ORIGINAL ARTICLE

Immunohistochemical Expression of CD-10, BCL-6 and MUM-1 Antibodies and Immediate Clinical Response in Patients of Diffuse Large B-Cell Lymphomas after Six Cycles of Chemotherapy

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Abstract

Objective: To determine the expression of CD-10, BCL-6 and MUM-1 in patients with diffuse large B-cell lymphoma (DLBCL) and its association with immediate clinical response after six cycles of CHOP chemotherapy.

Study Design: Analytical study.

Place and Duration of Study: Armed Forces Institute of Pathology (AFIP), Rawalpindi in collaboration with Nuclear medicine, Oncology and Radiotherapy Institute (NORI), Islamabad from September 2010 to September 2011.

Methodology: CD-10, BCL-6 and MUM-1 antibodies were applied on cases diagnosed as DLBCL. Immediate clinical response was noted after 6 cycles of chemotherapy with the help of oncologist and divided into complete response, partial response, stable disease and relapse/ progression. Patient's age, results of expression of CD-10, BCL-6 and MUM-1 and results of immediate clinical response to chemotherapy were noted. Regarding analysis of prognostic markers (CD-10, BCL-6 and MUM-1), chi-square test was used for immediate clinical response to chemotherapy in DLBCL.

Results: CD-10 was positive in 40% cases, BCL-6 in 58.7% cases and MUM-1 was positive in 46.7% cases. About 41.3% of patients showed complete response, 10.6% partial response, 17.3% stable disease and 30.8% showed relapse/ progression. CD-10 expression in DLBCL was associated with better immediate clinical response ($p = 0.011$) whereas MUM-1 expression in DLBCL was associated with poor immediate clinical response ($p < 0.0001$). However, there was no statistically significant association of BCL-6 with immediate clinical response ($p = 0.22$).

Conclusion: DLBCL shows expression of CD-10, BCL-6 and MUM-1 in nearly fifty percent of the cases. CD-10 is associated with good whereas MUM is associated with poor response. However, there was no association of BCL-6 with immediate clinical response.

Key Words: *Diffuse large B-cell lymphoma. Immunohistochemistry. Immediate clinical response. CHOP chemotherapy.*

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma worldwide representing about 25 to 30% of malignant lymphomas.[1] Although DLBCL is usually considered as a specific category, the diversity in the clinical presentation, morphology, genetics and molecular alterations strongly suggests that these tumors represent a heterogeneous group of neoplasms rather than a single clinicopathological entity.[2] In fact, the biological and clinical heterogeneity of diffuse large B-cell lymphomas (DLBCLs) has already been recognized in World Health Organization (WHO) classifications.[3]

During the last decade, most studies dealing with the heterogeneity of DLBCL have focused on morphologic features, individual protein expression, or molecular alterations. The significance of morphological variants remains controversial. The low reproducibility of histopathologic criteria and the lack of objective immunophenotypic or genetic features have made it impossible to build up new categories on this basis. In this regard, the expression of individual antigens related to different stages of B-cell differentiation, including CD-10, BCL-6 and MUM-1 may help to define groups of DLBCL with different clinical and pathological characteristics.[4]

Different molecular markers in DLBCL have different prognostic significance. CD-10, a neutral endopeptidase is a good prognostic factor. BCL-6, a transcriptional repressor is expressed in 50% of DLBCL and is a good prognostic factor. MUM-1, a transcriptional factor is an unfavourable prognostic factor. CD-10, BCL-6 and MUM-1 are now considered as prognostic markers.

DLBCL manifests a variable response to conventional CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisolone) based regimens. Prognostic markers are thought to play an important role in clinical response to chemotherapy. DLBCL expressing CD-10 and BCL-6 generally gives a better response than DLBCL not expressing these antigens. On the other hand DLBCL expressing MUM-1 depicts poor response.[5,6]

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In 1999, an international working group (IWG) of clinicians, radiologists, and pathologists with expertise in the evaluation and management of patients with non-Hodgkin's lymphoma (NHL) published guidelines for response assessment and outcome measurement. According to this system after completion of six cycles of chemotherapy, immediate clinical response should be determined to look for presence or absence of residual disease. Clinical response is divided into complete response (CR), partial response (PR), stable disease and relapse/progression.[7] Generally speaking patients showing complete or partial response are labeled as responders while patients showing stable disease or relapse/progression are labeled as non responders. These recommendations were adopted rapidly and widely by clinicians and regulatory agencies. Patient is placed in complete response category when there is complete disappearance of all detectable clinical evidence of disease which was present before the start of treatment and in partial response category when there is at least 50% decrease in all detectable clinical evidence of disease. When the patient can neither be assigned complete response and partial response category nor relapse/progression category, then he or she is labeled as having stable disease. By relapse/progression means that there are new sites of involvement or previously involved sites increased in size despite of the treatment.[8]

The objective of this study was to determine the expression of CD-10, BCL-6 and MUM-1 in patients diagnosed as DLBCL and to correlate the expression of these antigens with immediate clinical response after six cycles of CHOP chemotherapy.

