RESEARCH COMMUNICATION

Prognostic Sub-Grouping of Diffuse Large B-Cell Lymphomas into Germinal Centre And Post Germinal Centre Groups by Immunohistochemistry after 6 Cycles of Chemotherapy

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Abstract

Introduction: Diffuse large B-cell lymphomas (DLBCL) can be divided into germinal centre (GC-DLBCL) and post germinal centre (post GC-DLBCL) groups by applying immunohistochemical antibodies. As these subgroups respond differently to chemotherapy, it is possible at diagnosis to select a poor prognostic subgroup for aggressive treatment. Objective: To determine the frequencies of GC-DLBCL and post GC-DLBCL in patients by immunohistochemistry (IHC) and the clinical response after six cycles of chemotherapy. Subjects and Methods: In this descriptive study conducted in AFIP and CMH, Rawalpindi and NORI, Islamabad, from September 2010 to September 2011, a total of 75 pretreatment cases of DLBCL diagnosed during the study period were included. Cases were segregated in to GC-DLBCL and post GC-DLBCL groups according to results of immunohistochemistry markers CD10, BCL6 and MUM1. Immediate clinical response was assessed after 6 cycles of chemotherapy. Response was divided into complete response, partial response, stable disease or relapse or progression. Results: The mean age was 54.2±15. Males were 53 (70.7%). Forty (53.3%) cases comprised the GC-DLBCL group; 25(62.5%) of them showed a complete response. Most patients of the post GC-DLBCL 19(54%) showed relapse/progression. Results of immediate clinical response in both prognostic subgroups were significant ($p<0.05$). Results regarding positivity with immunohistochemical antibodies CD10 (p 0.011), BCL6 (p 0.013) and MUM1 (p 0.000) regarding immediate clinical response were also significant. Conclusion: GC-DLBCL group shows better response to CHOP chemotherapy regimen. Immunohistochemistry should be used to further classify DLBCL as this can enable us to select aggressive group for aggressive treatment. This manuscript is important because the study is the first to becarried out exclusively in Pakistan or our part of the world.

Keywords: Prognostic subgroups - diffuse large B-cell lymphoma - immunohistochemistry - chemotherapy

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma worldwide representing about 25 to 30 percent of malignant lymphomas (Karin, 2006). Prior to being named DLBCL in REAL classification, this was included under several different descriptors in prior classifications. Although REAL classification was subsequently updated as World Health Organization (WHO) classification in 2001, the status of DLBCL remained largely unchanged. In current WHO classification of 2008 (Jaffe et al., 2008) for Non Hodgkin Lymphoma, DLBCL has again been acknowledged as a heterogeneous group of neoplasm and trend is to split various types of DLBCL according their clinical behavior.

It was established soon in the patients of DLBCL that despite of having the same age, gender, stage and undergoing the same chemotherapy regimen the patients responded differently (Lossos et al., 2006; Haarer et al., 2006). In order to standardize the clinical response and to predict the accurate long term survival, International Prognostic Index (IPI) was devised (Sehn et al., 2007). 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 and are also important to classify DLBCL into GC-DLBCL and post GC-DLBCL are CD10, BCL6 and MUM1. These

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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 (Choi et al.,2009).

Immunohistochemistry (IHC) has emerged as an efficient tool because by using CD10, BCL6 and MUM1 antibodies, these antigens can be detected and DLBCL can be divided into GC-DLBCL and post GC-DLBCL groups. 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 (Winter et al., 2006). MUM-1, a transcriptional factor is an unfavorable prognostic factor. By IHC we can identify at diagnosis prognostically more aggressive subgroup of DLBCL for aggressive treatment (Muris et al., 2006). Studies have revealed that both subgroups depict different immediate clinical response when compared with each other. Generally GC-DLBCL responds better to chemotherapy than post GC-DLBCL (Haarer et al., 2006; Choi et al., 2009). Other studies do not show any difference in clinical response (Iliac et al., 2009). 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 (Zaja et al., 2006).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 and also in other under developed countries (Aftab et al., 2006; Abbasi et al., 2010) .

