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Original Article

Immunohistochemical Expression of BCL6 in Nodal and Extranodal Cases of Diffuse Large B Cell Lymphoma-A Study in a Tertiary Care Hospital in Islamabad

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ABSTRACT

Background: Diffuse Large B Cell Lymphoma (DLBCL) is a common non Hodgkin lymphoma with a high mortality rate. BCL6 is the common oncogene that is mutated in DLBCL. Special therapeutic agents have been designed to target the mutated BCL6 gene. Therefore, DLBCL is a curable malignancy, and patients who are positive for BCL6 gene expression have better prognosis and survival, as they benefit from targeted therapy against BCL6 mutations.

Objectives: To determine BCL6 expression in DLBCL in nodal and extranodal cases.

Material & Methods: This descriptive cross-sectional study was carried out in the Pathology department of Pakistan Institute of Medical Sciences Islamabad. Duration of study was 3 years and 2 months, from 1st October 2014 to December 2017. Lymph node biopsies and surgical specimens with clinical suspicion of a mass were processed through various steps; slides were prepared and stained with H&E. A comprehensive panel of antibodies including LCA, Pan B (CD-20), Pan T (CD-3), Ki-1 (CD- 30), (CD 15), (CD 10), (BCL 2), and MIB-1 (Ki-67) was applied on cases with morphology of lymphoproliferative disease. LCA, CD20 positive cases, and those cases with Ki 67 of more than 40% were selected for BCL6 protein expression Immuno-staining with BCL6 was done after numerous successive steps. Immuno-expression of BCL6 in all slides was assessed and noted in Annexure. Data were analyzed using SPSS version 21.

Results: Patients included were 57. The mean age was 50.1 years. Males were 54%. The frequency of the BCL6 gene expression was 33%. The majority of the nodal cases were negative for BCL6 31%. Positive nodal cases were 16%. The majority of the extranodal cases were also negative for BCL6 36%. The positive extra nodal cases were 17%.

Conclusion: BCL6 immunoexpression has a fair presence in DLBCL. It can be used as an adjunct tool in evaluating patients of DLBCL. Patients with positive BCL6 gene mutation can benefit from BCL6 specific target chemotherapy.

Key Words: BCL6, DLBCL, prognosis

Introduction

DLBCL is a usual lymphoid malignancy comprising 25-30 % of all lymphomas worldwide.[1] It is the commonest variety of non-Hodgkin lymphoma in all nations and all age levels and accounts for 25% of all lymphoma cases .[2] DLBCL is the seventh leading cancer in both males and females. In developing countries, the incidence is even higher with limited available data.

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The median age of presentation is around the sixth to the seventh decade and rarely, it can present in young adults and children .[3]

DLBCL usually presents as a locally aggressive disease with poor clinical outcomes. Most patients give a history of lymphadenopathy. On examination, cervical, axillary, or inguinal nodes are enlarged which are painless but rapidly increasing in size.[2] However, in 1/4th of cases, sites other than lymph nodes can also be involved, like skin, lungs, gastrointestinal tract and genitourinary tract, and even skeleton.[4]

Regarding etiological factors, genetic susceptibility also has a positive role in the development of lymphomas. Patients with a strong family history of hematopoietic malignancies have a twofold increased risk of developing DLBCL.[5] DLBCL as a group is very heterogeneous both in terms of clinical behavior and

morphology. The reason behind its heterogeneity is the varied range of molecular events.[6]

The pathogenesis of DLBCL involves the malignant transformation of the B lymphocytes within the germinal centers of lymph nodes. One of the most common genetic mutations in DLBCL is the translocation or hyper mutation of BCL6 gene. Around 70% of patients with DLBCL show point mutations in the BCL6 gene.[6]

BCL6 is a proto-oncogene that is situated on chromosome 3q27. It has a vital role in the development of germinal centers in lymph nodes. This gene is also responsible for repressing other target genes that cause DNA damage.[6] DLBCL is curable, and patients who are positive for BCL6 gene expression have better prognosis and survival. For the confirmation of the diagnosis of DLBCL, it is important to confirm the lineage of B cells. B cell lineage is usually identified by CD20, PAX5, and CD79a on Immunohistochemistry. CD20 positive B cells can successfully be targeted by anti CD20 therapy and most commonly used anti CD20 therapy is rituximab.[7]

Studies on gene expression analysis have shown that targeted therapies have improved the overall survival of the patients. Special therapeutic agents have been designed to target the mutated BCL6 gene and destroy tumor cells. Therefore, by directly targeting mutated genes such as BCL6, can lead to reprogramming of the tumor cells.[7]

