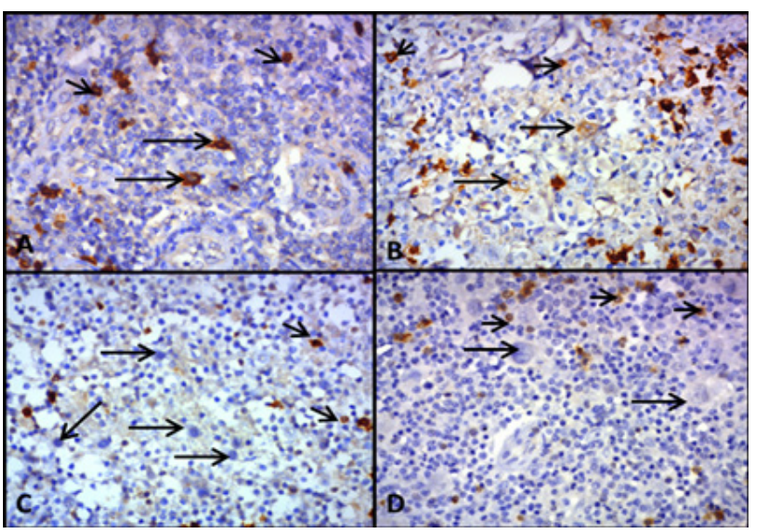

Fig. 8: Photomicrographs of sections from lymph nodes showing CD79a immune expression in: (A) LP cells (long arrows) in NLPHL and scattered small B-lymphocytes (short arrows) (X400), (B) HRS cells with characteristic binucleation (long arrows) in MC-CHL and scattered reactive B-lymphocytes (short arrows) (X400), (C) scattered reactive lymphocytes (short arrows) with negativity in HRS cells (long arrows) from a case of NS-CHL (X400) and (D) scattered reactive lymphocytes (short arrows) with negativity in HRS cells (long arrows) from a case of MC-CHL (X400).

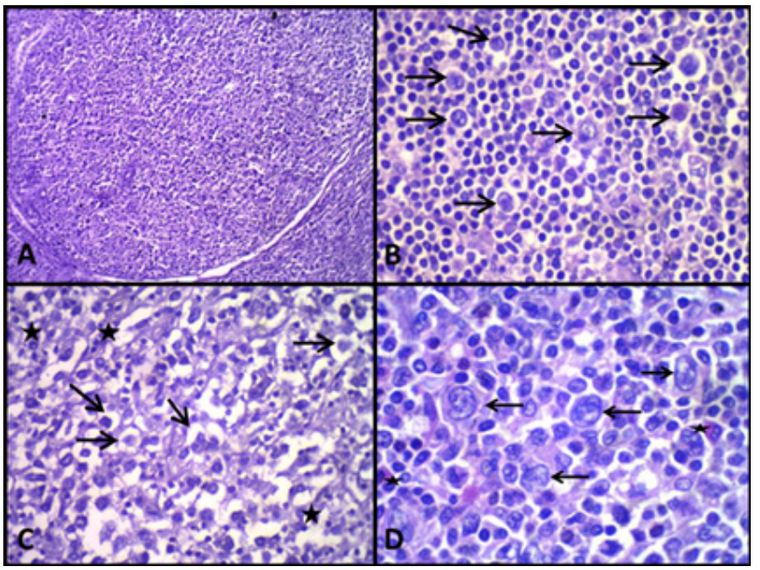

Fig. 9: Photomicrographs of sections from lymph nodes showing histopathology of the three CHL cases with atypical immune-staining features (H&E) (A) Attempt at nodule formation with scattered large neoplastic cells (X100), (B) Closer view showing abundant HRS like cells (arrows) with reactive small lymphocytes (X400), (C) sheets of lacunar like cells with characteristic perinuclear halo (arrows) within reactive inflammatory background (stars) and (D) Large neoplastic cells with convoluted nuclei and prominent nucleoli (arrows) with reactive background showing lymphocytes, histiocytes and eosinophils (stars) (X1000).