After the completion of treatment immediate clinical response is assessed according to the criteria devised by International Working Formulation group (Cheson et al., 2007; Cheson, 2008). According to this criterion immediate clinical response is stratified into complete response, partial response, stable disease and relapse/ progression. 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 that there are new sites of involvement or previously involved sites increased in size despite of the treatment (Cheson et al., 2007; Cheson, 2008).

In this study cases of nodal DLBCL diagnosed by light microscopy and immunohistochemistry (by using anti CD-20 antibody) have been be selected. DLBCL is divided into GC-DLBCL and post GC-DLBCL by using antibodies to CD-10, BCL-6 and MUM-1. Immediate clinical response of each subgroup to chemotherapy regimen is assessed after six cycles of chemotherapy as patients come for follow up in oncology department of Combined Military Hospital (CMH), Rawalpindi and Nuclear Medicine Oncology and Radiotherapy Institute (NORI), Islamabad. The rationale of this study is to classify DLBCL into GC-DLBCL and post GC-DLBCL groups by immunohistochemistry, to determine frequency of GC-DLBCL and post GC-DLBCL groups among patients diagnosed as DLBCL and to determine which subgroup gives better immediate clinical response to chemotherapy. This study is significant because it represents Pakistani population and biological behavior of DLBCL in this country and there is no such study published from this part of world.

Materials and Methods

Objective

To determine frequency of GC-DLBCL and post GC-DLBCL in patients of DLBCL by immunohistochemistry (IHC) and the immediate clinical response after six cycles of chemotherapy.

Setting, study was conducted at ; Oncology Department, Combined Military Hospital (CMH) and Nuclear Oncology Radiotherapy Institute (NORI), Paksitan.

Duration of study, total duration of study was one year (September 2010 to September 2011)

Sample size, 75 cases

Sampling Technique, it was convenience non probability sampling. All the patients who were diagnosed as DLBCL at histopathology department, AFIP and who underwent CHOP therapy either from NORI, Islamabad or CMH, RWP were included in the study. However, it was strictly taken into the consideration that the patient fulfills the inclusion criteria.

Sample selection:

Inclusion criteria:

- A total of at least 75 pretreatment cases of nodal DLBCL diagnosed by light microscopy and immunohistochemistry (by using anti CD 20 antibody).
- All ages and both genders.

Exclusion criteria:

- Samples inadequate for light microscopy and immunohistochemistry.
- Primary extranodal DLBCL.
- Patients who died before the start of treatment and during treatment.

Study Design, it was a descriptive study. Patients diagnosed as DLBCL were segregated into GC-DLBCL and post GC-DLBCL by applying immunohistochemistry antibodies. Afterwards with the help of oncologist the immediate clinical response was assessed according to criteria of International working group after six cycles of chemotherapy (no special treatment was given. Only those patients were included who undergo routine treatment of DLBCL).

Data Collection, approval of ethical committee of AFIP was taken. A total of 75 cases of DLBCL were included in the study by following strictly inclusion and exclusion criteria. After receiving the lymph node specimen, it was

sliced and kept for fixation in 10% buffered formalin for 24 hours and then processed. DLBCLs were diagnosed keeping in view the diffuse effacement of lymph node architecture by large monomorphic population of large atypical lymphoid cells. Afterwards immunostain for CD20 was applied and cases were labeled as DLBCL if cells were positive for CD20 antibody.

For prognostic sub grouping of DLBCL, three immunohistochemistry markers, CD10, BCL-6 and MUM1 were used. After immunohistochemistry process was completed slides were ready for result interpretation. CD10, BCL6 and MUM1 were labeled as positive if at least more than 30% of cells were positive for the respective antibodies. Cases of DLBCL were sub grouped as GC-DLBCL and post GC-DLBCL according to the results of immunohistochemistry. DLBCL was labeled as GC-DLBCL if either only CD10 was positive or both CD10 and BCL-6 were positive. If DLBCL was only positive for MUM1 and negative for both CD10 and BCL-6, it was called as post GC-DLBCL. If on the other hand CD10 was negative and BCL-6 was positive, then results of MUM1 were carefully interpreted. If MUM1 was positive, the case was labeled as post GC-DLBCL and if MUM1 was negative, case was labeled as GC-DLBCL.

