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Original article

Inverse Correlation of Concurrent BCL2, and C MYC Expressions and CD30 Positivity as Regard the Prognostic Significance in Diffuse Large B-Cell Lymphoma

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Abstract

Background **:** C MYC, BCL2 and CD30 play an important role in initiation and propagation of diffuse large B-cell lymphoma (DLBCL). Protein expression of these marker were used in the diagnosis, predicting the prognosis and as a therapeutic target in DLBCL. ***Purpose*****:** This study aimed to assess the prognostic impact and therapeutic utility of expression patterns of CD30, BCL2, MYC in DLBCL. **Patients and Methods:** This retrospective study was carried out on DLBCL diagnosed at Oncology Centre Mansoura University (2016-2019). The study was approved by IRB of Mansoura faculty of Medicine Code Number: R.23.01.2043, 21/03/2023. ***Results*****:** BCL2, C MYC, concurrent BCL2, C MYC and CD30 overexpression was present in 76%, 36%,and 26% of the studied cases respectively. Concurrent expressions of BCL2, C MYC showed significant association with the presence relapse (P= 0.003). Concurrent expression of BCL2, C MYC revealed significant association with the progressive therapy response (P=0.003). In addition, C MYC, expression was significantly associated with the presence of extra-nodal presentation (P=0.03). CD30 protein overexpression was significantly associated with stage II and III (P=0.03). CD30 positivity showed inverse correlation with the concurrent expression of BCL2, C MYC (Correlation Coefficient=--.355, P=0,02). Also, CD30 positivity and concurrent expression of BCL2, C MYC reflected significant prognostic impact on overall survival (OS) and disease free survival (DFS) (all P=0.00).***Conclusion:*** concurrent C MYC, BCL2 overexpression is associated with a lower OS, and DFS. CD30 positivity is associated with a better OS, and DFS which can be utilized in management plan of DLBCL.

Keywords: DLBCL; C MYC; BCL2; CD30; survival.

Introduction

Among non-Hodgkin lymphoma, The most common type is Diffuse large B-cell lymphoma (DLBCL) [(1)]. According to revision of WHO 2016, NHL is classified into different histologic variants, immunohistochemical and molecular subgroups and different subtypes/entities [(2)]. Also, there is still marked biological and clinical heterogeneity[(3)].

DLBCL as any type of cancer has defects at any point along the multiple carcinogenic pathways, leading to malignant transformation of the affected cells, tumor metastasis and resistance to anticancer drugs. Apoptosis has a pivotal role not only in initiation of cancer but also in the treatment as a target of many treatment modalities[(4)].

Previously the prognosis of DLBCL was determined by the international prognostic index (IPI), but recent clinical trials depend upon the genetic and proteomic testing in predicting the prognosis and facilitate the optimum selection of individualized treatment [(5)].

B-cell leukemia–lymphoma-2(BCL2), located on chromosome region 18q21, it codes for a protein that is expressed normally by resting T and B cells, but not by normal germinal center cells or cortical thymocytes. It is an important anti-apoptotic protein which regulates cell death. It is characteristic of up to 90% of follicular lymphomas (FLs). Also, BCL2 translocations are also seen in a subset of DLBCLs[(6,7)]. BCL2 protein expression and its effect on the survival of DLBCL patients are controversial in the rituximab era. Some studies found that the addition of rituximab to standard chemotherapy overcame the adverse prognostic influence of BCL2 expression[(8,9)].

The MYC protein is a transcription factor which regulates more than 15% of all cellular genes to promote cellular proliferation via metabolic and angiogenic mechanisms. MYC translocation is a characteristic feature for Burkitt's lymphoma (BL) and is mandatory for the diagnosis [(10)]. However, MYC gene aberrations are not limited to BL because DLBCL and other lymphomas can also harbor this genetic abnormality. In DLBCL, MYC aberration is found in less than 10% of the cases at diagnosis and in almost 20% at first relapse[(7)].

In contrast to BL, MYC aberration in DLBCL usually occurs with complex karyotypes and BCL2 & BCL6 rearrangements, defining the so-called "double-hit" and "triple-hit" DLBCL[(11)]. The MYC aberration is associated with a more aggressive phenotype and poor outcome, including shorter progression-free and overall survival (OS)[(12)].

One of the most consistent predictors of outcome is the International Prognostic Index (IPI) with a proposed revision to R-IPI for patients treated with Rituximab, cyclophosphamide, Hydroxydaunorubicin, Oncovin (Vincristine), Prednisone, R-CHOP. Many attempts have been made to

find biomarkers that can improve the outcome prediction beyond the IPI [13,14].

