Original Article

Frequency of Immune-Histochemical Markers-Based Diffuse Large B Cell Lymphoma Subgroups

Raazia Mahmood[1], Ayesha Safdar[1], Maria Khan[1], Ayesha Sajjad[1], Maria Tasneem Khattak[2] and Iqbal Muhammad Khan[1]

ABSTRACT

Objective: To assess the frequency of diffuse large B cell lymphoma subgroups by immune-histochemical markers.
Study Design: A Descriptive Cross-Sectional Study
Place and Duration of Study: This study was conducted at the Department of Histopathology. Rehman College of Dentistry / Rehman Medical Institute (RCD/RMI), Peshawar from August 2022 to February 2023.
Methods: DLBCL was diagnosed using a"panel of lymphoid antibodies that comprised CD20, CD79a, CD3, Ki-67, and Pax5". On silane-coated slides, slices were mounted for immunohistochemical staining. To analyze the data, IBM SPSS version 23 was used.
Results: In the current study, 80 patients were enrolled. The male patients were 45 (56.25%), and females were 35 (43.75%). Our study's mean age (SD) was 42 (4.43) years. Of 80 cases, 37 (46.25%) cases were observed as a germinal centres, while 43 (53.75%) were observed as non-germinal centre-like subgroups. In our study, 45 (56.25%) cases were observed as extranodal while 35(43.75%) cases were observed as nodal lymphoma.
Conclusion: Our study concludes that the frequency of the non-germinal sub-group of diffuse large B-cell lymphoma is higher than the germinal centre-like sub-group.
Key Words: Subgroups; Diffuse large B cell lymphoma; Immune-histochemical markers

Citation of article: Mahmood R, Safdar A, Khan M, Sajjad A, Khattak MT, Khan IM. Frequency of Immune-Histochemical Markers-Based Diffuse Large B Cell Lymphoma Subgroups. Med Forum 2023;34(10):109-113. doi:10.60110/medforum.341024.

INTRODUCTION

According to the World Health Organization, Hodgkin's (HL) and non-Hodgkin's lymphomas (NHL) are the two types of lymphoid neoplasms[1]. NHL may also be divided into mature B, mature T, and mature NK cell neoplasms. B-cell NHL is more prevalent than T-cell NHL, making up 80–85% of all instances of NHL, with T-cell NHL making up the rest of the 10%[2]. In adults, diffuse large B-cell lymphoma (DLBCL) accounts for 30–50% of all NHL and is the most prevalent lymphoid neoplasm. A lymphoid malignancy with a diffuse or nodular development pattern, DLBCL is characterized by medium- to large-sized B lymphoid cells[3].

[1.] Department of Histopathology. Rehman Medical Institute, Peshawar.
[2.] Department of Histopathology. Rehman College of Dentistry / Rehman Medical Institute (RCD/RMI), Peshawar.

Correspondence: Maria Tasneem Khattak, Associate professor of Histopathology, Rehman College of Dentistry/ Rehman Medical Institute (RCD/RMI), Peshawar.
Contact No: 03339471472
Email: accesstomaria@yahoo.com

Received: June, 2023
Accepted: July, 2023
Printed: October, 2023

The tumour cell size is determined by comparing it to the typical macrophage nucleus or the size of a normal lymphocyte, which should be greater. The site and molecular subtype determine clinical signs and symptoms, behaviour, and prognosis in DLBCL[4]. Using gene expression profiling (GEP), DLBCL may be divided into two unique molecular forms. One is germinal centre B-cell (GCB), and the other is activated B-cell (ABC). Both have varying predictions and treatment outcomes. Immunohistochemistry (IHC) methods have been proposed to predict the molecular forms. Hans' method, which primarily relies on three immunohistochemical stains—CD10 (which emphasizes the GCB subtype), Bcl6 (which links to both the GCB and the ABC subtype), and MUM1 (which highlights the ABC subtype)—is used routinely[5]. Three immunohistochemical stains (for GCB, ABC subtype, and CD20) allow the Hans algorithm to classify DLBCL NOS cases into two categories. The ABC subtype of DLBCL reacts more effectively to recently suggested therapeutic drugs such as "bortezomib, lenalidomide, or ibrutinib". In contrast, the GCB type is more chemosensitive and has superior median survival rates. According to the research done, ABC is more common than GCB. For ABC, Lu TX et al[6]. We found that the 5-year overall survival rate for 49% germinal centre lymphoma was 70.2%, while the 5-year overall survival rate for 51% non-germinal centre lymphoma was 18.4%. P was less than 0.001.

