Original Article

# FREQUENCY OF CO-EXPRESSION OF c-Myc AND BCL-2 IN DIFFUSE LARGE B-CELL LYMPHOMAS

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## ABSTRACT

**Objective:** The objective of study is to evaluate the frequency of co-expression of c-Myc and BCL-2 in Diffuse large B-cell lymphoma.

**Material and Methods:** After taking ethical approval cross sectional study was conducted at Chughtai Lab Lahore on 100 cases. The specimens were thoroughly examined under supervision of a consultant histopathologist with at least 5 years of post-fellowship experience. Representative sections were taken. Standard H&E slides were made and observed under microscope. Representative section was selected and c-Myc, BCL-2 immunohistochemical stains were performed. The expression of c-Myc and BCL-2 was documented by the researcher and the supervisor. The findings were entered in the Performa given at the end. Data was entered and analyzed using SPSS version 23. Post stratification chi-square test was applied by taking P value of $< 0.05$ as significant.

**Results:** It was noted that mean age of the study population was 41.76±14.72 years at the time of enrollment. Majority 53(53%) cases were male while 47(47%) were female cases in this study. It is evaluated that 26(26%) of cases has positive coexpression c-Myc and BLC-2 while 74(74%) cases has negative co-expression c-Myc and BLC- 2.

**Conclusion:** Given the prognostic value and potential need for a change in therapeutic approach, protein expression analysis for c-Myc and BCL-2 should be performed on all patients with DLBCL for subcategorization, risk stratification and consideration for targeted therapy

**Key Words:** Lymphoma, c-Myc, BLC-2, Double expressor (DEL).

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## INTRODUCTION

In western countries, DLBCL (Diffuse large B-cell lymphoma) are the most common types of non-Hodgkin's lymphoma, which accounts up to 30% of all cases of all non-Hodgkin's lymphomas [1]. The percentage of these lymphomas is much higher in developing countries.

The refinement of classification and heterogeneity of large cell lymphomas has markedly improved with the advent of genetic studies and understanding of proteomic structures of such cells. The molecular features of various types of lymphomas, identified through genetic studies and proteomic structure have also helped to identify the aggressive variants of the lymphomas. Two distinct subtypes of DLBCL have been identified through gene expression analysis; B cell activated type and Germinal Centre B cell. The cells of origin were at the crux of this classification. Unfortunately, this classification has limited significance of predicting prognosis in individual cases.

c-Myc, an oncogenic transcript or gene, is conceded as the most common type of genetic abnormality in malignancy. In about 70% cases of all human malignancies, there is over-expression of c-Myc genes [2]. In 7-15% cases of DLBCL, finding of c-Myc rearrangements noted which is linked with poor prognosis. Activation of c-Myc expression as a result of amplification of repeated mutation of micro RNA has led to greater protein expression in large percentage, between 30-50% of DLBCL [2].

Apart from c-Myc gene, BCL-2 has been the centre of interest for the researchers as prognostic biomarker. This gene (BCL-2) is linked with chemotherapy resistance. The term Double Expressor lymphoma (DEL) is given to the lymphomas with dual expression of c-Myc and BCL-2 genes [3]. Approximately 12% DLBCL cases show dual expression [4]. The lack of chromosomal rearrangements can also lead to over expression of c-Myc and BCL-2 markers. The DLBCL, with co-expression of c-Myc/BCL2, has no standard treatment regime. As it has very aggressive clinical course, its 5-year survival rate is only 40% approx [5]. The combined EPOCH-R (etoposide, cyclophos-phamide, doxorubicin, vincristine, prednisolone and rituximab) in dose adjusted manner has shown good efficacy in cases of double-protein co-expression DLBCL, but not in overall survival [6]. Allogenic stem cell transplant is also in trials. The purpose of this study is to assess the frequency of protein expressions secondary to c-Myc and BCL-2 genes.

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## MATERIAL AND METHODS

The data set compromised of 100 consecutive patients, who were diagnosed with DLBCL for the first time in Chughtai Institute of Pathology between 2017 and 2019. The approval of the study was given by the Research Ethics Committee of Chughtai Institute of Pathology. The samples of paraffin embedded blocks of tumours were utilised for immunohistochemical analysis by applying monoclonal antibodies. The samples were fixed in 10% Neutral buffered formalin and were paraffin embedded (Figure-I).