Only those patients were included which received CHOP chemotherapy, as CHOP-R is given to much selected patients because it is very expensive and it is impossible to carry out study on CHOP-R in limited time period. Patients were followed up throughout the treatment through their treatment files and at the end of six cycles of chemotherapy (3 months), their treatment files were retrieved from the record and immediate clinical response was noted with the help of consultant oncologists of the oncology centres. Patient's clinical response was divided into complete response, partial response, stable disease and relapse/progression. Patients showing complete response (CR) or partial response (PR) were considered responders. Likewise patients showing stable disease (SD) or relapse/progression were considered non-responders. This response was calculated by oncologists keeping in view the response criteria devised by international working group and as also discussed in introduction.

Data Analysis, statistical analysis was done using SPSS version 14.0. Mean and SD was calculated for quantitative variables like, patients age. Frequencies and percentages were calculated for qualitative variables like subgroup of DLBCL (GC-DLBCL or post GC-DLBCL), 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 GC-DLBCL and post GC-DLBCL subgroups. p-value was calculated by using chi-square at 95% confidence interval.

Results

A total of 75 cases of DLBCL were included in the study. To begin with 120 cases were included but 25 patients died before the start of treatment, 10 patients died during treatment and 10 did not undergo treatment. The mean age of patients of DLBCL was 54.2 ±15 years (Mean ±SD). The age ranged from 12 to 80 years. Distribution of patients in various decades was as follows: 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. Most of the patients 62 (82.6%) were above the age of 40 years. Most frequent decade was seventh decade (32%) followed by sixth decade (30.7%).

A total of 53 (70.7%) patients were males and 22 (29.3%) were females. Most of the patients in both the

Table 1. Immediate Clinical Response in Both Prognostic Subgroups of DLBCL

Immediate clinica Response	GC-DLBCL	Post GC-DLBCL	P values
Complete response	25 (62.5%)	6 (18%)	0.001
Partial response	7 (17.5%)	1 (3%)	0.034
Stable disease	4 (10%)	9 (25%)	0.109
Relapse/Progression	4 (10%)	19 (54%)	0.003
Total	40	35	

Table 2. Relationship of Immunohistochemistry Results (IHC) with Immediate Clinical Response

Groups	CD10		BCL6		MUM1	
	Ve+	Ve-	Ve+	Ve-	Ve+	Ve-
GC-DLBCL subgroups:						
CR	18 (60%)	6 (60%)	20 (63%)	4(50%)	0	24(60%)
PR	4 (13%)	2 (20%)	3 (9.4%)	3(38%)	0	6 (15%)
SD	4 (13%)	2 (20%)	6 (19%)	0	0	6 (15%)
Relapse/progression	4 (13%)	0	3 (9.4%)	1(13%)	0	4 (10%)
Total	30	10	32	8	0	40
Post GC-DLBCL subgroups:						
CR	0	6 (17%)	3(25%)	3(13%)	6(17%)	0
PR	0	1(2.9%)	0	1(4.4%)	1(3%)	0
SD	0	9(26%)	2(17%)	7 (31%)	9(26%)	0
Relapse/progression	0	19(54%)	7(58%)	12(52%)	19(54%)	0
Total	0	35	12	23	35	0

Table 3. Comparison of GC-DLBCL Responders with Post GC-DLBCL Responders

GC-DLBCL	Post GC-DLBCL	p-value
Total no. of patients		0.000
40	35	
Total number of responders (patients showing CR and PR)		
32	7	
Total number of responders (patients showing CR and PR)		
32	7	

Table 4. Comparison of GC-DLBCL Responders and Non Responders

	Responders[1]	Non Responders[2]	p-value
CD10 positive GC-DLBCL:			
Total patients (30)	22	8	(0.011)
BCL6 positive GC-DLBCL:			
Total patients (32)	23	9	(0.013)
MUM1 positive post GC-DLBCL:			
Total patients (35)	7	28	(0.000)

[1]patients showing CR and PR, [2] patients showing SD and relapse/progression)

