Prognostic value of CD10, BCL6 and MUM1 in diffuse large B-cell lymphoma

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is considered the commonest subtype of non-Hodgkin lymphoma (NHL) in the world. It is a refractory disease with a high mortality rate due to frequent relapses. Several prognostic parameters are now widely studied for risk stratification and achieving a better outcome.
Objectives: In this study, we aim to assess the prognostic value of immunohistochemical expression of CD10, BCL6, and MUM1 independently as surrogate markers for cell of origin (COO) classification of DLBCL and their correlation with clinicopathological characters and survival.
Patients and methods: This is a retrospective study conducted on 63 cases of DLBCL, NOS. Full-faced sections were constructed and immunostained for CD10, BCL6, and MUM1.
Results: CD10 expression was associated with early-stage (P=0.003), normal serum LDH level (P=0.022), absence of B symptoms (P=0.019), low international prognostic index (IPI) and age-adjusted-IPI (P=0.001) and also associated with longer progression free survival (PFS) (P=0.006). BCL6 expression was associated with centroblastic variant (P=0.005), good ECOG performance status (P=0.038) and low IPI (P=0.004) and also associated with better overall survival (OS) (P=0.028) and PFS (P=0.018). MUM1 expression was associated with advanced-stage (P=0.002) while no significant association was detected with other clinicopathological parameters or survival.
Conclusion CD10, BCL6, and MUM1 can be used independently as prognostic immunohistochemical markers for DLBCL that may denote the clinical behavior of the disease and further patients' outcomes.

Key words: CD10, BCL6, MUM1, DLBCL, survival.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is considered the commonest subtype of non-Hodgkin lymphoma (NHL) in the world and accounts for 25%–35% of all adult NHLs (**Teras et al., 2016**). It is a refractory disease with a high mortality rate due to frequent relapses (**Crump et al., 2017**). The International Prognostic Index (IPI) is considered the most widely used parameter for predicting outcomes in patients with DLBCL, NOS. However, it is not sufficient for precise detection of treatment outcomes as being dependent on only clinical findings and not reflecting the biological features of

DLBCL cells (**Shehata et al., 2019**).CD10, BCL6, and MUM1 are the immunohistochemical markers that compromise the Hans' algorithm that is employed in dividing DLBCL according to the cell of origin (COO) into germinal center B-cell type (GCB) and activated B-cell type (ABC) with different prognostic outcomes.

CD10 is a membrane metalloproteinase that is found in a variety of lymphoid, stromal cells, and epithelial cells **(Fabiani et al., 2005**). BCL6 is a zinc-finger transcriptional repressor that prevents premature B-cell activation and differentiation **(Basso and Dalla-Favera, 2012**). MUM1 is Multiple Myeloma Factor 1 or Interferon Regulatory Factor 4 (IRF4) transcriptional protein expressed in plasma cells and a small number of germinal center B cells (**Dwivedi *et al.*, 2015**).

This study aims to assess the immunohistochemical expression of CD10, BCL6, and MUM1 in DLBCL, NOS specimens and correlate their expression with the clinicopathological features of DLBCL, NOS patients as well as the patients' survival.

Patients and Methods

The study was conducted retrospectively on sixty-three specimens of diffuse large B-cell lymphoma, NOS. The formalin-fixed paraffin-embedded blocks were obtained from the archive of the surgical pathology lab of South Egypt Cancer Institute (SECI), Assiut University Hospital, Faculty of Medicine between 2011 and 2018. Only cases with available clinical data including follow-up data for at least one year after diagnosis and receiving CHOP therapy were included in this study. This study was approved by the ethical committee of South Egypt Cancer Institute. All available H&E and IHC stained slides were reviewed and reclassified based on the 2016 WHO classification criteria**(Swerdlow SH, 2017).**

Immunohistochemistry (IHC)

3-µm-thick sections were cut followed by deparaffinization and rehydration and blocking of endogenous peroxidase activity by 3% H2O2. Immersing the slides in citrate buffer (pH 6.0) and heating at 80°C in a microwave for 9 min was done for antigen retrieval. Then incubation with the primary antibodies (CD10,Rabbit polyclonal Antibody, Catalog number # PA5-29354**,**dilution 1:100; BCL6, Monoclonal antibody, clone BL6.02 (PG-B6p)m, Catalog number # MA5-11493**,**dilution 1:100; MUM1 Monoclonal Antibody (4G10), Catalog number# MA5-15639,dilution 1:100; Thermo Fisher Scientific Corporation, Fremont, California, USA).Secondary staining kits were used according to the manufacturer's instructions (Thermoscientific Corporation, Fremont, California,USA).

