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ORIGINAL RESEARCH

Comparison of Immunohistochemical Markers in ABC and GCB Subtypes in Diffuse Large B-Cell Lymphoma

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma worldwide, representing approximately 30–40% of all cases in different geographic regions. There is further division of DLBCL according to cell-of-origin into germinal center B-cell like (GCB) and activated B-cell like (ABC) subtypes, with about 10–15% of cases being unclassifiable. Patients with the GCB subtype usually have better prognosis than patients with the ABC subtype.

Objective: The present study was undertaken with an objective to find the incidence of germinal center B-cell-like (GCB) lymphoma Vs activated B-cell-like (ABC) lymphoma and to evaluate the role of immunohistochemistry in the diagnosis and prognosis.

Materials and Methods: A Three-year prospective study was undertaken in a tertiary health care setting. A total number of 81 lymph node biopsies were evaluated and 25 cases were diagnosed as DLBCL based on anatomical architectural alteration and a baseline IHC panel of CD3, CD20, LCA, Ki67 followed by CD5, CD10, CD15, CD30, CD23, BCL2, BCL6, MUM1, ALK, CyclinD1.

Result: Incidence of ABC is more common in the study setting, frequency being 21(84%) and GCB 4 (16%). ABC variant is more common in males, but statistically not significant ($p=0.053$). Nodal involvement in ABC is 84% and in GCB it is 15.79% ($P=1.000$), not significant. Frequency of extranodal involvement is more in ABC. GCB is associated with CD10 ($p<0.01$) and BCL6 ($p<0.05$). ABC is associated with BCL2 ($P<0.05$) and MUM1 (highly significant $p=0.000$). There is no significant difference in mitotic counts between males and females ($p>0.05$). There is no significant correlation between age and mitotic count ($r=0.02$, $p>0.05$).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

Conclusion: The incidence of ABC is more prevalent with nodal involvement being most common. There is strong association of markers like CD10 and BCL6 with GCB type and BCL2 and MUM1 with ABC type.

Key words: Diffuse large B-cell lymphoma, Immunohistochemistry, Germinal center B-cell type Activated B-cell type.

Introduction

Diffuse large-B-cell lymphoma (DLBCL) is a heterogeneous category that includes most diffuse lymphomas composed of large, transformed B cells. A neoplastic large B cell is generally defined based on the nuclear size, which is greater than twice the size of a normal lymphocyte or equal to or greater than the nucleus of a macrophage [1]

Most DLBCLs express variable numbers of pan-B-cell antigens (CD19, CD20, CD22, CD79a, PAX-5), with the exception of plasmablastic type or those with ALK expression.[2] CD5 expression is seen infrequently and is reported to be an adverse prognostic indicator [3]. DLBCLs have clonally rearranged immunoglobulin genes that are mutated, often with ongoing mutations [4-7]. Cases with both bcl-2 and c-myc translocations are associated with adverse prognosis [8,9]

The most widely cited literature is that of Hans et al. [10], which utilized antibodies to CD10, Bcl-6, and MUM-1 to distinguish two clinically significant groups that is germinal center B-cell–like (GCB) and activated B-cell–like (ABC). DLBCLs are aggressive lymphomas that develop in both children and adults [11] about one-half of them being found to be in stage III or IV at diagnosis. The NHL classification reports an overall 5-year survival rate of 46%[12]. Clinical factors are also very important in prognostication of DLBCL, with the International Prognostic Index (IPI) score used often. The IPI score is based on an assessment of age (≤60 vs. >60), performance status, lactate dehydrogenase (LDH) (≤normal vs. >normal), extranodal sites (0-1 vs. >1), and stage (I/II vs. III/IV) [13]. Bcl-2 expression has been associated with an adverse prognosis in DLBCL, whereas strong Bcl-6 expression was predictive of a better prognosis in some studies. [14-19]

WHO classification of DLBCL variants and subtypes
Diffuse large B-cell lymphoma not otherwise specified (DLBCL-NOS)
Common morphological variants
Centroblastic
Immunoblastic
Anaplastic
Rare morphological variants
Molecular Subgroups
Germinal center B-cell-like
Activated B-cell-like
Immunohistochemical subgroups
CD5+DLBCL
Germinal center B-cell-like
Nongerminal center B-cell-like
Diffuse large B-cell lymphoma subtypes
T-cell/histocyte rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

EBV-positive DLBCL of the elderly
Other lymphomas of large B-cells
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
ALK-positive large B-cell lymphoma
Plasmablastic lymphoma
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
Primary effusion lymphoma
Borderline B-cell lymphomas
B-cell lymphoma, unclassifiable, with features between DLBCL and Burkitt lymphoma
B-cell lymphoma, unclassifiable, with features between DLBCL and classical Hodgkin's lymphoma

Objective

To find out the proportion of germinal center B-cell-like (GCB) lymphoma and activated B-cell-like (ABC) lymphoma among cases of diffuse large B-cell lymphoma (DLBCL), and to evaluate the role of immunohistochemistry (IHC) in their diagnosis and prognosis.

