ORIGINAL ARTICLE

Immunohistochemical Expression of CD10, BCL6 and MUM1 in Differentiating Diffuse Large B Cell Lymphoma Subtypes

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Abstract

Objective: To determine the immunohistochemical expression of CD10, BCL6 and MUM1 in differentiating diffuse large B cell lymphoma subtypes.

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi, from November 2014 to May 2015.

Methodology: Newly diagnosed cases of DLBCL on H&E stain as well as IHC markers, according to WHO blue book 2008, were included in the study. Patients' gender, age and site of lymphoma were noted. DLBCL subtypes (GCB and activated type or non-GCB) were assessed based on IHC expression of CD10, BCL6 and MUM1 and the results were recorded. The data were analyzed by using computer software program SPSS version 20. Descriptive statistics, frequencies and percentages were calculated.

Results: Out of 96 patients, 79 (82%) were male and 17 (18%) were female. Mean age was 54.66 ±16.73 years. Thirty-six (37.5%) cases showed positivity for CD10 and BCL6 both (GCB type), whereas MUM1 was positive in 60 (62.5%) cases (non-GCB type or activated type). A significant statistical association was seen between expression of IHC markers (CD10, BCL6 and MUM1) and DLBCL subtypes (GCB and non-GCB type, $p<0.001$).

Conclusion: In Pakistani population, the frequency of non-GCB type expressing MUM1 is 62.5%, which is quite high as compared to western countries. It needs to be further explored, because it represents high-risk subsets in which alternative strategies for diagnosis and management should be planned.

Key Words: *BCL6. CD10. Diffuse large B cell lymphoma. Immunohistochemical expression. MUM1.*

INTRODUCTION

Lymphomas comprise a group of heterogeneous tumors, basically divided into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Lymphomas constitute about 3.37% of all malignant diseases throughout the world. The incidence of lymphoma in USA is 16.11, in UK is 12.92 and in Japan is 5.48 per 100,000 men and women per year. Many environmental and social factors also contribute in pathogenesis of lymphoma such as viruses, autoimmune disorders, smoking, alcohol, sun exposure and high body mass index.[1] NHLs are further divided into B and T cell lymphomas mainly. B cell NHL comprises about 80-85% and T cell NHL only comprises about 10-15% cases. In Pakistan, NHL incidence is more in northern areas than in southern areas.[2]

Diffuse large B-cell lymphoma (DLBCL) is the most common and aggressive type of NHL consisting of about 30-40% of all newly diagnosed cases of lymphomas.[3,4] DLBCL can be further classified into two subtypes – GCB and non-GCB (activated B cell) types by IHC markers.[5] GCB types have good prognosis and failure-free 3 year survival than non-GCB types.[3] The GCB type DLBCL expresses CD10 and BCL6 while non-GCB type DLBCL expresses MUM1.[3,6] CD10 is a membrane zinc dependent metallo-endopeptidase expressed on membranes of B cells.[7] BCL6 is a zinc finger sequence specific transcriptional factor expressed on nucleus of germinal center B cells.[6] MUM1 is Multiple Myeloma Factor 1 or Interferon Regulatory Factor 4 transcriptional protein expressed on the nucleus of lymphoid cells.[8]

Functionally, the B- and T-cell neoplasms can be divided into three categories: indolent, aggressive and very aggressive lymphomas. In general, indolent lymphomas are not curable; but on the other hand, characterized by a slowly progressive clinical course during which spontaneous remissions sometimes can be observed. The aggressive and very aggressive lymphomas have a more rapid clinical course, but are potentially curable with modern chemotherapy.[4]

Due to marked variation in results and geographical distribution of the disease in various regional and international studies, this classification significantly affects outcome, as GCB types have better prognosis than non-GCB types. It is also helpful in predicting effect of therapy.

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The aim of this study was to determine frequency of GCB and non-GCB types of DLBCL, based on immunohistochemistry (IHC) expression of CD10, BCL6 and MUM1 in Pakistani population.