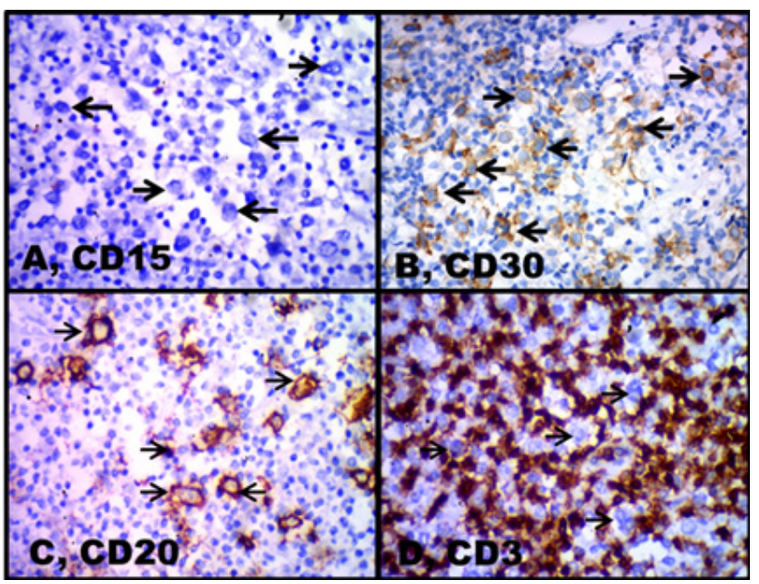

Fig. 10: Photomicrographs of sections from lymph nodes showing immunostaining features of the three CHL cases with atypical immune-staining features: (A) CD15 negativity in HRS cells (arrows), (B) CD30 positivity in the large neoplastic cells as well as scattered granulocytes (arrows), (C) CD20 positivity in the large neoplastic cells as well as few reactive lymphocytes in the background (arrows), (D) CD3 diffuse positivity in the reactive background lymphocytes with negativity in neoplastic cells (arrows). (X400)

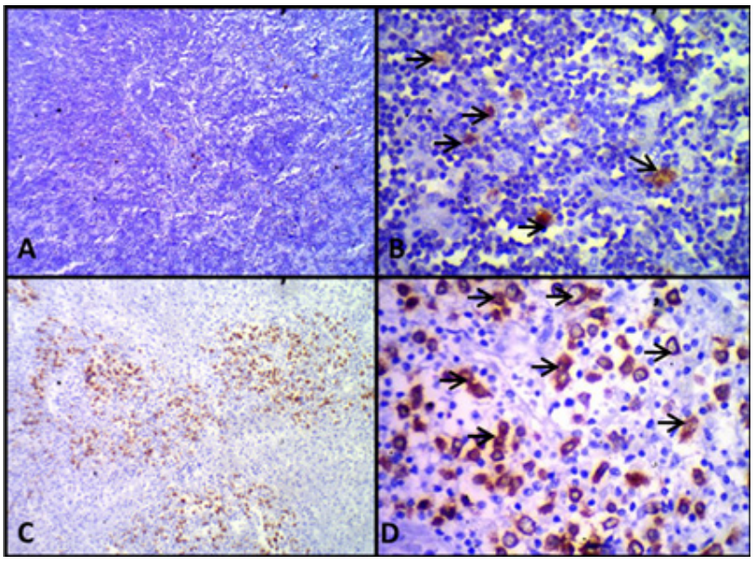

Fig. 11: Photomicrographs of sections from lymph nodes showing GATA3 immune expression in the three CHL with atypical Immune-staining features; (A) <30% of neoplastic cells show nuclear expression (seen as brown dots representing positive nuclei) (X100), (B) Closer view (X400). CD79a immune expression in some neoplastic cells (arrows) in GZL; (C) Diffuse cytoplasmic expression in >50% of the neoplastic cells and (D) Closer view showing positivity in the large neoplastic cells as well as small reactive lymphocytes (arrows) (X400).

