RESEARCH ARTICLE

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C-MYC and BCL2: Correlation between Protein Over-Expression and Gene Translocation and Impact on Outcome in Diffuse Large B Cell Lymphoma

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Abstract

Background: Due to lack of availability of gene expression profiling (*GEP*) for most developing countries and clinicians; the immunohistochemistry (IHC) is mostly used in the clinical application. The aim of our study is to check the possibility of using IHC to detect MYC and BCL2 in our patients with diffuse large B-cell lymphoma (DLBCL) instead of *GEP* to stratify them into high and low-risk groups. This will help in a proper treatment choice of subsequent improvement in the survival outcome. **Method:** During the study period, 90 DLBCL patients were eligible. MYC and BCL2 evaluated by IHC and gene rearrangement by real-time PCR (RT-PCR) and correlated with clinical-pathological features and survival. **Results:** Through IHC, the expression of MYC, BCL2, and double expression was detected in 35.6%, 46.7% and 30% of patients, respectively. While by RT-PCR, it was 4.53±0.74 for MYC compared with 2.18±0.78 for BCL-2. Most patients with BCL2+/MYC+; double-expressor and double-hit lymphomas (DEL and DHL) had high stage (III, IV), more extra-nodal involvement, (P value <0.001) and intermediate to high International Prognostic Index (IPI) risk profile (P-value <0.001). The median overall survival was 14 months and 6 months for DEL and DHL, respectively. While all patients with DHL died during the follow-up period, the median PFS were only 2 months for DEL. There was a statistically significant correlation between mRNA of MYC and BCL2 with their protein expression (p<0.001). **Conclusion:** Our results confirmed the unique characters and poor outcome associated with DEL and DHL mandated the need for more intense therapy and not the standard protocol. Moreover, the significant correlation between protein overexpression and gene rearrangement may open the door for the possibility to use IHC instead of RT-PCR in developing countries.

Keywords: Double expressor lymphoma- double hit lymphoma- Immunohistochemistry- MYC- BCL2- RT-PCR

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous clinical, pathological, immunophenotypic, and genetic disease. Based on gene signatures and cell of origin (COO), it is classified into germinal center B-cell (GCB)–like with favorable prognosis and unfavorable activated B-cell (ABC)–like phenotypes (Barrans et al., 2012).

Double-hit lymphomas (*DHL*) are a subtype of *DLBCL*, characterized by translocations involving the *MYC* gene combined with either translocation of the *BCL2* or *BCL6* gene. When the three translocations occurred and detected at the same time called triple-hit lymphomas (THL). Whereas, detection of protein overexpression (not gene translocation), is called double-expressor lymphoma (DEL).

The diagnosis of DHL is only determined following the results of a cytogenetic test, such as fluorescence in situ hybridization (FISH). They comprise 15% of B-cell lymphoma with clinical features intermediate between DLBCL and Burkitt´s lymphoma (BL). While DEL accounted for 20% to 30% (Jaffe et al., 2008; Aukema et al., 2011).

The World Health Organization (WHO) updated to recognize the co-expression of *MYC* and *BCL2* proteins as a new adverse prognostic marker. In case of DHL or DEL, the prognosis is poor after the standard chemotherapy protocol, R-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone, and Rituximab chemotherapy)

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with less than 30% long-term survivors (Hu et al., 2013).

Currently, the best treatment regimen for DHL is unknown, however, more intensive treatment protocols such as EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus Rituximab) may give better results than the standard one (Oki et al., 2014).

IHC is the most significant supplementary tool for the evaluation of lymphoma because of its practicability and low cost. In contrast, molecular cytogenetic techniques are expensive and not available in most clinical laboratories.

The aim of this study is to evaluate the association of protein overexpression and gene translocation of *MYC* and *BCL2* in *DLBCL* and also to address the correlation with clinical-pathological features and survival outcome.

Materials and Methods

Patients, study design, and ethical aspect

The present retrospective study included 90 de novo DLBCL patients diagnosed at the Pathology Department, Zagazig University during the period from January 2011toMarch 2015. They received the classic management, R-CHOP at Medical Oncology and Clinical Oncology Departments. The diagnosis was based on the 2016 WHO classification criteria (Swerdlow, 2016). By the use of medical files of patients; the demographic data, clinical-pathological features, laboratory workup, and follow up period were recorded retrospectively. The study included 15 lymphoid tissue samples from reactive lymph nodes in age and sex-matched patients which represented control samples. All specimens were divided into two parts; the first one was frozen at−80°C until used for detection of *MYC* and *BCL2* gene expression by RT-PCR in Medical Biochemistry Department. The second was immediately fixed in 10% formalin for histopathologic examination in the Pathology Department, Zagazig University.