CD30 has been identified as a cell-surface marker of Reed-Sternberg and Hodgkin cells of classical Hodgkin lymphoma. CD30 is expressed by several types of T- and B-cell non-Hodgkin lymphoma, anaplastic large cell lymphoma (ALCL), primary mediastinal large B-cell lymphoma (PMBCL), and Epstein-Barr virus (EBV)–driven clonal lymphoproliferative disorders, as well as in reactive conditions, such as infectious mononucleosis [15]

CD30 is normally expressed by T and B immunoblasts in the parafollicular region and the peripheral rim of germinal centers. The pattern of CD30 expression makes it an ideal target for monoclonal antibody therapy in patients with CD30+ lymphomas [16]

In the era of targeted therapy for DLBCL, CD30 has emerged as an important molecular target. Brentuximabvedotin (a drug combining an anti- CD30 monoclonal antibody and the antitubulin agent monomethyl auristatin E), was reported to achieve an objective clinical response in patients with CD30+ DLBCL, showing a promising approach that may increase the response rate and prolong the survival time in patients with relapsed or refractory CD30+ DLBCL [17]

Approximately 40% of patients with DLBCL suffer relapse and eventually die although there are major advances in treatment strategies, especially the addition of the anti-CD20 monoclonal antibody rituximab to the standard cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisone (CHOP) [18,19]. This explains the ultimate need to have prognostic models which guide risk-justified treatment selection.

Purpose:

The aim of the present study was to comprehensively assess the prognostic impact of protein expression patterns of CD30, BCL2, MYC in DLBCL patients treated in a large, prospective randomized clinical trial.

Patients and Methods:

This retrospective study was carried out on Diffuse large B cell lymphoma specimens received at the Oncology Centre Mansoura University during the period from January (2016) to January (2019). Clinical and follow up data were retrieved from patient's medical records for at least 3 years duration. Each specimen was coded and patient's name was not shown for ethical reasons. This study had been accepted by IRB Mansoura Faculty of Medicine, Mansoura University, Egypt, Code Number: R.23.01.2043, 21/03/2023.

Sections of 4 um thickness have been cut from formalin fixed paraffin embedded blocks for routine H&E, others were prepared on charged slides for immunohistochemistry. Microscopic examinations of tumor slides were done on an Olympus CX31 light microscope. Pictures were obtained by a PC-driven digital camera (Olympus E-620) using computer software (Cell*, Olympus Soft Imaging Solution GmbH).

Immunohistochemistry:

Immunohistochemical analysis for Bcl2, C-myc and CD30 was performed on tumor sections. Commercially available monoclonal antibody against **Bcl2** (sc-7382, dilution 1:100, Santa Cruz Biotechnology Inc., USA), **CMYC** (sc-40, dilution 1:100, Santa Cruz Biotechnology Inc., USA), and **CD30** (clone M0751, Dako Corporation, Carpinteria, CA, USA, RTU). Detection kits used was high sensitive kits (DakoCytomation envision +dual link system peroxidase code K4061) using DAB as chromogene. Immunostaining required pretreatment with 1 mM EDTA (at pH 8.0) for 20 minutes in microwave oven. Sections from tonsil were used as an external positive control for BCL2, CD30 and C MYC. As for negative controls, sections were stained without the addition of a primary antibody.

Immunohistochemical Analysis

As for the immunohistochemistry assessment, Slides were scanned by X40 magnification. Ten cellular areas selected (i.e. the so-called hot spots) and evaluated at X400 magnification by two pathologists. A cutoff value for positivity of each marker was used as following: MYC was >40% nuclear positivity on tumor nuclei whereas BCL2 protein was >70% of tumor cells with positive cytoplasmic staining reaction and CD30 was >20% of tumor cells with positive membranous staining[5,16,20]. The reproducibility of protein expression of each BCL2, CD30 and MYC and co-expression of MYC-positive/BCL2-positive proteins by IHC was determined by comparing the results obtained by three/ five different pathologists (Figure1, 2).

Statistical analysis:

All parameters included age, gender, staging, clinical response, relapse Bcl2, C-MYC and CD30 expression- were evaluated by statistical analysis. The statistical analysis of data was done by using statistical package for social science (SPSS) program version 20 **(**Armonk, NY: IBM Corp**)** [21]. Descriptive statistics were done. The presented data was non parametric. The used tests were; Chi-square test for categorical variables, to compare between different groups. Student T test for numerical variables. Spearman's bivariant correlation test used to discriminate the relation between the three markers expressions. Survival analysis of recurrence free survival and overall survival was done using Kaplan-Meier analysis and log rank test was used for comparison between groups. Probability (p) values < 0.05 were considered significant.

Results

This retrospective study included 42 cases of DLBCL that were diagnosed and immunophenotyped at the Oncology Centre Mansoura University during the period from January (2016) to January (2019) and followed up for 3 years.