Table 1: Relation between GATA3 and different parameters (n=70)

	Total (n = 70)		GATA3				Test of sig.	*P value*
			Negative (n = 21)		Positive (n = 49)			
	No.	%	No.	%	No.	%		
Age (years)	35.69 ± 14.1		41.33 ± 18.77		33.27 ± 10.90		t= 1.841	0.077
Sex								
Male	41	58.6	14	66.7	27	55.1	X^2= 0.810	0.368
Female	29	41.4	7	33.3	22	44.9		
Site								
Head and neck	52	74.3	15	71.4	37	75.5		
Axillary	9	12.9	2	9.5	7	14.3	X^2= 4.098	^{MC}p= 0.249
Inguinal	7	10.0	2	9.5	5	10.2		
Abdominal	2	2.9	2	9.5	0	0.0		
Subtypes								
NLPHL	10	14.3	10	47.6	0	0.0		
NS-CHL	24	34.3	2	9.5	22	44.9		
MC-CHL	22	31.4	2	9.5	20	40.8	X^2= 35.207*	^{MC}p <0.001*
LR-CHL	12	17.1	6	28.6	6	12.2		
LD-CHL	2	2.9	1	4.8	1	2.0		
CD79a								
Negative	53	75.7	11	52.4	42	85.7	X^2= 8.883*	0.003*
Positive	17	24.3	10	47.6	7	14.3		

X^2: Chi square test, MC: Monte Carlo, FE: Fisher exact test, t :Student t – test
p: *p value* for comparing between the different groups
*: Statistically significant at $p \leq 0.05$

DISCUSSION

Hodgkin lymphoma has an estimated annual incidence of 83,000 cases world-wide and mortality of 23,000 deaths in 2020[17]. In Egypt, HL is counted as one of the common hematologic malignancies, accounting for about 1% of cases diagnosed annually. According to its incidence it was ranked the nineteenth and according to mortality it was ranked the twenty-first among all cancers diagnosed in Egypt in 2020[18].

Unfortunately, Africa shows a high mortality rate, as a recent research stated that African countries -including Egypt- should pay more attention to HL management including preventive measures, early diagnostic strategies, appropriate therapeutic regimens and rehabilitation[19]. Luckily, its therapy represents one of the most optimistic and successful stories in medicine as currently, more than 80% of newly diagnosed cases are likely to be cured[2]. So, early accurate diagnosis and therapy could completely eradicate mortality and even morbidity from this neoplasm.

The seventy HL cases included in the statistics of this study were subtyped according to the availability into five groups representing the five HL subtypes; ten NLPHL, twenty-four NS, twenty-two MC, twelve LR and two LD cases. Their distribution percentage was 14%, 34%, 32%, 17% and 3% respectively. This distribution is somehow similar to the established data concerning the subtype's incidence that states that, the commonest subtype is nodular sclerosis, followed by mixed cellularity, then NLPHL and lymphocytic rich. Lymphocytic depletion has the lowest incidence[20].

According to age distribution among HL cases included in this study, the commonest decades were from 21-30 and from 31-40 years, as 62.8% of the cases lied in these decades. Statistical analysis stated that there was no significant difference in age distribution among different HL subtypes as the mean age among HL subtypes was 33.3, 33.2, 34.2, 47.7 and 33 in NLPHL, NS, MC, LR and LD subtypes respectively. But it showed that there was slight increase in the mean age of LR-CHL subtype. This finding is in accordance to that stated by an Egyptian study carried in 2016 by Zeggai *et al*[21], which stated that most of the HL cases were found between 20-39 years.