Molecularly targeted therapies and BCL6 inhibitors such as BCL6 peptide inhibitors have shown to cause rapid cell death within 24hours within vitro and vivo. BCL6 peptide inhibitors are highly specific in destroying DLBCL, without causing inflammatory disease, which usually results from BCL6 depletion.[7]

BCL6 expression status will provide an option for specific target therapy against BCL6 mutations in DLBCL. Therefore, patients who are positive for BCL6 gene mutations can benefit from specific targeted therapy such as BCL6 peptide inhibitors against the BCL6 gene.[7] This will remarkably reduce the burden of disease and improve patient survival and life expectancy.

Applying the immunoexpression of BCL6 in DLBCL is fairly a simple, quick, less expensive and easily approachable procedure as compared to genetic analysis or DNA sequencing studies. [8] Immunohistochemistry (IHC) has been in the use of pathological diagnostic regimes for decades .[9] IHC markers such as BCL6 have considerable prognostic and tumor predictive role in DLBCL.

Objective

To determine the expression of BCL6 in DLBCL in nodal and extra nodal cases.

Materials and Methods

This descriptive cross-sectional study was carried out in the Pathology department of Pakistan Institute of Medical Sciences, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad. Duration of study was 3 years and 2 months, from 1st October 2014 to December 2017. The sample size was 57 cases. The sample size was calculated by WHO sample size calculator by using the following formula on the basis of frequency of BCL6 in DLBCL. [4]

n=Z (1-P) /d2 (where Z= Z1-a/2) taking following parameters:

- Confidence level = 95%
- Anticipated population proportion (P) = 70%
- Absolute precision required (d) = 12%
- Sample size (n) = 57

Sampling technique used was Random consecutive sampling. Biopsies and resection specimens of male and female patients of all ages with a histopathological diagnosis of DLBCL were included. Patients with any co morbidity are eligible to be selected. Cases of Hodgkin lymphoma and patients with reactive conditions of the lymph node were excluded. All lymph node biopsies and surgical resection specimens (in 10% buffered formalin) received in the Department of Pathology. Pakistan Institute of Medical Sciences Islamabad with the clinical impression of DLBCL were collected. Patient's data along with registration number and relevant details were entered on the Performa. After gross examination, the tissues were processed in automatic tissue processor and blocks were prepared, followed by cutting, slide preparation and staining with hematoxylin and eosin stain.Tissue processing was carried out in automated tissue processor LEICA TP-1020. Sections of three to four micron thickness were cut from paraffin-embedded brain biopsy specimens. These sections were mounted on slides and then stained with H&E in a tissue stainer Shandon Varistan 24-4. Microscopy of all cases was carried out on Olympus CX 22 LED series microscope; by a postgraduate resident along with supervisor and diagnosis on (H&E) slides were made. A panel of immunohistochemical markers consisting of LCA, CD20, CD3, Ki67 applied to cases having a morphology of DLBCL. LCA, CD20 positive cases, and those cases with a Ki 67 of more than 40% were selected for BCL6 protein expression.

For IHC staining, antigens in individual cells of a tissue section are detected by using monoclonal or polyclonal antibodies targeted against that specific antigen. IHC staining was done, three to five micron thick paraffin-embedded tissue sections were cut, after the initial selection of a slide by microscopic examination. BCL6 immunostaining was done and the entire section was screened to find the region with maximum positive nuclear staining of for BCL6. The percentage of positively stained nuclei was scored in the region using 40X objectives. Quotients (positively stained tumor cells/total counted tumor cells) were calculated as percentage & rounded off to the nearest integer. The BCL 6 nuclear staining in DLBCL was reported positive when >10% of the tumor nuclei were stained. [9] Data was analyzed using SPSS version 21. The categorized variables were analyzed as percentages and frequency. The quantitative numerical variables were analyzed as mean and SD. Frequency of BCL6 was calculated. P-value of less than 0.05 was considered significant.

Results

Mean age was 50.1±16.6 years. Most of the patients were in their 5th, 6th & 7th decade of life. Majority of the cases were above 40 years of life, however, 12/57 (21%) were found in the younger age group between 21 to 40 years of age. Out of these 57 cases of DLBCL, 31 were males (54%) while 26 were females (46%). (Table 1)

Most common site of nodal presentation of DLBCL in this study was cervical lymph nodes 14/57 (24.5%) and most common extra nodal site was spinal cord 5/57 (8.7%). DLBCL was seen as extra nodal in the majority of the cases 30/57 (53.0%) and it was found nodal type in 27/57 (47.0%). The expression of BCL6 was positive in 19 (33%) cases of DCBCL while 38 (67%) were negative. (Table 2)

Majority of the nodal cases were negative for BCL6 18/57 (31%) and the positive nodal cases for BCL6 were 9/57(16%).Majority of the extra nodal cases were also negative for BCL6 20/57 (36%) and the BCL6 positive extra nodal cases were 10/57(17%). (Table 3).