Materials and Methods

A Three-year prospective study was conducted at the department of Pathology in a tertiary health care setting.

Selection of cases

A total number of 81 lymphoma cases were evaluated. Due to poor status of patient and loss of follow up 25 cases were subjected to IHC panel and were diagnosed as DLBCL based on anatomical, architectural alteration and a baseline IHC panel of CD3, CD20, LCA, Ki67 followed by CD5, CD10, CD15, CD30, CD23, BCL2, BCL6, MUM1, ALK, CyclinD1.

Exclusion criteria

1. All cases showing reactive hyperplasia of lymph node and lymphadenitis due to tubercular etiology were excluded.
2. Paraffin embedded tissue blocks with sections from the non-representative areas and sections with quantitatively inadequate material were not included in the study.
3. H & E stained sections showing extensive histo-morphological artifacts such as cautery/crush artefacts were also excluded from the study.

Clinical data

The relevant clinical information with respect to all the cases for the study was collected from the requisition forms sent with the specimen. Data collected was entered in MS Excel v 2016 and imported to licensed version of Stata v 12.1 SE. All qualitative variables were summarized as percentages and all quantitative variables were summarized in terms of mean and standard error of mean. Inferences regarding association if any were drawn using various statistical tests like Chi-square test (for proportions), Mann-Whitney U test (for mean) and Spearman rank correlation test (for measuring association).

Result

ABC was found to be more common than GCB type in DLBCL cases (21 cases out of 25, i.e. 84%). It was seen that ABC variety was seen more commonly in male patients (17 cases of 21, i.e. 94.4%) though this was not found to be statistically significant (p=0.053). Nodal

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

involvement in ABC was 84% and in GCB it was 16%, but it was not statistically significant (p=1.000) (Table 1). On follow up of the patients for 1 year it was seen that out of 84% cases of ABC 77% showed poor response to chemotherapy, 12% showed variable response and 11% succumbed to the disease. Out of 16% cases of GCB, 69% showed a good response to chemotherapy, 19% showed bad response due to lack of treatment and 12% showed variable response.

Sl. No.	Parameter	Total (%) n=25	ABC (%) n=21	GCB (%) n=4	p value
	Age[#]	54.9 ± 3.5	55.3 ± 4.1	53 ± 4.2	0.557
	Gender *Male* *Female*	 18 (72%) 7 (28%)	 17 (81%) 4 (19%)	 1 (25%) 3 (75%)	0.053
	Nodal involvement	19	16	3	1.000
	Extra-nodal involvement	6	5	1	0.553

Table 1: General characteristics of the cases of DLBCL and ABC and GCB variants

[*] *Significant at p<0.05;* [#] *Expressed in terms of Mean ± SE*

GCB was found to be associated with CD10 (p<0.01) and BCL6 (p<0.05). ABC was found be associated with BCL2 and MUM1 (P<0.05). There was no significant difference in mitotic counts between males and females(p>0.05). There was no significant correlation between age and mitotic count (r= -0.02, p>0.05).

Sl. No.	Type of marker	ABC (%) n=21	GCB (%) n=4	p value
	CD10	1 (0.05%)	3 (0.75)	0.007[*]
	BCL6	5 (0.24%)	4 (1%)	0.010[*]
	BCL2	16(0.76%)	0(0.00%)	0.010[*]
	MUM1	20(0.95%)	0(0.00%	0.000[*]
	Ki67[#]	0.80 ± 0.03	0.6 ± 0.13	0.351

Table 2: Immunohistochemistry profile of ABC and GCB type of DLBCL (n=25)