Sex distribution in this study showed that male sex predominated with its percentage being 58.6% among the seventy HL cases. Statistical analysis revealed that NS-CHL and LD-CHL subtypes showed female predominance as females represented 66.7% and 100% of cases respectively. On the other hand, NLPHL, MC and LR-CHL showed male predominance with males representing 80%, 68.2% and 83.3% of the cases respectively. These findings are similar to that stated by a study carried in Egypt in 2016, which showed that female sex predominated in NS-CHL and LD-CHL, while male sex predominated in MC-CHL and LR-CHL subtypes[21].

The commonest site to be affected by HL according to this study is the head and neck including; cervical, posterior triangle, submandibular, submental, intra-parotid and supraglottic. Statistical analysis revealed no significant difference in site distribution among different HL subtypes. However slight differences could be seen, as among NLPHL 90% were in head and neck which was the highest percentage, followed by MC (77.3%), NS (70.8%), LR (66.7%) and LD (50%). This finding is similar to most researches carried in various geographical territories worldwide that stated that the commonest site to be affected by HL is laterocervical[22].

The immune staining still takes the upper hand in diagnosing HL as flow cytometric use in diagnosing HL is of limited value, that's because HRS cells usually escape detection using this technique[23]. Also, molecular studies as conventional Polymerase Chain Reaction (PCR) are of limited use in diagnosing HL and usually the analysis results in false negative results due to few neoplastic cells in relation to the abundant reactive background cells[1].

GATA3, a lineage specifier, is critical in regulating self-renewal of hematopoietic stem cells[24], and controlling lymphoid cell differentiation[25]. It has been reported that GATA3 promotes T cell specification by repressing B cell potential in pro-T cells[26], while decreased GATA3 expression is essential for early B cell commitment[27]. The aberrant expression of GATA3 was observed in HRS cells in HL cell lines in 2010 by Stanelle *et al*, who stated that GATA3 controls cytokine expression and signaling in HL which in turn plays an essential role in the pathogenesis of HL[28].

GATA3 results in this study varied among HL cases. Expression of GATA3 was null in LP cells in all the cases of NLPHL denoting that this marker could be used as an exclusion marker for NLPHL. This finding is similar to that stated by other researchers as Kezlarian *et al*, who stated that all the cases of NLPHL were completely negative for GATA3[29]. In most of the cases of NLPHL there were rosettes surrounding the large LP cells in the form of GATA3 positive small lymphocytes. This finding is diagnostic as it is in accordance with a research that stated that NLPHL cases uniformly showed T-follicular helper cells (TFH) -which are a subset of CD4 positive T-lymphocytes- rosettes around LP neoplastic cells[9]. Another research stated that TFH expresses GATA3 even with change in cytokine profile[30].

GATA3 expression was seen in nuclei of HRS cells in cases of CHL at the following positivity rates: 91.7% in NS, 90.9% in MC and 50% in both LR and LD subtypes. These findings were similar to that concluded in another research carried in 2019, which stated that positivity for GATA3 in CHL cases was 87% in NS and 70% in both MC and LR subtypes[29].

Statistical analysis showed that the specificity of GATA3 for differentiating CHL from NLPHL was 100%; as none of the NLPHL cases showed positivity for GATA3 in the large neoplastic cells. This finding is in accordance with the findings of a previous study carried by Kezlarian

B *et al*, which stated that GATA3 could be a useful marker in excluding NLPHL diagnosis with 100% negative predictive value[29].

Yet sensitivity of GATA3 nuclear expression among the CHL biopsies was 81.67% as eleven out of the sixty CHL included biopsies were negative for GATA3 expression. This finding supports that this marker is a good positive confirmatory test for CHL, but its negativity doesn't rule out CHL. This finding is in accordance with the findings of a study carried in 2019, stating that 20% of CHL can be negative for GATA3 IHC marker and so its negativity can't exclude CHL diagnosis[29].