METHODOLOGY

The study was conducted at Armed Forces Institute of Pathology (AFIP), Rawalpindi in collaboration with Nuclear-medicine, Oncology and Radiotherapy Institute (NORI), Islamabad, from September 2010 to September 2011. This was a descriptive case series study. Approval of study was taken from Institutional Review Board (IRB) of AFIP and NORI. Informed consent was obtained from all the patients included in this study. A total of 75 pretreatment cases of DLBCL diagnosed on the basis of light microscopy and IHC (by applying CD-20 antibody) during the study period were included. Sampling technique was convenience non-probability. Nodal DLBCL diagnosed by light microscopy and immunohistochemistry (by using anti CD-20 antibody) of all ages and both genders were included. Samples inadequate for light microscopy and immunohistochemistry, primary extranodal DLBCL and patients who died before the start of treatment and during treatment were excluded.

The specimens were collected from pathology department. Each was given a case number and medical record number and demographic details of patients were recorded. The specimen were fixed in 10% buffered neutral formalin. After appropriate gross examination, sections were processed and stained with haematoxylin and eosin (H&E). Microscopic features were noted. Only those cases were included which were diagnosed as DLBCL. Immunohistochemistry markers CD-10, BCL-6 and MUM-1 were applied. Results of immunohistochemistry were noted. Immediate clinical response was assessed with the help of oncologist after six cycles of CHOP chemotherapy. Response was divided into complete response, partial response, stable disease or relapse or progression. Mean and SD were calculated for quantitative variable like patient age. Frequencies and percentages were calculated for qualitative variables like result outcome of IHC for anti CD-10, BCL-6 and MUM-1 and results of immediate clinical response to chemotherapy.

Regarding analysis of prognostic markers, chi-square test was used for clinical response to chemotherapy in DLBCL. The p-value was calculated by using chi-square at 95% confidence interval and considered significant at $p < 0.05$.

RESULTS

A total of 75 cases of DLBCL were included in the study. The mean age was 54.2 ± 15 years. Age ranged from 12 to 80 years. There were 3 (4%) patients in second decade, 4 (5.3%) in third decade, 6 (8%) in fourth decade, 12 (16%) in fifth decade, 23 (30.7%) in sixth decade, 24 (32%) in seventh decade and 3 (4%) in eight decade. A total of 53 (70.7%) patients were males and 22 (29.3%) were females.

CD-10, BCL-6 and MUM-1 were considered positive when more than 30% of the cells were positive (Figures 1 and 2). CD-10 was positive in 30 (40%) cases and negative in 45 (60%) cases. BCL-6 was positive in 44 (58.7%) cases and negative in 31 (41.3%) cases. MUM-1 was positive in 35 (46.7%) cases and negative in 40 (53.3%) cases.

A total of 31 (41.3%) patients showed complete response, 8 (10.6%) partial response, 13 (17.3%) stable disease and 23 (30.8%) showed relapse/progression.

Out of 30 cases, which were CD-10 positive, 18 (60.1%) showed complete response and 4 (13.3%) showed partial response whereas 4 patients each (13.3%) showed stable disease and relapse/progression.

Out of 44 BCL-6 positive cases of DLBCL, 23 cases (52.5%) showed complete response, 3 (6.81%) partial response, 8 (18.7%) stable disease and 10 (22.7%) relapse/progression.

Out of these 35 MUM-1 positive cases of DLBCL, 6 (17.1%) showed complete response, 1 (3%) partial response, 9 (25.7%) stable disease and 19 (54.2%)

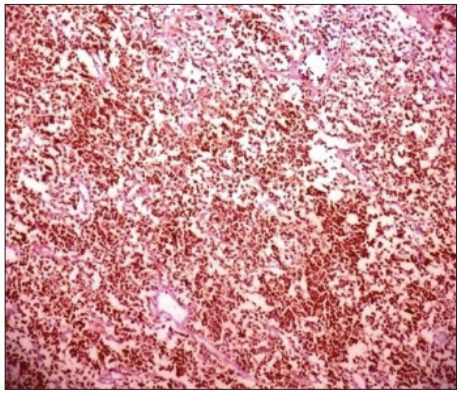

Figure 1: CD-10 positive cells in DLBCL (40x).