The demographic and clinical data of the included cases were illustrated in table (1). The mean age of the study cases were 53.02± 13.5, 27 cases were ≤ 60 years old, 15 cases were > 60 years old. Twenty eight cases were male and 16 cases were female. Most of the cases were stage III, IV (18, 15

cases) respectively. Positivity for BCL2, C MYC, and CD30 were detected in 32, 15, 11/42 cases respectively. As regard the clinical response to treatments, it was regressive, stationary, and progressive in 13, 12, and 17 cases respectively. Twenty two cases were relapsed during the period of follow up (Table 1, Figure 1, 2).

The association of BCL2 protein cytoplasmic positivity and the clinicopathological parameter was investigated and demonstrated in table 2. There were no significant association between BCL2 expression and any of the clinicopathological parameters a part from relapse (p= 0.045). BCL2 positivity showed predominance among male cases, stage III, IV, and progressive therapy response but didn't reach the significance level. On the other hand lower relapse rate was noticed among positive BCL2 cases.

The association of c-MYC protein nuclear positivity and the clinicopathological parameter was investigated and demonstrated in table 2. There were no significant association between C-Myc nuclear expression and any of the clinicopathological parameters a part from extra-nodal representation (*p*= 0.03) clinical response (*p*= 0.001) and relapse (*p*= 0.001). C-Myc positivity showed predominance among male cases, and stage III, IV but didn't reach the significance level. C-Myc positivity significantly associated with progressive clinical course and high relapse rate.

The association of co-expression of MYC-positive/BCL2-positive proteins and the clinicopathological parameter was investigated and demonstrated in table 3. There were no significant association between co-expression of MYC-positive/BCL2-positive and any of the clinicopathological parameters a part from clinical response (p= 0.003) and relapse (p= 0.003). co-expression of MYC-positive/BCL2-positive showed predominance among male cases, and stage III, IV but didn't reach the significance level. Co-expression of MYC-positive/BCL2-positive significantly associated with progressive clinical course and high relapse rate.

The association of CD30 protein positivity and the clinicopathological parameter was investigated and demonstrated in table 4. There were no significant association between CD30 expression and any of the clinicopathological parameters a part from stage (p= 0.03). CD30 positivity showed predominance among male cases, good performance status, absence of bulky disease or B symptoms, regressive and stationary clinical response and lower relapse rate but didn't reach the significance level. CD30 positivity significantly associated with stage II, III.

Spearman's bivariant correlation of CD30,BCL2,C MYC &and Co-expression of BCL2 and C MYC was illustrated in table5 and revealed that CD30 protein positivity was significantly correlated with negative C-Myc and concurrent of MYC /BCL2-negative (P= 0.032, 0,02) respectively. Also, CD30 protein positivity was insignificantly correlated with positive BCL2(p=0. 7)

Survival analysis were demonstrated using Kaplan-Meier curves. Patient were monitored during the period of follow up

(27.3 M±11.1M). Univariate analysis by log rank test of the impact of, C MYC, CD30, and concurrent BCL2-C/ MYC expressions on the overall survival and disease free survival- revealed statistical significant impact with P values range from (0.0000-0.001). However, BCL2 expressions didn't show significant impact on neither OS (P=0,088) nor DFS(P=0,513) figures 3, 4, 5, 6

Table 1: Clinicopathological characteristics of the studied cases

Clinicopathological Variables		N	%
Age	**(M±SD)**	**53.2±13.4**	
	≤60	27	64
	>60	15	36
Gender	Male	28	62
	female	16	38
Stage	I	4	9
	II	5	12
	III	18	43
	IV	15	35
Performance status	<2	29	69
	≥2	13	31
Extra nodal site	No	31	74
	1 site	9	21
	≥2	2	5
R-IPI	Good(≤2)	24	57
	Poor(>2)	18	43
B symptom	Yes	11	26
	No	31	74
Bulky disease	Yes	8	19
	No	34	81
BM/CNS Involvement	Yes	4	9.5
	No	38	90.5
BCL2	Negative	10	24
	positive	32	76
C myc	Negative	27	64
	positive	15	36
Co expression C myc&BCL2	Negative	31	74
	positive	11	26
CD30	Negative	31	74
	Positive	11	26
Therapy response	Regressive	13	31
	Stationary	12	29
	Progressive	17	40
relapse	Yes	20	48
	No	22	52
Survival	Survived	25	59.5
	dead	17	40.5

Table 2: Clinicopathological relation to Bcl2, C MYC expressions in studied cases