The GATA3 could also be helpful in planning therapy. Since 2015, this transcription factor -GATA3- has been targeted in the therapy of asthma. Recently in 2019, a specific cell-permeable construct called SB010, a DNAzyme designed as single stranded synthetic anti-sense DNA formed of 34-base, specifically binds to GATA3 messenger RNA and cleaves it[31]. This therapeutic role may be of use in GATA3 positive HL cases.

Cytoplasmic expression of CD79a beautifully highlighted the LP cells in all biopsies of NLPHL, denoting that these cells are of B cell origin. This finding supports the previous researches carried in 2014 by Goel *et al* that stated that CD79a is diffusely and strongly positive in the LP cells of NLPHL[32].

Statistical analysis showed that most of the cases of CHL (53 out of sixty) were negative for CD79a, yet seven cases out of the included sixty cases of CHL (11.7%) showed some positivity to CD79a that denotes that still some HRS cells keep some B cell features. However, this positivity was of weaker intensity than that seen in reactive lymphocytes nearby. This finding is supported by a research carried in 2020 that also denoted that some HRS cells retain B cell characteristics and expresses weakly B cell markers including CD79a. However, it stated that the percent of CD79a positive CHL cases were 36.4%[33]. This difference in positivity percentage of the CHL cases to CD79a may be attributed to the age of the cases included in this study as most of the cases are between 20-39 years, while a previous study reported that patients older than 50 years of age had a higher proportion of CD79a-positive cells[34].

Specificity of CD79a in diagnosing NLPHL in this study was 88.33% as fifty-three biopsies of CHL were negative to this marker out of the sixty included CHL biopsies. However its sensitivity in diagnosing NLPHL was 100% as all the included cases were positive for this marker.

Despite the well-established immunostaining panel used in diagnosing HL, yet pathologists are still puzzled with few cases that aren't fitting in a specific category. Three cases in this study belong to this category. The histopathological features were highly suspicious for CHL with abundance of lacunar cells as well as HRS cells, associated with incomplete fibrous tissue strands and areas of abundant neoplastic cells with sparse inflammatory cells. However, CD15 was negative in the large neoplastic cells in the three cases while CD30 was positive in the large neoplastic cells and scattered granulocytes in the three of them. CD20 was expressed in the large neoplastic cells as well as in few background lymphocytes. CD3 was expressed in abundance in the background lymphocytes, but completely negative in the large neoplastic cells.

The previous findings point out to grey zone lymphoma (GZL) which was first described in 1998 to address lymphomas at the border of CHL and other entities. Then this term extended to lymphomas between CHL and Diffuse Large B-Cell Lymphoma (DLBCL). The last updated and revised WHO classification of Hematopoietic and Lymphoid Tissue proposed GZL to include B-cell lymphoma unclassifiable (BCLu) that show features intermediate between DLBCL and CHL (BCLu-DLBCL/CHL). The WHO adopted this gray zone category in the revised classification in order to maintain purity of well-defined categories, as well as to allow further clinical studies on this category[8].

Histopathologically, BCLu-DLBCL/CHL shows sheet-like growth formed of pleomorphic neoplastic cells within fibrous stroma. Most of these neoplastic cells resemble lacunar cells and HRS cells. There is usually sparse inflammatory cell infiltrate made up of few scattered eosinophils, lymphocytes and histiocytes[35].

According to a recent study carried in 2019, CHL is defined by the presence of HRS cells showing down regulation of B cell markers as CD20 -being either weak or completely negative- and strong CD15 and CD30 expression. On the other hand, in GZL, HRS cells show strong CD20 expression, lack CD15 with absent or weak CD30 expression[36].

The GATA3 and CD79a expression in the three GZL cases was as follows: two cases showed GATA3 positivity in the nuclei of the large neoplastic cells (<30%) interpreted as moderate GATA3 positivity, however CD79a was also positive in the large neoplastic cells. One case from the three GZL cases showed complete negativity to GATA3 marker and diffuse positivity to CD79a in the large neoplastic cells.