The current study was piloted to assess the occurrence of diffuse large B cell lymphoma subgroups by immune-histochemical markers[7].

METHODS

This descriptive cross-sectional study was carried out at the Department of Histopathology, RMC/RMI, Peshawar. Our study was six months, from August 2022 to February 2023. The study approval was properly taken from the ethical and research committee of the hospital. The sample size of our study was 80 patients based on the WHO sample size calculator. The inclusion criteria of our study were all patients of both genders aged 18-80 years, assessment of all nodal and extranodal disease and patients of all incisional, core and excisional biopsies.

In contrast, the criteria for exclusion were all the patients with HIV positivity and patients with DLBCL as a secondary disease. Sections were obtained after grossing all specimens. Following that, hematoxylin and eosin were used to evaluate the morphology of the tissue sections that had been formalin-fixed and paraffin-embedded. On silane-coated slides, slices were mounted for immunohistochemical staining. For quality assurance, appropriate positive controls were used on the same slides. All large biopsy patients received negative controls by appropriate antigen retrieval. DLBCL was diagnosed using a “panel of lymphoid antibodies that comprised CD20, CD79a, CD3, Ki-67, and Pax5". Then, three antibodies were employed for the subgroups of DLBCL, including the "monoclonal antibodies CD 10 (Clone 56C6 Cell Marque), BCL-6 (Clone BL6-02 Neo Mark), and IRF-4 (MUMIp) (Clone MUMIp Santacruz)". Patients who are negative for CD10, positive for Bcl-6 and negative for MUM1 are considered members of the germinal centre-like subgroup by employing the Hans algorithm. Instances with CD10 expression by more than 30% of cells are also considered members of this subgroup. All the additional instances are classified as belonging to the non-germinal centre subgroup. To analyze the data, IBM SPSS version 23 was used. Mean and standard deviation were determined for continuous variables like age, whereas percentages and frequencies were calculated for categorical parameters like sex and subgroups. To examine the influence of effect modifiers, the data were stratified by age and sex.

RESULTS

In the current study, a total of 80 patients were enrolled. The male patients were 45 (56.25%), and females were 35 (43.75%). (Figure 1) Our study's mean age (SD) was 42 (4.43) years, with a maximum age of 78 and a minimum age of 18. Out of 80 cases, 37 (46.25%) cases were observed as germinal centres, while 43 (53.75%) cases were observed as non-germinal centre subgroups (Figure 2). Based on gender stratification, the germinal centre subgroup was observed in 21(56.76%) male participants and 16(43.24%) female participants. In the non-germinal centre subgroup, males were 22 (51.16%), while females were 21 (48.84%). Based on age stratification, the germinal centre subgroup was observed in 20 (54.05%) cases aged 50 years, while 17 (45.95%) cases were observed with age 50. In the non-germinal centre subgroup, 23 (53.49%) patients were observed at age 50, whereas 20 (46.51%) patients were observed at age 50. (Table 1)

