Original Article

Frequency of Subgroups of Diffuse Large B-Cell Lymphoma by Immunohistochemistry

Uzma Bukhari, Fouzia Lateef, Saba Jamal

ABSTRACT

OBJECTIVE: To determine the frequency of subgroups (germinal centre and non germinal centre) of Diffuse large B cell lymphoma (DLBCL) in a tertiary care hospital.

MATERIALS AND METHODS: It is a descriptive cross sectional study performed in histopathology department, clinical laboratories Dr. Ziauddin Hospital Karachi. 62 cases of Diffuse large B cell lymphoma were collected during fifteen (15) months period from April 2011 to June 2012. All resected specimens and small biopsies were grossed; sections were processed overnight and stained by Hematoxylin and Eosin (H&E) for morphologic evaluation. The panel of lymphoid antibodies included CD20, CD79a, CD3, Ki-67 & Pax5 were used for the diagnosis of DLBCL. Then monoclonal antibodies CD 10, BCL-6 and MUM-1 were applied for subgrouping of DLBCL.

RESULTS: In a total of 62 cases of Diffuse large B cell lymphoma, 27 (44%) cases were categorized as germinal centre like subgroup and 35 (56%) were categorized as non germinal centre like subgroup. Patient's ages ranged from 04 to 95 years. 58% patients were males and 42% were females with male to female ratio1.4:1. 36(58%) cases were extranodal and 26(42%) were nodal lymphoma. Non germinal center like subgrpoup was preponderance in both nodal and extranodal lymphoma as 15(58%) and 20(56%) respectively.

CONCLUSION: This study reports an increased number of patients of non germinal centre subgroup of Diffuse large B cell lymphoma. Male to female ratio was 1.4:1. Mean age of patient was 45 years.

KEY WORDS: Lymphoma, Diffuse Large B Cell Lymphoma, Germinal centre like subgroup, Non germinal centre like subgroup.

INTRODUCTION

Diffuse Large B-Cell Lymphoma (DLBCL), NOS constitutes 25-30% of adult non-Hodgkin's lymphomas in western countries and a higher percentage in developing countries.[1] The conventional treatment of DLBCL is Anthracycline based (CHOP) chemotherapy. Despite the use of anthracycline based chemotherapy, durable remission is achieved in only 40% to 50 % of patients.[2] Therefore, it is important to identify at diagnosis those patients, who may benefit from more aggressive or experimental therapies.[3]

A study by Alizadeh et al[4] suggested that DLBCL could be divided into prognostically significant subgroups according to gene expression profiling by DNA microarray. One type demonstrated gene expression characteristic for germinal center B- cell and was called germinal center B cell (GCB) like DLBCL, the other showed gene expression normally induced during in vitro activation of peripheral blood B cells and was termed activated B- cell (ABC) like or non germinal center (Non GCB) DLBCL. The results of this study were confirmed by a few other investigations.[5, 6, 7] Patients with GCB- like DLBCL demonstrated significantly better overall survival rates as compared to those with the ABC like subgroups.[3]

The method of gene expression profiling has proven to be impractical as clinical tool in every day practice. Thus the investigators paid more attention of to a cheaper, simpler and more available technique immunohistochemistry.[2] Three antigens (CD10, Bcl-6 and MUM1) have shown promise in their ability to predict the gene expression profile (GEP) subtypes of DLBCL.[8]

Hans et al,[3] Haarer CF et al,[8] Anderson JJ et al, [9] and Berglund M et al [10] have found that a 3-antibody panel of immunohistochemical stains can be used to sub classify DLBCL into prognostically significant germinal center B cell (GCB) and non germinal center B cell (non GCB) subtypes. According to a recently reported study [8] 49% germinal center lymphoma with 5 years overall survival was 70.2 % and 51% non germinal centre lymphoma with 5 years over all survival was 18.4 %. The P. Value was <0.001.

This study was carried out on Large B Cell Lymphoma to identify the magnitude of prognostic subtypes by immunohistochemistry, so that more aggressive therapy could be initiated and the patient could be counselled for survival rate.

