

# Prognostic Significance of BCL2 Protein in Diffuse Large Cell Lymphoma of Head and Neck; Relation to Response to Chemotherapy

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**How to cite this paper:** Elhendawy, H.A., Ibrahim, A.T., Elmahdi, H.S. and Omar, A.M. (2020) Prognostic Significance of BCL2 Protein in Diffuse Large Cell Lymphoma of Head and Neck; Relation to Response to Chemotherapy. *Open Journal of Pathology*, 10, 76-92.

<https://doi.org/10.4236/ojpathology.2020.102008>

**Received:** February 27, 2020

**Accepted:** April 6, 2020

**Published:** April 9, 2020

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## Abstract

Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease that displays a highly variable clinical outcome. It is a neoplasm of large transformed B cells with a diffuse growth pattern. DLBCL is the most common type of non-Hodgkin's lymphoma (NHL) (31% of all cases). Approximately half of patients with DLBCL are cured with current chemotherapy regimens. The purpose of this study was to evaluate BCL2 expression in 45 patients diagnosed with DLBCL of head and neck region and correlate the level of its immunohistochemical expression with different clinicopathological variables with emphasis upon patients' age, gender, nodal or extra-nodal location of lymphoma, patients' response to chemotherapy, progression-free survival (PFS) and overall survival (OS). A retrospective analysis of 45 patients diagnosed to have DLBCL. A cut off value of  $\geq 50\%$  protein expression denoted BCL2 positivity. Out of 45 cases, 36 cases (80%) revealed BCL2 positive expression and 9 cases (20%) were BCL2 negative. We found statistically significant differences in BCL2 expression regarding different patients' responses to chemotherapy, patients' OS and PFS ( $p \leq 0.05$ ). No statistically significant differences in BCL2 expression regarding the patients' Ann Arbor clinical stage, age group and tumor site (nodal or extra-nodal,  $p > 0.05$ ) using the Chi-square test. BCL2 expression was analyzed in relation to 5 years OS and PFS using Kaplan Meier curves and Log Rank test for survival analysis. Cases that demonstrated BCL2 positivity revealed shortened OS and PFS with highly statistically significant differences among the studied variables ( $p = 0.000$ ). We also found that patients who respond well to the chemotherapeutic regimen had negative BCL2 expression, the differences were statistically signifi-

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cant ( $p = 0.015$ ). In conclusion, BCL2 expression could be considered a predictor for patients' chemotherapeutic response, OS and PFS.

### Keywords

Diffuse Large B-Cell Lymphoma, BCL2, Chemotherapy, OS, PFS

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## 1. Introduction

The head and neck region is one of the most common sites for the extra-nodal lymphomas. Most of the primary oral and para oral NHLs is DLBCL histological type [1] [2]. DLBCL is a heterogeneous disease that shows various outcomes [3]. It is a neoplasm of large transformed B cells with a diffuse growth pattern. Generally, DLBCL is a common histological type of NHL (31% of all cases) [4]. Almost 50% of DLBCL cases are cured with CHOP standard regimen (CTX, Adriamycin, VCR, prednisolone) [5] [6]. However, the remaining 50% are chemoresistant and this regimen is not able to cure them [7]. The latter group usually recurs after CHOP-induced remission with a short disease-free interval [8]. Other strategies in treatment as a combination between chemotherapeutics and mononuclear antibodies, or intensive chemotherapeutics associated with transplantation of bone marrow may be effective to them [9]. Thus, it would be extremely beneficial if we could detect previously which patients need more intensive treatments. Nowadays, researchers have been trying to find prognostic factors that help in predicting who will experience good or unsatisfactory response.

Apoptosis is one of the mechanisms which affect chemotherapy response and has been thoroughly investigated. Evidence has been accumulated in recent years showing that many and perhaps all chemotherapeutic agents affect tumor cell killing by inducing apoptosis [8]. Intrinsically chemoresistant tumors are unable to activate the apoptotic machinery and may therefore resistant to induction of cell death by chemotherapeutic agents. The BCL2 protein is considered to be an important multidrug resistance agent. BCL2 is a member of a related and interacting family of proteins, some of which (e.g., Bcl-xl) are antiapoptotic [10]. The prognostic impact of BCL2 overexpression is seen in various studies. Some studies have shown no difference, whereas others have shown reduced survival [11]. In the post rituximab era, the prognostic significance of BCL2 expression has been found to be controversial, the prognostic significance of BCL2 shown in some studies, while others not showing any prognostic significance [12]. The association between BCL2 expression and disease-free survival has been assessed in several studies [8] [13]. In aggressive NHL, these studies have led to the belief that BCL2 family proteins have an important role in chemosensitivity or chemoresistance of DLBCL. The purpose of our study was to evaluate BCL2 expression in DLBCL of the head and neck region and correlates its expression with different clinicopathological variables with emphasis upon patients' chemothe-

therapeutic response, PFS and OS.