METHODOLOGY

It was a descriptive cross-sectional type study carried out at Histopathology Department at Armed Forces Institute of Pathology, Rawalpindi, from November 2014 to May 2015. Approval was taken from Institutional Ethical Committee. Sample size was calculated using WHO sample size calculator. Newly diagnosed cases of DLBCL at AFIP, were included by consecutive, non-probability sampling technique, between ages of 15-80 years, irrespective of the histological type and grade of the tumour. Scanty tissue sample, inadequately fixed samples, specimens of patients already on treatment or treated and inconclusive results were excluded from the study.

IHC assays for CD10, BCL6 and MUM1 were done by using Dako's kit, on separate tissue sections as per the manufacturer's guidelines. IHC results were interpreted on high power field objective (40X). Moderate to strong staining of equal to or more than 30% of tumor cells were considered as positive. To reduce bias, results were verified by the supervisor with 25 years plus experience. All details were put into the already formulated proforma.

The collected information was analyzed by using SPSS version 20. The quantitative variable, e.g. age, was presented as mean ± standard deviation. Frequency and the percentage were calculated for age groups, gender, expression of IHC markers (CD10, BCL6 and MUM1) and DLBCL subtypes (GCB and non-GCB type). Effect modifiers like age groups, gender and expression of IHC markers, were controlled through stratification. Post-stratification, Chi-square test was applied to determine the statistical association between IHC expression of markers and DLBCL subtypes. P-value less than or equal to 0.05 was taken as significant.

RESULTS

During the study period, a total of 96 cases of DLBCL were inducted. The distribution of cases according to different age groups, gender, expression of IHC markers (CD10, BCL6 and MUM1) and DLBCL subtypes (GCB and non-GCB type) is summarized in Table I.

The age of patients ranged from 16 to 80 years with a mean age of 54.66 ±16.73 years. Most of the patients were over 50 years of age (59%, n=57). There were 79 (82%) males and 17 (18%) females with a male to female ratio of 4.5:1. Thirty-six (37.5%) cases showed positivity for CD10 and BCL6 both, making DLBCL of GCB type as shown in Figures 1a-d. MUM1 was positive in 60 (62.5%) cases, making non-GCB type of DLBCL as shown in Figure 2a-d.

A significant statistical association was seen between expression of IHC markers (CD10, BCL6 and MUM1) and DLBCL subtypes (GCB and non-GCB type, p< 0.001).

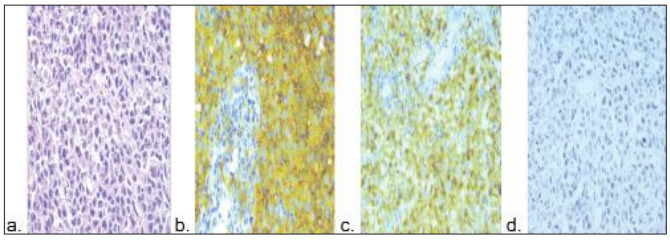

Figure 1: Photomicrographs of GCB Type DLBCL. **(a)** H&E staining (40X), **(b)** CD10 membranous staining, **(c)** BCL6 nuclear staining, **(d)** MUM1 negative staining.

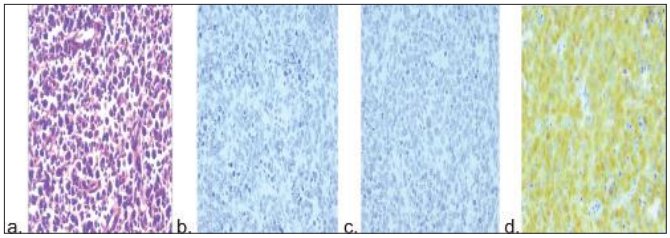

Figure 2: Photomicrographs of Non-GCB Type DLBCL. **(a)** H&E staining (40X), **(b)** CD10 negative staining, **(c)** BCL6 negative staining, **(d)** MUM1 nuclear staining.

Table I: Distribution of cases according to effect modifiers (mean age 54.66±16.73 years, n=96).