IHC evaluation of CD10, BCL6, and MUM1

For each case, the fields with a higher percentage of stained tumor cells were used for the analysis. The positivity was identified as brown complete membranous staining for CD10 and nuclear staining for both BCL6 and MUM1. The intensity of the staining was not used to determine the positivity. The interpretation of each marker was done by a semiquantitative method through the examination of the whole immunostained slide. A cut-off point of 30 % is used for all three markers as either positive (more than 30 % positive tumor cells) or

negative (less than 30 % positive tumor cells) (Cho *et al.*, 2018).

Statistical analysis

The statistical software package SPSS version16 was used for all analyses. Descriptive statistical analysis was done for clinicopathological variables that include: Age, Gender, histological variant, serum LDH level, B symptoms, stage according to Ann Arbor criteria, ECOG performance status, BM involvement, IPI score, response to treatment, relapse/progression. Continuous variables were statistically described in terms of median (range), while categorical variables were presented as numbers and percentages. **Chi-Square ($\chi 2$) test** was used for comparing categorical data. **Exact test** was used instead when the expected frequency is <5. Progression-free survival (PFS) and overall survival (OS) were calculated using **Kaplan-Meier survival curves** and the significance between the survival curves was evaluated by **Log-rank test**. PFS was considered as the period from initiating therapy to progression of the disease in patients with partial or complete remission; OS was the time from initiating therapy till the patient dies or till the time of the last follow-up visit. All *P* values were two-tailed and considered statistically significant if ≤ 0.05.

Results

Clinicopathological characters

The median age of the patients was fifty-two years old. Out of 63 cases, forty-two cases were presented with nodal involvement while the remaining cases were at extranodal sites. As regard tumor size; 59 cases were less than 10 cm while the remaining cases were more than 10cm. The histological variants included 71.6 % centroblastic variant, 7.7 % immunoblastic variant and 20.7% anaplastic variant. The clinical stages of the patients were grouped into early-stage (I-II) and advanced stage (III-IV) with most of the cases (70%) presented with advanced stage. BM involvement was reported in 32 % of cases. Thirty-seven patients showed abnormal serum LDH level and twenty-two cases revealed the presence of B symptoms. The ECOG PS was grouped into good PS with a score 0-1 (54%) and poor PS with a score ≥ 2 in the remaining cases. As regarding IPI; 52.5%of patients had low to intermediate risk while the rest of the patients had intermediate to high risk. Regarding Age-Adjusted IPI (AA-IPI); most patients (74.7%) had intermediate to high risk. Follow-up data were available for 61 out of 63 patients. The median follow-up period of our study was 35 months, ranged from 4 to 128 months. The median OS was 32(ranged from 4 to 102). The median PFS was 35 (ranged from 4 to 128). At the end of the study, the total number of deaths was 12 cases and the number of the relapsed cases was 16.

Immunohistochemical results of CD10, BCL6, and MUM1 and their association with clinicopathological parameters

Seventeen out of 63 cases of DLBCL, NOS (26.9%) were positive for CD10 while 46 cases (73.1%) were negative. Regarding IHC results of BCL6, 37 cases (58.7%) were positive and 26 cases (41.3%) were negative. MUM1 was positive in 41 cases (65%) and negative in 22 cases (35%) **(Figure 1).**

Positive expression of CD10 was associated with female gender (*P*=0.049), early stage (I, II)

(*P*=0.003), normal LDH level (*P*=0.022), absence of B symptoms (*P*=0.019), low IPI and AA-IPI (*P*=0.001).About the IHC expression of BCL6, there was a significant association between positive BCL6 expression and centroblastic variant (*P*=0.005), good ECOG PS (*P*=0.038), and low IPI (*P*=0.004).Regarding the IHC expression of MUM1, the only significant association was found between positive MUM1 expression and advanced stage (III, IV) (*P*=0.002) while no significant association was detected with other clinicopathological parameters. **Table (1)**