## 2. Materials and Methods

### 2.1. Patients Selection

This retrospective study was conducted from January 2014 to January 2019 (5 years interval). Patients who were included to our study diagnosed with primary DLBCL that were aroused from the head and neck region. All cases included to our study had complete medical records and with confirmed diagnosis of DLBCL by sample immunophenotyping. After exclusion of cases had insufficient biopsy specimen and those with missing medical records, Forty-five paraffin blocks for DLBCL cases with thorough medical and clinicopathological data were retrieved from archives of the Pathology laboratory and Oncology unit of Oncology Center, Faculty of Medicine, Mansoura University. Patients' follow up data were obtained from the medical reports belong to the internal medicine department. All patients who were included in our study were treated with R-CHOP-like therapies (rituximab, cyclophosphamide, prednisone, doxorubicin hydrochloride and vincristine). After completion of treatment, patients were followed every 3 months intervals. The follow up comprised clinical examination and periodic ultrasonography for the head and neck region. In addition, chest X-ray, bone scan, and abdominal ultrasonography were performed when relapse was suspected. Overall survival (OS) and disease-free survival (DFS) data were retrieved from patients' medical reports. Five years OS was calculated from the dates of diagnosis to death or to the dates of the last follow up. PFS was calculated from the dates of first progression, death, relapse or the last follow up. Previous clinicopathological and follow-up data were obtained after getting approval from the institutional review board. This study was approved by the local ethical committee.

### 2.2. Immunohistochemical Staining

All selected blocks were cut at 4  $\mu\text{m}$  thickness. The slices were placed on coated slides. After xylene deparaffinization, the sections were rehydrated in descending grades of alcohol followed by water. Antigen retrieval was performed by using 0.01 M citric acid buffer (pH = 6.0) and heated for 10 minutes in the microwave. The sections were then incubated in a blocking medium (3%  $\text{H}_2\text{O}_2$ ) for five minutes followed by washing with distilled water. Rabbit monoclonal anti-human antibody (clone; EPR 5111, 1:50, dilution, Abcam, 1 Kendall Square, Suite B2304, Cambridge, MA 02139-1517, USA (was used against BCL2 antibody. Assessment of BCL2 positivity was performed by staining sections of hyperplastic human tonsillar tissue that used as a positive control at the same time and under the same conditions. Negative control slides obtained by replacement of the primary antibodies by plain phosphate buffer saline. Immuno-detection was executed using Power-stain TM1.0 poly HRP DAB kit for mouse + rabbit (Cat No 52-0017, Genemed Biotechnologies, Inc., 458 Carlton Ct., South San Francisco, CA 94080, USA). Immune staining was performed based on the manufacturer's

instructions. Immunoreaction was visualized by adding DAB Counterstaining of slides that were performed with the Mayer hematoxylin.

### 2.3. Immunostaining Evaluation

Immunohistochemical results were evaluated semiquantitatively. The BCL2 positivity was determined by cytoplasmic staining (brown) of neoplastic cells. The internal control was T cells. The percentage of positive cells in the whole section after the exclusion of the areas of reactive T cells was determined. It was scored negative if 5% or less of neoplastic cells were stained. In this study, the value of BCL2 was considered weak positive if 6 to less than 50% were brown stained. For statistical analysis, we considered negative and weak positive (low expression) and high expression if  $\geq 50\%$  of tumor cells were brown stained [14]. The histopathological and immunohistochemical evaluation was done by two pathologists independently and blindly. The images were acquired utilizing a Nikon Eclipse microscope equipped with a 5-megapixel cooled CCD camera and the Image-Pro + AMS7 software.

### 2.4. Statistical Analysis

Statistical analysis of the data was done by using the Excel program and the Statistical Package for Social Science (SPSS) version 22 program. The description of the data was done in the form of mean (+/-) Standard Deviation (SD) for quantitative data, while in the form of frequency and proportion for qualitative data. The analysis of the data was done by one way ANOVA, to test statistical significant difference between groups and Wilcoxon multiple comparisons test for comparison of each two groups. Pearson correlation co-efficiency test was used to test the association between the different variables. A chi-square test was used to compare between two groups for qualitative data. The construction of survival curves was conducted using the Kaplan-Meier method, and the significance was assessed with the log-rank test. Univariate and multivariate survival analyses were performed with the Cox proportional hazards model to detect an independent prognostic factor. Using the univariate and multivariate analysis, the outliers were detected and cleaned from the data set. A P-value less than or equal to 0.05 was considered statistically significant.