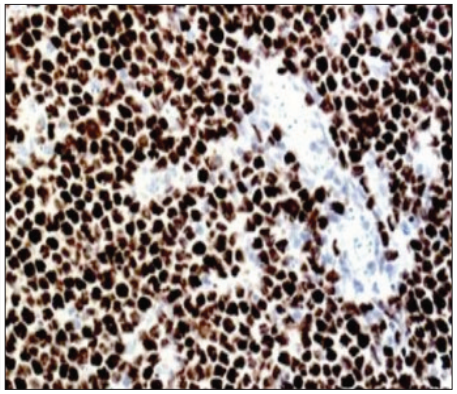

Figure 2: BCL-6 positive cells in DLBCL (200x).

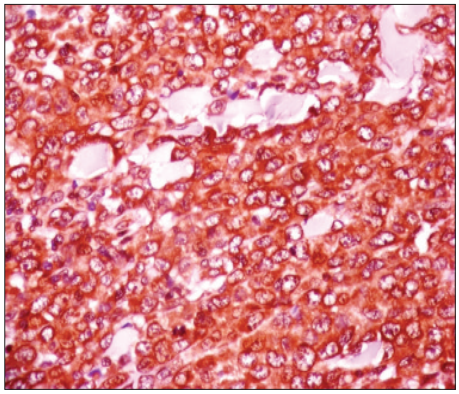

Figure 3: MUM-1 positive cells in DLBCL (400x).

Table I: Comparison of responders and non-responders in CD-10 positive and BCL-6 positive cases of DLBCL.

	CD-10 positive responders (patients showing CR and PR)	CD-10 positive non-responders (patients showing stable disease and relapse/progression)	p-value
Total patients (30)	22 (73.33%)	8 (26.67%)	0.011
	BCL-6 positive responders (patients showing CR and PR)	BCL-6 positive DLBCL (patients showing stable disease and relapse/progression)	p-value
Total patients (44)	26 (59.09%)	18 (40.91%)	0.22

CR = Complete response, PR = Partial response.

Table II: Comparison of post-responders and non-responders in MUM-1 positive DLBCL patients.

	MUM-1 positive responders (patients showing CR and PR)	MUM-1 positive non-responders (patients showing stable disease and relapse/progression)	p-value
Total patients (35)	7 (20%)	28 (80%)	< 0.0001

CR = Complete response, PR = Partial response.

relapse/progression. Out of these 40 MUM-1 negative cases, 24 (60%) showed complete response, 6 (15%) partial response, 6 (15%) stable disease and 4 (10%) relapse/progression.

Out of 3 patients in second decade, 2 (66.6%) showed complete response and 1 (33.4%) stable disease. There was no patient in category of partial response or relapse/progression. Out of a total of 4 patients in third decade, 2 (50%) showed complete response and 1 (25%) each showed partial response and stable disease respectively. In the fourth decade, 3 (50%) showed complete response and 3 (50%) stable disease. There were 12 patients in fifth decade. Out of these 12, 6 (50%) fell in the category of complete response, 1 (8.5%) partial response, 3 (25.5%) stable disease and 2 (16%) relapse/progression. A total of 23 patients were in 6th decade, out of which 11 (48%) showed complete response, 4 (17.3%) stable disease and 8 (34.7%) relapse/progression. In seventh decade, out of 24 patients, 7 (29.2%) showed complete response, 3 (12.5%) partial response, 2 (8.3%) stable disease and 12 (50%) relapse/progression. There were only 3 cases in 8th decade, out of which 2 (67%) showed partial response and 1 (33%) relapse/progression.

Out of 53 males, 23 (43.3%) showed complete response, 2 (3.7%) partial response, 8 (15.5%) stable disease and 20 (37.5%) relapse/progression. On the other hand, out of 22 females, 8 (36.3%) showed complete response, 6 (27.2%) partial response, 5 (22.7%) stable disease and 3 (13.8%) relapse/progression.