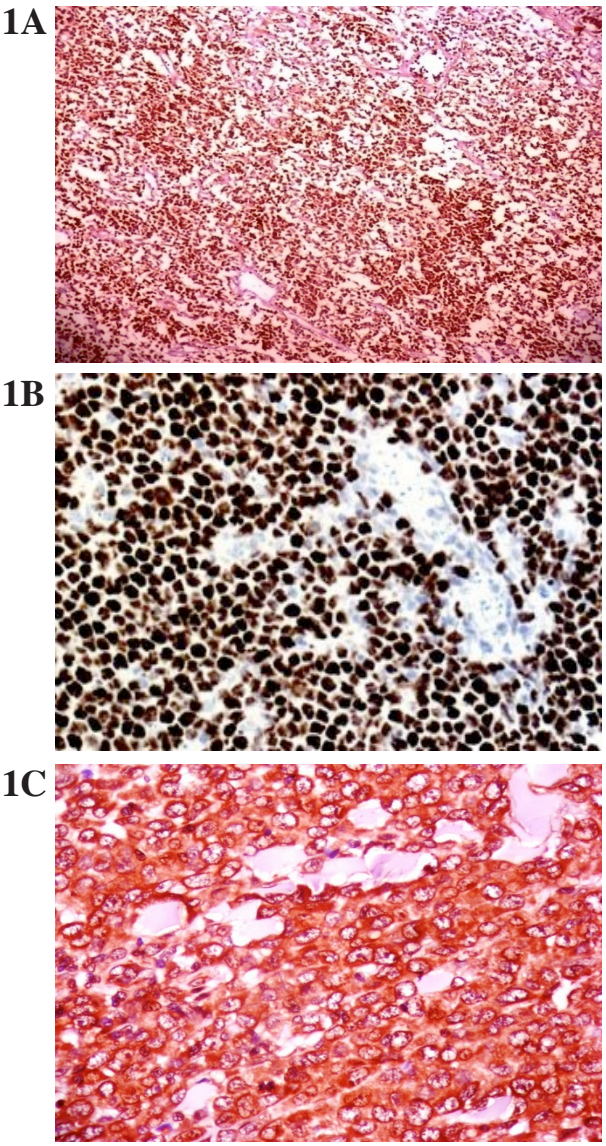

Figure 1. 1A: CD10 Positive Cells in GC – DLBCL, 1B: BCL6 Positive Cells in GC-DLBCL, 1C: MUM1 Positive Cells in Post GC-DLBCL.

genders were above the age of 40 years. Out of 53 male patients 45 (85%) were above the age of 40 years and out of 22 female patients, 17 (77.2%) were above the age of 40 years.

CD10, BCL6 and MUM1 were considered positive when more than 30% of the cells were positive (Fig1). CD10 was positive in 30 (40%) cases and negative in 45 (60%) cases. BCL6 was positive in 44 (58.7%) cases and negative in 31 (41.3%) cases. MUM1 was positive in 35 (46.7%) cases and negative in 40 (53.3%) cases.

On the basis of results of immunohistochemistry markers, DLBCL was divided in GC-DLBCL and post GC-DLBCL. A total of 40 (53.3%) cases belonged to GC-DLBCL group and 35 (46.7%) cases to post GC-DLBCL group.

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 40 patients of GC-DLBCL group 25 (62.5%) showed complete response, 7 (17.5%) partial response, 4 (10%) stable disease and 4 (10%) relapse/progression. Out of 35 cases of post GC-DLBCL group, 6 (18%) showed complete response, 1 (3%) partial response, 9 (25%) stable disease and 19 (54%) relapse/progression (Table 1).

Out of 30 cases which were CD10 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. A total of 45 cases of DLBCL were negative for CD10. Out of ten CD10 negative GC-DLBCL cases, 6 (60%) showed complete response,2 (20%) partial response and 2 (20%) stable disease. There was no patient in relapse/progression. Out of 35 CD10 negative cases which belonged to post GC-DLBCL, 6 (17.1%) showed complete response, 1 (2.9%) partial response, 9 (25.7%) stable disease and 19 (54.3%) relapse/progression (Table 2a and 2b).