## 3. Results

### 3.1. Clinicopathological Characteristics of Studied Cases

A total of 45 patients of DLBCL were identified and included in the study. Regarding gender, our study included 24 males (53.3%) and 21 females (46.7%) with male to female ratio 1.14:1. The research included 35 cases (77.8%) were younger than 60 years old, while the rest were older than 60 years old (10 cases, 22.2%). DLBCLs that aroused from lymph nodes of head and neck region comprised 28 cases (62.2%), while the rest aroused from extranodal location (**Table 1**). The majority of the studied cases presented as localized disease in stage I and

**Table 1.** Clinico-pathological features of studied cases.

Clinic-pathological variables		Frequency	Percent
Gender	male	24	53.3%
	female	21	46.7%
Age group	<60 years	35	77.8%
	≥60 years	10	22.2%
Tumor location	Nodal	28	62.2%
	Extranodal	17	37.8%
Ann Arbor clinical stage	Stage I & II	33	73.3%
	Stage III & IV	12	26.7%
Tumor recurrence	yes	18	40%
	no	27	60%
Deaths	yes	9	20%
Patient response to chemotherapy	Response by <3 courses	14	31.1%
	Response by ≥3 courses	16	35.6%
	chemoresistant	15	33.3%

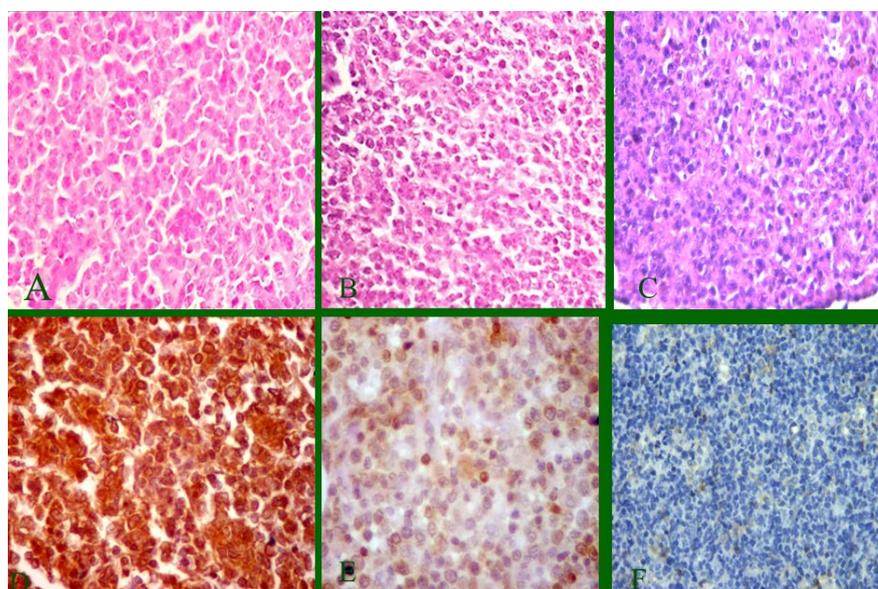
II according to Ann Arbor clinical staging system (33 cases, 73.3%). DLBCLs of advancing clinical stages (III and IV) demonstrated in 12 cases (26.7%).

All participant of the study received chemotherapy. Patients' response to chemotherapeutic treatment was assessed during the follow up visits in accordance with international working group recommendations for response criterion for NHL. Patients were divided into 3 groups according to their response to the administered chemotherapy as follow: respond to less than 3 chemotherapeutic courses (14 cases, 31.1%), respond to three or more chemotherapeutic courses (16 cases, 35.6%) and chemoresistant group (15 cases, 33.3%). Recurrence of the tumor during follow up demonstrated in 18 cases (40%). Nine of the followed cases (20%) were died during follow up. Patients' clinicopathological data is shown in **Table 1**.

### 3.2. BCL2 Expression in Relation to Different Clinicopathological Variables

We selected a cutoff of ≥50% protein expression for BCL2 positivity. In the total cases BCL2 positive rate was 80% (36 out of 45 cases; **Figures 1(D)-(F)**). The relationship between different clinicopathological variables and BCL2 expression was assessed using Chi-square test. BCL2 expression had no statistically significant associations with tumor location either nodal or extranodal ( $p = 0.758$ ), Ann Arbor clinical stage ( $P = 0.736$ ) and patient's age ( $p = 0.37$ ). **Table 2** illustrates the data.

BCL2 expression was positively expressed in 17 out of the 18 cases (94.4%) that had recurrence during follow up. Nineteen out of 27 non-recurring cases (70.4%) had positive BCL2 expression. Chi-square test revealed a statistically



**Figure 1.** Hematoxylin and eosin staining showed DLBCL with large cells with abundant eosinophilic cytoplasm, vesicular nuclei and single or multiple nucleoli (photo A, B and C  $\times 400$ ). BCL2 immunostaining showed diffuse strong staining ( $>50\%$  of neoplastic cells, photo D  $\times 400$ ), focal expression ( $6\%$ :  $<50\%$  of neoplastic cells show positive low BCL2 expression, photo E  $\times 400$ ), and negative BCL2 staining ( $5\%$  or less) of neoplastic large cells with positive staining of reactive T cells (positive internal control (photo F  $\times 400$ )).