MATERIAL & METHOD

It is a descriptive cross sectional study performed in histopathology department, Clinical Laboratories Dr. Ziauddin Hospital Karachi, over a period of fifteen months from April 2011 to June 2012. All specimens were grossed and sections were taken. Then the sections of formalin fixed paraffin embedded tissues were stained by Hematoxylin and Eosin (H&E) for morphologic evaluation. For immunohistochemical staining, sections were mounted on silane coated slides. Specific positive controls were run on same slides for quality control. Negative controls were applied with all large biosy cases according to relevant antigen retrieval.

The panel of lymphoid antibodies included CD20, CD79a, CD3 , Ki-67 & Pax5 were used for the diagnosis of DLBCL. Then for the subgrouping of DLBCL three antibodies were used, these include monoclonal antibody CD 10 (Clone 56C6 Cell Marque), monoclonal antibody BCL-6 (Clone BL6-02 Neo Mark) and monoclonal antibody IRF-4 (MUMIp) (Clone MUMIp Santacruz). Using the Hans algorithm[3] (Figure I), cases with CD10 expression by >30% of cells are regarded as germinal center like subgroup as well as cases that are CD10 negative, Bcl-6 positive and MUM1 negative. All other cases are regarded as of non- germinal center like subgroup.The data was analyzed for continuous variables such as age. Percentages were computed for categorical variables such as sex and subgroups. The data was stratified according to age and sex to observe the impact of effect modifiers.

RESULTS

Out of 62 cases of Diffuse large B cell lymphoma (Figure II), CD-10 (>30%) was positive in 24 cases of germinal center like subgroup (Figure III). Bcl-6 was positive in 26 cases of germinal center subgroup (Figure IV) and 27 non germinal center subgroup, while MUM-1(Figure V) was positive in germinal and non germinal center subgroups as 14 and 33 cases respectively. (Table I)

In a total of 62 cases, 27(44%) were categorized as germinal center and 35(56 %) were non-germinal center like subgroup. Males 34(55%) out numbered females 28(45%) with male to female ratio 1.4:1. Non germinal center subgroup was reported in 18 (53%) males and 17(61%) females while Germinal center subgroup was seen in 16(47%) males and 11(39%) females.

Patient's ages ranged from 04 to 95years, with the mean age of 45 years. Median age was 50 years. Out of 27 cases of germinal center subgroup, 14(52%) cases were in less than 50 years age group, while 13 (48%) cases were in more than 50 years age group. In non germinal center subgroup almost an equal number of cases were seen in less than 50 years and more than 50 years age group as 18(51%) and 17 (49%) respectively.

In current study 36(58%) cases were extranodal and 26(42%) were nodal lymphoma. Non germinal center like subgroup was preponderance in both nodal and extranodal lymphoma as 15(58%) and 20(56%) respectively.

FIGURE I: HANS ALGORITHM TODETERMINE GERMINAL CENTER (GC) OR NON–GERMINAL CENTER (NON-GC) LYMPHOMA[1,3]

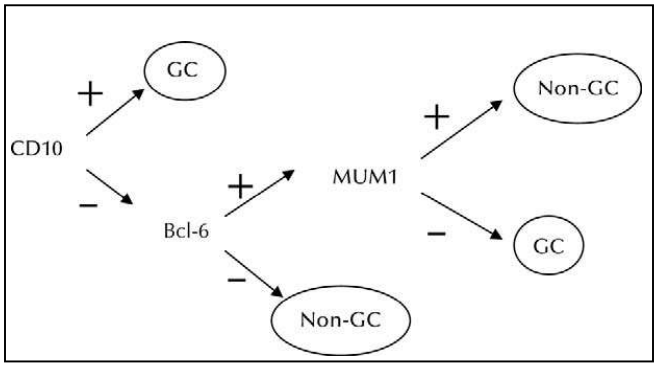

FIGURE II:
DIFFUSE LARGE B CELL LYMPHOMA (H&E)

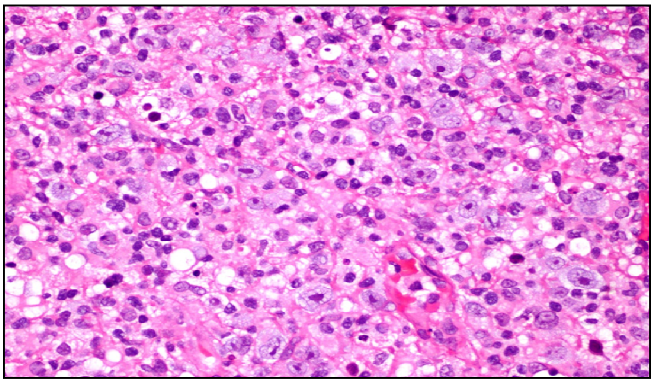

FIGURE III: GERMINAL CENTRE LIKE SUB-GROUP, CD10-POSITIVE (>30%)