The patient's data and names were protected without patient identifiers. The institutional review board (IRB) approved the study.

Histopathology and IHC

Hematoxylin and eosin (H and E) stained slides were evaluated. IHC analysis was performed using the polymer Envision detection system; the Dako EnVision ™ kit (Dako, Copenhagen, Denmark). Antibodies that used were: *CD20* (clone L26, Dako, Carpinteria, CA), *CD79a* (clone JCB117, Dako, ready to use), *CD10* (clone 56C6, ready to use, Dako), *Bcl6* (*PGB6-P* clone, ready to use, Dako), *Bcl2* (clone 124, ready to use, Dako), and *Myc* (clone EP121, ready to use, Biocare). Diaminobenzidine was used as the chromogen and hematoxylin as the counterstaining.

IHC assessment

The expressions of *CD20*, *CD79a*, and *CD10* were assessed for positivity or negativity. The WHO classification defines over-expression of *MYC* protein ≥ 40%, *BCL2* protein ≥ 50% (Hu et al., 2013), and Ki67 index ≥90% (Tang et al., 2017).

RT- PCR analysis for MYC and BCL2 gene expression
RNA and cDNA preparation

Total RNA was isolated from tissue samples using RNeasy (Qiagen, Valencia, CA) according to the manufacturer's instructions. The reverse transcription reaction was done using a reverse transcription kit (Reverse Transcriptase, Roche Diagnostics) following the manufacturer's protocol.

Quantitative RT- PCR

MYC and *BCL2* expressions were detected as previously described by Xia et al., (2015). β-actin was used as a reference gene. Amplification for MYC and *BCL-2* were performed in a total volume of 20 µL containing 10µL of kit-supplied QuantiTect™ SYBR® Green RT-PCR Master mix (Applied-Biosystems), 0.4 µL of each primer, 2 µL of cDNA and 7.2 µL ddH_2O. Primer pair sequences for the *MYC* gene were; c-*Myc*-F: CCTCCACTCGGAAGGACTATC; c-*Myc*-R: TGTTCGCCTCTTGACATTCTC, for BCL-2 were; BCL2-F; GTGGATGACTGAGTACCTGAACC; BCL-2-R: AGACAGCCAGGAGAAATCAAAC and for β-Actin were β -Actin-F: CCTGGCACCCAGCACAAT; β -Actin-R: GGGCCGGACTCGTCATAC. The PCR cycling parameters were set as follows: 95 C for 30 s followed by 40 cycles of PCR reaction 95 °C for 5 s and 60°C for 34 s. The amplification was carried out using Real-time PCR (StratageneMx3005P-qPCR System). Relative changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Treatment protocol and response evaluation

The eligible patients followed the chart as illustrated in Figure 1A.

Statistical Analysis

All statistics were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium). Parametric and non-parametric t-tests were used for comparison of two independent groups. Progression-free survival (PFS) was defined as the time interval from the first diagnosis until disease progression, while overall survival (OS) was defined as the time from the initial diagnosis to death or the last follow up. PFS and OS were estimated according to Kaplan-Meier and compared by the log-rank test. Multivariate analyses were performed with the use of a Cox regression model to estimate hazard ratios for an evolving event. All P values are based on 2-tailed statistical analysis, considering P values <0.05 as significant.

Results

Patients and clinical-pathological parameters

Of the 90 patients; 49 were males with a mean age 57.82±14.11 (range 25–90). Extra-nodal involvement was observed in about 29% of cases. According to the Ann Arbor Staging system, stage I included 14 patients, stage II (n =25), stage III (n=35) and stage IV (n=16). All cases were DLBCL with diffuse growth pattern,

Table1. Clinical-pathological Features, Immunohistochemical Markers and Outcome in Studied Patients with NHL.