**Table 2.** BCL2 expression in relation to different clinicopathological variables.

variables	Groups	Positive BCL2	Negative BCL2	total	Chi-square test P-value
Tumor site	Nodal	22 (78.6%)	6 (21.4%)	28	0.758
	Extranodal	14 (82.4%)	3 (17.6%)	17	
Ann Arbor stage	I & II	26 (78.8%)	7 (21.2%)	33	0.736
	III & IV	10 (83.3%)	2 (16.7%)	12	
Age group	$<60$ years	27 (77.1%)	8 (22.9%)	35	0.37
	$\geq 60$ years	9 (90%)	1 (10%)	10	
recurrence	Present	17 (94.4%)	1 (5.6%)	18	0.048
	Free	19 (70.4%)	8 (29.6%)	27	
Died/alive status	Died	9 (100%)	Zero	9	0.094
	Alive	27 (75%)	9 (25%)	36	
Patient Response to chemotherapy	- Respond to $< 3$ courses	8 (57.1%)	6 (42.9%)	14	0.033
	- Respond to $\geq 3$ courses	14 (87.5%)	2 (12.5%)	16	
	- Resistant	14 (93.3%)	1 (6.7%)	15	

Chi-square test; p-value significant if  $\leq 0.05$ .

significant difference in BCL2 expression according to incidence of recurrence ( $P = 0.048$ ).

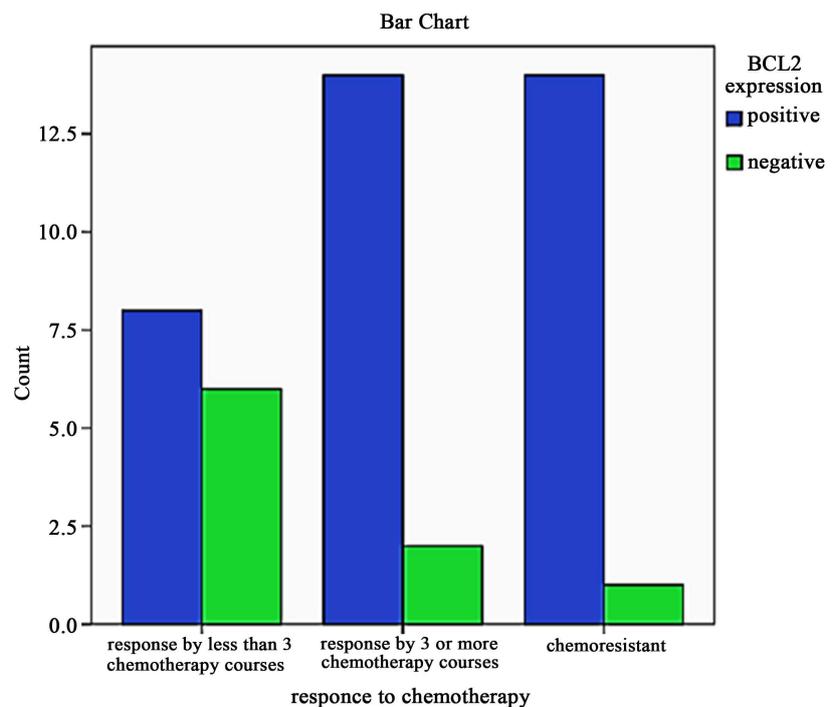
All cases that died during follow up demonstrated positive BCL2 expression (9

cases, 100%). On the other hand, BCL2 was positively expressed in 27 (75%) out of 36 alive cases. Although Chi-square test revealed no statistically significant difference in BCL2 expression between the two groups (died and alive), but the P-value ( $p = 0.09$ ) approximates the value required to reach significance.

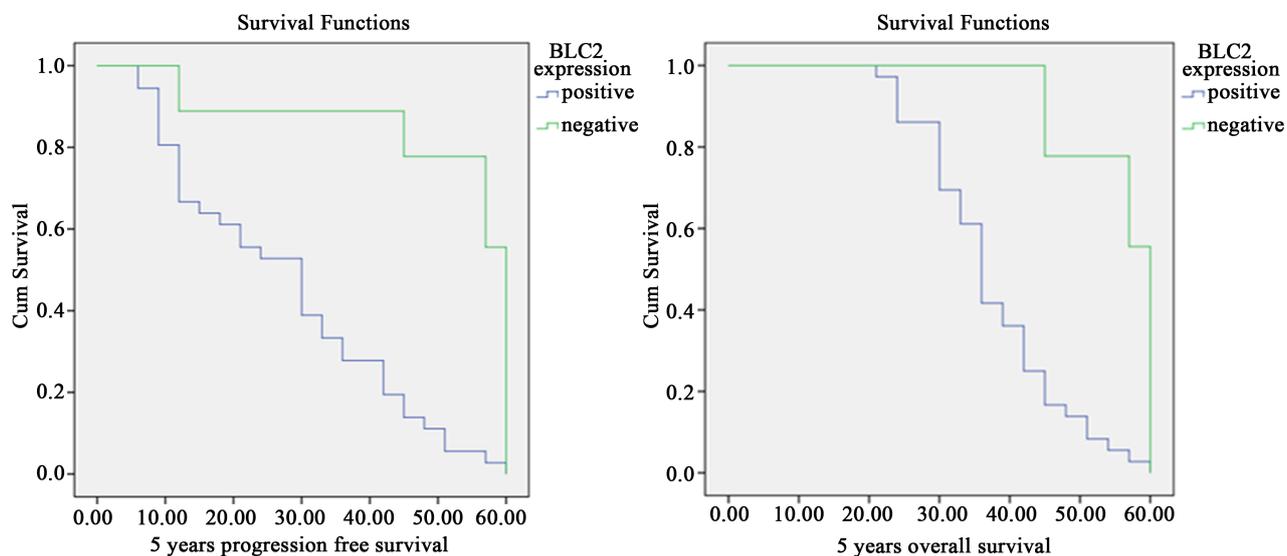
BCL2 expression was varied among the three different patients' responses to chemotherapy. Fourteen out of 15 chemoresistant cases (93.3%) had positive BCL2 expression. Fourteen out of 16 Patients (87.5%) who received and responded to  $\geq 3$  chemotherapeutic courses had positive BCL2 expression. Eight out of 14 patients (57.1%) who responded to less than three chemotherapeutic courses had positive BCL2 expression. Chi-square test revealed a statistically significant difference in BCL2 expression among the mentioned three groups of patients according to their response to the taken drugs ( $P = 0.033$ ; **Figure 2**). BCL2 expression in the studied DLBCLs could predict the patients' chemotherapeutic response (ANOVA test P-value = 0.015).