[*] *Significant at p<0.05;* [#] *Expressed in terms of Mean ± S*

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

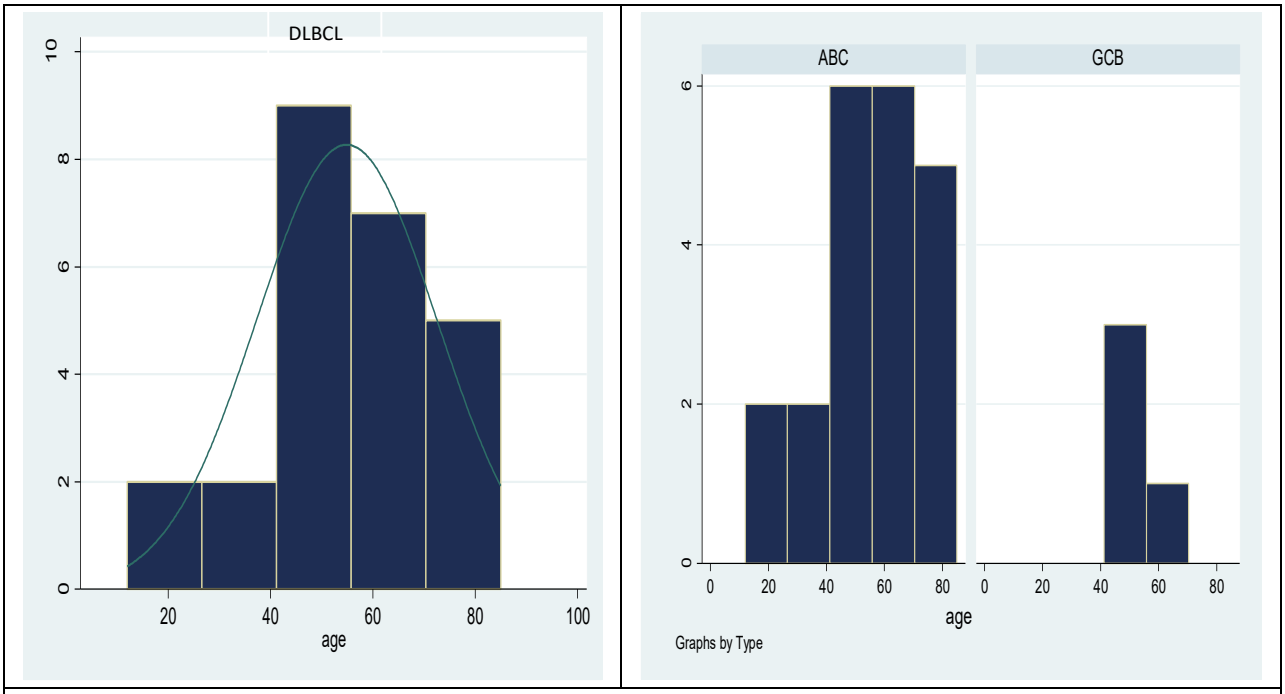


Fig 1: Age distribution of the patients of (a) DLBCL (b) ABC and GCB