Characteristics		All patients (N=90)	
		No.	(%)
Age	Mean ± SD	57.82 ±14.11	
(Years)	Median (Range)	58 (25-90)	
Sex	Male	49	(54.4)
	Female	41	(45.6)
Extranodal involvement			
Absent		64	(71.1)
Present		26	(28.9)
Stage	Stage I	14	(15.6)
	Stage II	25	(27.8)
	Stage III	35	(38.9)
	Stage IV	16	(17.8)
IPI risk group	Low	14	(15.6)
	Low-Intermediate	23	(25.6)
	High-Intermediate	33	(36.7)
	High	20	(22.2)
Response	PD	27	-30
	SD	15	(16.7)
	PR	28	(31.1)
	CR	20	(22.2)
	OAR	48	(53.3)
	NR	42	(46.7)
*Follow-up	Mean ± SD	24.78 ±9.38	
	Median (Range)	29 (4-36)	
Relapse		(N=20)	
	Before 24 month	8	-40
	After 24 month	12	-60
Progression		(N=42)	
	Within 6 month	30	(71.4)
	After 6 month	12	(28.6)
Mortality	A live	66	(73.3)
	Died	24	(26.7)
Ki 67	Low	23	(25.6)
	High	67	(74.4)
BCL2	Negative	48	(53.3)
	Positive	42	(46.7)
MYC	Negative	58	(64.4)
	Positive	32	(35.6)
Co-expression	Non-expressor	43	(47.8)
	BCL2 expressor	15	(16.6)
	MYC expressor	5	(5.6)
	Double expressor	27	(30)
Bouble hit	Absent	83	(92.2)
	Present	7	(7.8)

abundant apoptosis, and frequent mitosis. All cases expressed CD20 and CD79a. All DHL patients showed CD10 positivity. In DEL, 85% was CD10 positive and

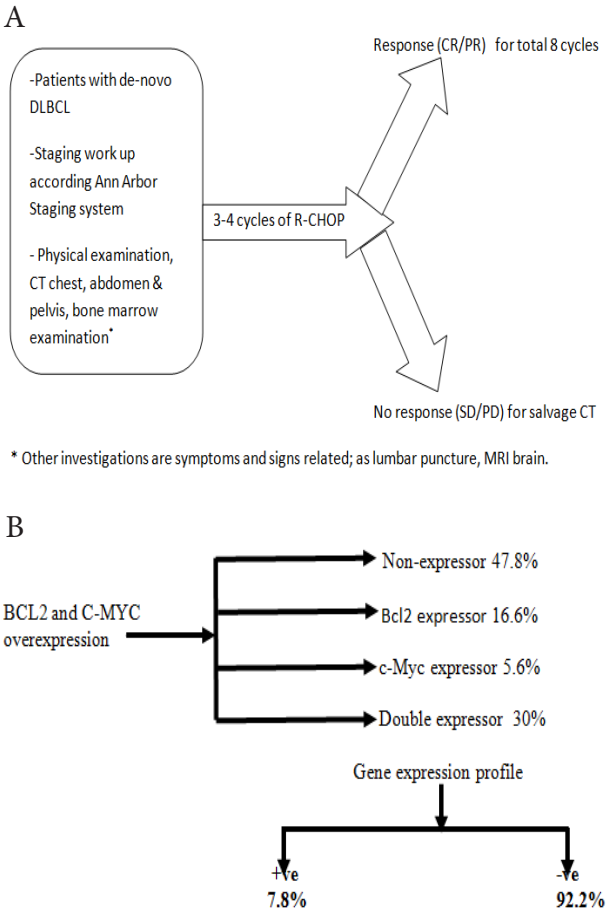

Figure 1. A, Treatment flow chart; B, Hans-algorithm for our patients; All cases express CD20 and CD79a. CD10 was 100% and 85% in double-hit and double-expressor lymphomas, respectively.

45% was *BCL6* positive.

Considering the treatment assessment, 22.2% of our patients achieved complete response (CR), 31.1% had a partial response (PR), and 16.7% had stable disease (SD), while the remaining showed progressive disease (PD). The main patients' characteristics showed in Table 1 and Figure 1B.

The clinical-pathological features of DEL/DHL

MYC and *BCL2* proteins were detected in 35.6% and 46.7% of patients, respectively. Co-expression was present in 30%. Most of the patients with DEL and DHL had an advanced stage (III, IV), intermediate to high IPI (P-value <0.001), and more extra nodal involvement. Table2.

The relation between DEL, DHL, and outcome

Patients with *MYC* and *BCL2* overexpression/gene translocation had a significantly poor outcome. All patients with DHL had DP while those with DEL showed PR and SD in 3.7% and 22.2%, respectively. For DEL patients, the OS was 14 months and PFS was 2 months compared with DHL; the OS was 6 months (all patients with DHL died during the follow-up period). Table 3,4 and Figure 2, 3.

mRNA levels of MYC and BCL-2 in patients with DLBCL

RT- PCR analysis showed that the *MYC* mRNA was 4.53±0.74 and BCL-2 mRNA was 2.18±0.78.