### 3.3. BCL2 Expression and Patients' Overall Survival

Patients' OS were analyzed in relation to status of BCL2 expression using Kaplan Meier method and log rank test was used for comparing the variables. The mean OS in patients with positive BCL2 expression was 37.5 months, while those who had negative BCL2 expression was 56 months. In other words, the mean OS was greater in BCL2 negative patients ( $n = 9$ ) as compared to the positive ones ( $n = 36$ , 56 Vs 37.5 months;  $p = 0.000$ ) **Table 3**, **Figure 3**. BCL2 expression in tumor could predict the 5 years OS ( $R^2 = 40.5\%$ ,  $p = 0.000$ , **Table 4**).



**Figure 2.** BCL2 expression in relation to different patients' responses to chemotherapeutic treatment.



**Figure 3.** BCL2 expression in relation to 5 years overall survival and 5 years progression free survival.

**Table 3.** Patients' overall survival in relation to BCL2 expression.

BCL2 expression	Mean <sup>a</sup>		Median				Log Rank test (Sig.)		
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error		95% Confidence Interval	
			Lower Bound	Upper Bound				Lower Bound	Upper Bound
positive	37.500	1.619	34.327	40.673	36.000	1.268	33.515	38.485	0.000
negative	56.000	2.121	51.842	60.158	60.000	0.000			
Overall	41.200	1.754	37.762	44.638	39.000	2.321	34.450	43.550	

**Table 4.** Prediction of OS by BCL2 expression.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	0.636 <sup>a</sup>	0.405	0.391	9.18416	0.405	29.214	1	43	0.000

a. Predictors: (Constant), BCL2 expression; Dependent Variable: 5 years overall survival.

Patients' OS was analyzed in relation to patient age, gender, tumor location nodal or extranodal, tumor clinical stage and different patient's responses to the taken chemotherapeutic drugs. None of these variables had effect on patient's OS except for patient's age and response to chemotherapy. In more words, mean survival was greater in patients under age of 60 years as compared to those above 60 years old ( $n = 42, 8$  Vs  $35.7$  months;  $p = 0.044$ ). Also, chemoresistant cases had shorter OS as compared to cases that respond to 3 or more chemotherapeutic courses and cases that respond to less than three courses ( $32.2$  Vs  $42.18$  Vs  $49.71$  months respectively;  $p = 0.000$ ).

### 3.4. BCL2 Expression and Progression Free Survival

Patient PFS was analyzed in relation to BCL2 expression using Kaplan Meier survival curves. The mean PFS in patients with positive BCL2 expression was 27.4 months as compared to those who had negative BCL2 expression 52.3 months, a high statistically significant difference in PFS according to the status of BCL2 expression (p-value = 0.000; **Table 5, Figure 3**).

Patients' PFS was analyzed in relation to patient age, gender, tumor location either nodal or extranodal, clinical stage and different patient responses to chemotherapy. All these independent variables had significant difference in relation to PFS except for gender and tumor location. In more words, patients who diagnosed with tumor in advanced clinical stage (III and IV), the age  $\geq 60$  years old, chemoresistant cases had shortened PFS when compared to patients who had tumors in localized clinical stage (I and II), patient age younger than 60 years, patients who respond to chemotherapy ( $P < 0.05$ , log Rank test; **Figures 4-7**). BCL2 expression in tumor could predict patients PFS ( $R^2 = 28.7\%$ , p-value = 0.000, **Table 6**).

Finally, we analyzed patients' OS in addition to PFS in relation to BCL2 expression, Ann Arbor clinical stage, incidence of recurrence and different patients' responses to chemotherapy as covariables using COX regression model. BCL2 expression and different patients' chemotherapeutic response were the only two independent variables that affect patients' OS and PFS ( $p < 0.05$ , **Table 7 and Table 8**).