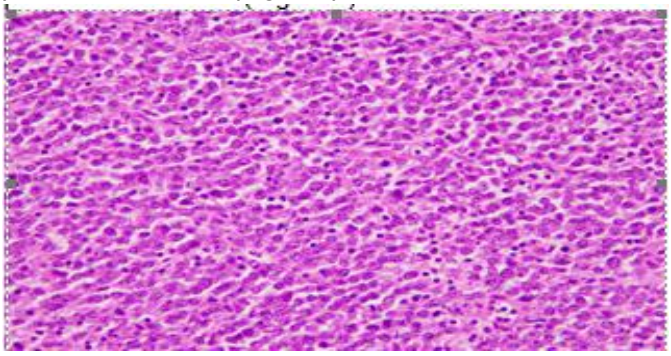

**Figure-I: DLBCL (sheets of cells with vesicular nuclei).**

The 'n' number was 100, out of which 43 were females and 57 were males, between the age ranges of 21-75 years.

For this research, if 40% of tumour cells expressed immunoreactivity to the Myc protein then it is categorized positive while the mark was set at 50 % for the expression of BCL-2 gene (Figure-II, III) [7].

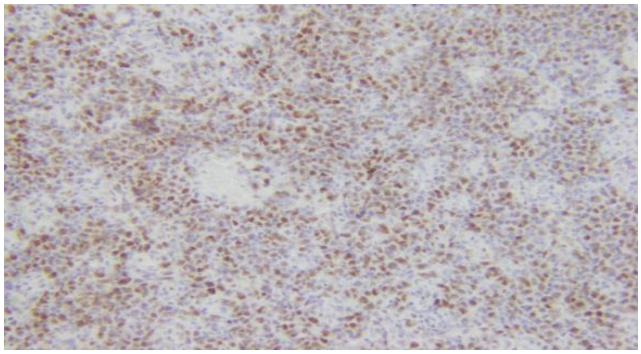

**Figure-II: c-Myc.**

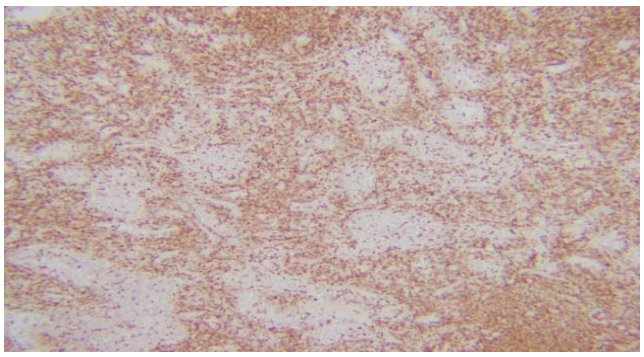

**Figure-III: BCL-2.**

The SPSS version 23 has been utilised for the purpose of data entry and analysis. Application of chi-square test conducted after stratification of data and P value of less than 0.05 is considered significant.

## RESULTS

It was noted that mean age of the study population was 41.76±14.72 years at the time of enrollment. Majority 53(53%) cases were male while 47(47%) were female cases in this study (Figure-IV).

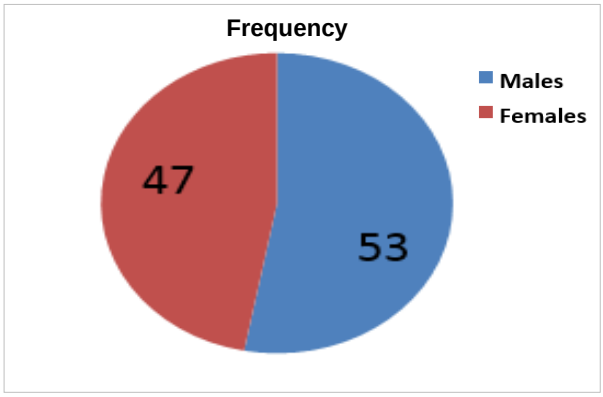


**Figure-IV: Gender distribution.**

It was evaluated that 26(26%) of cases had positive co-expression c-Myc and BLC-2 while 74(74%) cases had negative co-expression c-Myc and BLC-2. (Table-I).

Data was stratified according to age and gender. It was found that 22(32.4%) cases had positive coexpression in age group 20-45 years of age and 4(12.5%) cases had positive co-expression in age group >50 years, showing significant difference (Table-II). It was observed that there was no significant difference for gender in the study participants (Table-III).