Table 2. Relation between Clinical-pathological Features and DEL/DHL*

Characteristics	BCL2/c-Myc Expression				p-value	Double hit		p-value
	Non-expressor (N=43)	BCL2 expressor (N=15)	c-Myc expressor (N=5)	Double expressor (N=27)		Absent (N=83)	Present (N=7)	
	No.(%)	No.(%)	No.(%)	No. (%)		No.(%)	No.(%)	
Age (years)								
Mean ± SD	54.45 ±13.91	53.5 ±12.94	54.4 ±15.12	66.18 ±11.78	0.002*	57.1 ±14.12	66.28 ±11.87	0.099*
Median (Range)	55 (25-80)	55.5 (27-72)	54 (34-76)	67 (40-90)		57 (25-90)	67 (45-79)	
Sex								
Male	22 (44.90%)	9 (18.30%)	3(6.10%)	15 (30.60%)	0.978‡	44 (89.80%)	5 (10.20%)	0.448‡
Female	21 (51.20%)	6 (14.60%)	2 (4.90%)	12 (29.30%)		39 (95.10%)	2 (4.90%)	
Extranodal involvement								
Absent	42 (65.60%)	9 (14.00%)	4 (6.30%)	9 (14.10%)	<0.001‡	63 (98.40%)	1 (1.60%)	0.002‡
Present	1 (3.80%)	6 (23.10%)	1 (3.80%)	18 (69.20%)		20 (76.90%)	6 (23.10%)	
Stage								
Stage I	12 (85.70%)	2 (14.30%)	0 (0%)	0 (0%)	<0.001§	14 (100%)	0 (0%)	0.005§
Stage II	17 (68%)	4 (16%)	2 (8%)	2 (8%)		25 (100%)	0 (0%)	
Stage III	12 (34.30%)	9 (25.70%)	2 (5.70%)	12 (34.30%)		32 (91.40%)	3 (8.60%)	
Stage IV	2 (12.50%)	0 (0%)	1 (6.30%)	13 (81.30%)		12 (75%)	4 (25%)	
IPI risk group								
Low	13 (85.70%)	2 (14.30%)	0 (0%)	0 (0%)	<0.001§	14 (100%)	0 (0%)	<0.001§
Low-Intermediate	15 (65.20%)	4 (17.40%)	2 (8.70%)	2 (8.70%)		23 (100%)	0 (0%)	
High-Intermediate	14 (42.40%)	8 (24.20%)	2 (6.10%)	9 (27.30%)		33 (100%)	0 (0%)	
High	2 (10%)	1 (5%)	1 (5%)	16 (80%)		13 (65%)	7 (35%)	
ki67								
Low	22 (95.70%)	1 (4.30%)	0 (0%)	0 (0%)	<0.001‡	23 (100%)	0 (0%)	0.184‡
High	21 (31.30%)	14 (20.90%)	5 (7.50%)	27 (40.30%)		60 (89.60%)	7 (10.40%)	

Double expressor/double hit lymphomas*

The quantitative expressions of *MYC* and *BCL-2* in tissue were significantly higher in DLBCL patients compared to the control ($P < 0.001$). There was a statistically significant correlation between protein overexpression and mRNA of both *MYC* and *BCL2* ($p<0.001$) Table5.

Discussion

DLBCL is the most common NHL, representing approximately 40% of all lymphoma all over the world. It is a heterogeneous disease with multiple biological distinct disorders. In the recent WHO revision of lymphoma classification and based on *GEP*, a new category is recognized as "high-grade B-cell lymphoma (*HGBL*)