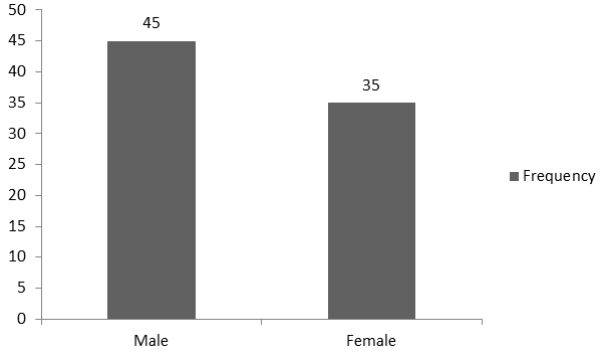

Figure No.1: Distribution of patients based on gender

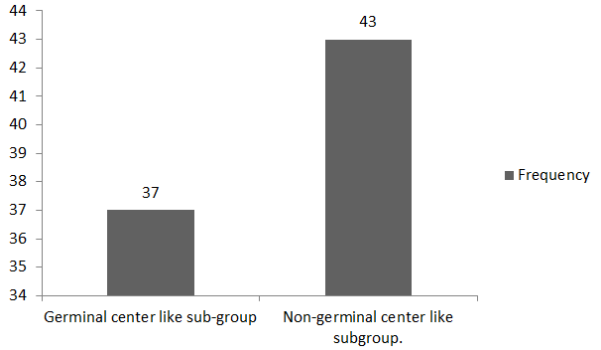

Figure No. 3: Frequency of Extranodal and Nodal lymphoma

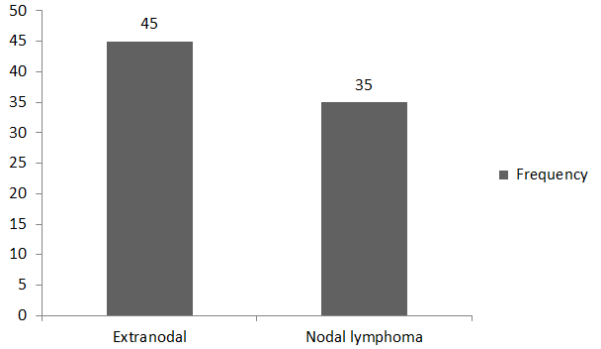

Figure No. 3: Frequency of Extranodal and Nodal lymphoma

In 33 (89.19%) germinal centre-like subgroup cases, CD-10 was observed as positive. In the germinal centre-like subgroup, Bcl-6 was observed as positive in 35 (94.59%) cases, while in the case of the germinal subgroup, it was observed as positive in 34 (79.07%) cases. MUM-1 was observed positive in 19 (51.35%) cases of germinal and 41 (95.35%) non-germinal centre

subgroups. In our study, 45 (56.25%) cases were observed as extranodal, while nodal lymphoma was observed in 35(43.75%) cases. The germinal centre subgroup was predominant both in nodal (n=21, 60%) and "extranodal lymphoma" (n=25, 55.56%). (Figure 3)

Table No.1: Stratification of gender and age concerning germinal and non-germinal subgroup

Characteristics	Germinal Centre Subgroup	Non-Germinal Centre Subgroup	Total
Gender			
Male	21 (56.76% of germinal)	22 (51.16% of non-germinal)	45 (56.25%)
Female	16 (43.24% of germinal)	21 (48.84% of non-germinal)	35 (43.75%)
Age			
≤ 50 years	20 (54.05% of germinal)	23 (53.49% of non-germinal)	43 (53.75%)
> 50 years	17 (45.95% of germinal)	20 (46.51% of non-germinal)	37 (46.25%)
Subgroup			
Germinal Centre	37 (100% of germinal)	--	37 (46.25%)
Non-Germinal Centre	--	43 (100% of non-germinal)	43 (53.75%)
Lymphoma Type			
Nodal	21 (of nodal)	14 (of nodal)	35 (43.75%)
Extranodal	16 (of extranodal)	29 (of extranodal)	45 (56.25%)

Table No.2: Immunohistochemical Marker Expression in Germinal and Non-Germinal Centre Subgroups

Marker	Germinal Centre Subgroup (n=37)	Non-Germinal Centre Subgroup (n=43)
CD10	Positive in 33 (89.19%)	Not applicable
BCL-6	Positive in 35 (94.59%)	Positive in 34 (79.07%)
MUM-1	Positive in 19 (51.35%)	Positive in 41 (95.35%)

Table No.3: Correlation of Immunohistochemical Marker Expression with Extranodal and Nodal Presentation in DLBCL Subgroups

Marker	Extranodal Lymphoma (n=45)	Nodal Lymphoma (n=35)
Germinal Centre Subgroup (n=37)		
CD10	Positive in 22 (48.89%)	Positive in 11 (31.43%)
BCL-6	Positive in 24 (53.33%)	Positive in 11 (31.43%)
MUM-1	Positive in 10 (22.22%)	Positive in 9 (25.71%)
Non-Germinal Centre Subgroup (n=43)		
CD10	Not applicable	Not applicable
BCL-6	Positive in 19 (42.22%)	Positive in 15 (42.86%)
MUM-1	Positive in 30 (66.67%)	Positive in 11 (31.43%)