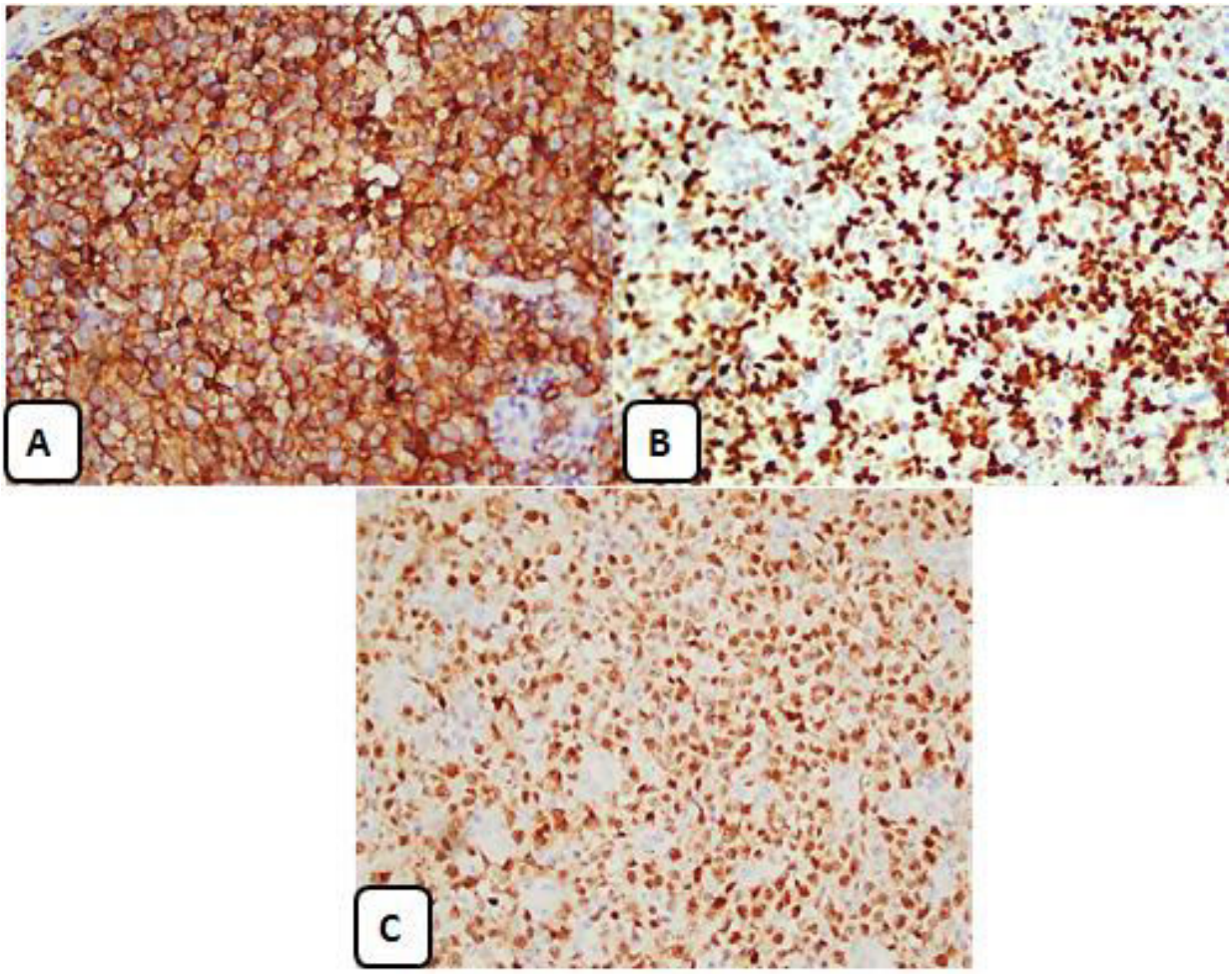

Figure (1): Positive immunohistochemicalexpression of (a) CD10, (b) BCL6, and (c) MUM1.

Table (1): Association between IHC expression of CD10, BCL6 and MUM-1 and the clinicopathological parameters.