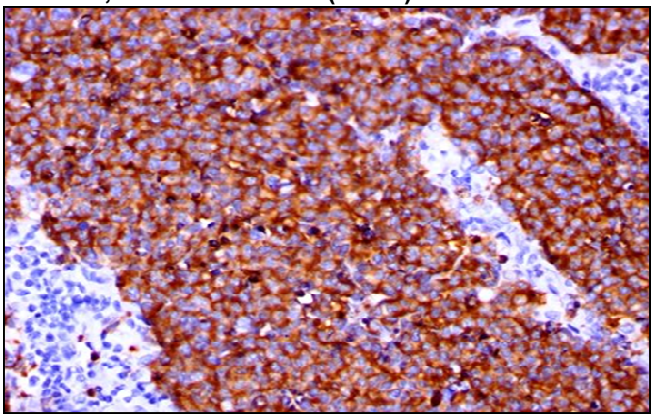

FIGURE IV: GERMINAL CENTRE LIKE SUB-GROUP, BCL-6 POSITIVE

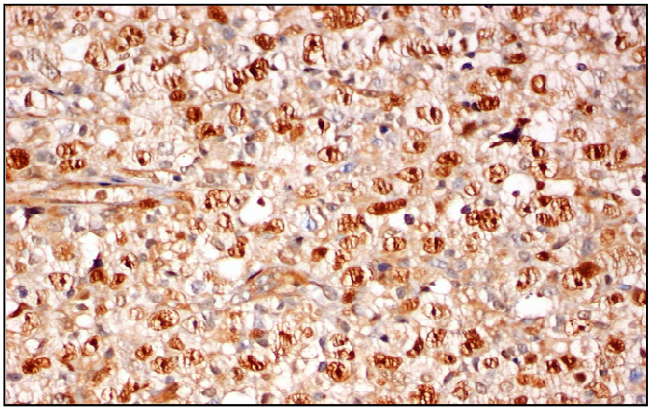

FIGURE V: NON-GERMINAL CENTRE LIKE SUB-GROUP, MUM-1 POSITIVE

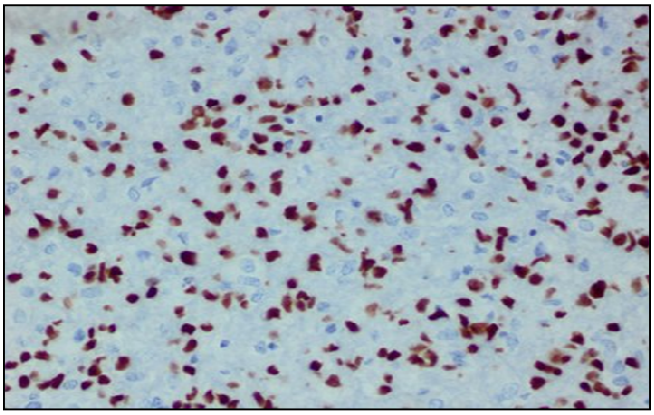

TABLE I: IMMUNOPHENOTYPIC FEATURES

Antibody	Germinal center like subgroup Positive cases	Non germinal center like subgroup Positive cases
CD10 (>30%)	24	--
Bcl-6	26	27
MUM-1	14	33

DISCUSSION

Diffuse large B cell lymphoma is one of the most frequently occurring of all non Hodgkin's lymphomas (NHL) and constitutes a great majority of all aggressive lymphoid neoplasms.[11] DLBCL is also the most frequent type of NHL in Pakistan.[12,13] What makes DLBCL an "emerging epidemic" remains to be unveiled, but at least several major factors, along with their interplay, are thought to be the culprits.[14]

The present study showed 27(44%) germinal centre subgroup and 35(56%) of non germinal centre subgroup of diffuse large B cell lymphoma. Our findings are in accordance with a local study[12] who reported 34 (55%) non GCB subgroup and 27(45%) GCB subgroup in 42 cases of DLBCL. Our results are also comparable with a study from Spain[15] who also reported 53% non GCB as compared to 47% GCB subgroups. Other Asian studies[16,17,18,19,20] also reported similar results. In contrast, in Western countries including Sweden[10] and USA[21] the frequency of germinal center like subgroup was 52%, 58% and 54% respectively. In most of the western studies frequency of the GCB subtype was slightly higher than that for the non-GCB subtype. The exact reason for difference in frequency of GCB and non GCB is not known.