Table 1: Summary of demographic features and presentation (n=57)

n	57
Age (years)	**50.1±16.6**
1-10	1(1.8%)
11-20	2(3.5%)
21-30	6(10.5%)
31-40	6(10.5%)
41-50	12(21%)
51-60	13(22.7%)
61-70	10(17.5%)
71-80	5(8.7%)
>80	2(3.5%)
Gender	**% age**
Male	31 (54%)
Female	26 (46%)
DIFFERENT SITES OF presentation of DLBCL (n=57) Site	**% age**
Ankle	2 (3.5%)
Axillary lymph nodes	4 (7%)
Cecum	1 (1.7%)
Cervical lymph nodes	14 (24.5%)
Gastric tissue	3 (5.2%)
Inguinal lymph nodes	4 (7%)
Lumbar area	3 (5.2%)
Lymph nodes(site not mentioned on request form)	4 (7%)
Mesenteric lymph nodes	1 (1.7%)
Nasopharynx	4 (7%)
Parotid	1 (1.7%)
Renal	1 (1.7%)
Retroperitoneal	3 (5.2%)
Spinal cord	5 (8.7%)
Spleen	4 (7%)
Thyroid	1 (1.7%)
Tonsil	2 (3.5%)

Table 2: Expression of BCL6 in DLBCL (n=57)

BCL6 expression	**Positive**	19 (33.0%)
	Negative	38 (67.0%)

Table 3. Comparison of BCL6 expression in nodal and extra-nodal cases

Site	Negative	Positive
Nodal	18 (31%)	9 (16%)
Extra Nodal	20 (36%)	10 (17%)

Nodal cases negative for BCL6 18/57 (31%) and BCL6 positive nodal cases were 9/57(16%). Extra nodal cases negative for BCL6 were 20/57 (36%) and the BCL6 positive extra nodal cases were 10/57(17%).

Discussion

DLBCL, a malignant tumor of B lymphocytes, is a common variety of non-Hodgkin lymphoma. It is prevalent in all geographical areas worldwide and all age categories. Many investigators in the recent past have divided DLBCL according to germinal center B cell-like and activated B cell-like subcategories to see their implications regarding the prognosis of patients.[10]

The present study aimed at measuring the frequency of the BCL6 gene in DLBCL cases and tried to relate its significance to prognosis. In this study, the mean age of patients was 50.1 ± 16.6 and the majority of these cases were above 40 years of age. A study by Akyurek N et al from Turkey witnessed slightly elder population with a median age of 54 years.[11]

Another study was conducted by Bellas C and colleagues from Spain in which 100 cases were selected where p was 0.05 statistically significant and the chi-square test was used. The results of this study also witnessed a similar trend with a median age of 61 years ranging from 18-88 years and 29% of the cases had BCL6 gene rearrangement.[12]

A hospital-based epidemiological study on DLBCL was conducted at Agha Khan University Hospital (AKUH) by Pervaiz S et al, reported the median age was 47.2 years. Bajwa a et al from Pakistan (AFIP), Rawalpindi reported mean age was 54.66 ±16.73 years.[13]

Shia A from Malaysia reported a more comparable mean age of 49 years in their study. The age of patients varies according to geographical regions and ethnic backgrounds as well as socioeconomic status. The western and developed world populations have a high average age than underdeveloped and developing countries, this could be one of the reasons that the average age of presentation patients is low in our study. Moreover, we noticed that a significant proportion of cases develop DLBCL in younger ages in the current study, verifying, the argument that it is not only the elder population's disease but young people can also be affected[14].

In the present study, male gender was slightly predominant. Akyurek N et al from Turkey reported a comparable gender distribution.[11] Bellas C et al from Spain witnessed that males were in a slight majority in their study.[12] Shia A et al from Malaysia also witnessed the male majority with (61.1%) cases in their study[14].