Variables	CD10		*P*-value	BCL6		*P*-value	MUM-1		*P*-value
	Negative (n=46) N (%)	Positive (n=17) N (%)		Negative (n=26) N (%)	Positive (n=37) N (%)		Negative (n=22) N (%)	Positive (n=41) N (%)	
Age groups									
< 60 years	29 (70.7)	12 (29.3)	0.577	14 (34.1)	27 (65.9)	0.117	14 (34.1)	27 (65.9)	0.860
≥ 60 years	17 (77.3)	5 (22.7)		12 (54.5)	10 (45.5)		8 (36.4)	14 (63.6)	
Sex									
Female	17 (60.7)	11 (39.3)	**0.049***	8 (28.6)	20 (71.4)	0.067	10 (35.7)	18 (64.3)	0.906
Male	29 (82.9)	6 (17.1)		18 (51.4)	17 (48.6)		12 (34.3)	23 (65.7)	
Tumor presentation									
Nodal	32 (76.2)	10 (23.8)	0.422	20 (47.6)	22 (52.4)	0.148	15 (35.7)	27 (64.3)	0.852
Extranodal	14 (66.7)	7 (33.3)		6 (28.6)	15 (71.4)		7 (33.3)	14 (66.7)	
Tumor size									
< 10 cm	42 (71.2)	17 (28.8)	0.567	25 (42.4)	34 (57.6)	0.637	20 (33.9)	39 (66.1)	0.606
≥10 cm	4 (100.0)	0 (0.0)		1 (25.0)	3 (75.0)		2 (50.0)	2 (50.0)	
Histological variants									
Centroblastic	31 (68.9)	14 (31.1)	0.406	13 (28.9)	32 (71.1)	**0.005***	18 (40.0)	27 (60.0)	0.457
Immunoblastic	5 (100.0)	0 (0.0)		4 (80.0)	1 (20.0)		1 (20.0)	4 (80.0)	
Anaplastic	10 (76.9)	3 (23.1)		9 (69.2)	4 (30.8)		3 (23.1)	10 (76.9)	
Stage grouping									
Early stage (I&II)	9 (47.4)	10 (52.6)	**0.003***	8 (42.1)	11 (57.9)	0.929	12 (63.2)	7 (36.8)	**0.002***
Advanced stage(III&IV)	37 (84.1)	7 (15.9)		18 (40.9)	26 (59.1)		10 (22.7)	34 (77.3)	
BM involvement									
Free	29 (67.4)	14 (32.6)	0.144	17 (39.5)	26 (60.5)	0.682	13 (30.2)	30 (69.8)	0.252
Involved	17 (85.0)	3 (15.0)		9 (45.0)	11 (55.0)		9 (45.0)	11 (55.0)	
Serum LDH level									

<500 ≥ 500	15(57.7) 31 (83.8)	11(42.3) 6 (16.2)	**0.022***	10(38.5) 16 (43.2)	16(61.5) 21 (56.8)	0.704	11(42.3) 11 (29.7)	15(57.7) 26 (70.3)	0.303
B symptoms Present Absent	 20 (90.9) 26 (63.4)	 2 (9.1) 15 (36.6)	 **0.019***	 12 (54.5) 14 (34.1)	 10 (45.5) 27 (65.9)	 0.117	 8 (36.4) 14 (34.1)	 14 (63.6) 27 (65.9)	 0.860
ECOG PS Good (0-1) Poor(2,3,4)	 25 (73.5) 21 (72.4)	 9 (26.5) 8 (27.6)	 0.921	 10 (29.4) 16 (55.2)	 24 (70.6) 13 (44.8)	 **0.038***	 11 (32.4) 11 (37.9)	 23 (67.6) 18 (62.1)	 0.643
IPI Low/ Intermediate (0-2) Intermediate /High (3-4)	 18 (54.5) 28 (93.3)	 15 (45.5) 2 (6.7)	 **0.001***	 8 (24.2) 18 (60.0)	 25 (75.8) 12 (40.0)	 **0.004***	 15 (45.5) 7 (23.3)	 18 (54.5) 23 (76.7)	 0.066
AA-IPI Low/ Intermediate (0-1) Intermediate /High(2-3)	 6 (37.5) 40 (85.1)	 10 (62.5) 7 (14.9)	 **0.001***	 6 (37.5) 20 (42.6)	 10 (62.5) 27 (57.4)	 0.723	 8 (50.0) 14 (29.8)	 8 (50.0) 33 (70.2)	 0.143
Response to therapy Responder Non-responder	 36 (80.0) 9 (20.0)	 16 (94.1) 1 (5.9)	 0.260	 20 (80.0) 5 (20.0)	 32 (86.5) 5 (13.5)	 0.506	 18 (81.8) 4 (18.2)	 34 (85.0) 6 (15.0)	 0.733