The current study showed 36(58%) cases of extranodal DLBCL and 26 (42%) nodal lymphoma. Our findings are in accordance with other studies of Pakistan[12,14] & a study of Korea[22] also reported preponderance of extranodal DLBCL. However our findings are contradictory with studies from USA[7] and Europe who reported a lower frequency of extranodal lymphoma i.e. Denmark: 40%[23], Turkey: 44.5%[24] & Italy: 50% [25.]Awareness of the possible existence of extranodal lymphoma correctly should lead to a rapid diagnosis. This fact should be recognized by physicians who are not hematologists or oncologists.

In current study most of the patients were categorized as non germinal center like subgroup in both nodal and extranodal lymphoma as 58% and 56% respectively. In contrast to our findings, several international studies have [26,27,28,29] reported a higher frequency of non-GCB phenotype in extranodal lymphomas i.e 96%, 83%, 100% and 89% of cases, respectively

There is a possibility that the difference in frequency of GCB and non-GCB subtypes of DLBCL in these populations might be based on other ethnic factors. However the exact etiological factors responsible for causation of GCB versus non GCB in Western and Asian populations are still unclear and the discrepancy may reflect distinct genetic behavior.

We found 55% males and 45% females with male-to female ratio of 1.4:1. These results are in accordance with a local study[12] and studies from Malaysia[17] USA[30] and Sweden[10] who also found predominance of males over females in diffuse large B cell lymphoma.

In our study non germinal center like subgroup was seen with higher frequency both in males 53% and females 61%. These results are somewhat in contrast with a local study by Naz et al[12] who reported a higher frequency (74%) non germinal center like subgroup in males and slightly higher frequency (53%) germinal center like subgroup in females. This difference could be due to their small (42 cases) sample size. Our results are in agreement with a study from Malaysia [17] but in contrast with a study from Sweden[10] who reported an equal number of males 50% in germinal and non germinal centre like subgroup. In their study a slightly higher number of females 52% were present in

germinal center like subgroup.

In our study patient's ages ranged from 04 to 95years, with the mean age of 45 years. Median age was 50 years. Our findings are comparable with local[12] and international studies[17,18,19] who reported median age as 49 years, 52 years, 55.5 years and 51 years respectively.

In present study no significant difference was seen regarding age groups in both germinal and non germinal center lymphoma. Almost similar number of patients were present above and below 50 years age group. When we compared our results with a local study[12] it was noted that there was a higher frequency of germinal center like subgroup in patients less than 60 years and non germinal center like subgroup was mostly seen in equal to or more than 60 years of age. This difference of age group is might be due to smaller (42 cases) sample size. A study from Spain[15] reported 22-93 years age range for germinal center like subgroup lymphoma and for non germinal center 24-85 years. A higher sample size (128 cases) could be the cause for this different finding.

In conclusion we found that the results for CD10, bcl-6, and MUM1 can be combined to divide DLBCL into GCB and non-GCB subgroups with an outcome similar to that predicted by cDNA microarray analysis. The results of our study showed a high frequency of aggressive non germinal center like subgroup lymphoma, which is equally present both in young and older age groups with an equal gender distribution. We could not predict patients outcome related to specific immunohistocemical markers because we had limitation of having follow-up data in our series; due to the study being performed in laboratory setting with availability of paraffin embedded tissue blocks or biopsies from different areas of country; where patients come for a short duration, but our study may be helpful in identifying the behavior of disease and prediction of patient's outcome at the time of diagnosis; when compared with previously described follow-up based studies worldwide[3,30.]

CONCLUSION

In our setup, because of the dearth of clinical based follow-up studies, there is dire need to generate data regarding survival, outcome and prognosis of patients in association with these markers. To verify our findings further population based studies are needed which will help to identify any etiological factors regarding sub grouping of DLBC.

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