Clinicopathological Variables		N	BCL2		P	C myc		P
			-ve (10)	**+ve (32)**		**-ve (27)**	**+ve (15)**	
Age	**(M±SD)**	**53±13**	**55±15**	**52±13**	**0.5**	**51±15**	**56±10**	**0.3**
	≤60	**27**	**5(50%)**	**22(69%)**	**0.4**	**19(70%)**	**8(53%)**	**0.2**
	>60	**15**	**5(50%)**	**10(31%)**		**8(30%)**	**7(47%)**	
Gender	**female**	**16**	**6(60%)**	**10(31%)**	**0.1**	**11(41%)**	**5(33%)**	**0.7**
	Male	**26**	**4(40%)**	**22(69%)**		**16(59%)**	**10(66%)**	
Stage	**I**	**4**	**0(00%)**	**4(13%)**	**0.2**	**4(15%)**	**0(0%)**	**0.08**
	II	**5**	**2(20%)**	**3(9%)**		**5(19%)**	**0(0%)**	
	III	**18**	**6(60%)**	**12(38%)**		**9(33%)**	**9(60%)**	
	IV	**15**	**2(20%)**	**13(41%)**		**9(33%)**	**6(40%)**	
Performance status	**<2**	**29**	**7(70%)**	**22(69%)**	**0.6**	**20(74%)**	**9(60%)**	**0.3**
	≥2	**13**	**3(30%)**	**10(31%)**		**7(26%)**	**6(40%)**	
Extra nodal site	**No**	**31**	**9(90%)**	**22(69%)**	**0.1**	**17(63%)**	**14(93%)**	**0.03**
	1 site	**9**	**0(0%)**	**9(28%)**		**8(30%)**	**1(7%)**	
	≥2	**2**	**1 (10%)**	**1 (3%)**		**2(7%)**	**0(0%)**	
R-IPI	**Good(≤2)**	**24**	**4(40%)**	**20(63%)**	**0.1**	**17(63%)**	**7(47%)**	**0.3**
	Poor(>2)	**18**	**6(60%)**	**12(38%)**		**10(37%)**	**8(53%)**	
	Yes	**4**	**0(0%)**	**4(12.5%)**		**3(11%)**	**1(7%)**	
Therapy response	**Regressive**	**13**	**3(30%)**	**10(31%)**	**0.6**	**9(33%)**	**4(27%)**	**0.001**
	Stationary	**12**	**4(40%)**	**8(25%)**		**12(45%)**	**0(0%)**	
	Progressive	**17**	**3(30%)**	**14(44%)**		**6(22%)**	**11(73%)**	
Relapse	**NO**	**20**	**2(20%)**	**18(56%)**	**0.04**	**18(77%)**	**2(13%)**	**0.001**
	YES	**22**	**8(80%)**	**14(44%)**		**9(33%)**	**13(87%)**	

*P value ≤0.05 is significant

Table 3: Clinicopathological relation to co-expression of Bcl2&C MYC in studied cases

Clinicopathological Variables		N	CD30		P
			-ve (31)	**+ve (11)**	
Age	**(M±SD)**	**53±13**	**54±13**	**50±14**	**0.3**
	≤60	**27**	**18(58%)**	**9(82%)**	**0.27**
	>60	**15**	**13(42%)**	**2(18%)**	
Gender	**female**	**16**	**14(45%)**	**2(18%)**	**0. 1**
	Male	**26**	**17(55%)**	**9(82%)**	
Stage	**I**	**4**	**3(10%)**	**1(9%)**	**0.03**
	II	**5**	**1(3%)**	**4(36.5%)**	
	III	**28**	**14(45%)**	**4(36.5%)**	
	IV	**15**	**13(42%)**	**2(18%)**	
Performance status	**<2**	**29**	**21(68%)**	**8(73%)**	**0.5**
	≥2	**13**	**10(32%)**	**3(27%)**	
Extra nodal site	**No**	**31**	**24(77%)**	**7(64%)**	**0.5**
	1 site	**9**	**6(19%)**	**3(27%)**	
	≥2	**2**	**1(3%)**	**1(9%)**	
R-IPI	**Good(≤2)**	**24**	**16(52%)**	**8(73%)**	**0.2**
	Poor(>2)	**18**	**15(48%)**	**3(27%)**	
	Yes	**4**	**3(10%)**	**1(9%)**	
Therapy response	**Regressive**	**24**	**9 (29%)**	**4 (36%)**	**0.1**
	Stationary	**7**	**7 (23%)**	**5(46%)**	
	Progressive	**11**	**15 (48%)**	**2 (18%)**	
Relapse	**NO**	**23**	**13 (42%)**	**7 (64%)**	**0.18**
	YES	**19**	**18 (58%)**	**4 (36%)**	
Survival	**Survived**	**25**	**13(42%)**	**7(64%)**	**0.2**
	dead	**17**	**18(58%)**	**4(36%)**	