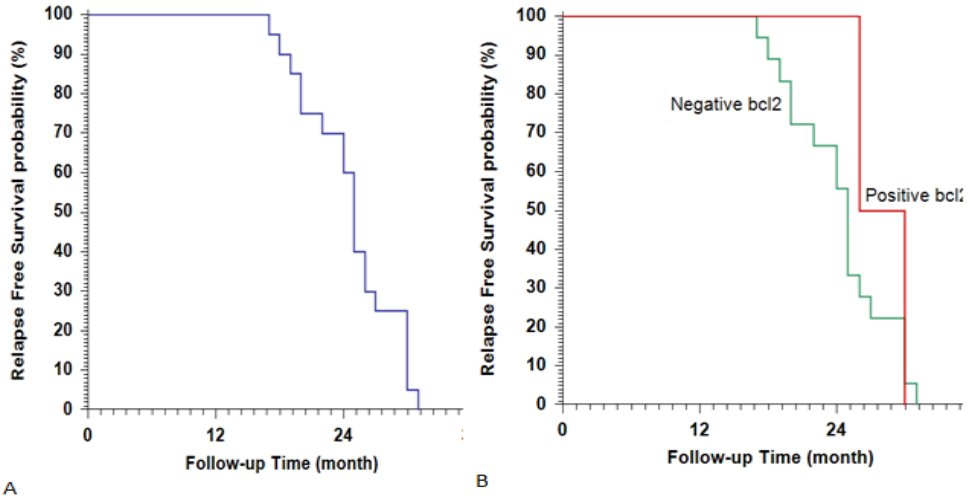

Figure 2. Kaplan-Meier Plot of Relapse Free Survival (RFS), (A) All studied patients; (B) stratified according to BCL2.

Table 3. Relation between Immunohistochemical Markers and Outcome

Outcome	Ki67		p-value	BCL2		p-value	c-Myc		p-value
	Low (N=23) No. (%)	High (N=67) No. (%)		Negative (N=48) No. (%)	Positive (N=42) No. (%)		Negative (N=58) No. (%)	Positive (N=32) No. (%)	
Response									
PD	1 (4.3%)	26 (38.8%)	<0.001‡	3 (6.3%)	24 (57.1%)	<0.001‡	4 (6.9%)	23 (71.9%)	<0.001‡
SD	1 (4.3%)	14 (20.9%)		3 (6.3%)	12 (28.6%)		8 (13.8%)	7 (21.9%)	
PR	15 (65.2%)	13 (19.4%)		24 (50%)	4 (9.5%)		26 (44.8%)	2 (6.3%)	
CR	6 (26.1%)	14 (20.9%)		18 (37.5%)	2 (4.8%)		20 (34.5%)	0 (0%)	
NR	2 (8.7%)	40 (59.7%)	<0.001‡	6 (12.5%)	36 (85.7%)	<0.001	12 (20.7%)	30 (93.8%)	<0.001‡
OAR	21 (91.3%)	27 (40.3%)		42 (87.5%)	6 (14.3%)		46 (79.3%)	2 (6.3%)	
Relapse	(N=6)	(N=14)		(N=18)	(N=2)		(N=20)		
Before 24 month	4 (66.7%)	4 (28.6%)	0.161‡	8 (44.4%)	0 (0%)	0.495‡	8 (40%)		---
After 24 month	2 (33.3%)	10 (71.4%)		10 (55.6%)	2 (100%)		12 (60%)		
RFS									
Mean RFS	22.17 month	25.79 month	0.020†	24.33 month	28 month	0.416†	24.70 month		
(95%CI)	(19.62-24.72)	(23.51-28.06)		(22.34-26.33)	(24.08-31.92)		(22.82-26.58)		
Median RFS	22 month	26 month		25 month	26 month		25 month		
24 month RFS	33.3%	71.4%		55.6%	100%		60%		
30 month RFS	0%	7.1%		5.6%	0%		5%		
PFS									
Progression	(N=16)	(N=26)		(N=27)	(N=15)		(N=33)	(N=9)	
Within 6 monh	9 (56.3%)	21 (80.8%)	0.158‡	15 (55.6%)	15 (100%)	0.003‡	21 (63.6%)	9 (100%)	0.041‡
After 6 month	7 (43.8%)	5 (19.2%)		12 (44.4%)	0 (0%)		12 (36.4%)	0 (0%)	
PFS									
Mean PFS	6.38 months	4.15 months	0.039†	6.37 months	2.53 months	<0.001†	5.82 months	2 months	<0.001†
(95%CI)	(5.66-7.09)	(3.15-5.15)		(5.66-7.08)	(2-3.07)		(5.11-6.53)	(1.35-2.62)	
Median PFS	6 months	3 months		6 months	3 months		6 months	2 months	
3 month PFS	100%	50%		96.3%	20%		84.9%	11.1%	
6 month PFS	43.8%	19.2%		44.4%	0%		36.3%	0%	
9 month PFS	6.3%	3.9%		7.4%	0%		6.1%	0%	
Mortality									
Alive	23 (100%)	43 (64.2%)	0.001‡	46 (95.8%)	20 (47.6%)	<0.001‡	56 (96.6%)	10 (31.3%)	<0.001‡
Died	0 (0%)	24 (35.8%)		2 (4.2%)	22 (52.4%)		2(3.4%)	22 (68.8%)	
OS									
Mean OS (95%CI)	36 month	27.37 month (24.54-30.19)	0.001†	35.17 month (34.01-36.32)	21.58 month (18.22-24.94)	<0.001†	35.33 month (34.40-36.26)	17.84 month (13.95-21.73)	<0.001†
Median OS	NR	NR		NR	20 month		NR	15 month	
24 month OS	100%	63%		95.8%	44%		96.6%	22.1%	
30 month OS	100%	63%		95.8%	44%		96.6%	22.1%	