Researches stated that most cases of GZL were young male patients between 20-40 years. Unfortunately, these patients had worse outcome when compared to that of CHL cases or DLBCL cases. Thus identification of such cases is important as it has been recommended that treatment for these cases should be dose-intensive regimens [1]. A research stated that the majority of GZL cases presented by an anterior mediastinal mass (63%), the remaining cases had either non-anterior mediastinal mass (12%) or no mediastinal involvement at all (25%)[37].

Gene expression profiling and mutational landscape between CHL and BCL, lead to postulation that stated

GZL as a biologic transition between the two large categories with a thymic B cell being the common cell of origin[38]. Principle component analysis performed on gene expression profiling in a recent research found that the expression level of known genes expressed by malignant cells (as JAK2 and STAT1) increased from CHL to GZL to BCL. Oppositely, the expression level of tumour microenvironment related genes (as CD3 and CD4) decreased upon the same pathway from CHL to BCL[37].

More recently, GZL has been further sub-classified into four subtypes named from group 0 to group3. Group 0 – at the beginning of the spectrum- corresponded to cases more near to CHL diagnosis but with strong and diffuse CD20 expression. Group 4 –at the end of the spectrum- corresponded to cases more near to BCL but with intense and diffuse CD30 expression in the neoplastic cells. Groups 1 and 2 corresponded to intermediate features. This study has concluded that group 0 is definitely a part of the GZL spectrum, while group 3 should be out of the spectrum as DLBCL[37].

A recent research stated that GZL is a highly variable neoplasm representing a diagnostic as well as therapeutic challenge due to its rarity. GZL represents clinical heterogeneity, highly aggressive nature and unfortunately lacks standardized therapeutic strategy, so prospective studies to evaluate novel therapeutic approaches are required[39,40].

CONCLUSION

GATA3 nuclear expression within large neoplastic cells coupled with the characteristic histologic findings is a good positive and confirmatory marker for CHL, yet negativity doesn't rule out CHL. And, GATA3 immune staining coupled with CD79a is a good panel to confirm NLPHL as GATA3 positivity completely excludes NLPHL with 100% positive predictive value. On the other hand, CD79a is positive in virtually all cases of NLPHL with the negative predictive value 100. Combining CD79a with GATA3 forms a complementary staining panel that can be used to classify and subtype cases suspicious for HL.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة نسيجية ومناعية هستوكيميائية؛ تم فيها الجمع بين GATA3 وCD79a كلوحة تكميلية واعدة لتشخيص وتمييز ليمفوما هودجكين

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مقدمة: سرطان الغدد الليمفاوية هودجكين هو أحد الأورام التي تظهر تشخيصًا واعدًا للغاية إذا تم تشخيصه مبكرًا وبدقة على الرغم من كونه ورمًا غير شائع إلا أنه لا يزال يمثل عبئًا اقتصاديًا من خلال زيادة معدلات الاعتلال لدى المرضى. يمثل ما يقرب من ١٥ ٪ من جميع أنواع السرطان لدى الشباب ، و ٨,٤ ٪ من حالات السرطان الجديدة سنويًا في الشعب المصري. يؤثر النوع الفرعي لسرطان الغدد الليمفاوية هودجكين بشكل مباشر على علاج هذا الورم ، حيث يظهر سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية مسار بطيء أكثر من سرطان الغدد الليمفاوية هودجكين الكلاسيكي ، ومع ذلك ينتكس بشكل متكرر. ولكن في المجمل يظهراستجابة جيدة للعلاج. أيضًا ، تختلف الأنظمة العلاجية تمامًا في كلا النوعين الفرعيين. وبالتالي ، فإن التمييز بين النوعين الفرعيين الرئيسيين من سرطان الغدد الليمفاوية هودجكين سيحسن نتيجة الحالات. تشخيص ورم الغدد الليمفاوية هودجكين في معظم الحالات غير واضح من خلال الصبغات الروتينية –هيماتوكسلين و ايوسين- وحده ، ويتطلب تحليلات مناعية إضافية قبل بدء العلاج. ومن بين تلك الدلالات المعتمدة CD١٥ و CD٣٠ و CD٢٠ و CD٣. حيث تؤكد الإيجابية لـ CD١٥ و ٣٠ مع سلبية لـ CD٢٠ في الخلايا السرطانية، جنبًا إلى جنب مع الإيجابية لكل من CD٢٠ و CD٣ في الخلفية التفاعلية مع غلبة CD٣تشخيص سرطان الغدد الليمفاوية هودجكين الكلاسيك. وعلى الجانب الآخر تؤكد الإيجابية لـ CD٢٠ مع السلبية ل CD١٥ و CD٣٠ في الخلايا السرطانية مع الإيجابية لكل من CD٢٠ و CD٣ في الخلفية التفاعلية تشخيص سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية. ومع ذلك ، لا تزال هناك بعض حالات سرطان الغدد الليمفاوية هودجكين تظهر أنماط مناعية متداخلة لا تنتمي لتشخيص محدد. وهذا يتطلب مزيدًا من الدراسات لاكتشاف العلامات المفيدة الأخرى في التمييز بين هذه الأنواع.