Table 4: Clinicopathological relation to CD30 in studied cases

Clinicopathological Variables		N	CD30		P
			-ve (31)	+ve (11)	
Age	(M±SD)	53±13	54±13	50±14	0.3
	≤60	27	18(58%)	9(82%)	0.27
	>60	15	13(42%)	2(18%)	
Gender	female	16	14(45%)	2(18%)	0. 1
	Male	26	17(55%)	9(82%)	
Stage	I	4	3(10%)	1(9%)	0.03
	II	5	1(3%)	4(36.5%)	
	III	28	14(45%)	4(36.5%)	
	IV	15	13(42%)	2(18%)	
Performance status	<2	29	21(68%)	8(73%)	0.5
	≥2	13	10(32%)	3(27%)	
Extra nodal site	No	31	24(77%)	7(64%)	0.5
	1 site	9	6(19%)	3(27%)	
	≥2	2	1(3%)	1(9%)	
R-IPI	Good(≤2)	24	16(52%)	8(73%)	0.2
	Poor(>2)	18	15(48%)	3(27%)	
	Yes	4	3(10%)	1(9%)	
Therapy response	Regressive	24	9 (29%)	4 (36%)	0.1
	Stationary	7	7 (23%)	5(46%)	
	Progressive	11	15 (48%)	2 (18%)	
Relapse	NO	23	13 (42%)	7 (64%)	0.18
	YES	19	18 (58%)	4 (36%)	
Survival	Survived	25	13(42%)	7(64%)	0.2
	dead	17	18(58%)	4(36%)	

Table 5 Spearman's bivariate correlation of CD30,BCL2, C MYC, &and Co-expression of BCL2 and C MYC in studied cases

Correlations

			CD30	bcl2	c-myc	Coexpress
Spearman's rho	CD30	Correlation Coefficient	1.000	-.048	-.331*	-.355*
		Sig. (2-tailed)	.	.761	.032	.021
		N	42	42	42	42
	BCL2	Correlation Coefficient	-.048	1.000	-.050	.333*
		Sig. (2-tailed)	.761	.	.753	.031
		N	42	42	42	42
	C-MYC	Correlation Coefficient	-.331*	-.050	1.000	.799**
		Sig. (2-tailed)	.032	.753	.	.000
		N	42	42	42	42
	Coexpress	Correlation Coefficient	-.355*	.333*	.799**	1.000
		Sig. (2-tailed)	.021	.031	.000	.
		N	42	42	42	42

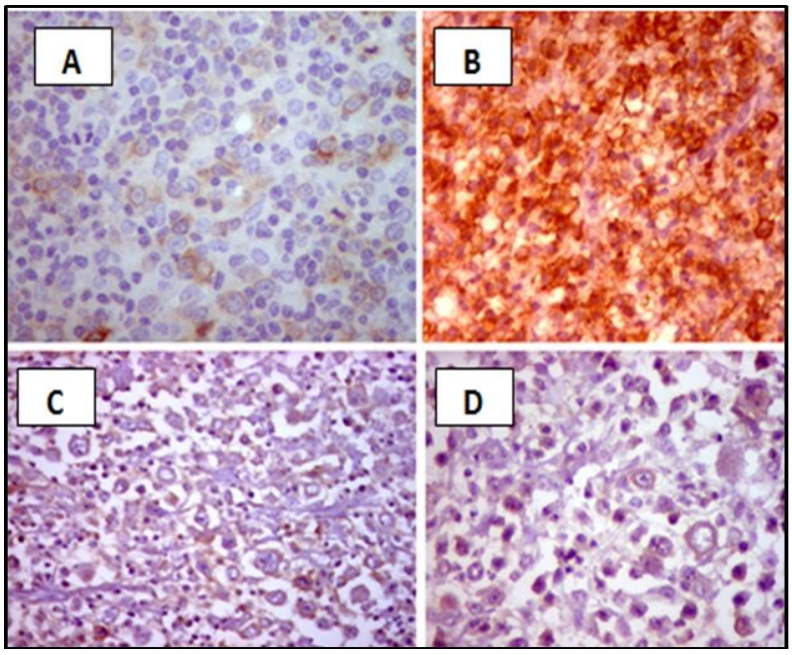

Figure (1):Immunohistochemistry expression of bcl2 in DLBCL nos (Ax400) showed scattered cytoplasmic staining in large lymphoma cells (<60%)., strong diffuse cytoplasmic staining in DLBCL (BX400) . C&D photos showed DLBCL nos with membranous and cytoplasmic staining for CD30 (x200,, x400 respectively)