Chi-square test was calculated and applied by keeping confidence interval at 95%. When we compared CD-10 positive responders with CD-10 positive non-responders, CD-10 expression in DLBCL was found associated with better immediate clinical response ($p = 0.011$, Table I). On the other hand, on comparing MUM-1 positive non-responders with MUM-1 positive responders, MUM-1 expression in DLBCL was found associated with poor immediate clinical response ($p < 0.0001$, Table II). Although more than half of BCL-6 positive cases were responders, we could not find any statistically significant association of BCL-6 with immediate clinical response ($p = 0.22$, Table I).

DISCUSSION

DLBCL is the most common lymphoid malignancy in adults accounting for 40% of all non-Hodgkin's lymphomas.[1] The percentage is higher in developing countries.[9,10] DLBCL has been a salient component of all lymphoma classification systems starting from Rappaport (1966)[11] classification to the latest WHO

classification of lymphoma (2008).[3] In current WHO classification of 2008 for non-Hodgkin lymphoma,[3] DLBCL has again been acknowledged as a heterogeneous group of neoplasm and trend is to split various types of DLBCL according to their clinical behaviour. After the real classification, the usual practice has been to diagnose DLBCL on the basis of morphological features and later on by confirming the B-cell lineage by applying CD-20 antibody. After diagnosis, routine treatment is started in the form of chemotherapy. Soon it was established in the patients of DLBCL that despite of having the same age, gender, stage and undergoing the same chemotherapy regimen the patients respond differently.[4,12] In order to standardize the clinical response and to predict the accurate long-term survival, International Prognostic Index (IPI) was devised. This included age, stage, serum LDH levels and performance status. Although a good standardization level was attained regarding long-term overall survival but results were not very encouraging regarding immediate clinical response. This stimulated the search to find out some factors associated with immediate clinical response. In this effort some genetic factors of prognostic importance were discovered. Although there were quite many genetic factors which had prognostic importance but the factors which are important from prognostic point of view are CD-10, BCL-6 and MUM-1. These factors are also known as IPI independent prognostic factors because when these are used alone without using the variables of IPI, the results are even more promising.[13]

Treatment is usually given in the form of 6 cycles of CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisolone) therapy one cycle each after 2 weeks. Although there is a recent addition in the treatment of DLBCL of CHOP-R (Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisolone - Rituximab) which is more efficient than CHOP regimen but it is very expensive.[14] As it costs about 12 lac Pakistani rupees for 6 cycles, majority of the patients in our setup cannot afford it. So still CHOP is the standard treatment of choice in most of the centres of this country.

Although DLBCL can be diagnosed in any age group, it is more common in 5th and 6th decade. The mean age in this study was 54.2 ± 15 years (Mean ± SD) with age range of 12 - 80 years. The results were comparable to studies by Mushtaq *et al*.[15], Jamal *et al*.[16], Veelken *et al*.[17] and Oh *et al*.[18] in which mean ages were 58 years (20 - 75 years), 55 years (19 - 78 years), 59 years (19 - 83 years) and 57 years (23 - 83 years) respectively. Other studies reveal comparatively higher mean ages. In studies by de Jong *et al*.[19], Fu *et al*.[20] and Sjo *et al*.[21] mean ages were 68 (22 - 93 years), 63 (14 - 90 years) and 65 (29 - 95 years) years respectively. Most of the patients in our study were in 6th and 7th decades (63%). The results were comparable with most of the above mentioned studies in which the most frequent decades were 6th and 7th decades. Generally, the paediatric DLBCL responds better to chemotherapy as compared to adult age group. Age is one of the prognostic factors in a sense that the patients above the age of 60 years generally show overall poor survival.

Globally, DLBCL is more common in males as compared to females. Similar trend was seen in this study in which 70.7% of patients were males and 29.3% were females. The results were comparable to the studies of Mushtaq *et al*.[15], Jamal *et al*.[16], de Jong *et al*.[19], Oh *et al*.[18] and Sjo *et al*.[21] in which males comprised 68%, 70%, 60%, 59% and 65% of the patients respectively. Few other studies like that of Borovecki *et al*.[22] and Veelken *et al*.[17] generally showed equal distribution of gender. It was noted that studies done on larger sample size showed male predominance whereas few studies which showed female predominance were done on comparatively lower sample size. As male gender predominance was noted in studies described above representing different ethnic groups so it can be said that there is a global trend of more males being diagnosed as DLBCL.