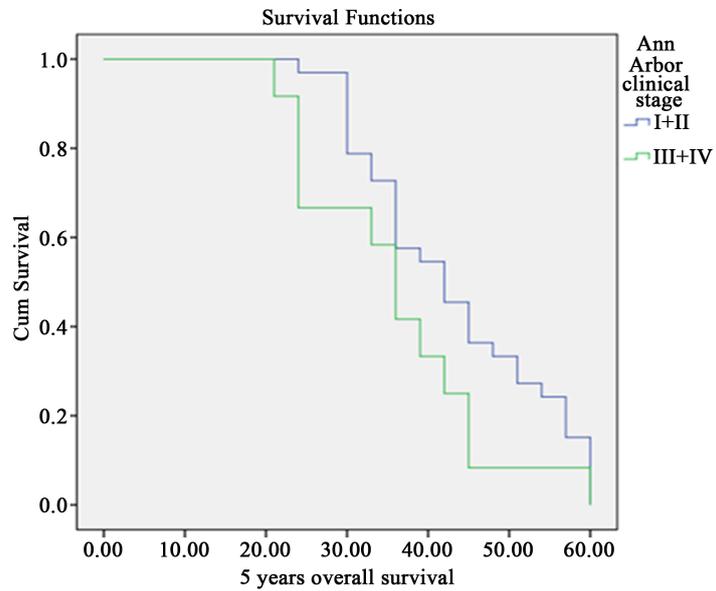
**Table 5.** BCL2 expression in relation to PFS.

BCL2 expression	Mean <sup>a</sup>				Median				Log Rank test P-value (sig.)
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval		
			Lower Bound	Upper Bound			Lower Bound	Upper Bound	
positive	27.417	2.689	22.146	32.687	30.000	4.387	21.401	38.599	0.000
negative	52.333	5.294	41.957	62.710	60.000	0.000			
Overall	32.400	2.807	26.899	37.901	30.000	4.311	21.550	38.450	

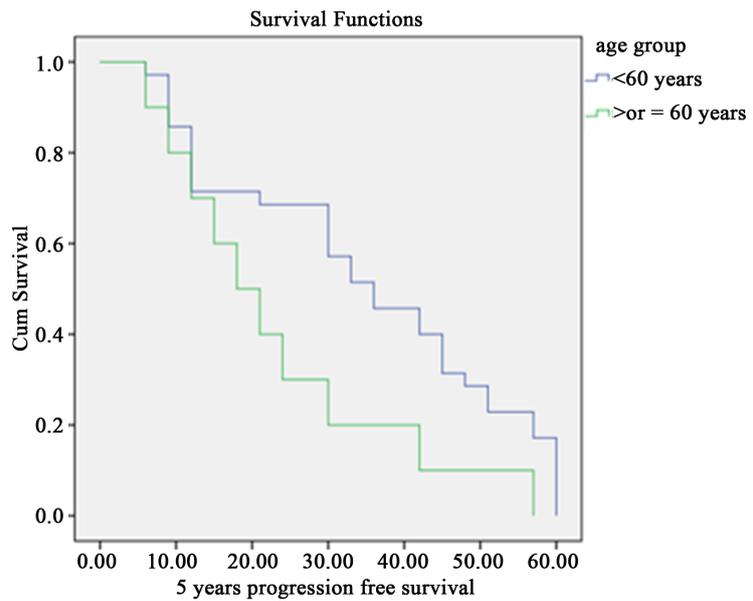
**Table 6.** Progression free survival in relation to BCL2 expression.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	0.535 <sup>a</sup>	0.287	0.270	16.08752	0.287	17.272	1	43	0.000
	Model		Sum of Squares	df	Mean Square	F			Sig.
	Regression		4470.050	1	4470.050	17.272			0.000 <sup>b</sup>
	Residual		11,128.750	43	258.808				
	Total		15,598.800	44					

ANOVA a. Dependent Variable: 5 years progression free survival; b. Predictors: (Constant), BCL2 expression.



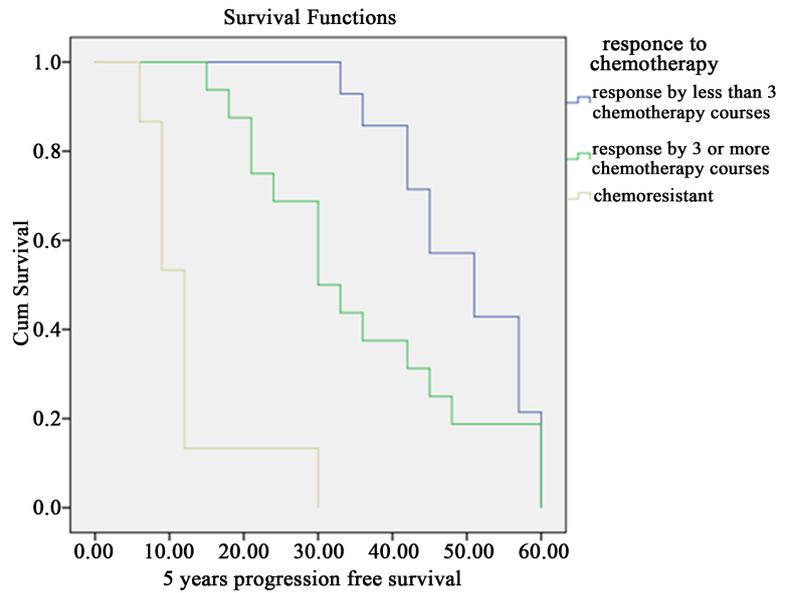
**Figure 4.** Progression free survival in relation to Ann Arbor clinical stage.