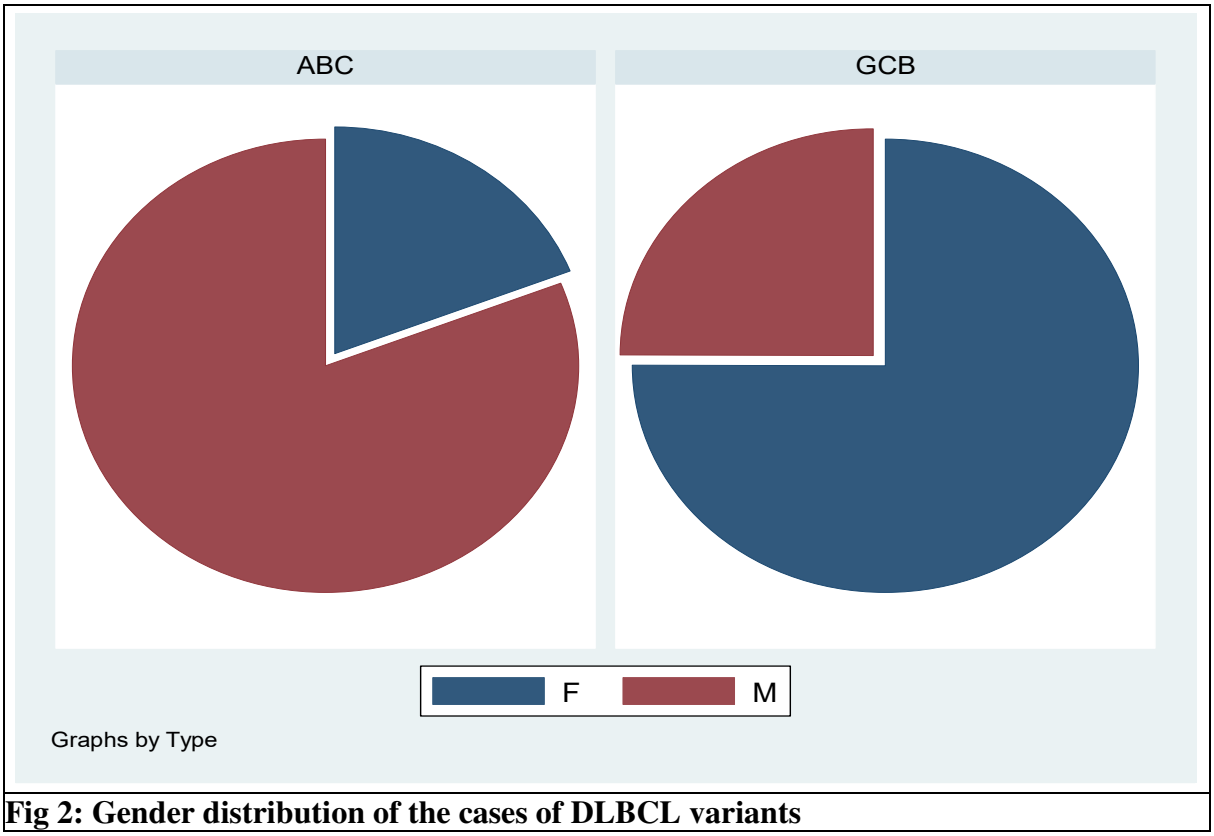


Fig 2: Gender distribution of the cases of DLBCL variants

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

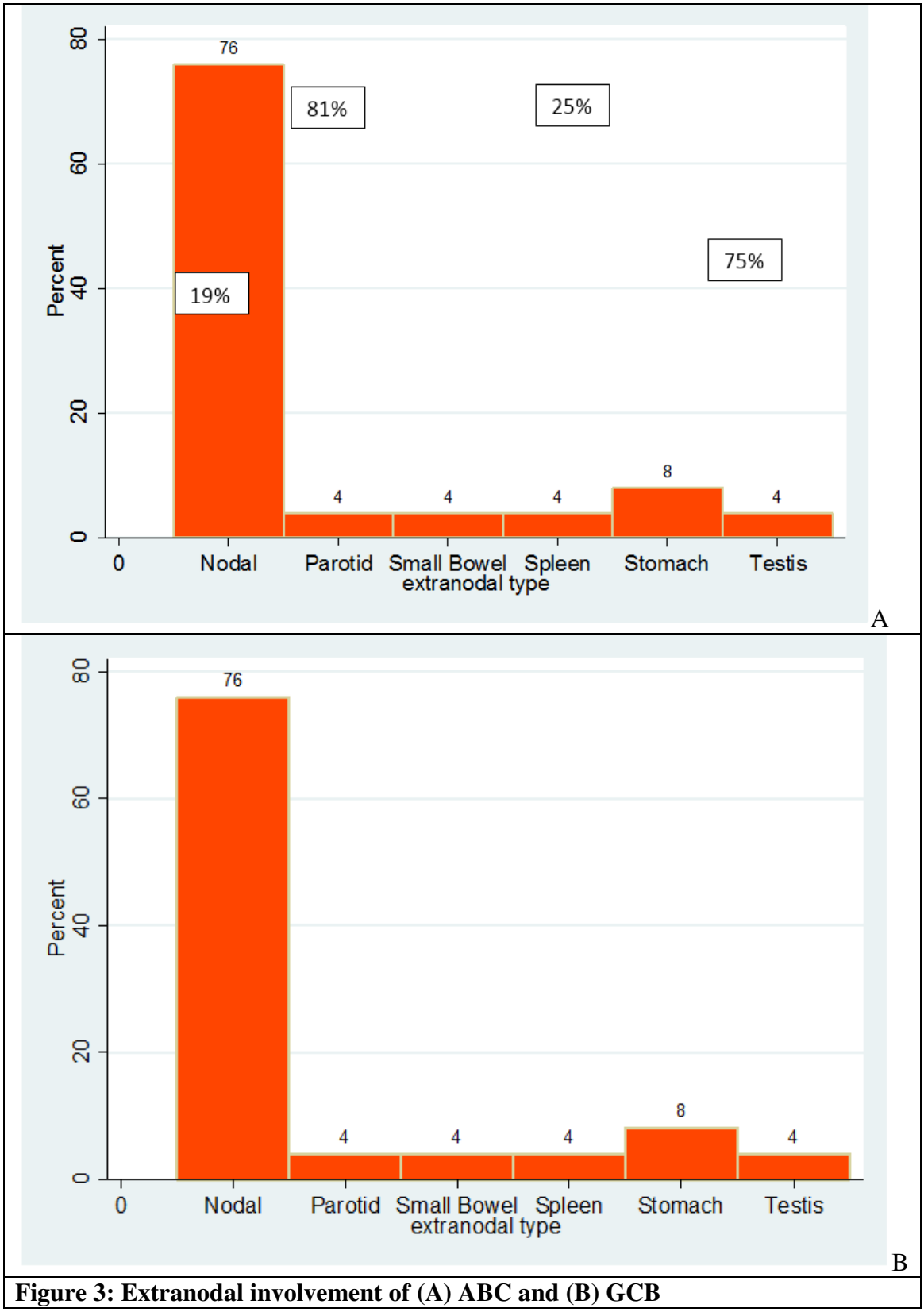


