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IMMUNOHISTOCHEMICAL PROFILE OF DIFFUSE LARGE B-CELL LYMPHOMA IN A PAKISTANI COHORT: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma with variable clinical presentation and prognosis. Immunohistochemical classification into germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subtypes is widely used for prognostic stratification. This study aimed to evaluate the immunohistochemical profile and demographic characteristics of DLBCL patients in Karachi, Pakistan.

Methods: This cross-sectional study included 116 patients diagnosed with DLBCL at Dow University of Health Sciences Hospital, Karachi, between 2015 and 2018. Immunohistochemical staining for CD10, BCL6, and MUM1 markers was performed on formalin-fixed paraffin-embedded tissue samples. The Hans algorithm was applied to classify cases into GCB and ABC subtypes.

Results: The mean age of patients was 49.79±16.41 years, with 60.3% males and 39.7% females. Based on the Hans algorithm, 70.7% cases were classified as GCB-DLBCL and 29.3% as ABC-DLBCL. CD10 was positive in 65.51% cases, BCL6 in 78.44%, and MUM1 in 59.48%. The majority of patients (69.9%) had received prior treatment, with 61.2% having undergone chemotherapy, 9.5% radiotherapy, and 12.1% both. No significant association was found between immunohistochemical subtype and age, gender, ethnicity, or treatment history ($p>0.05$).

Conclusion: Our study reveals a higher prevalence of the GCB subtype among DLBCL patients in Karachi, Pakistan, which differs from patterns reported in some other populations. These findings contribute to the growing body of evidence suggesting regional variations in DLBCL subtype distribution and highlight the importance of local epidemiological data for tailoring treatment approaches.

Keywords: Diffuse large B-cell lymphoma, Immunohistochemistry, Hans algorithm, CD10, BCL6, MUM1, Pakistan

INTRODUCTION

Diffuse Large B-cell Lymphoma (DLBCL) is the most prevalent subtype of non-Hodgkin lymphoma (NHL), accounting for approximately 30-40% of all adult NHL cases worldwide [1]. In Pakistan, NHL represents the third most common malignancy in men and the sixth most common in women, with a concerning increase in DLBCL cases reported in recent years [2,3].

DLBCL is a heterogeneous entity with variable clinical presentation, morphology, immunopheno type, and outcomes. The World Health Organization (WHO) classification recognizes multiple subtypes and variants of DLBCL [4]. Gene expression profiling has identified distinct molecular subtypes, including germinal center B-cell-like (GCB) and activated B-cell-like (ABC) DLBCL, which differ in their cell of origin (COO), pathogenesis, and clinical outcomes [5]. The ABC subtype is generally associated with inferior prognosis compared to the GCB subtype [6].

Since gene expression profiling is not routinely available in clinical practice due to cost and technical constraints, several immunohistochemical (IHC) algorithms have been developed as surrogates. The Hans algorithm, which uses CD10, BCL6, and MUM1 markers, is widely employed to classify DLBCL into GCB and non-GCB (ABC) subtypes [7]. This classification has important prognostic implications and may guide therapeutic decisions.

While extensive research on DLBCL has been conducted in Western populations, data from South Asian countries, particularly Pakistan, remain limited. Understanding the immunohistochemical profile and demographic characteristics of DLBCL in the Pakistani population is crucial for developing appropriate diagnostic and therapeutic strategies. This study aimed to evaluate the immunohistochemical profile of DLBCL patients at a tertiary care hospital in Karachi, Pakistan, and classify them according to the Hans algorithm.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted at the Dow Diagnostic Research and Reference Laboratory (DDRRL) and Dow University of Health Sciences (DUHS), Karachi, Pakistan, from November 2015 to April 2018. The study was approved by the Institutional Review Board of DUHS, and written informed consent was obtained from all participants.

Study Population

A total of 116 patients with histologically confirmed DLBCL were included in the study. Inclusion criteria were: (1) confirmed diagnosis of DLBCL with sufficient tumor material for immunohistochemistry, and (2) provision of written informed consent. Patients with non-DLBCL diagnoses, insufficient tissue samples, or history of organ transplantation were excluded.\

Histopathological Analysis

Tissue specimens were fixed in 10% neutral buffered formalin for 24 hours, processed, and embedded in paraffin. Sections of 2-4 μm thickness were cut and stained with hematoxylin and eosin (H&E). The diagnosis of DLBCL was confirmed by experienced pathologists according to the WHO classification criteria.