**Table-I: Co-expression of c-Myc and BCL-2.**

| Co-expression | Frequency | Percent |
|---|---|---|
| **Yes** | 26 | 26.0 |
| **No** | 74 | 74.0 |
| | 100 | 100.0 |

**Table-II: Co-expression of c-Myc and BCL-2 with respect to age.**

| | | Co-Expression of c-Myc and BCL-2 | | P-value |
|---|---|---|---|---|
| | | Yes | No | |
| **Age Group** | 20-45 years | 22 | 46 | 0.027 |
| | | 32.4% | 67.6% | |
| | >50 years | 4 | 28 | |
| | | 12.5% | 87.5% | |
| **Total** | | 26 | 74 | 100 |
| | | 26.0% | 74.0% | 100 % |

**Table-III: Co-expression of c-Myc and BCL-2 with respect to gender.**

| | | Co-expression of c-Myc and BCL-2 | | P-value |
|---|---|---|---|---|
| | | Yes | No | |
| **Gender** | **Male** | 16 | 37 | 0.36 |
| | | 30.2% | 69.8% | |
| | **Female** | 10 | 37 | |
| | | 21.3% | 78.7% | |
| **Total** | | 26 | 74 | 100 |
| | | 26.0% | 74.0% | 100% |

## DISCUSSION

The DLBCL is a quite heterogenous group of tumors and this heterogeneity is based on various clinicopathological characteristics, genetic alterations and response to treatment and prognosis. Though response to standard treatment is noted in almost half of the cases of DLBCL but in remaining half, resistance to treatment is still a major concern. The mortality rate is up to 40% within the first 2 years of diagnosis. The identification of alternative treatment strategies is the focus for the patients with worse prognosis. Assessment of prognostic value of individual biomarkers has been the focus of numerous studies. The more recent studies have also identified the significance of molecular classifications for DLBCL in prognosis and response to treatment.

In 2000, first time ever gene expression profiling was utilised in the study of DLBCL. As a result of this study, two major categories of cell of origin were identified based on prognostic outcomes; activated B-cell (ABC) and germinal centre B-cell (GCB). Hans *et al*. proposed the algorithm with application of BCL6, CD10 and multiple myeloma oncogenes (MUM-1) to classify DLBCL into prognostically significant subtypes like GCB and ABC.

The translocation of chromosome is another important prognostic and pathogenic feature of B-Cell lymphomas. The most common chromosomal translocations identified in DLBCL are related with BCL2, BCL6 and Myc loci [7,8,9]. Perry *et al*, after studying 105 cases of DLBCL deduced that co-expression of BCL-2 and c-Myc genes were linked with poor survival and this finding was independent of any other related factor. This study was conducted on patients who were given R-CHOP therapy [10].

In our study, BCL2 expression was detected in 51% of cases and c-Myc expression was 27% which is comparable with the previous studies [11,12,13] Johnson *et al*. conducted a study in 2012 on 167 patients and was found 21% co-expression of c-Myc and BCL2 which is more in line with our study [11]. In Pakistan, similar study was conducted by Armed Forces Institute of Pakistan in Rawalpindi on 74 diagnosed cases and their results were 14% which were comparatively lower than results of our study [12]. Another study conducted by Atif A. Hashmi from Liaquat National Hospital, Karachi on 109 diagnosed cases. His results showed 35.8% co-expression of c-Myc and BCL2 [13].

A study conducted by Haung JZ established worse survival outcomes secondary to chromosomal translocations 14; 18, in DLBCL [14]. As like of previous research studies BCL2 rearrangements have limited predictive value in overall survival of the patients who were treated with rituximab plus CHOP [15,16].

Velera *et al*. conducted the gene analysis studies on 219 patients diagnosed with DLBCL. In this study they noted 4% of the cases were either doble or triple hit, means concurrent genetic abnormalities in BCL2 and/or BCL6 genes while in 3 % cases only Myc rearrangements were the sole cause of abnormalities, i.e., Myc single hit [17]. c-Myc translocation is a key transcription factor which promotes cell cycling and tumor proliferation.

The DLBCL, with co-expression of c-Myc/BCL2, has no standard treatment regime [18]. Owing to their worst prognosis, it is better to differentiate Double Expressor Lymphomas from DLBCL group so that aggressive treatment regimens should be given. Allogenic stem cell transplant is also in trials and Alex F Herrera also studied some cases [19,20].

Our study clearly indicates the importance of comprehensive immunohistology and molecular profiling of MYC and BCL2 genes in identifying alternative treatment approaches in patients with DLBCL. Combining the molecular profiling with advent of novel agents has not only improved the outcomes but paving way for future research.

## CONCLUSION

Given the prognostic value and potential need for a change in therapeutic approach, protein expression analysis for c-Myc and BCL-2 should be performed on all patients with DLBCL for subcategorization, risk stratification and consideration for targeted therapy.