Figure 3: Extranodal involvement of (A) ABC and (B) GCB

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

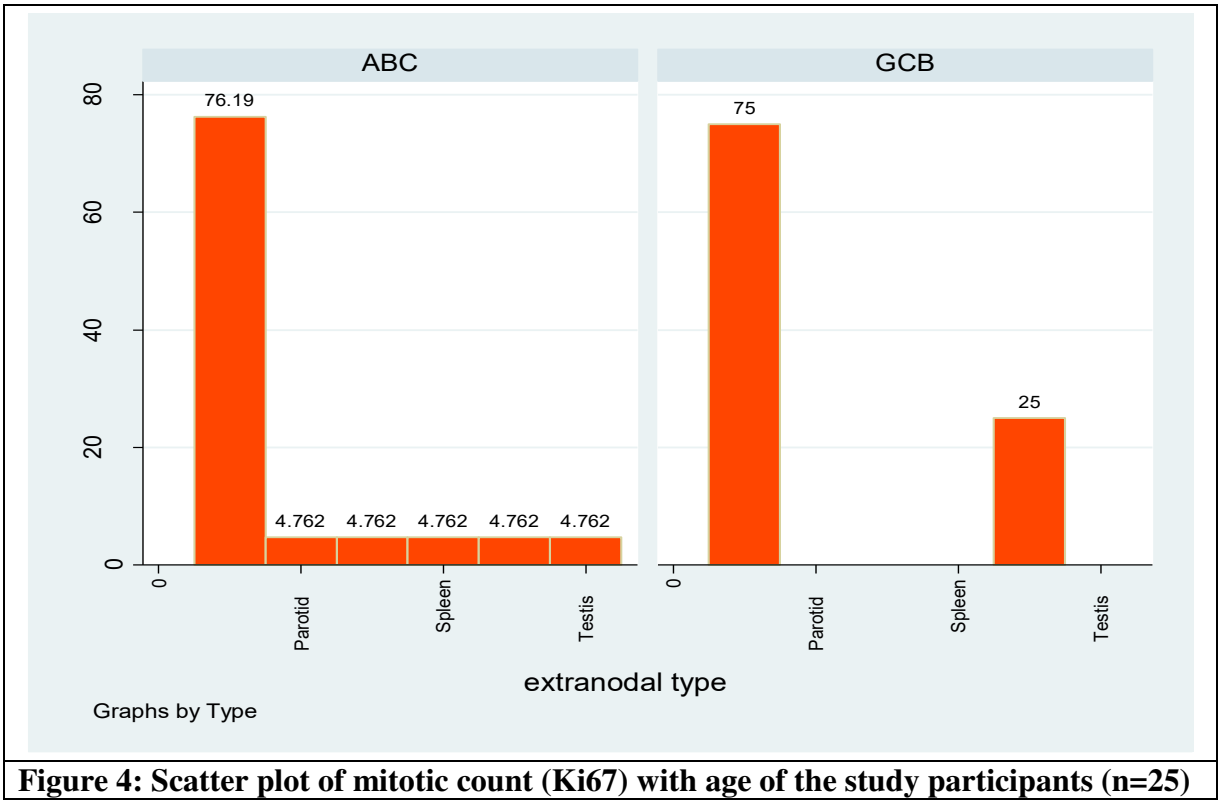
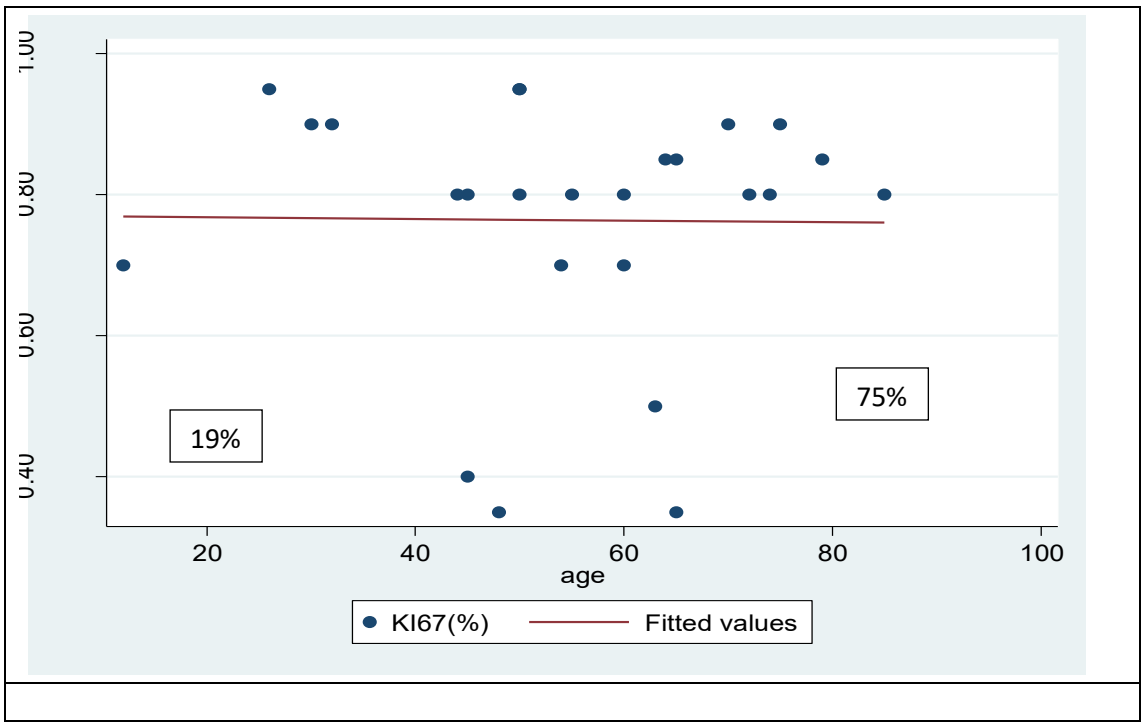