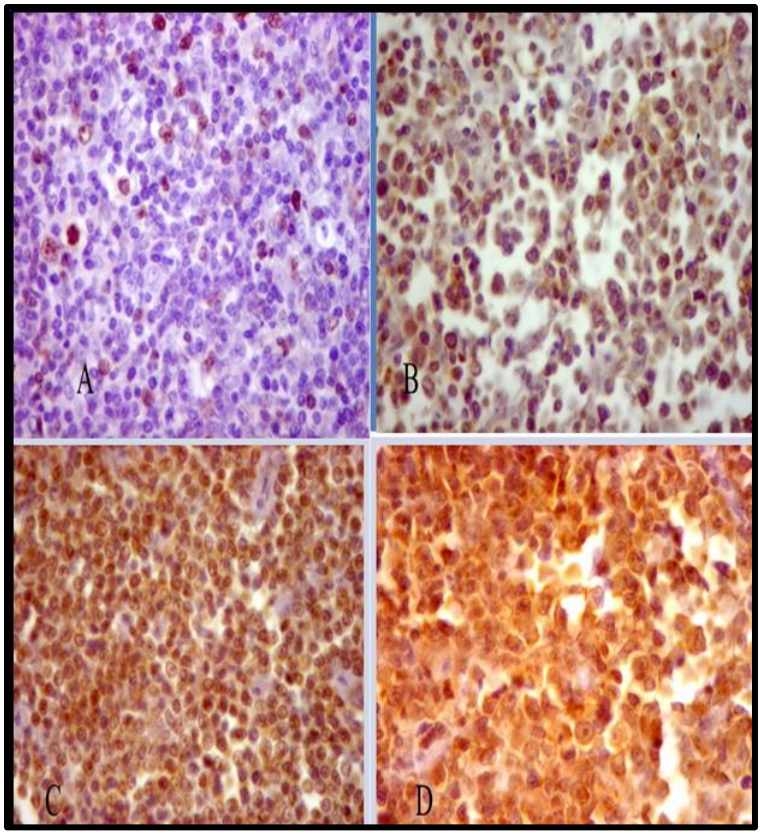

Figure (2):Immunohistochemistry expression of c-myc in DLBCL nos (Ax200) showed scattered nuclear staining in large lymphoma cells (<40%). In photos B&C&D, DLBCL nos showed diffuse nuclear staining for c-myc (x200, x200, x400 respectively)

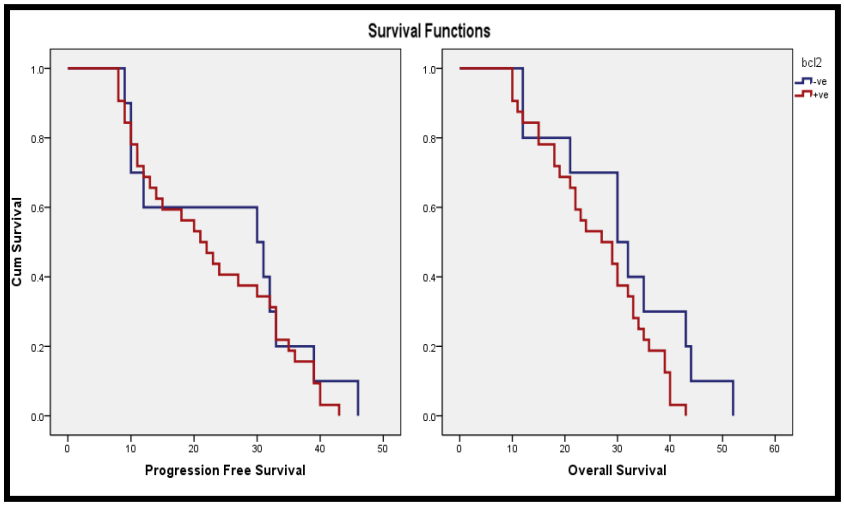

Figure (3): Survival in relation to Bcl2 expression (not significant by log Rank P 0.513, 0.088)

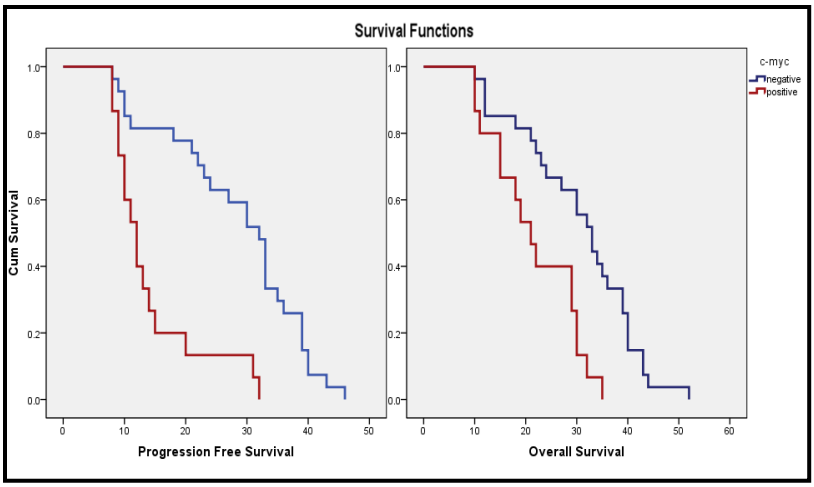

Figure (4): Survival in relation to C myc expression (Significant by log Rank P= 0.000, 0.001)