الهدف من البحث: يهدف العمل الحالي إلى التحقق من صلاحية الدلالات المناعية GATA٣ و CD٧٩a في تشخيص سرطان الغدد الليمفاوية هودجكين. كما يهدف إلى التدقيق في الاستخدام المحتمل لـ GATA٣ في التمييز بين سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية و سرطان الغدد الليمفاوية هودجكين الكلاسيكي.

مواد وأساليب العمل: تم تنفيذ العمل الحالي على سبعين حالة من سرطان الغدد الليمفاوية هودجكين وثلاث حالات سرطان الغدد الليمفاوية هودجكين الكلاسيكي لا تستوفي المعايير المناعية لتشخيص الورم. تم جمع جميع الحالات بأثر رجعي من أرشيف قسم علم الأمراض ، معهد البحوث الطبية، جامعة الإسكندرية وأرشيف مختبرات خاصة خلال الفترة من أبريل ٢٠١٩ إلى أبريل ٢٠٢٠. و قد تم تقسيم السبعين حالة حسب التوافر إلى الخمسة أنواع الفرعية لسرطان الغدد الليمفاوية هودجكين وتم تصنيف الحالات الثلاث الأخرى على أنها حالات سرطان الغدد الليمفاوية هودجكين الكلاسيكي ذات نمط مناعي لا يتناسب مع نمط سرطان الغدد الليمفاوية هودجكين الكلاسيكي المعتمدة وقد تم استبعادهم من التحليل الإحصائي. تم إخضاع المسلسل المكون من مقاطع بارافين بسمك ٥ ميكرون من جميع الكتل التي تم الحصول عليها إلى: ١) صبغة H&E الروتينية: لمراجعة التشخيص والتأكد من كفاية العينة، ٢) مراجعة و / أو إعادة صبغ CD١٥ و CD٣٠ و CD٢٠ و CD٣، ٣) الصبغة المناعية GATA٣، ٤) الصبغة المناعية CD٧٩a، تم الكشف عن تعبير

