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ORIGINAL ARTICLE

Frequency of Diffuse Large B- Cell Lymphoma, Activated B- Cell Type

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ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogenous neoplasm of lymphoid series cells constituting 30%–40% of non-Hodgkin lymphoma in adults. DLBCL presents significant heterogeneity
Aim: To determine the frequency of activated B-cell subtype of DLBCL by using Hans algorithm.
Methods: It was designed as a cross sectional study conducted in a duration of six months (5th Jun 2018 to 7th January 2019). All data was collected by using a Performa. The clinical parameters like age and gender were recorded. The histopathological method used was paraffin embedding and hematoxylin–eosin staining. The immunohistochemical technique used on serial sections was immune-enzymatic soluble complex method.
SPSS version 23 was used for data entry and analysis. Mean and standard deviation was determined for quantitative variables like age and tumor size. Calculation of qualitative variables like gender, tumor site (nodal as well as extra-nodal) and immunohistochemical staining was done in the form of frequencies and percentages.
The mean age of the patients was 48.55±15.41 years. Majority of them were male with frequency of 69(71.9%) and female were 27(28.1%). Mean size of lymphoma was 12.44±4.24cm in the patients. Most of the cells were nodal with frequency of 58(60.4%) and a few extra nodal with frequency of 38(39.6%). It was noticed that germinal centre B-cell type was in 53(55.2%) and activated B-cell type lymphoma was present in 43(44.8%).
Conclusion: It is observed that frequency of the activated B–cell type lymphoma are less common in patients with lymphoma.
Keyword: Lymphoma, Germinal, carcinoma

INTRODUCTION

WHO classifies lymphoid neoplasms as Hodgkin's (HL) and Non-Hodgkin's lymphomas (NHL). NHL are further sub-categorized as mature B,mature T and NK cell neoplasms[1]. B-cell NHL are common than T-cell NHL comprising 80-85% of all cases of NHL , later constituting the remaining 10%[2]. Diffuse large B-cell lymphoma (DLBCL), is the most common lymphoid neoplasm in adults attributing to 30-50% of all NHL[3].

DLBCL is considered a lymphoid malignancy of medium to large sized B lymphoid cells, with a diffuse or nodular growth pattern. The size of the tumor cells is measured by comparing it with the nucleus of normal macrophage or with the size of normal lymphocyte as it should be larger than both.Clinical presentation, behavior and prognosis of DLBCL depend upon site and molecular sub-type[4,9].

DLBCL can be grouped into two distinct molecular forms: germinal center B-cell (GCB) and activated B-cell (ABC) by gene expression profiling (GEP). Both have different prognosis and response to treatment. Immunohistochemistry (IHC) algorithms have been suggested to anticipate the molecular forms. Hans algorithm is mostly used in routine and is based on three immunohistochemical stains, CD10 (highlights GCB subtype), Bcl6 (relates to both GCB and ABC subtype), MUM1(highlights ABC subtype). Hans algorithm can subdivide DLBCL,NOS into two groups depending upon the positivity of these three immunohistochemical stains,:GCB and ABC subtype [5, 6, 7] GCB type being more chemosensitive with better median survival rates and ABC subtype of DLBCL responds better to newly proposed therpeutic agents like bortezomib, lenalidomide or ibrutinib[5,7].

The various studies carried out show ABC to be more prevalent than GCB. A prevalence of 61.3% for ABC has been reported by Lu TX et al. in China[5]. A somewhat similar prevalence has been reported in Pakistan by U Hassan et al. (47%)[8] and U Bukhari et al (56%)[10].

GCB and ABC subtypes of DLBCL have different response to treatment, therefore, further segregation of DLBCL into GCB and ABC is necessary. There is variability in the results obtained by the studies conducted nationally and internationally[5,8].

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The aim of our study is to find actual frequency of ABC subtype in our population.

MATERIALS AND METHODS

This cross-sectional study was conducted at Chughtai Lab, Lahore which was completed in 6 months. The sample size is estimated to be 96 cases, using 95% confidence interval and 7% margin of error taking expected percentage of ABC subtype as 47% in DLBCL[8]. Non-probability consecutive sampling technique was used.
Inclusion criteria: Patients of both gender age ranging between 20-80 years, all nodal and extra-nodal disease assessment, block for review and immunohistochemistry from outside laboratories and all incisional, core and excisional biopsies.
Exclusion criteria: All autolysed/unfixed samples (samples in weak formalin).
Data collection: After approval from institutional ethical committee , the biopsy specimen fulfilling the inclusion criteria was enrolled in the study. Non-GCB was confirmed as per operational definition. All data was collected by using a performa. The requisition forms sent from surgery department would be retrieved along with other relevant investigations. The clinical parameters like age and gender was recorded. The histopathological method used was paraffin embedding and hematoxylin–eosin staining. The immunohistochemical technique used on serial sections was immune-enzymatic soluble complex method. The antibodies used were monoclonal, mouse anti-human protein against MUM-1, CD-10 and Bcl-6 from Dako.
Data analysis procedure: SPSS version 23 was used for data entry and analysis. Mean and standard deviation was determined for quantitative variables like age and tumor size. Calculation of qualitative variables like gender, tumor site (nodal as well as extra-nodal) and immunohistochemical staining was done in the form of frequencies and percentages. Stratified analysis was conducted for effect modifiers like age, gender and tumor site. Application of chi-square test was done after stratification by taking P value of 0.05 as significant.