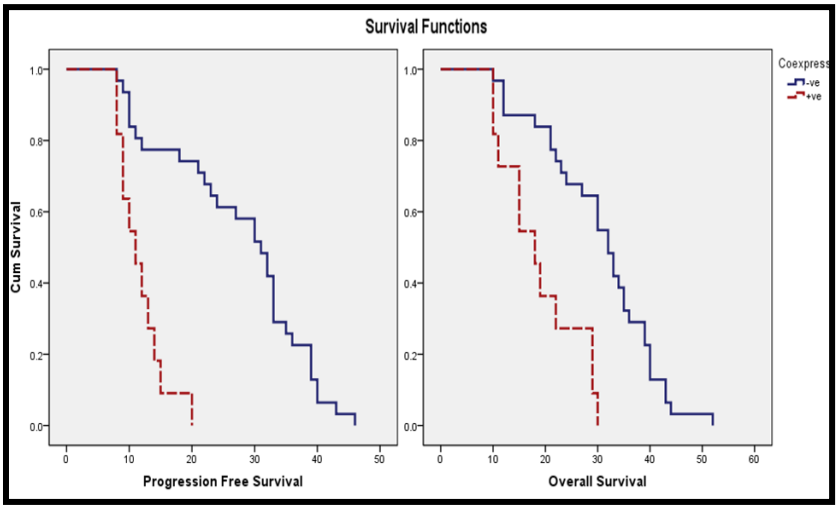

Figure (5): Survival in relation to C myc/Bcl2 coexpression (Significant by log Rank P 0.000, 0.000)

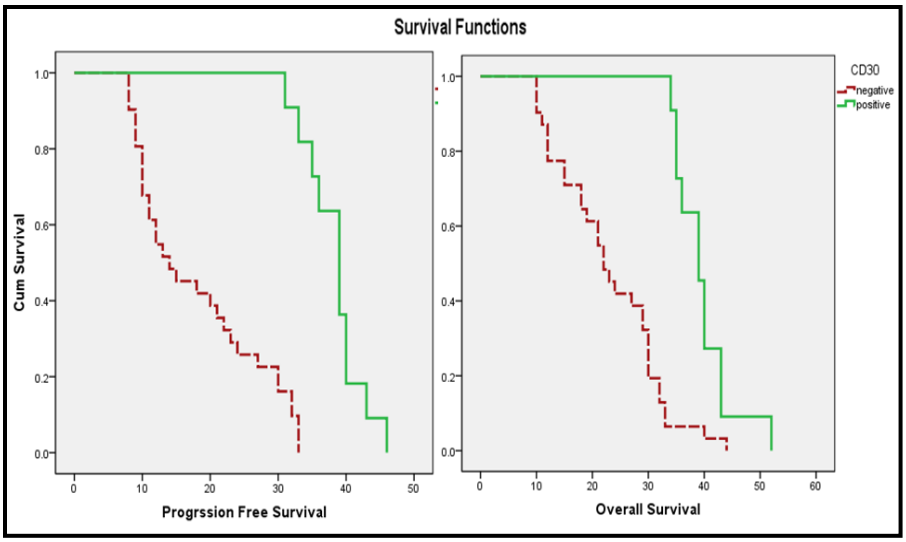

Figure (6): Survival in relation to CD 30 coexpression expression (Significant by log Rank P 0.000, 0.000)

Discussion

For better and accurate treatment plan, it is necessary to understand the oncogenesis and progression of NHL in general and DLBCL in particular for this study. So, we select different biomarker encountered in the pathogenesis of DLBCL and then targeted different pathways in this field aiming for stop the progression and better outcome. Our study is to investigated the expression of BCL2, C-myc, co-expression of BCL2, C MYC, and CD30- as they are vital station in the progression of DLBCL and also, study the association of expression patterns of these markers with clinicopathological characteristics of DLBCL and their impact on either OS, or DFS. Using these results as a guide lines on selecting the patient who is candidate for targeted therapy is our aim. In this study, we depend upon IHC in evaluation of the biomarker expressions as it is the most applicable in our centers. In addition, Immunohistochemistry is not just any more a useful and necessary diagnostic aid and helps in sub-typing different types of NHL but also used in predicting the prognosis and help in making treatment plan decision especially the challenged new strategies either targeted or immunotherapy [22,23].

Advances on the understanding of the genetic landscape and molecular features of DLBCL have identified high-risk subsets with poor outcomes to chemo-immunotherapy that are actively being studied in clinical trials [24]. Genetic assay is not routinely used in the clinical setting due to cost and technical issues, including a slow turnaround time of 2–3 weeks. Therefore, IHC remains widely adopted in clinical practice. In addition, IHC is sufficiently concordant with gene expression profile (GEP) in DLBCL as both represent complex molecular entities. Therefore, IHC could serve as a reasonable surrogate markers for determining underlying important genetic alterations that affect prognosis of DLBCL patients [25].