GATA٣ على أنه لون بني في نوى الخلايا السرطانية. لم يتم احتساب الإيجابية في نوى الخلايا الصغيرة على أنها إيجابية. كان التسجيل قوياً عندما كان أكثر من ٣٠٪ من الخلايا السرطانية موجبة بينما كانت متوسطة عندما كانت أقل من ٣٠٪ من الخلايا السرطانية إيجابية وسلبية عندما كانت جميع الخلايا السرطانية سلبية تمامًا.
تم الكشف عن تعبير CD٧٩a على أنه تلون بني في السيتوبلازم للخلايا السرطانية. مرة أخرى لم يتم احتساب أي تعبير في الخلايا الأخرى. كان التعبير إيجابيا عندما كان التعبير في أكثر من ٥٠٪ من الخلايا السرطانية.
النتائج: أظهرت النتائج أنه تم التعبير عن GATA٣ في تسعة وأربعين حالة من أصل ستين حالة تم تشخيصهم سرطان الغدد الليمفاوية هودجكين الكلاسيكي وكانت سلبية تمامًا في جميع حالات سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية. وتشير هذه النتائج إلى أن خصوصية GATA٣ في التمييز بين نوعين سرطان الغدد الليمقاوية هودجكين هي ١٠٠٪. كما أن قيمته التنبؤية الإيجابية هي ١٠٠ حيث أن احتمال تشخيص سرطان الغدد الليمفاوية هودجكين الكلاسيكي كان ٤٩ حالة مقابل لا شيء بين حالات سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية. كانت جميع حالات سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية إيجابية ل CD٧٩a، بينما فقط سبع حالات من حالات سرطان الغدد الليمفاوية هودجكين الكلاسيكي من أصل ستين كانت إيجابية. وقد أثبتت هذه النتائج أن حساسية CD٧٩a في التفريق بين النوعين ١٠٠٪.و قيمته التنبؤية السلبية هي ١٠٠ لأن احتمال استبعاد سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية كان ١٠٠٪. وأيضا كانت هناك حالتان من الثلاث حالات لسرطان الغدد الليمفاوية هودجكين الكلاسيكي -الغير متمشية مع الأنماط المناعية لسرطان الغدد الليمفاوية هودجكين الكلاسيكي- موجبة لـ GATA٣ على الرغم من كونها سلبية تمامًا لـ CD١٥. كما كانت الحالات الثلاث إيجابية لـ CD٧٩a ، وبالتالي تم وضع الحالات الثلاث في فئة سرطان الغدد الليمفاوية ذو المنطقة الرمادية.

الاستنتاج:

١. يعد تعبير GATA٣ النووي اختبارًا تأكيديًا جيدًا لسرطان الغدد الليمفاوية هودجكين الكلاسيكي ، ولكنه ليس سلبيًا جيدًا ، حيث أن سلبيته لا تستبعد هذا الورم.
٢. يعد GATA٣ مقترنًا بـ CD٧٩a اختبارًا جيدًا لتشخيص حالات سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية، حيث أن ايجابية الورم لGATA٣ تنفي تماما احتمالية سرطان الغدد الليمفاوية هودجكين السائدة الخلايا الليمفية العقدية والإيجابية ل CD٧٩a تؤكد التشخيص.
٣. بعض حالات سرطان الغدد الليمفاوية هودجكين الكلاسيكي تظهر إيجابية ل GATA٣ رغم سلبيتها ل CD١٥ كما رأينا في حالتين من الثلاث حالات لسرطان الغدد الليمفاوية هودجكين الكلاسيكي ذات أنماط مناعية غير متمشية مع الأنماط المعتمدة.

التوصيات:

- دراسة التعبير المناعي لGATA٣ في الحالات التي يشتبه في أنها سرطان الغدد الليمفاوية هودجكين الكلاسيكي ولكنها سلبية ل CD١٥ للتأكيد على احتمالية استخدامه في تشخيص حالات سرطان الغدد الليمفاوية هودجكين الكلاسيكي.
- دراسة مدى امكانية استغلال التعبير المناعي لGATA٣ في الاستراتيجيات العلاجية بسبب التطبيق الأخير للعلاج الموجه نحو عامل النسخ هذا في أمراض أخرى مثل الربو.
- دراسة فئة سرطان الغدد الليمفاوية ذو المنطقة الرمادية على نطاق أوسع للتأكد من مدى فعالية ال GATA٣ مقترنًا بـ CD٧٩aفي تشخيص هذه الفئة.