with *MYC* and *BCL2* and/or *BCL6* rearrangements, with removal the category of unclassified lymphoma (Li et al., 2018).

MYC is a transcription factor located on chromosome 8 (8q24), regulates the expression of several target genes involved in DNA damage and repair. Cells with *MYC* translocations usually have *TP53* mutations allowing them to escape apoptosis and survive (Sehn et al., 2005; Barrans et al., 2010). *BCL2* and *BCL6* are anti-apoptotic factors deregulated in *DLBCL* via chromosomal translocation or gene rearrangement (Swerdlow et al., 2008; Lenz et al., 2008). Ki67 is usually associated with the advanced/aggressive disease however; its impact on survival outcome is controversy (Miller et al., 1994; Bryant et al., 2006).

In our study and among DEL patients, the morphological pattern of diffuse growth was the commonest (99%) with a "starry sky" pattern of 20% of the cases, which represented the main features of BL. In the DHL group, the typical morphology was a diffuse monomorphic pattern of numerous apoptotic bodies (Oliveira et al., 2017).

Most of our patients with DEL or DHL had a high stage (III, IV), intermediate to high IPI, and higher extra- node involvement (P <0.001). These results matched with many previous studies (Oliveira et al., 2017; Snuderl et al., 2010; Riedell et al., 2018; Friedberg, 2017, Reagan et al., 2017).

Moreover, protein overexpression of *MYC*, *BCL2*, and co-expression was detected in 35.6%, 46.7%, and 30% of patients, respectively. 7.8% of our patients were

Table 4. Relation between DEL and DHL

Outcome	BCL2/c-Myc Expression					Double hit		p-value
	Non-expressor (N=43) No. (%)	BCL2 expressor (N=15) No. (%)	c-Myc expressor (N=5) No. (%)	Double expressor (N=27) No.(%)	p-value	Absent (N=83) No. (%)	Present (N=7) No. (%)	
Response								
PD	0 (0.0%)	4 (26.7%)	3 (60%)	20 (74.1%)	<0.001§	20 (24.1%)	7 (100%)	0.001‡
SD	2 (4.6%)	6 (40.0%)	1 (20%)	6 (22.2%)		15 (18.1%)	0 (0%)	
PR	23 (53.5%)	3 (20.0%)	1 (20%)	1 (3.7%)		28 (33.7%)	0 (0%)	
CR	18 (41.9%)	2 (13.3%)	0 (0%)	0 (0%)		20 (24.1%)	0 (0%)	
NR	2 (4.7%)	10 (66.7%)	4 (80%)	26 (96.3%)	<0.001§	35 (42.2%)	7 (100%)	0.004‡
OAR	41 (95.3%)	5 (33.3%)	1 (20%)	1 (3.7%)		48 (57.8%)	0 (0%)	
Relapse	(N=18)	(N=2)				(N=20)		
Before 24 month	8 (44.4%)	0 (0%)			0.495‡	8 (40%)		---
After 24 month	10 (55.6%)	2 (100%)				12 (60%)		
RFS								
Mean RFS	24.33 month	28 month			0.416†	24.70 month		
(95%CI)	(22.34-26.33)	(24.08-31.92)				(22.82-26.58)		
Median RFS	25 month	26 month				25 month		
24 month RFS	55.6%	100%				60%		
30 month RFS	5.6%	0%				5%		
Progression	(N=25)	(N=8)	(N=2)	(N=7)				
Within 6month	13 (52%)	8 (100%)	2 (100%)	7 (100%)	0.005§			
After 6 month	12 (48%)	0 (0%(	0 (0%)	0 (0%)				
PFS								
Mean PFS	6.60 months	3.38 months	3.50 months	1.57 months	<0.001†			
(95%CI)	(5.91-7.29)	(3.02-3.373)	(2.52-4.48)	(1.18-1.97)				
Median PFS	6 months	3 months	3 months	2 months				
3 month PFS	100%	37.5%	50%	0%				
6 month PFS	48%	0%	0%	0%				
9 month PFS	8%	0%	0%	0%				
Mortality								
Alive	43 (100%)	13 (86.7%)	3 (60%)	7 (25.9%)	<0.001§	66 (79.5%)	0 (0%)	<0.001‡
Died	0 (0%)	2 (13.3%)	2 (40%)	20 (74.1%)		17 (20.5%)	7 (-100%)	
OS								
Mean OS	36 month	30.64 month	26.40 month	14.95 month	<0.001†	31.57 month	6.57 month	<0.001†
(95%CI)		(27.54-33.75)	(17.41-35.39)	(11.93-17.96)		(29.68-33.46)	(4.98-8.16)	
Median OS		NR	35 month	14 month		36 month	6 month	
24 month OS	100%	85.7%	53.3%	12.4%		78.9%	0%	
30 month OS	100%	85.7%	53.3%	12.4%		78.9%	0%	