The molecular features of the underlying lymphoma such as cell of origin (and MYC/BCL2 protein expressions- are considered a more objective means to screen for double or triple hit lymphoma. Some experts recommend the identification of MYC and BCL2 protein overexpression by IHC to limit who should be tested for MYC/BCL2/BCL6 translocations by FISH due to the lower cost (estimated at 4–5 folds less than FISH studies) and nearly universal availability of IHC staining. However, using IHC expression of MYC and BCL2 is confounded by the recent understanding that isolated dual protein expression without underlying chromosomal rearrangements is a distinct and adverse prognostic factor in DLBCL, NOS [(26)].

The clinical data can't be assessed in our study due to small sample size because we exclude cases with incomplete data or poorly formed paraffin blocks that can't be recut and immunohistochemically evaluated. This notice can explain the difference as regard younger age predominance, male predominance which is not uniformly consistent with other studies and met analysis in DLBCL [(5)].

In the present study, the prevalence of BCL2 expression in this study was 76% of the studied cases the result which are strongly different from other studies because of different cut off value of positivity range from 30% to 70% or the study depend on the detection of gene rearrangement [(5,27)]. BCL2 expression didn't show significant association with the clinicopathological characteristics of the studied cases apart from association with the presence of relapse (P=0.04) **Table 2.** This result is concordant with many studies that used either IHC or gene analysis in evaluating BCL2 positivity [(5,27,28)]. Blocking the mitochondrial apoptotic pathway by BCL2 protein explains the poor prognostic influence and drug resistance association of high expression of BCL2 [(28,29)]. There was no statistically significant impact of BCL2 expression on OS, or DFS using log rank test and Kaplan-Meier curves. There is no long explanation of this unexpected finding other than wide variation of the cut off value of positivity or BCL2 and the detection of overexpression by IHC not by genetic analysis.

As regard the C MYC expression in the studied cases, the prevalence of C MYC expression in this study was 36% the result which are strongly different from Ting et al,[(5)] which represent 5.8% because they consider positivity for extra gene copies by FISH. However in the present study, IHC is the used tool for detection of over expression beside the selection of the cases according to the clinical data availability [(5)]. However, Xia and. Zhang [(12)] showed almost close result to our study 40% overexpression of C MYC [(12)]. C MYC overexpression didn't show significant association with the clinicopathological characteristics of the studied cases apart from association with the presence of relapse, progressive therapy response and the presence of extra-nodal presentation (P=0,001, 0,001, and 0.03 respectively) table 2. This result reflect the poor

prognostic effect of C MYC overexpression, which is concordant with literature and explained by its oncogenic effect [5–7,12]. In solidarity with these significant association with poorly prognostic clinicopathological parameters, log rank test determine significant impact of C-myc overexpression with OS and DFS (log Rank P= 0.001, 0.000 respectively) **Figure 4.**

C MYC act in synergism with BCL2 in driving the pathogenesis of DLBCL, where C MYC promote the cell cycle and increase the oncogenic stress of the cell that stimulate apoptosis by P53 which is antagonized by BCL2 activation and progression of DLBCL.[7,12,30].

In this study, there was concurrent overexpression of Cmyc, and BCL2 in 11 cases (26% of the studied cases). Statistically Significant association of concurrent overexpression of both proteins with progressive therapy response and the presence of relapse (P=0.003, 0.003) table 3. Log rank test determine significant impact of concurrent overexpression with OS and DFS (log Rank P= 0.00, 0.000) figure 5. All these result support that overexpression of both MYC and BCL2 proteins can predict poor survival in DLBCL which is concordant with many studies either using IHC or FISH [7,30].

In continuity of research about the prognostic marker and influencer of the therapy response in DLBCL. the biological role of CD30 in DLBCL is not fully understood however it has favourable outcome association [16]. In this study, there was CD30 positivity in 11 cases (26%). No significant association of CD30 positivity with any of the clinicopathogical parameters which is in agreement with Hu et al.[16]. However we found significant association with advanced stages (P=0.03) which was concordant with that demonstrated in the meta-analysis of Rodrigues-Fernandes et al 2021 [31]. Kaplan-Meier curves revealed significant impact of CD30 positivity on OS, DFS (P=0.000, 0.000) table 4, figure 6. This prognostic impact of CD30 positivity on the survival support the result of many studies and meta-analysis no matter what cut off value was used[16,17,31]. Another result support the favorable outcome of CD30 positivity in DLBCL is the inverse correlation with the concurrent expression of Cmyc and BCL2 determined by Spearman's bivariate correlation (Correlation Coefficient=--.355, P=0,02).

Conclusion

C MYC overexpression alone or concurrent with BCL2 overexpression are associated with a lower OS, and DFS. However BCL2 overexpression alone without C MYC overexpression didn't reveal that. CD30 positivity is associated with a better OS, and DFS. IHC can be applied for assessing the biomarker expression for prediction of the prognosis and selecting optimum individualized therapy.

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