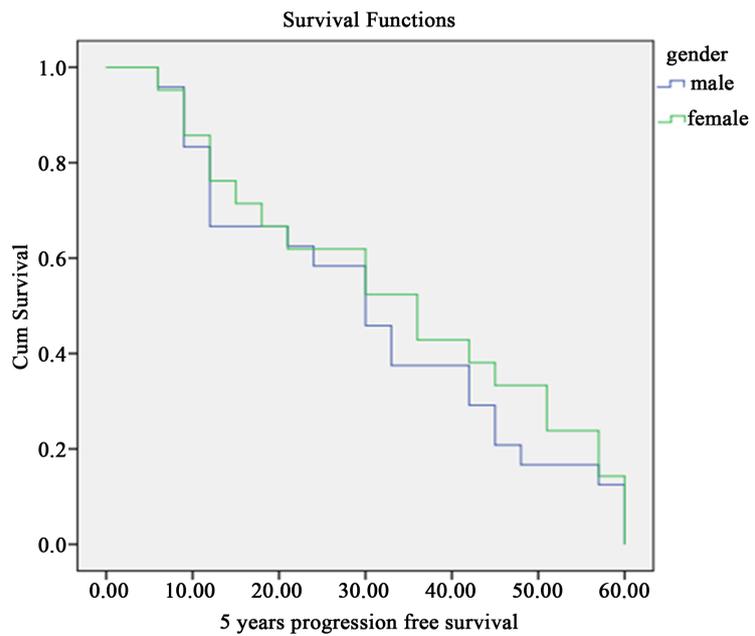
**Figure 5.** Progression free survival in relation to patient age.

**Table 7.** Analysis of OS in relation to covariables (COX regression model).

	Variables in the Equation					
	B	SE	Wald	df	Sig.	Exp (B)
stage	1.007	0.642	2.457	1	0.117	2.736
BCL2	1.215	0.450	7.303	1	<b>0.007</b>	3.369
recurrence	0.344	0.498	0.477	1	0.490	1.411
chemotherapy			7.659	2	<b>0.022</b>	
Chemotherapy (1)	1.816	0.736	6.096	1	0.014	0.163
Chemotherapy (2)	1.763	0.643	7.512	1	0.006	0.172



**Figure 6.** Progression free survival in relation to patients' chemotherapeutic response.



**Figure 7.** Progression free survival in relation to patient gender.

**Table 8.** Analysis of PFS in relation to covariables (COX regression model).

Variables in the Equation						
	B	SE	Wald	df	Sig.	Exp (B)
stage	0.518	0.553	0.880	1	0.348	0.595
BCL2	1.329	0.474	7.858	1	<b>0.005</b>	3.777
recurrence	12.894	64.441	0.040	1	0.841	398,108.921
chemotherapy			9.489	2	<b>0.009</b>	
Chemotherapy (1)	2.203	0.824	7.141	1	0.008	0.111
Chemotherapy (2)	2.391	0.777	9.478	1	0.002	0.092

## 4. Discussion

Hodgkin's lymphomas habitually include lymph nodes of the neck and mediastinum, while extranodal lymphomas represent just 5% from claiming HLs for illustration in the tonsils. On contrast, roughly 30% about NHLs demonstrate heterogeneous extranodal manifestations, for example, such that in the major salivary glands, paranasal sinuses, mandible, maxilla and Waldeyer's ring [15] other than the gastrointestinal tract, the head and neck are commonly involved as an extranodal site in NHL, affecting 11% - 33% of patients [16]. Clinically, head and neck lymphomas absent the definite characteristics that might empower attribution to a particular lymphoma type without biopsy and histological proof. In particular, with respect to lymphomas having aggressive behavior, prompt histological proof will be essential for patient management, early treatment initiation and frequently for the outcome [17].

DLBCL is an aggressive type of NHL in which the IPI is used as a prognostic score in the clinical setting to determine outcomes. Patients who have similar IPI scores have varied outcomes, molecular signatures have been studied to prognosticate patients with DLBCL. Studies have revealed that the expression of certain genes is associated with poor outcomes [12] [18]. BCL2 is an anti-apoptotic protein that also has an antiproliferative effect and is a powerful prognostic marker before rituximab. Studies showed that the addition of rituximab has eliminated the negative impact of the BCL2 expression [10] [12] [13] [19]. However, the prognostic value of BCL2 protein in DLBCL is still controversial [14] [15] [20] [21], reflecting the heterogeneity of the disease and different molecular techniques used. BCL2 overexpression has been identified with different methods, such as IHC, Western blot, chimeric genomic hybridization, fluorescence in situ hybridization, and cDNA microarray. Studies using different techniques have shown varied results [22] [23] [24] [25].