RESULTS

Mean age of the patients was 48.55±15.41 years (table1). Most of the patients were male with frequency of 69(71.9%) and female

were just 27(28.1%)[table2]. Mean size of lymphoma was 12.44±4.24cm in the patients (Table 3). Most of the cells were nodal with frequency of 58(60.4%) and few extra nodal with frequency of 38(39.6%) (Table 4). it was noted that germinal centre B- cell type was in 53(55.2%) and activated B-cell type was in 43(44.8%) (Table 5).

After stratification for age, gender, size and site of lymphoma it was noticed that there was no significant difference for the activated B cell subtype as noted in the table6-8. But a significant difference was noticed in the site of the activated B celll subtype in the lymphoma p-value was <0.05 (Table 9).

Table 1: Distribution of the Mean Age in the Study Group

N	96
Mean	48.55
Std. Deviation	15.41

Table 2: Distribution of the Gender in the Study Group

	Frequency	**%age**
Male	69	71.9
Female	27	28.1
Total	96	100

Table 3: Distribution of the Mean Size of Lymphoma in the Study Group

N	96
Mean	12.4479
Std. Deviation	4.24728

Table 4: Distribution of the Site of Lymphoma in the Study Group

	Frequency	**%age**
Nodal	58	60.4
Extra nodal	3839.6	
Total	96	100

Table 5: Distribution of DLBCL type

	Frequency	%age
GCB	53	55.2
ABC	43	44.8
Total	96	100

Table 6: Stratification of DLBCL type for Age

Age group	**Type of DLBCL**		**P value**
	GCB	**ABC**	
20-25 years	**27(52.9%)**	**24(47.1%)**	0.68
>50 years	**26(57.8%)**	**19(42.2%)**	
Total	**53(55.2%)**	**43(44.8%)**	96(100%)

Table 7: Stratification of DLBCL type for Gender

Gender of patients	**Type of DLBCL**		**P value**
	GCB	**ABC**	
Male	**40(58%)**	**29(42%)**	**0.49**
Female	**13(48.1%)**	**14(51.9%)**	
Total	**53(55.2%)**	**43(44.8%)**	**96(100%)**

Table 8: Stratification of DLBCL type for Size of Lymphoma

Size of lymphoma	**Type of DLBCL**		**P value**
	GCB	**ABC**	
4-15cm	**39(54.9%)**	**32(45.1%)**	**0.55**
>15cm	**14(56%)**	**11(44%)**	
Total	**53(55.2%)**	**43(44.8%)**	**96(100%)**

Table 9: Stratification of DLBCL type for Site of Lymphoma

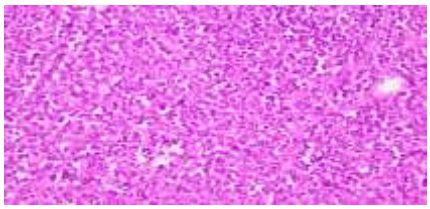
Site of lymphoma	**Type of DLBCL**		**P value**
	GCB	**ABC**	
Nodal	**39(67.2%)**	**19(32.8%)**	**0.03**
Extra nodal	**14(36.8%)**	**24(63.2%)**	
Total	**53(55.2%)**	**43(44.8%)**	**96(1000%)**

DISCUSSION

As there have been advances in knowledge of cancer pathogenesis, multiple targeted therapies have been proposed and it is expected that therapy will alter the individual biology. Researchers have defined the molecular charecteristics and diversity in major lymphoma types, and many new agents are in development. However, patient and material selection is very important for research purposes . Studies have shown that diagnosed cases of DLBCL respond to (R-CHOP) rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone with or without the proteasome inhibitor bortezomib (VELCADE®).However, the response of non-GCB subtype is poor as compared to GCB type to the aforementioned agents,the mechanism being the activation of the nuclear factor-κB pathway which skips inhibition by bortezomib in ABC subtype[11].

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) constituting 30-50% of all NHL charecterized by large sized neplastic B lymphoid cells, with a diffuse or nodular growth pattern (figure1). The size of the tumor cells is measured by comparing it with the nucleus of normal macrophage or with the size of normal lymphocyte as it should be equal to or larger than the macrophage nucleus or more than two times the size of a normal lymphocyte. The salient morphological variants of DLBCL are centroblastic, immunoblastic, T-cell/ histiocyte-rich, and anaplastic. It has a wide age range, has nodal/extranodal presentation and exhibits marked morphological variation. It is an aggressive lymphoid malignancy with short term survival, if left untreated. However,bone marrow involvement is merely seen at in 20–30% of patients at the time of diagnosis. Therefore, the prognosis and survival rate of patients diagnosed as DLBCL has been improved with treatment modalities like rituximab, from 80% to 90% in the patients who have low-risk disease[12,13].