Effect modifiers	Cases (n)	Percentage (%)	DLBCL Subtype		P-value
			GCB	Non-GCB	
Age groups (years) (n=96)					
16-50	39	41	18 (46.2%)	21 (53.8%)	0.147
51-80	57	59	18 (31.6%)	39 (68.4%)	
Gender (n=96)					
Male	79	82	31 (39.2%)	48 (60.8%)	0.448
Female	17	18	5 (29.4%)	12 (70.6%)	
Positive IHC (n=96)					
CD10	36	37.5	36 (37.5%)	0	0.000
BCL6	36	37.5	36 (37.5%)	0	
MUM1	60	62.5	0	60 (62.5%)	

DISCUSSION

Distribution of lymphomas shows marked heterogeneity in relation with race, geographical area and their characteristics vary in different regions of the world.[6] In a Chinese study, 124 cases of DLBCL were classified into GCB type 22.1% (n=27), and non-GCB type 78.2% (n=97) using algorithm of Hans *et al*.[9] In the same study, 114 cases of West were classified as GCB 52.6% (n=60) and non-GCB 47.4% (n=54) using algorithm of Hans *et al*.[9] In a Japanese study, GCB type was 46% (n=18), and non-GCB type was 54% (n=21) using Hans algorithm.[10]

The lymphomas and other histopathological diagnoses are correlated with clinical scenario, morphology and immunohistochemical profile for an accurate diagnosis. No single IHC marker is specific for any tumour. Therefore, especially in case of lymphomas, the diagnosis rely with IHC panel and clinical scenario and genetic mutations. Thus, this classification uses all provided and available information to diagnose lymphoma subtypes, including histomorphology, immunophenotype, genetics and clinical history.[11] Treatment decisions are usually depend on clinical scenario and stage, but main decision is based on the histopathological subtype of lymphoma. Hence, the most significant step in deciding the therapy and prediction of benefit of treatment is with correct histopathological diagnosis. Most lymphoma types can have multiple management protocols. However, the treatment of DLBCL subtypes is mainly chemotherapy in combination with drugs against specific receptor at the tumor cells.[12]

Hans algorithm has been widely used to stratify DLBCLs into GCB types and non-GCB types, using immunohistochemical markers CD10, BCL6 and MUM1. This method is helpful in identifying subgroups to predict clinical outcomes in DLBCL patients undergoing frontline therapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or rituximab-CHOP (R-CHOP) chemotherapy.[13]

In this study, majority of the cases has the non-GCB phenotype, i.e. 62.5% and GCB phenotype was found in 37.5% patients. These findings correlate with the study of China and Spain, conducted by Haung *et al*. and Batlle-Lopez *et al*.,[14,15] who reported the percentage of GCB DLBCL 37.75% and 42.27%, respectively. Hwang *et al*. observed similar results in a study conducted in Korea.[16]

In this study, positive CD10 and BCL6 expression was found in 37.5% each and MUM1 was positive in 62.5% cases. These findings are supported by a study conducted by Davies *et al*. who reported 39%, 38% and 65% prevalence of CD10, BCL6 and MUM1, respectively.[17] Moreover, they found 35% GCB type and 65% non-GCB type DLBCLs, comparable to this study.

The authors found significantly different results from studies. Visco *et al*. conducted at USA,[18] and Nyman *et al*. conducted at Oxford UK,[13] reporting only 46.40% and 52% non-GCB types of DLBCLs respectively, using Hans algorithm. However, non-GCB type DLBCL group in our study is 62.5%, which is much more than the above mentioned studies.

Recent studies using comparative genomic hybridization have demonstrated that patients with GCB phenotype express a higher number of distinct chromosomal abnormalities than those with non-GCB phenotype, and these subtypes may, therefore, represent different clinical entities.[18] Now in latest WHO classification of DLBCL in 2016, the above mentioned entities are included. Therefore, this study is very much credible and authentic to consider DLBCL subtypes as GCB and non-GCB (activated type).

CONCLUSION

In Pakistani population, the frequency of GCB type DLBCL expressing CD10 and BCL6 is 37.5%, and non-GCB type DLBCL expressing MUM1 is 62.5%. It is much higher than western countries and needs to be explored, because it represents high-risk subsets in which alternative strategies should be planned. Improved prognostication and the availability of predictive biomarkers will be crucial to allow the possibility of individualized risk-adapted therapy.

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