Immunohistochemistry

Immunohistochemical staining was performed on 2-4 μm thick tissue sections using antibodies against CD10, BCL6, and MUM1/IRF4. After deparaffinization and rehydration, antigen retrieval was performed using heat-induced epitope retrieval in EnVision™ FLEX Target Retrieval solution. Endogenous peroxidase activity was blocked using hydrogen peroxide. Primary antibodies were applied, followed by HRP-labeled polymer, and visualization with DAB chromogen. Slides were counterstained with hematoxylin, dehydrated, and mounted.

Immunohistochemical staining was evaluated by two independent pathologists. Positivity was defined as staining in ≥30% of tumor cells for each marker. The intensity of staining was graded as: 1 (weak), 2 (moderate), or 3 (strong). Cases were classified as GCB or ABC subtype according to the

Hans algorithm: cases positive for CD10 or cases negative for CD10 but positive for BCL6 and negative for MUM1 were classified as GCB; all other cases were classified as ABC.\

Statistical Analysis

Data were analyzed using SPSS version 22.0. Descriptive statistics were calculated for demographic and clinical characteristics. Frequencies and percentages were determined for categorical variables, while means and standard deviations were calculated for continuous variables. Chi-square test was used to assess associations between immunohistochemical subtypes and demographic or clinical factors. A p-value <0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

A total of 116 patients with DLBCL were included in the study. The mean age was 49.79±16.41 years (range: 16-84 years), with a relatively even distribution across age groups (Table 1). Males comprised 60.3% (n=70) of the cohort, and females 39.7% (n=46).

The ethnic distribution reflected the diverse population of Karachi: 37.9% (n=44) were Urdu-speaking, 26.7% (n=31) Sindhi, 13.8% (n=16) Punjabi, 12.1% (n=14) Pathan, 5.2% (n=6) Balochi, 2.6% (n=3) Saraiki, and 0.9% each (n=1) were Memon and Bengali.

Regarding treatment history, 61.2% (n=71) of patients had received chemotherapy, 9.5% (n=11) had undergone radiotherapy, 12.1% (n=14) had received both chemotherapy and radiotherapy, while 17.2% (n=20) had not received any prior treatment. Surgical intervention was reported in 31.9% (n=37) of cases.

Table 1: Demographic and Clinical Characteristics of DLBCL Patients (n=116)

Characteristic	**n (%)**
Age (years)	
16-50	58 (50.0)
51-85	58 (50.0)
Gender	
Male	70 (60.3)
Female	46 (39.7)
Ethnicity	
Urdu-speaking	44 (37.9)
Sindhi	31 (26.7)
Punjabi	16 (13.8)
Pathan	14 (12.1)
Balochi	6 (5.2)
Saraiki	3 (2.6)
Memon	1 (0.9)
Bengali	1 (0.9)
Treatment History	
Chemotherapy	71 (61.2)
Radiotherapy	11 (9.5)
Both	14 (12.1)
None	20 (17.2)
Surgical Intervention	
Yes	37 (31.9)
No	79 (68.1)

Immunohistochemical Profile

Based on the Hans algorithm, 82 cases (70.7%) were classified as GCB-DLBCL and 34 cases (29.3%) as ABC-DLBCL (Table 2). Analysis of individual markers revealed CD10 positivity in 65.51% (n=76) of cases, BCL6 positivity in 78.44% (n=91), and MUM1 positivity in 59.48% (n=69).

The distribution of staining intensity varied across markers. For CD10, 42.24% (n=49) showed weak intensity (grade 0-1) and 57.75% (n=67) showed moderate to strong intensity (grade 2-3). For BCL6, 36.20% (n=42) showed weak intensity and 63.79% (n=74) showed moderate to strong intensity. For MUM1, the distribution was equal, with 50% (n=58) showing weak intensity and 50% (n=58) showing moderate to strong intensity.

Regarding the extent of staining, most cases showed staining in 0-40% of tumor cells for all three markers: 69.82% (n=81) for CD10, 81.89% (n=95) for BCL6, and 86.20% (n=100) for MUM1. Extensive staining (41-90% of tumor cells) was observed in 30.17% (n=35) for CD10, 18.10% (n=21) for BCL6, and 13.79% (n=16) for MUM1.