Figure 1



The disease has been recognized as heterogeneous at clinical, pathological, morphological, molecular, and at immunophenotypic levels. To identify the heterogeneity at molecular level, gene expression profiling (GEP) study has been performed by Alizadeh et al. and which revealed that DLBCL can be sub-categorized into the prognostically important subtypes such as germinal center B-cell-like (GCB), activated B-cell-like (ABC), or type 3 GEPs. As their name indicate, their genetic profile is similar to the normal germinal centre B cells and activated B cells, whereas type 3 group is not well defined. The ABC subtype and the type 3 group have been assembled together as the non-GCB, as both have same prognostic implication, while the patients labeled as DLBCL GCB subtype have a better prognosis and overall good survival rate[14,15].

Different studies have been carried out during the last decade for identification of the molecular subtypes of the DLBCL that are of clinical and prognostic significance. In the year 2004, Hans et al. found that the molecular subtypes can also be anticipated using panel of three immunostains, that is CD10, BCL-6, and MUM1, and these stains corresponded to the genetic profile in 71% of germinal centre B cell type and 88% of non-germinal centre B cell type (Fig. 2,3 &4). The present study was performed in the uniform series of DLBCL patients treated with RCHOP. Different alogrithms were used to startify DLBCL into prognostically valuable subtypes. However, Hans algorithm proved to be the most significant[16,17] .The current study showed that GCB subtype of DLBCL is more oftenly encountered than the non-GCB subtype, just like in the west. However, ABC subtype outnumbers the GCB subtype in the Asian countries. Environmental and genetic factors are probably responsible for the variation in frequency of different DLBCL subtypes as they influence its pathogenesis, which strongly suggests that more research toward the pathogenesis of lymphoid neoplasm is needed. Another reason for variation could be due to the difference in selection criteria of patients in the studies. Several studies

have examined the immmunophenotypes in extranodal cases only and have shown that most extranodal DLBCL belong to the non-GCB phenotype. Therefore, inclusion of patients with extranodal disease could be the reason for higher percentage of non-GCB in Asian countries[18].

Figure 2(CD-10) Figure 3(CD-10)

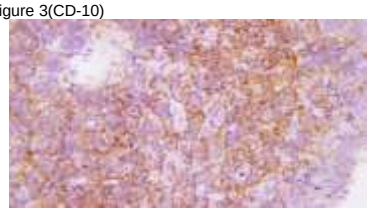


Figure 4(BCL6)

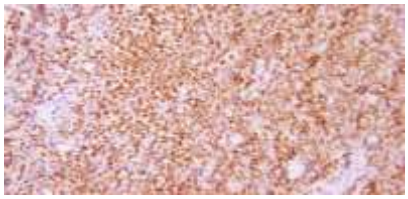
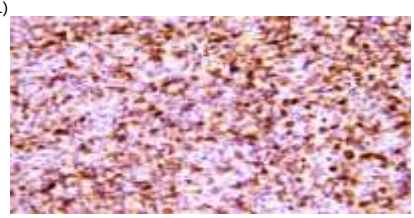


Figure 5(MUM1)



It has been confirmed through various studies that GCB subtype has better survival than non-GCB subtype; nevertheless, no significant difference was found between the two subtypes in our study with respect to the response to the treatment and survival that is similar to a study of Benesova et al. The cause of nonsignificance could be owing to the small sample size and treatment of patients through RCHOP because it has been revealed through some studies that RCHOP significantly improves the clinical outcome in DLBCL patients. A study done by Nyman et al. has shown that the supplementary advantage of RCHOP had been mainly observed among the patients with non-GCB DLBCL[19].

According to one of the studies, 44 patients (53%) came out to have GCB immunophenotype and 39 patients (47%) showed non-GCB phenotype. However, there was no marked disparity between the two groups regarding overall survival rates. This study revealed similar results[20]. In another study 29% of the selected population showed GCB phenotype and 71% showed the non-GCB phenotype[21]. Choi et al, established a new alogrithm according to which 34 cases (27.4%) were analyzed as GCB and 90 (72.6%) as the non-GCB subtype. Both of the above mentioned algorithms established the fact in Chinese patients that the GCB subtype of DLBCL was less frequently seen than the non-GCB subtype ($P < .0001$) which was different from the current study[22].

This study has limitation of the sample size due to time constraint. Hence in order to get more accurate results, it is recommended that this study should be conducted on a larger scale with increased number of cases.

CONCLUSION

It is observed that germinal center subtype DLBCL is more common as compared to the non germinal center subtype DLBCL

It gives compliance to the studies already carried out at the different centers and geographical areas.

Conflict of interest: Nil

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