Double expressor/double hit lymphomas*

Table 5. The Relation between mRNA MYC, BCL-2 Levels and their Protein Expression

	mRNA Myc levels	p
+veMyc protein expression (n=32)	4.53±0.74	<0.001
- veMyc protein expression (n=58)	0.95±0.41	
	mRNA BCL-2 levels	
+veBCL-2 protein expression (n=42)	2.18±0.78	<0.001
- veBCL-2 protein expression (n=48)	0.88±0.11	

DHL identified by RT-PCR, which inconsistent with many previous data. In a retrospective study done by Green et al., (2012) on 193 newly diagnosed DLBCL to evaluate *MYC* and *BCL2* using *IHC* and *FISH* revealed that 6% of patients were diagnosed as *DHL* by *FISH* and 29% as *DEL* using *IHC*. In addition, Yan et al., (2014) in another retrospective study on 336 patients of de novo DLBCL treated with CHOP±R to evaluate the prognostic value of *MYC*, *BCL2*, *BCL6* using *IHC* or *FISH*. The results showed that protein overexpression of *MYC*, *BCL2*, and *BCL6*

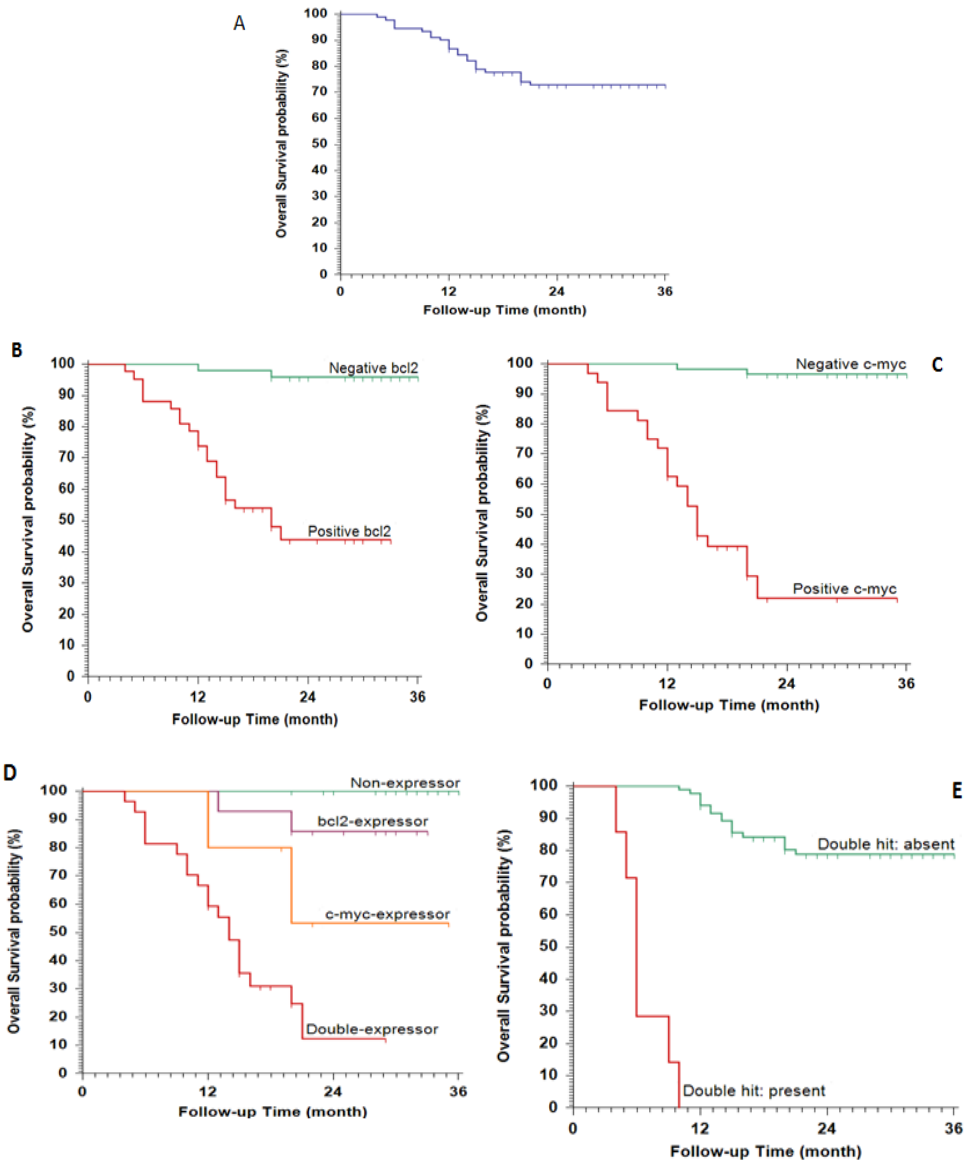

Figure 3. Kaplan-Meier Plot of Overall Survival (OS), (A), All studied patients; (B), stratified according to BCL2; (C), stratified according to c-Myc expression; (D), stratified according to BCl2/c-Myc expression; (E), stratified according to double hit.

were ≥40%, ≥70%, and ≥50%, respectively.

In the present study, the median OS are 6 months for DHL patients compared with 36 months in the absence of the translocation. These results are corresponding to the previous study done on 57 patients with high-grade NHL revealed shorter median OS for DHL compared with non-DHL (8·2 vs. 56·8 months, P < 0·001) (Lands burg et al., 2014). Moreover, all our patients with DHL died through our study. In another retrospective study done on 120 Brazilian patients with aggressive NHL by Oliveira et al., (2014), reported a poor outcome associated with DHL where most of the patients died within 6 months.

2-year OS for DEL was 14 months. Similar findings were reported by Savage et al., (2009) when evaluated the outcome of 135 patients with DLBCL post-treatment with R-CHOP. The 5-year OS was significantly worse in *MYC* positive (33%), with a high incidence of CNS relapse, compared with *MYC* negative cases (72%). Another retrospective studied on 69 eligible patients, Aggarwal et al., (2016) reported poor outcome for patients with DEL compared with non-DEL.