Figure 4: Scatter plot of mitotic count (Ki67) with age of the study participants (n=25)



r= -0.02; p>0.05

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

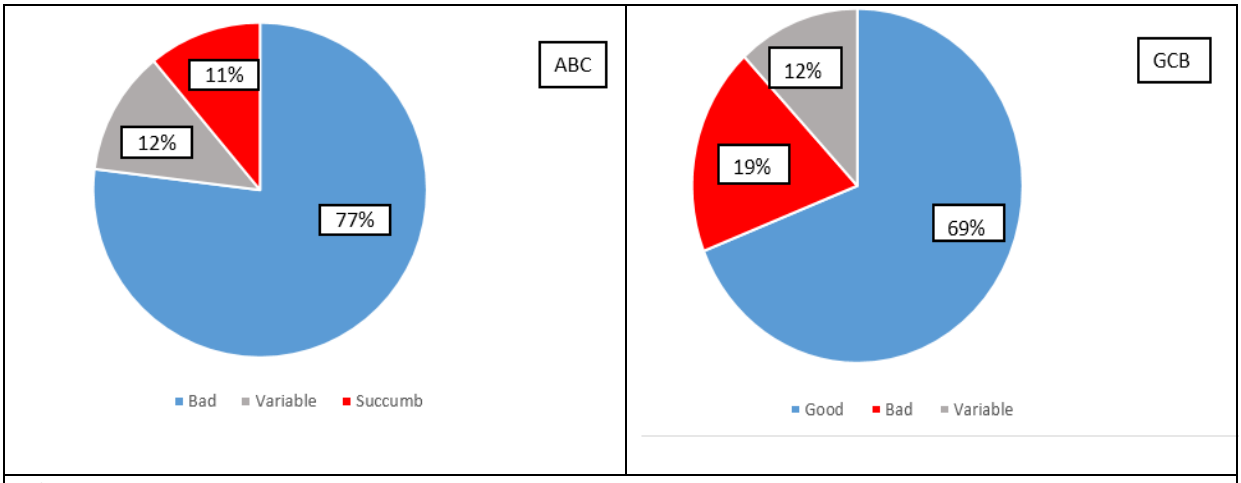


Fig-5 Follow up

DLBCL-TESTIS
GCB-TYPE

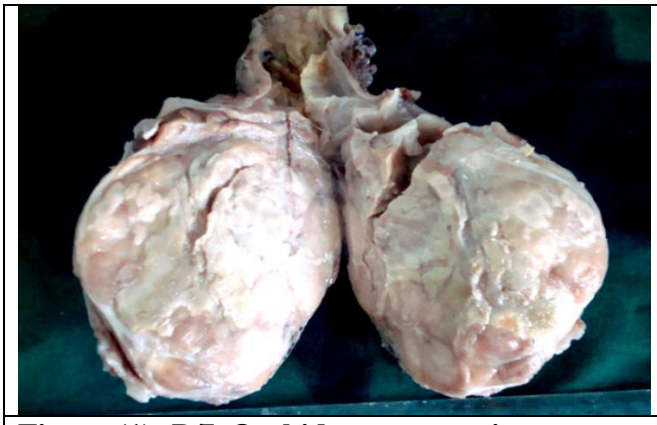


Figure (1): B/L Orchidectomy specimen

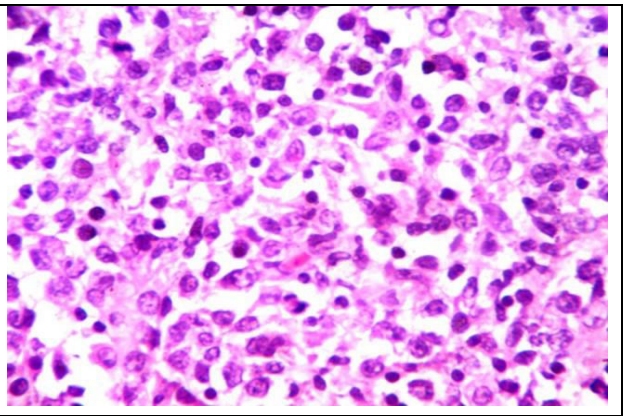


Figure (2): Neoplastic cells in diffuse sheets

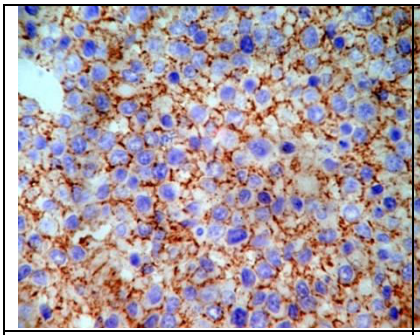


Figure (3): Membranous staining for CD20

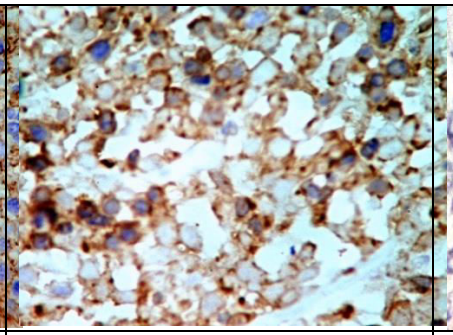


Figure (4): Nuclear staining for CD10

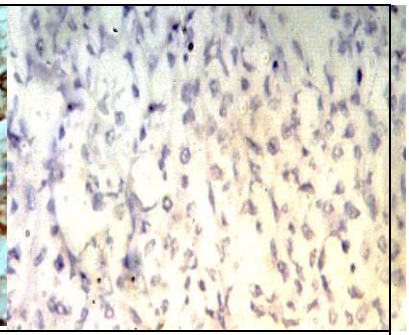


Figure (5): MUM1-Negative

DLBCL-SPLEEN
ABC-TYPE

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

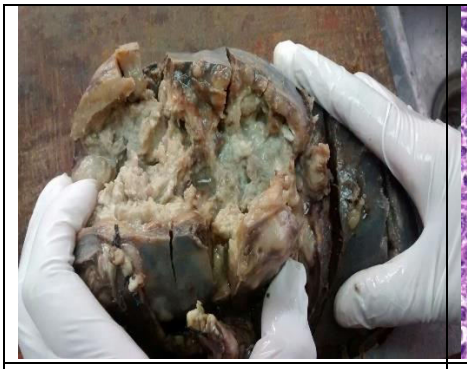


Figure (1): Spleen with splenic abscess

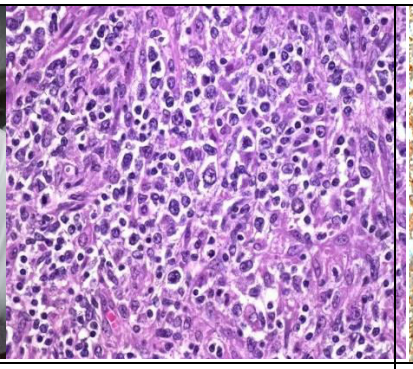


Figure (2): Large neoplastic cells

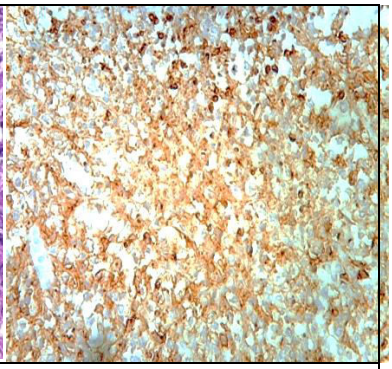


Figure (3): Membranous staining for CD45

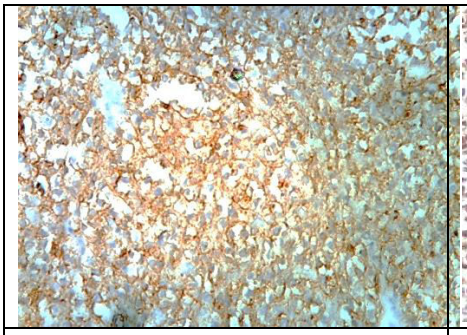


Figure (4) Membranous staining for CD10

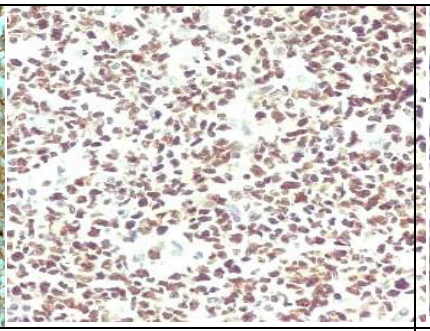


Figure (5) Nuclear staining for MUM1

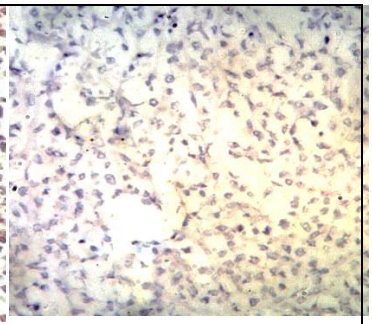


Figure (6): BCL6-Negative

Discussion

The heterogeneity seen in DLBCL is reflected at multiple levels, from the macroscopic view [20] with different clinical presentations to a microscopic view of the tumor microenvironment [21] and furthermore, to a subcellular level with differences in protein expressions [22][23]. The possibility of using immunohistochemical staining for DLBCL subdivision and prognostication was re-confirmed by Berglund et al.