Out of 44 BCL6 positive cases of DLBCL,32 (72.7%) cases belonged to GC-DLBCL and 12 (27.3%) to post GC-DLBCL. 20 cases (62.5%) of GC-DLBCL which were Bcl6 positive showed complete response, 3 (9.4%) partial response, 6 (18.7%) stable disease and 3 (9.4%) relapse/progression. Three cases (25%) of post GC-DLBCL which were Bcl6 positive showed complete response, 2 (16.7%) stable disease and 7 (58.3%) relapse/progression. Out of 31 BCL6 negative cases, 8 (26%) belonged to GC-DLBCL and 23 (74%) to post GC-DLBCL. A total of 4(50%) GC-DLBCL patients which were BCL6 negative showed complete response, 3 (37.5%) partial response and 1 (12.5%) relapse/progression. Three cases (13%) of post GC-DLBCL which were BCL6 negative showed complete response, 1(4.4%) partial response, 7 (30.5%) stable disease and 12 (52.1%) relapse/progression (Table 2a and 2b).

All 35 MUM1 positive cases belonged to post GC-DLBCL. Out of these 35, 6 (17.1%) showed complete response, 1 (3%) partial response, 9 (25.7%) stable disease and 19 (54.2%) relapse/progression. All 40 MUM1 negative cases belonged to GC-DLBCL. Out of these 40, 24 (60%) showed complete response, 6 (15%) partial response, 6 (15%) stable disease and 4 (10%) relapse/progression (Table 2a and 2b).

Chi square test was calculated and applied by keeping confidence interval at 95%. With regard to immediate clinical response, the later was better in GC-DLBCL group (80%) as compared to post GC-DLBCL (21%), when responders (patients showing complete response and partial response were labeled as responders) of both groups were compared (p 0.000) as shown in table 3. CD10 expression in GC-DLBCL group was associated with better immediate clinical response (p 0.011) as shown in table 4. Likewise BCL6 expression in GC-DLBCL group was also associated with better clinical response (p 0.013) as shown in table 5. On the other hand MUM1 expression in post GC-DLBCL group was associated with poor immediate clinical response (p 0.000) as shown in table 6.

Discussion

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma worldwide representing

about 25 to 30 percent of malignant lymphomas (Karin, 2006). 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 (Xu et al., 2006). In fact, the biological and clinical heterogeneity of diffuse large B-cell lymphomas (DLBCLs) has already been recognized in World Health Organization (WHO) classifications (Jaffe et al., 2008).

Although DLBCL can be diagnosed in any age group, it is more common in 5th and sixth decade. The mean age in our study was 54.2±15 yrs (Mean±SD) with age range of 12-80 years. The results were comparable to many studies (Jamal et al., 2006; Oh et al., 2006; Veelken et al., 2007; Mushtaq et al., 2008) 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. Age is one of the prognostic factors in a sense that the patients above the age of 60 years generally show poor overall survival. This was established based on studies which used IPI as the index to predict overall survival in patients of DLBCL after chemotherapy. There is a difference between long term overall survival and immediate clinical response. Since we have assessed immediate clinical response in this study, so age does not seem to influence the results of immediate clinical response once DLBCL is divided into its prognostic subgroups based on immunohistochemistry results of CD10, BCL6 and MUM1. This is because of the fact that prognostic subgroups of DLBCL are IPI independent factors (Veelken et al., 2007; Sjö et al., 2007). Patients of all age groups belonging to GC-DLBCL group generally show good response to chemotherapy as compared to those patients which belong to post GC-DLBCL (Sjö et al., 2007). The latter findings were obtained irrespective of the age whether above or below the age of 60 years. Even within the same age group patients belonging to either GC-DLBCL or post GC-DLBCL respond differently (Sjö et al., 2007). This is very much evident from our study. Out of 25 patients belonging to second through fifth decades, 14 (56%) belonged to GC-DLBCL and out of the latter 10 (71%) showed complete response, 1(7%) partial response and 3 (22%) stable disease. Likewise out of 11 patients which belonged to post GC-DLBCL, only 3 (27%) showed complete response and 1 (9%) showed partial response whereas 5 (45%) patients showed stable disease and 2 (19%) relapse/progression. Similar results were achieved in patients belonging to sixth, seventh and eight decades in which patients belonging to GC-DLBCL showed better response as compared to post GC-DLBCL. The results were comparable to the studies of Veelken et al., 2007; Borovecki et al., 2008; Fu et al., 2008; De Jong et al., 2009 in which ages did not seem to contribute significantly to prognosis once DLBCL was divided in GC-DLBCL or post GC-DLBCL by immunohistochemistry. Most of the patients in both subgroups of DLBCL belonged to 6th and 7th decades. Similar results were obtained in other studies (Fu et al., 2008; De Jonget al., 2009). So there seems no difference between either of the prognostic subgroups regarding age.