Qualitative data are presented as n (%). Chi-square analysis or Fisher Exact test were used for comparing qualitative variables. Significance defined by $p < 0.05$.

Survival analysis

Regarding the survival analysis according to each protein expression of Hans, no significant difference in OS was found according to CD10 expression yet CD10 expression was associated with better PFS (3-years OS, 88.2±7.8%VS 77.3±6.8% respectively, *P*=0.311) (3-years PFS, 100% VS 71.7±7.3% % respectively, *P* =0.006).

BCL6 expression was associated with better OS and PFS (3-years OS, 88.1±5.7% VS 69.4±9.8% respectively, *P*=0.028) (3-years PFS, 85.1±6.2% VS 70.6±10.1% respectively, *P* =0.018).However, no significant difference in both OS and PFS between MUM1 positive and negative cases (3-years OS, 83.5±6.3%VS 76.0±9.5% respectively, P=0.269) (3-years PFS, 76.9±7.2% VS 84.7±8.2% respectively, *P*=0.444).**Figure (2&3).**

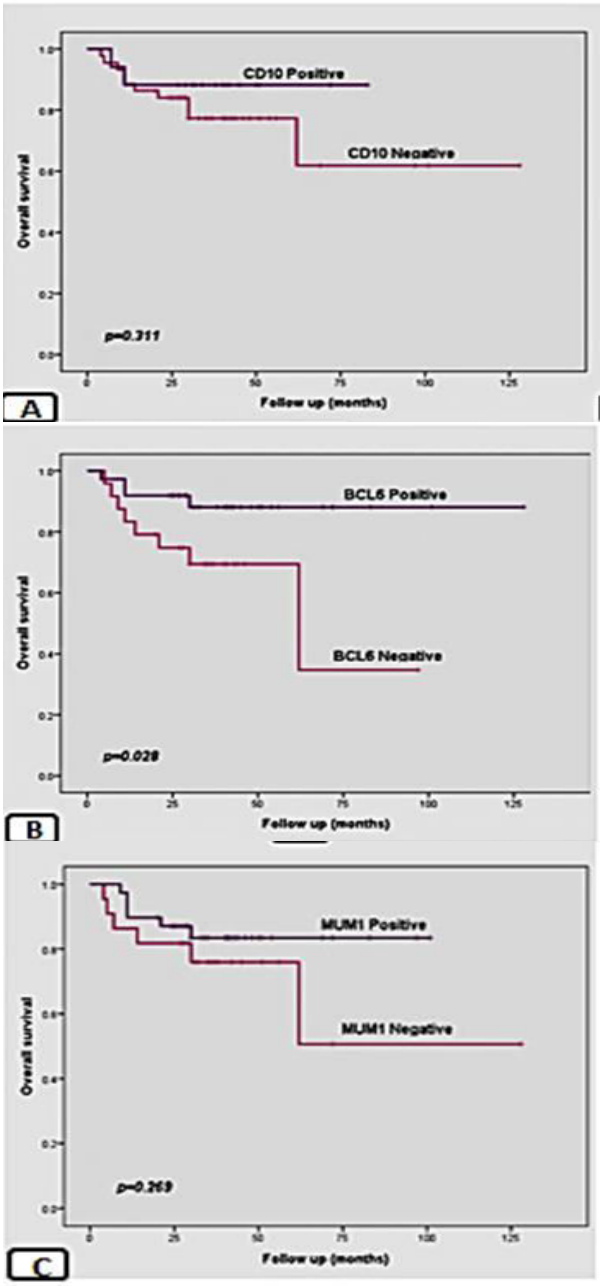

Figure (2): Kaplan-Meier curves for analysis of the expression of each protein of Han's algorithm (A: CD10, B: BCL6 and C: MUM1) and its relation to OS