DISCUSSION

One of the non-Hodgkin's lymphomas (NHL) with the highest incidence rate and making up the vast majority of aggressive lymphoid neoplasms is diffuse large B cell lymphoma[8]. The most prevalent form of NHL in Pakistan is DLBCL. Although the exact cause of DLBCL's "emerging epidemic" status is still unknown, at least a few key factors and how they interact are believed to be responsible[9]. In the current study, a total of 80 patients were enrolled. The male patients were 45 (56.25%), and females were 35 (43.75%). These findings are consistent with local research that found diffuse large B cell lymphoma is more common in men than women[10]. Our study's mean age (SD) was 42 (4.43) years, with a maximum age of 78 and a minimum age of 18. Of 80 cases, 37 (46.25%) were germinal centres, while 43 (53.75%) were non-germinal centre-like subgroups[11]. Our results are consistent with a local investigation that found 42 instances of DLBCL in 34 (55%) non-GCB subgroups and 27 (45%) GCB subgroups. Our findings are close to those of research from Spain, which reported 53% non-GCB subgroups compared to 47% GCB subgroups[12]. The findings of other Asian investigations were likewise comparable. Contrary to this, the prevalence of the germinal center-like subgroup was 52% and 58%, respectively, in Western nations like Sweden (20) and the USA[13]. The "prevalence of the GCB subtype was often somewhat greater than that of the non-GCB subtype in Western research. It is unknown why GCB frequency varies from non-GCB frequency. In our study, 45 (56.25%) cases were observed as extranodal while 35(43.75%) cases were observed as nodal lymphoma. Non germinal center subgroup was predominant both in nodal (n=21, 60%) and extranodal lymphoma (n=25, 55.56%)[14]. Our results are consistent with previous research from Pakistan[12,14] and Korea, which likewise found that extranodal DLBCL predominated. Our results, yet, are in conflict with investigations from the USA and Europe which showed lower frequencies of extranodal lymphoma. Correct recognition of extranodal lymphoma as a potential condition should result in an early diagnosis. Physicians who are neither hematologists nor oncologists should be aware of this fact. On the basis of gender stratification, the germinal center subgroup was observed in 21(56.76%) male participants while it was observed in 16(43.24%)

female participants[15]. In case of non germinal center subgroup, males were 22 (51.16%) while females were 21 (48.84%). On the basis of age stratification, germinal center subgroup was observed in 20 (54.05%) cases having age <50 years while 17 (45.95%) cases were observed with age >50 years[16]. In case of non germinal center subgroup, 23 (53.49%) patients were observed with age <50 years whereas 20 (46.51%) patients were observed with age <50 years. (Table 1) In 33 (89.19%) cases of germinal center like subgroup, CD-10 (>30%) was observed as positive. In cases of germinal center-like subgroup, Bcl-6 was observed as positive in 35 (94.59%) cases, while in cases of non-germinal center subgroup, it was observed as positive in 34 (79.07%) cases[17]. MUM-1 was observed positive in 19 (51.35%) cases of germinal and 41 (95.35%) cases of non-germinal center subgroups. These findings differ considerably with a local research by Naz et al[19]. who found that men had a greater frequency of the non-germinal center subgroup (74%) while females had a little higher frequency of the germinal center subgroup (53%) in that study. Their low sample size of 42 cases may be the cause of this difference. Our findings concur with those of a Malaysian research[19]. By comparing our findings to those of different study we discovered that there was a larger frequency of the germinal centre-like subgroup in patients who were younger than 60 years old, while the non-germinal centre-like subgroup was more often seen in patients who were either equal to or older than 60 years old[20]. This discrepancy in age group may result from a lower sample size (42 instances). According to Spanish research the age range for germinal center-like subgroup lymphomas is 22–93 years, whereas the age range for non-germinal center lymphomas is 24-85 years[21].

CONCLUSION

Our study concludes that the frequency of the non-germinal centre-like sub-group of diffuse large B cell lymphoma is higher than the germinal centre-like sub-group. Additional population-based investigations that can identify any etiological factors relating to diffuse large B cell lymphoma sub-grouping are required to confirm our findings and provide further evidence for our conclusions.

Author's Contribution:

Concept & Design of Study:	Raazia Mahmood
Drafting:	Ayesha Safdar, Maria Khan
Data Analysis:	Ayesha Sajjad, Maria Tasneem Khattak, Iqbal Muhammad Khan
Revisiting Critically:	Raazia Mahmood, Ayesha Safdar
Final Approval of version:	Raazia Mahmood

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None
Ethical Approval: No. RMI/RMI-REC/Article Approval/55 dated 25.02.2021

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