Globally DLBCL is more common in males as compared to females. Similar trend was seen in our study in which 70.7% of patients were males and 29.3% were females. The results were comparable to the studies of Jamal et al.,(2006); Oh et al.,(2006), Sjö et al.,(2007) Mushtaq et al.,(2008); de Jong et al., (2009) in which males comprised 68%, 70%, 60%, 59% and 65% of the patients respectively. As male gender predominance was noted in studies described above (Oh et al., 2006; Sjö et al., 2007; Fu et al.,2008) representing different ethnic groups so it can be said that there is a global trend of more males being diagnosed as DLBCL(Sjö et al., 2007). There was no significant difference in both genders in terms of their division into GC-DLBCL and post GC-DLBCL groups. The results were comparable to the studies of Saad et al., 2010, Fu et al., 2008. Generally there is no significant difference in clinical response between both genders. In our study most of the patients in both the genders were above the age of 40 years and a little more than half of the patients in both genders belonged to GC-DLBCL, however the difference was not large enough.

Antigens of prognostic importance against which immunohistochemistry antibodies were used are CD10, BCL6 and MUM1. CD10 was positive in 40% of cases and negative in 60% of cases in our study. The results were almost similar to the study of Lene et al which revealed 41% positive expression of CD10 (Sjö et al., 2007). All other studies (Oh et al., 2006; Veelken et al., 2007; Wagner et al., 2007; Borovecki et al., 2008; Fu et al., 2008; De Jong et al., 2009; Saad et al.,2010) revealed lower percentages of CD10 expression in 19%, 28%, 22%, 20%, 19%, 30% and 30% of cases respectively.

BCL6 expression is considered necessary for formation of germinal centres. Like CD10, its expression is generally associated with good prognosis and good immediate clinical response. In our study BCL 6 expression was seen in 58.7% of the cases. Similar and high expressions were found in international studies [Sjö et al., 2007 (65%) ;Wagner et al., 2007 (73%); De Jong et al., 2009 (56%)]. Low BCL6 expression was noted in other studies [Oh et al., (2006) (39%) ; Veelken et al., (2007) (28%) ; Borovecki et al., (2008) (47%); Fu et al., (2008) (23%) ; Saad et al., (2010) (48%)].

MUM1 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 (Muris et al., 2006). MUM1 was expressed in 47% of the cases in our study. Similar or higher expression was noted in studies by Sjö et al.,(2007) (54%) ; Wagner et al.,(2007) (80%) ; Borovecki et al.,(2008) (94%); Fu et al.,(2008) (50%); De Jong et al.,(2009) (47%). Lower expression was noted in studies by Oh et al.,(2006) (31%) ; Veelken et al.,(2007) (30%) ; Saad et al.,(2010) (32%).

On the basis of IHC results DLBCL cases were divided into GC-DLBCL and post GC-DLBCL.GC-DLBCL was the most frequent group in our study (53.3%). GC-DLBCL was the most frequent group in studies by Fu et al.,(2008) (53%); Saad et al.,(2010) (52%) . On the other hand post GC-DLBCL was the most frequent group in

studies by Oh et al.,(2006) (58%); Veelken et al.,(2007) (66.7%); Wagner SD et al.,(2007) (63%); Borovecki et al.,(2008) (81%); De Jong D et al.,(2009) (58%); Seki et al.,(2009) (51.8%). There is no specific reason known for this different distribution of prognostic subgroups. It may be due to different genetic makeup and specific genetic aberrations pertaining to different populations.