Through the measuring of the cell proliferation rate by the percentage of Ki67 labelling index, as a selective parameter of DLBCL patients for further cytogenetic tests is an area of controversy. A study done by Kalaw et al., (2012) concluded that 90% as a cut-off value was not indicative for *MYC* translocations.

Hence, DHL is a temporary title for aggressive B-cell lymphomas, diagnosed not only by a unique aggressive clinical course associated with disease progression but also by its gene rearrangements. Those patients require a new treatment regimen based on gene repression of possible genetic mechanisms, or certain protocol that may eliminate the effect of the cytogenetic aberrations.

Meanwhile, our results showed a statistically significant association with protein overexpression detected by IHC and gene translocation identified by mRNA for both *MYC* and *BCL2*. Owing to this high specificity between IHC and cytogenetic tests by FISH, the economic problems with developing countries, and

the importance of risk stratification, can we replace IHC instead of *GEP*?

Conclusions and Recommendation

Our findings confirm that regardless of the way of detection of *MYC* and *BCL2* either by *IHC* or *FISH*, they associated with unique pathological features (high mitotic rates and starry sky appearance) and poor outcome. Because the IHC is a conventional and accessible method of assessment in the developing countries like Egypt, it may replace the cytogenetic study and can act as selection criteria for further cytogenetic testing. Regarding the unsatisfying response, still, the era of treatment of DLBCL is attractive to further research. Based on the progress of molecular findings, tailored therapy or risk-adapted therapy is mostly the best moving forward.

Limitations

The retrospective study depends totally on documentation by medical staff, so it is almost always criticized due to insufficient data. Also, the small sample size may represent an obstacle to get clearer and power data.

Conflict of interest

The authors certify that there is no potential or actual conflict of interest related to this research.

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