[24] However, the value of an immunohistochemical based algorithm has been questioned since studies have failed to replicate earlier results when patients are treated with rituximab, whereas the GEP model seems to still apply [25]. Nevertheless, by the refinement of already existing algorithms and description of new ones, immunohistochemical staining can still be a useful tool for subdividing DLBCL. Choi et al. have proposed an algorithm based on GCET1, CD10, BCL6, MUM1 and FOXP1 with 93% concordance with GEP classification of DLBCL[26] and the newly described Tally algorithm is based on CD10,GCET1, MUM1, LMO2 and FOXP1 [27]. Nyman et al. have proposed a simplified algorithm based only on MUM1 and FOXP1 [28] (Fig6).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

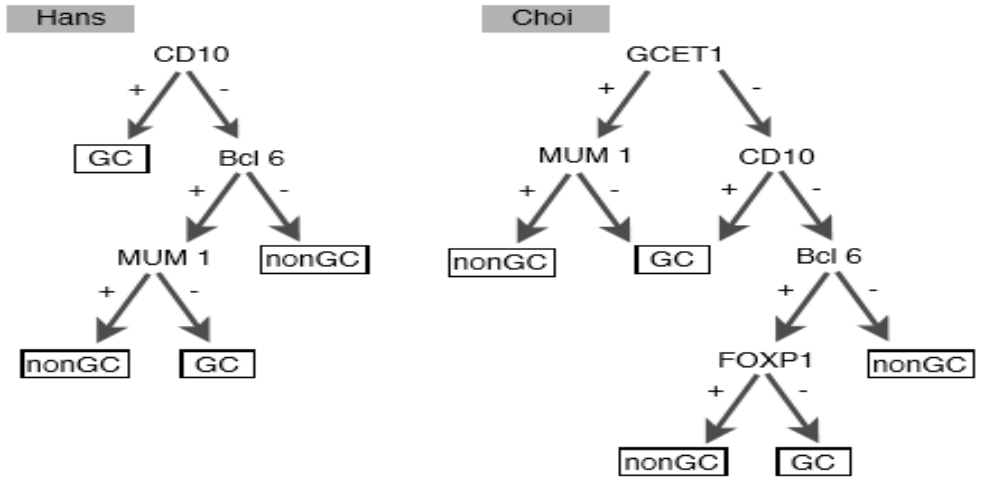


Figure 6

In contrast to known literature my study shows more incidence of ABC variant. The purpose of either individual biomarkers or pattern-based biomarkers models is to provide a basis for predicting survival, choice of initial treatment, stratification of patients in clinical trials, accurate communication among healthcare providers and uniform reporting of outcomes. In our study DLBCL accounts for about 40% of all lymphomas and prognosis is very variable. It is generally aggressive but potentially curable. About 40% of adult DLBCL patients respond well to therapy and have prolonged survival, the remainder die of the disease.

Adverse prognostic indicators include:(1). A poor International Prognostic Index based on clinical factors (2) A high proliferation rate (3) BCL-2 expression. The standard treatment of DLBCL has evolved to include the anti–B-cell antibody rituximab and CHOP (R-CHOP). In view of the improved survival achieved at present by the addition of rituximab, the results indicate that the negative prognostic impact of BCL2 expression was more pronounced in the patients treated with CHOP therapy than in those treated with R-CHOP

Patients were followed for 6 months and it was found that GCB patients were found to be more chemoresponsive as compared to ABC type. The course of ABC variant is more aggressive and less chemosensitive. Even though many patients with DLBCL can be cured of the disease, successful treatment fails for a proportion of patients. It is therefore of utmost interest to not only find new treatment strategies but also define new prognostic markers that can help clinicians to guide more intense treatments to those who would benefit the most.

Conclusion

ABC type of DLBCL was found to be more prevalent in the present study. Nodal involvement was seen to be commoner than extranodal involvement. There was strong association of markers like CD10 and BCL6 with GCB type. Markers like BCL2 and MUM1 were found to be strongly associated with ABC type. There was no association of proliferative activity with the type of DLBCL

Conflict of interest

None

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

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ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

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