Most CD10 positive DLBCL which belonged to GC-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 CD10 negative GC-DLBCL patients showed response either in the form of complete response or partial response. CD10 expression in GC-DLBCL group was associated with better clinical response (p 0.011). Few other studies also revealed significant results of CD10 regarding immediate clinical response i.e. studies by Oh et al.,(2006) (p 0.09); De Jong et al.,(2009) (p 0.019); Seki et al.,(2009) (p 0.022); Saad et al.,(2010) (p 0.007). Non significant results were obtained in studies of Sjö et al., (2007); Veelken et al., (2007) (p 0.7); Borovecki et al.,(2008) (p 0.146); Fu et al., (2008). According to our study CD10 expression was associated with better clinical response.

Most of the BCL6 positive GC-DLBCL patients showed response in the form of complete response or partial response. Majority of the BCL6 positive post GC-DLBCL showed no response. Bcl6 expression in GC-DLBCL group was associated with better clinical response (p 0.013) in our study and studies by Saad et al.,(2010) (p 0.007); Sjö et al.,(2007) (p 0.003); Borovecki et al.,(2008) (p 0.030); Malumbres et al.,(2008) (p 0.01); De Jong et al.,(2009) (p 0.013); Seki et al., (2009) (p 0.021). According to our study expression of BCL6, like CD10 was associated with good clinical response.

All MUM1 positive cases belonged to post GC-DLBCL group. Majority of MUM1 positive DLBCL showed no response either as stable disease (25.7%) or relapse/progression (54.2%). MUM1 expression in post GC-DLBCL group was associated with poor immediate clinical response (p 0.000) in our study and studies by De Jong et al., (2009) (p 0.003); Seki et al.,(2009) (p 0.011). Most of the studies showed non significant results. The latter included studies by Oh et al., (2006) (p 0.5); Sjö et al., (2007); Veelken et al., (2007) (p 0.9); Borovecki et al.,(2008) (p 0.513); Fu et al., (2008); Saad et al.,(2010) (p 0.9). So the MUM1 positive cases were associated with poor immediate clinical response in our study.

Majority of GC-DLBCL patients showed complete response (62.5%), followed by partial response (17.5%), whereas stable disease and relapse/progression was seen in 10% of the patients each. On the other hand majority of post GC-DLBCL patients showed relapse/progression (54%) and stable disease (25%). Only 18% showed complete response and 3% partial response. With regard to immediate clinical response, the later was better in GC-DLBCL group (80%) as compared to post GC-DLBCL (21%), when responders (patients showing complete response and partial response were labeled as responders) of both groups were compared (p 0.000). Significant results were also obtained in study by Haarer et al., (2006) (p 0.03); Oh et al., (2006) (p 0.048); Sjö et al.,(2007) (p 0.020); Rimsza et al.,(2008) (p <0.05), Choi et al.,(2009) (p 0.001); De Jong et al., (2009) (p 0.007); Seki et al.,(2009) (p 0.05); Saad et al., (2010) (p 0.007). On the other hand non significant results were obtained in studies by Borovecki et al., (2008) (p 0.146); Fu et al., (2008) & Veelken et al.,(2007) (p 0.71). So according to our study, GC-DLBCL gives a better immediate clinical response to chemotherapy as compared to post GC-DLBCL. So results of this study proves the hypothesis as the DLBCLs have variable expression of prognostic markers (CD10, BCL6 and MUM1) which divide them into GC-DLBCL and post GC-DLBCL and the former group (GC-DLBCL) has better immediate clinical response as compared to post GC-DLBCL.

This study is significant because it represents Pakistani population and biological behavior of DLBCL in this country and there is no such study published from this part of world.

In conclusion, there is not much difference in frequency of both GC–DLBCL and post GC-DLBCL subgroups. GC-DLBCL group shows better response as compared to post GC-DLBCL. DLBCLs have variable immunohistochemical expression of prognostic markers which divide them into further subgroups (GC-DLBCL and post GC-DLBCL). Immunohistochemistry should be used to further classify DLBCL into GC-DLBCL and post GC-DLBCL as this classification can enable us to select aggressive group of DLBCL for aggressive treatment in future.

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