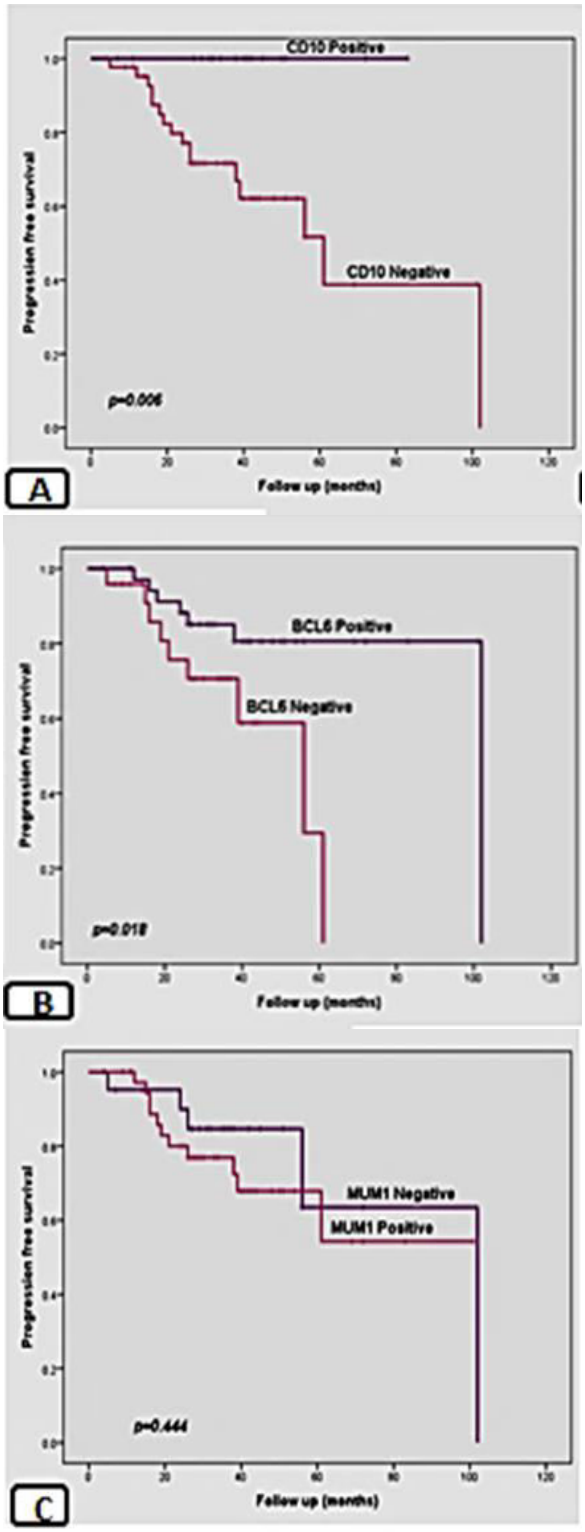

Figure (3): Kaplan-Meier curves for analysis of the expression of each protein of Han's algorithm (A:CD10, B: BCL6 and C: MUM1) and its relation to PFS

Discussion

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL(**Teras et al., 2016**). Relapsed or refractory disease is considered the most common cause of death in DLBCL (Crump *et al.*, 2017) and so, several prognostic parameters are now widely studied to achieve a better understanding of the biological and molecular features of DLBCL.

In this study, we aimed to assess the immunohistochemical expression of CD10, BCL6, and MUM-1independently. All IHC stains were performed on full-faced sections of 63 samples of DLBCL, NOS obtained from the registry of SECI. CD10 was positive in 26.9% of cases and BCL6 was positive in 58.7% of cases. These results were correlated with the original study done by **Hans et al(2004)** in which CD10 and BCL6 positivity was recorded in 28 % and 56 % of patients respectively.

Higher expression of MUM-1 was detected in our study (65%) which also found in other studies as that done by Lu et al where MUM-1 positivity detected in 65.9%(2016) and **Bajwa, et al (2017)** where positive MUM-1 expression was found in 62.5% of cases. This high expression may hinder the prognostic performance of MUM-1 and because of this, a higher cut-off value of 80 % for MUM-1 was applied in the Choi algorithm to achieve higher specificity for the ABC phenotype **(Choi et al., 2009**).