## AUTHOR CONTRIBUTION

**Azra Bashir:** Original concept, study design, data analysis, paper write up and critical revision.

**Saima Batool and Anam Khan:** Data analysis and result interpretation.

**Faria Waqar Khan and Maimoona Aslam:** Result interpretation and discussion.

**Akhtar Sohail Chughtai:** Approval and final critical revision.

## REFERENCES


1. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (4th Ed). Lyon, France: International Agency for Research on Cancer, 2008 Swerdlow SH, Campo E, Harris NL, *et al*. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (5th Ed). Lyon, France: International Agency for Research on Cancer, 2016.
2. Xia B, Zhang L, Guo SQ, Li XW, Qu FL, Zhao HF, et al. Coexpression of MYC and BCL-2 predicts prognosis in primary gastrointestinal diffuse large B-cell lymphoma. World J Gastroenterol. 2015; 21(8): 2433.
3. Horn H, Ziepert M, Becher C, Barth TF, Bernd HW, Feller AC, et al. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood. 2013; 121(12): 2253-63.
4. Gandhi M, Petrich Am. Current concepts in double-hit lymphoma. Am J of Hematol/ Oncol. 2016; 12(2):
5. Dodero A, Guidetti A, Tucci A, Barretta F, Novo M, Devizzi L, *et al*. Dose-adjusted EPOCH plus rituximab improves the clinical outcome of young patients affected by double expressor diffuse large B-cell lymphoma. Leukemia. 2019; 33(4):1047-51.
6. Wilson WH, Dunleavy K, Pittaluga S, Hegde U, Grant N, Steinberg SM, *et al*. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. J Clin Oncol. 2008; 26: 2717–24
7. Savage KJ, Karsan A, Slack GW, Toze CL, Sehn LH, Mourad YR, et al. Outcome of patients with double-hit lymphomas treated with CODOX-M/IVAC+ R followed by hematopoietic stem cell transplantation in British Columbia.
8. Mohammed AA, Rashed HE, Abdelrahman AE, Obaya AA, Toam M, Nour HM, et al. C-MYC and BCL2: Correlation between protein over-expression and gene translocation and impact on outcome in diffuse large B cell lymphoma. Asian Pac J Cancer Prev. 2019; 20(5): 1463.
9. Huang S, Nong L, Wang W, Liang L, Zheng Y, Liu J, et al. Prognostic impact of diffuse large B-cell lymphoma with extra copies of MYC, BCL2 and/or BCL6: comparison with double/triple hit lymphoma and double expressor lymphoma. Diagn Pathol. 2019; 14(1): 81.
10. Riedell PA, Smith SM. Double hit and double expressors in lymphoma: Definition and treatment. Cancer. 2018; 124(24): 4622-32.
11. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, *et al*. Concurrent expression of MYC and BCL2 in diffuse large B- cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol. 2012; 30(28): 3452.
12. Naseem M, Asif M, Khadim MT, Ud-Din H, Jamal S, Shoaib I. The frequency of double expresser in selected cases of high grade diffuse large B-cell lymphomas. Asian Pac J Cancer Prev. 2020; 21(4): 1103.
13. Hashmi AA, Iftikhar SN, Nargus G, Ahmed O, Asghar IA, Shirazi UA, *et al*. Double-expressor phenotype (BCL-2/ c-Myc Co-expression) of diffuse large B-Cell lymphoma and its clinicopathological correlation. Cureus. 2021; 13(2): e13155.
14. Huang JZ, Sanger WG, Greiner TC, Staudt LM, Weisenburger DD, Pickering DL, *et al*. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-Cell gene expression profile. Blood. 2002; 99: 2285-90
15. Smith SM. Aggressive B-cell lymphoma: The double-hit and double-expressor phenotypes. Clin Adv Hematol & Oncol. 2017; 15(1): 40-42.
16. Perry AM, Alvarado-Bernal Y, Laurini JA, Smith LM, Slack GW, Tan KL, *et al*. MYC and BCL 2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. Bri J Haematol. 2014; 165(3): 382-91.
17. Valera A, López-Guillermo A, Cardesa-Salzmann T, Climent F, González-Barca E, Mercadal S, *et al*. MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Haematologica. 2013; 98: 1554-62.
18. Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, Balasubramanyam A, *et al*. MYC/ BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: A report from the international DLBCL Rituximab-CHOP consortium program. Blood. 2013; 121: 4021-31.
19. Copie-Bergman C, Gaulard P, Leroy K, Briere J, Baia M, Jais JP, *et al*. Immuno-fluorescence in situ hybridization index predicts survival in patients with diffuse large B-cell lymphoma treated with R-CHOP: A GELA study. J Clin Oncol. 2009; 27: 5573-9.
20. Herrera AF, Song JY, Griffin GK, Nikolaenko L, Mei M, Bedell V, *et al*. Double-hit and double-expressor lymphomas are not associated with an adverse outcome after allogeneic stem cell transplantation. Blood. 2016; 128(22): 830.


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