In fact, some studies suggest that patients with BCL2 gene rearrangements have better survival. In studies using Southern blot analysis, the presence of BCL2 gene rearrangement did not appear to be predictive of survival except for few studies showed worse survival [22] [23] [24] [25]. Multiple studies have looked at the expression of BCL2 using immune stains and some have found no difference in OS [22], while other studies found that BCL2 expression was prognostic in DLBCL and associated with poor outcomes [18] [26].

In this project, we studied the expression of BCL2 in DLBCLs. BCL2 in addition to being an antiapoptotic protein; has a role in resistance to chemotherapeutic agents. Eighty percent of the studied DLBCL cases demonstrated positive BCL2 expression. Akay OM *et al.* (2014) [27] reported the same percentage of BCL2 expression in the worked cases of DLBCL. They also found that BCL2 overexpression (more than 80%) was associated with slow response or resistance to chemotherapy and a negative influence on OS [27]. Akay OM *et al.* (2014) concluded that rearrangements of BCL2 and expression of this oncogenic protein may serve as prognostic markers in DLBCL patients [27].

Positive BCL2 expression significantly associated with shortened OS and PFS,

the incidence of recurrence and chemoresistant patient response. The incidence of patients' death also was positively correlated with positive BCL2 expression, but the statistically significant difference is not reached ( $P = 0.09$ ). In accordance with these findings, high BCL2 expression was associated with short survival in many studies [11] [18] [27] [28]. Expression of BCL2 is a prognostic factor that is independent of IPI score and other indicators of poor clinical outcomes in patients with several types of cancer [18] [24] [27] [28] including aggressive NHL, acute myelogenous leukemia [11] and prostatic adenocarcinoma [27]. BCL2 is an independent prognostic factor on DFS [29]. Patients with DLBCL who express BCL2 proteins are high-risk groups with poor OS and they can't achieve better response to the usual regimen [30] [31]. The OS for patients with DLBCL had poor survival when BCL2 was overexpressed, poor survival seen in both ABC and GCB subtypes when BCL2 positive [22] [26] [31] [32]. This is in concordance with the study by Rantanen *et al.* which showed that BCL2 positivity had a bearing on survival and was associated with poor outcomes, especially in those who had received anthracycline-based chemotherapy [33]. Results different from these were seen in the study by Iqbal *et al.* [9] [11] which showed that BCL2 overexpression was associated with poor survival in the non-GCB and not the GCB subtype. However, BCL2 overexpression was not associated with poor survival in DLBCL as a whole. These studies were in the prirituximab era [9] [11].

Contradictory to our findings, Otilia Labău, Gabriela Mutiu did not find a statistically significant relation between BCL2 expression with response to chemotherapy [6] [8]. In another study by Mounier *et al.* (2003), an analysis of the GELA trial demonstrated that the benefit of rituximab appeared limited to patients with lymphoma that overexpressed bCL2 on IHC [13] [19]. The outcomes of patients with DLBCL with BCL2 overexpression have been varied with various studies [16] [22] [27] [32].

In DLBCL, the inhibitory action of BCL2 on apoptosis is hypothesized as a cause of chemotherapy resistance and this notion was supported by several clinical studies in the prirituximab era demonstrating an inverse correlation between BCL2 protein expression and survival. Studies carried out in the post-rituximab era, however, raise the question of whether BCL2 remains a biomarker of treatment failure and many studies have demonstrated that this is no longer the case. Patients in both CHOP and R-CHOP arms had better survival when BCL2 negative [10] [12] [26] [32].

The causes of variation in the results are due to variable techniques were used to assess BCL2 expression in various studies that is a factor that causes inconsistent survival results in these studies. Survival could be modified by several contributory factors as CD10, BCL6 MUM-1 [27] bak, bax, BCL Xs [27] [33] [34] that associated with poor outcomes in DLBCL. Here, in our study, we studied BCL2 only.

## 5. Summary and Conclusion

DLBCL is a heterogeneous disease that displays a highly variable clinical out-

come. This study was conducted to evaluate BCL2 expression in 45 patients diagnosed with DLBCL of the head and neck region and correlate the level of its immunohistochemical expression with different clinicopathological variables. Positive BCL2 expression was significantly associated with poor OS, PFS and chemoresistant patient response ( $P \leq 0.05$ ). No statistically significant differences in BCL2 expression regarding the patients' Ann Arbor clinical stage, age group and tumor site (nodal or extra-nodal, ( $p > 0.05$ ) using the Chi-square test. In conclusion, BCL2 expression could be considered a predictor for patients' chemotherapeutic response, OS and PFS.

## Fund

Nil.

## Conflicts of Interest

No conflict of interest.

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