In this study, we assessed the association between the expression of each marker and variable clinicopathological parameters including patients' survival. Our data revealed that positive CD10 expression was associated with early-stage, better PS, low IPI, normal LDH level, and absence of B

symptoms. This is going in concordance with a study by **Lu et al (2016)**. But, we found no significance between CD10 positive expression and achieving remission after therapy. This is in agreement with **Xu et al(2001).**

As regard survival, CD10 expression was associated with longer PFS, but no significant difference in OS rate between patients with CD10 positive and negative expression which agreed with **Zhang et al (2012)** and **Peng et al (2017)**.

On the contrary, some previous studies conducted that CD10 expression may be associated with poor clinical outcomes **(Uherova et al., 2001, Xu et al., 2001**). This difference in these studies may be due to different number of cases where Uherova et al utilized only 28 cases of DLBCL, different methodology as both used flow cytometric immunophenotyping or different cut-off values where Xu et al used a threshold of 10 % to estimate the positivity of CD10 and so, some authors presumed that the use of CD10 alone may be not reliable to predict survival **(Asaad et al., 2016)**.

BCL6 positive expression was associated with good PS and low IPI which similar to results conducted by **Coutinho et al(2013)** while no correlation was found with other clinical parameters including response to therapy which is in agreement with studies done by **Mahmoud et al (2011)**,**Yan et al(2014)** and **Lu et al (2016)**. We also found that BCL6 expression was significantly associated with better OS and PFS which is in agreement with **Bodoor et al (2012)** and **Devin et al (2019).**

However, these results were in contrast to **Jovanovic et al (2015)** who had found that BCL6 expression was associated with poorer prognosis, and **Dwivedi et al (2015)** who couldn't found any difference in survival concerning to expression of BCL-6.

Although the presence of *BCL6* rearrangement was more frequently observed in the non-GCB DLBCL **(Jesse et al., 2010**)and was included in the triple hit lymphoma (THL) along with MYC and BCL2 with further poorer clinical outcome; several studies reported the absence of any correlation between *BCL6* rearrangement and BCL6 protein expression **(Jesse et al., 2010, Shustik et al., 2010**). Jovanovic et al also proposed that the prognostic value of BCL6 protein expression in DLBCL might be depending on the type of treatment used **(Jovanovic et al., 2015**).

As regards MUM1, the only positive correlation was detected between positive MUM1expression and advanced stage while no significant difference was detected in relation to the remaining clinicopathological parameters, which came in agreement with **Ola et al (2015)** and **Lu et al (2016)**.MUM1 expression denotes the terminal B cell differentiation toward plasma cells and the association of MUM1 expression and advanced stage explained by the fact that MUM1 expression is associated with constitutive activation of NF-κB pathway with subsequent expression of other NF-κB targeted genes which may be leading to chronically activated B cell receptor (BCR) signaling (**Lenz et al., 2008**). Hassan et al., 2014 reported that MUM1 expression was associated with poor clinical response **(Hassan et al., 2014)**. This was in contrast to our study where there was no significant difference in response to therapy as regard MUM1 expression. Also, we

found no significant difference in survival rates between patients with MUM1 positive and negative expression which agreed with **Bodoor et al (2012)** and **Oh et al (2011)**, But against the study done by **Lu et al (2016)** who found that MUM1 expression was a significant predictor of worse OS and PFS.The study done by Van Imhoff et al found that a cutoff value of 30% for MUM-1 had no prognostic value while using a higher cutoff value of 70% might be required to achieve higher specificity and improve the prognostic performance of MUM-1 (**van Imhoff et al., 2006**). In conclusion, CD10, BCL6, and MUM-1 can be used independently as prognostic immunohistochemical markers for DLBCL that may denote the clinical behavior of the disease and further patients' outcomes.

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Conflict of interest

The authors declared no conflicts of interest.

Author contribution

This work was carried out in collaboration between all authors. Author SSA conduct the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors AMA, FAMB, MAFA, and EHY wrote the protocol and reviewed the manuscript. All authors read and approved the final manuscript.

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