Antigens of prognostic importance against which immunohistochemistry antibodies were used are CD-10, BCL-6 and MUM-1. CD-10 is a metalloendopeptidase. It is expressed on surface of wide variety of normal and neoplastic cells. It is very frequently exposed on surface of cells of germinal centre origin.[23] Generally, its expression is associated with good prognosis.[24] CD-10 was positive in 40% of cases and negative in 60% of cases in this study. The results were almost similar to the study of Sjo *et al*.[21] which revealed 41% positive expression of CD-10. All other studies revealed low CD-10 expression i.e. studies of de Jong *et al*.[19], Oh *et al*.[18], Veelken *et al*.[17], Borovecki *et al*.[22], Wagner *et al*.[25] and Saad *et al*. revealed CD-10 expression in 28%, 22%, 20%, 19%, 30% and 30% respectively.

BCL-6 is a zinc finger protein. It is a transcriptional repressor.[23] Its expression is thought to be necessary for formation of germinal centres. Like CD-10, its expression is generally associated with good prognosis and good immediate clinical response. In this study, BCL-6 expression was seen in 58.7% of the cases. Similar and high expressions were found in the study by de Jong *et al*.[19] (56%), Sjo *et al*.[21] (65%) and Wagner *et al*.[25] (73%). Low BCL-6 expression was noted in studies by Oh *et al*.[18] (39%), Veelken *et al*.[17] (28%), Borovecki *et al*.[22] (47%) and Saad *et al*. (48%)[22].

MUM-1 is lymphoid specific member of the interferon regulatory factor family of transcription factors.[24] It is normally expressed in plasma cells and is a potential marker of post GC cells. Many studies have shown that its expression is associated with a worse overall survival and clinical response. MUM-1 was expressed in 47% of the cases in this study. Similar or higher expression was noted in studies by de Jong *et al*.[19] (47%), Sjo *et al*.[21]

(54%), Wagner *et al*.[25] (80%) and Borovecki *et al*.[22] (94%). Lower expression was noted in studies by Veelken *et al*.[17] (30%), Oh *et al*.[18] (31%) and Saad *et al*. (32%)[26].

Most CD-10 positive DLBCL showed response either as complete response (60.1%) or partial response (13.3%). Remaining patients showed no response either as stable disease (13.3%) or relapse/progression (13.3%). On the other hand, very few CD-10 negative DLBCL patients showed response either in the form of complete response or partial response. CD-10 expression in DLBCL group was associated with better clinical response (p 0.011). Few other studies also revealed significant results of CD-10 regarding immediate clinical response i.e. studies by de Jong *et al*.[19] (p 0.019), Oh *et al*.[18] (p 0.09), Saad *et al*.[26] (p 0.007) and Seki *et al*.[26] (p 0.022). Non-significant results were obtained in studies of Sjo *et al*.[21], Veelken *et al*.[17] (p 0.7) and Borovecki *et al*.[22] (p 0.146). So according to this study CD-10 expression was associated with better clinical response.

More than half of the BCL-6 positive DLBCL patients showed response in the form of complete response or partial response. Although more BCL-6 positive cases showed better response, still the association of positive expression of BCL-6 with immediate clinical response was not significant (p 0.22). BCL-6 expression was associated with better clinical response in studies by de Jong *et al*.[14] (p 0.013), Lene *et al*.[21] (p 0.003), Borovecki *et al*.[22] (p 0.030), Saad *et al*. (p 0.007) and Seki *et al*[26]. (p 0.021). On the other hand, results similar to this study were seen in studies by Oh *et al*.[18] (p 0.25) and Veelken *et al*.[17] (p 0.3).

Majority of MUM-1 positive DLBCL showed no response either as stable disease (25.7%) or relapse/progression (54.2%). Opposite was true for MUM-1 negative cases, majority of which showed response either as complete response (60%) or partial response (15%). MUM-1 expression in DLBCL group was associated with poor immediate clinical response ($p < 0.0001$) in this study and studies by de Jong *et al*.[19] (p 0.003) and Seki *et al*.[26] (p 0.011). Most of the studies showed no significant results. The latter included studies by Sjo *et al*.[21], Oh *et al*.[18] (p 0.5), Veelken *et al*.[17] (p 0.9), Borovecki *et al*.[22] (p 0.513) and Saad *et al*. (p 0.9). So the MUM-1 positive cases were associated with poor immediate clinical response in this study.

CONCLUSION

DLBCL shows expression of CD-10, BCL-6 and MUM-1 in nearly fifty percent of the cases. CD-10 is associated with good whereas MUM is associated with poor response. However, we did not find any association of BCL-6 with immediate clinical response.

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