In this study, the presentation of DLBCL was nodal in (47.0%) cases and extranodal in (53.0%) cases. Thus, the extranodal presentation out numbering the nodal presentation. In the study by Akyurek N et al, from Turkey the presentation was nodal in (62.0%) and extranodal in (38.0%) [11]. Similarly, Bellas C et al from Spain witnessed (50.0%) nodal and (50.0%) extranodal presentation of DLBCL[12]. A similar trend was also noted by Pervaiz S et al from Pakistan in their study, (42.2 %) nodal and (58.8%) extra nodal presentation of DLBCL[13]. These reports regarding the presentation of DLBCL are continuous with the current study findings.

The site of DLBCL was cervical in the majority of current study cases, other frequent sites were axillary, spinal cord, thyroid, stomach, parotid, tonsils, spleen, renal, lumbar, inguinal, cecum, mesentery, retroperitoneal and ankle. Many others have also witnessed a similar trend of the site of DLBCL, a recent study from Spain reported stomach, breast, testis, small intestine, lung, mediastinum, liver, central nervous system, skin and thyroid as the main sites of lymphoma [12]. Pervaiz S et al in their study reported cervical lymph nodes as the most frequent nodal site of presentation[13]. The most frequent extranodal site was the gastrointestinal tract (GIT), followed by the head and neck. There were 33.0% cases with a positive finding of the BCL6 gene in the present study. Bajwa A et al from AFIP, Rawalpindi, Pakistan concluded in their study that 37% patients were BCL6 positive and 62% were MUM1 positive.[13] Shia A et al from Malaysia witnessed even a higher presence, 29 out of total 53 cases (55.7%), were positive for BCL6 immunoexpression.[14]

A study from Turkey studied the prognostic significance of MYC, BCL2, and BCL6 rearrangements in patients with DLBCL; they witnessed greater rearrangement of BCL6 gene (29.0%) in their study. Another study from Spain also witnessed an exactly similar trend with (29.0%) rearrangement of BCL6[12]. A study reported from the USA reported an even higher BCL6 mutation (61.0%), in their cases of DLBCL.[15] These findings regarding BCL6 gene detection in DLBCL cases is of great significance. Many previous studies have demonstrated that higher mRNA or protein expression had better overall survival. One small-scale previous study also established the relationship between BCL6 mRNA and survival. BCL6 gene expression can stratify patients into good and poor prognostic groups, even in the low and high IPI risk groups.[15]

When selective analysis to associate study parameters with BCL6 was done, it was noted that age and presentation had no effect on presence or absence of

the gene, however, it was noted that proportion wise males were more likely to have positive BCL6 status in this study. BCL6 rearrangement showed no significant association with clinical features, including age, sex, and nodal/extra nodal primary site of presentation in a study from turkey[11]. There are many investigators who report that DLBCL exhibit significant diversity in their clinical presentation, cytomorphology, immunophenotype, and genotype. However, in the present study, we could not see any clinical parameters statistically associated with the presence of BCL6.

Evidence suggests that DLBCL is a highly curable disease and many studies on BCL6 gene expression analysis have shown that targeted therapies have proven to improve the overall survival of the patients. The function of special therapeutic agents is to target the mutated BCL6 gene and destroy the tumor cells. Moreover, immediate cell death within 24 hours with vitro and vivo molecular targeted therapies and BCL6 inhibitors have been proven and witnessed. In instances when BCL6 genes deplete, there are chances of inflammatory disease, however, its peptide inhibitors are highly specific in destroying DLBCL without causing any such complication.

Conclusion

Based on the findings of current study, a fair stratum (33.0%) of DLBCL patients showed the presence of BCL6 gene. Male gender was in slight dominance and also showed a greater proportion of BCL6. BCL6 is considered as an important marker in predicting response to chemotherapy and long term survival. It is of importance for the treating clinician and in the coming years can assist in modified treatment of DLBCL.

Strength and limitations of Study

The current study has many advantages as this is one of its own kind of trial done in the local settings. A reasonable sample of 57 cases of diffused large B cell lymphoma was selected. Data regarding demographic features of patients, their clinical presentation and findings of BCL6 gene was collected in the study which is one of the few attempts done on this topic in the local as well as national level settings. There were few limitations of the study as well which were mainly related to the observational design of the trial. Moreover, due to time constraints, the patients could not be followed after diagnosis. And the information regarding their final outcome in terms of recovery or death was not gathered which could have helped in measuring the survival period in patients who had the presence of the BCL6 gene. Various risk factors for DLBCL like family history, occupational history, exposure to radiations, HIV status or history of immunosuppression were not added due to lack of clinical data. Histologic subtypes and various other common pathological parameters like tumor size, morphology and additional associated pathologies could be added.

Conflict of interest: None

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