Table 2: Immunohistochemical Profile of DLBCL Cases (n=116)

Parameter	n (%)
DLBCL Subtype	
GCB	82 (70.7)
ABC	34 (29.3)
CD10 Expression	
Positive	76 (65.51)
Negative	38 (32.75)
Inconclusive	2 (1.72)
BCL6 Expression	
Positive	91 (78.44)
Negative	25 (21.55)
MUM1 Expression	
Positive	69 (59.48)
Negative	46 (39.65)
Inconclusive	1 (0.9)

Association of DLBCL Subtype with Clinical Parameters

No significant association was found between DLBCL subtype and age group (p=0.467), gender (p=0.630), treatment history (p=0.169), surgical intervention (p=0.211), or ethnicity (p=0.222). Similarly, no significant associations were observed between individual marker expression (CD10, BCL6, or MUM1) and demographic or clinical parameters, except for MUM1 expression, which showed a significant association with surgical intervention ($p<0.001$) and ethnicity ($p<0.001$).

DISCUSSION

This study provides insights into the immunohistochemical profile and demographic characteristics of DLBCL patients in Karachi, Pakistan. Our findings reveal a predominance of the GCB subtype (70.7%) over the ABC subtype (29.3%) based on the Hans algorithm. This distribution differs from some Western studies reporting a more balanced distribution or even a predominance of the ABC subtype [6,7].

The predominance of the GCB subtype in our cohort is consistent with some studies from Asian populations. For instance, a study from China reported GCB and ABC subtypes at 66.3% and 33.7%, respectively [8], while a study from Japan found GCB and ABC subtypes at 62.6% and 37.4%, respectively [9]. However, it contrasts with studies from other regions, such as North India, where the ABC subtype was more prevalent (60.4%) than the GCB subtype (39.6%) [10]. These variations highlight the potential influence of genetic and environmental factors on DLBCL pathogenesis across different populations.

In our study, CD10 positivity was observed in 65.51% of cases, which is higher than reported in most studies. Hans et al. [7] reported CD10 positivity in 30% of DLBCL cases, while other studies have reported rates ranging from 30-45% [11,12]. This higher frequency of CD10 expression might contribute to the predominance of the GCB subtype in our cohort, as CD10 positivity directly classifies cases as GCB according to the Hans algorithm.

BCL6 positivity was observed in 78.44% of our cases, which is comparable to rates reported in other studies (70-80%) [7,11]. MUM1 positivity was found in 59.48% of cases, which is also within the range reported in the literature (50-75%) [7,12].

The mean age of our cohort (49.79 years) was relatively younger compared to Western studies, where the median age at diagnosis is typically in the sixth or seventh decade [1]. This younger age at presentation has been observed in other studies from developing countries and may reflect demographic differences or possibly earlier onset of disease due to environmental or genetic factors [13].

The male predominance (60.3%) in our study is consistent with global trends, as DLBCL generally affects men more frequently than women [1]. The ethnic distribution in our cohort reflects the diverse population of Karachi, with Urdu-speaking and Sindhi communities representing the largest groups.

No significant association was found between DLBCL subtype and age, gender, ethnicity, or treatment history. This lack of association has been reported in some studies [7], while others have found correlations between subtype and certain demographic features [6]. The significant association between MUM1 expression and both surgical intervention and ethnicity is interesting and warrants further investigation in larger cohorts.

Our study has several limitations. First, the sample size, while substantial for a single-center study, may not be large enough to detect subtle associations between variables. Second, we used the Hans algorithm for classification, which, while widely accepted, has been shown to have limitations in accurately replicating gene expression profiling results. Third, we did not assess clinical outcomes, which would have provided additional insights into the prognostic significance of the immunohistochemical subtypes in our population.

CONCLUSION

Our study demonstrates a predominance of the GCB subtype among DLBCL patients in Karachi, Pakistan, with high rates of CD10 and BCL6 positivity. The demographic profile reveals a relatively younger age at presentation compared to Western populations, with a male predominance. These findings contribute to the growing body of evidence suggesting regional variations in DLBCL subtype distribution and highlight the importance of local epidemiological data for tailoring treatment approaches. Future studies with larger cohorts and long-term follow-up are needed to better understand the clinical implications of these findings in the Pakistani population.

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