Comparative analysis of immunohistochemical features of nodal versus extranodal diffuse large B cell lymphoma with respect to cell-of-origin classification

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ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL) represents a heterogeneous group of lymphoid malignancies with distinct oncogenic events and clinical behavior that cannot be unraveled by morphology and immunophenotype alone. Simple biological segregation such as the Hans classifier helps to explain the heterogeneous responses to standard treatment and provides a rationale for the investigation of novel targeted therapies. Aims and Objectives: In this study, we tried to estimate immunohistochemical (IHC) features of nodal and extranodal DLBCL using cell-of-origin classification (Hans Algorithm) with the markers CD10, Bcl-6, MUM1. Materials and Methods: Blocks of the patients with a histological diagnosis of DLBCL over the past 3 years were retrieved and submitted for IHC analysis to classify into germinal center B cell type (GCB) and non-GCB type. Results: Mean age for nodal DLBL was 48.19 ± 14.68 years, extranodal were 55.7 ± 13.22 years. Mean age for GCB were 56.6 ± 15.66 years whereas for non-GCB were 51.45 ± 12.85 years. Among nodal lymphomas cervical was the most common site and among extranodal lymphomas, intestinal lymphomas were commonest (including colorectal). Relative proportion of GCB among extranodal was 28.78%, whereas in nodal DLBCL it was 16.67%, relative risk of getting GCB type DLBCL was 1.72 times higher in extranodal compared to nodal DLBCL (P = 0.081). Total positivity of MUM1 was 17%, whereas for Bcl6 and CD10 it was 29% and 15% respectively. Ki67 was considerably higher in GCB type and for extranodal DLBCL in our study. Conclusion: Proportion of extranodal GCB type DLBCL compared to nodal DLBCL is considerably higher in our study population, though it varies greatly among Asian and world data. Uniform meta-analysis and systematic review is necessary to stratify.

KEY WORDS: Diffuse Large B Cell Lymphoma; Immunohistochemistry; Non-Hodgkin; B-cell Lymphoma 6 Protein; Human

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of Non-Hodgkin lymphoma (NHL), and accounts for 30–40% of all adult NHL. Although DLBCL is a potentially curable disease with current therapy, 40% of patients will die of relapsed or refractory disease. DLBCL accounts for one-third of all NHLs in Western studies and constitutes 60–70% of all B cell lymphomas in Asia. The prevalence in India has been documented to be 38%. Gene expression profiling (GEP) of DLBCL showed the molecular subtypes of germinal center B-cells (GCB) and activated B-cells (ABC) to have different overall survival (OS) curves with standard regime of therapy. The Leukemia Lymphoma Molecular Profiling Project applied the same microarray...
GEP to an expanded cohort and identified three subgroups within DLBCL: ABC, GCB, and unclassified subgroups.

The DLBCL subtypes of GCB or non-GCB were categorized using CD10, BCL6, and MUM1 according to the Hans algorithm.[4-6] Hans classifier is a valid method to evaluate the prognosis of DLBCL-Not otherwise specified (NOS). The clinicopathological and molecular genetic diversity of DLBCL was reflected in the 2008 WHO classification of lymphomas. Furthermore, the updated revision in 2016 described many subgroups and entities based on distinct morphological, immunophenotypical, molecular, and clinical parameters.[7] DLBCL represents a heterogeneous group of lymphoid malignancies with distinct oncogenic events and clinical behavior that cannot be unraveled by morphology and immunophenotype alone.[8,9] Simple biological segregation such as the Hans classifier helps to explain the heterogeneous responses to standard treatment and provides a rationale for investigation of novel targeted therapies.[10] There are few studies in East India showing Cell of origin classification applied on extranodal DLBCL, and wide variation of data is noted among authors.

In this study, we estimated the relative proportion of GCB and Non-GCB type DLBCL among nodal and extranodal DLBCL in our institute along with their relative demographic profile such as age, sex, and site distribution.

MATERIALS AND METHODS

In this observational cross-sectional study, 100 Patients with a histological diagnosis of DLBCL submitted for immunohistochemical (IHC) analysis in our department over 3 years (2017–2020). After receiving the approval of the Institutional Ethical committee (MC/KOL/IEC/ NON-SPON/861/01/2021 dated 04/01/2021), investigators retrieved records and contact numbers of previously diagnosed DLBCL in our department. Informed consent is taken over the phone or by recall visits. After receiving consent, paraffin blocks are retrieved from archives. IHC is done by standard operating protocol followed in our laboratory using moist heat-based antigen retrieval by pressure cooker and antigen expression detection by polymer detection method. CD10, Bcl-6, MUM-1 and ki-67 antigen expression were detected. Known positive control was run in parallel. Ki-67 positivity was expressed in percentage of 100 tumor cell nuclei counted. For CD10, Bcl-6, and MUM-1 at least ≥30% positivity in tumor cell nuclei was taken as Positive.[10] Findings were noted and presented in tables and graphs. Simple statistical calculation was done in SPSS version 11 software.

RESULTS

In this study, we evaluated 100 cases of histology-proven DLBCLs in our institute over the period of 3 years. Mean age of study population was 53.38 years (±14.96 Standard deviation). Age range was varied from 14 to 80 years. Mean age for nodal DLBL were 48.19 ± 14.68 years whereas for extranodal were 55.7 ± 13.22 years. Mean age for GCB were 56.6 ± 15.66 years whereas for non-GCB were 51.45 ± 12.85 years [Figure 1].

Sex ratio was M: F = 2.125:1. There were 31 cases of Nodal lymphoma and 69 cases of extranodal lymphomas. Using the Hans algorithm we categorized 26 cases of GCB and 71 cases of non-GCB while 3 could not be categorized. Total positivity of MUM1 was 17%, whereas for Bcl6 and CD10 it was 29% and 15% respectively. Among nodal lymphomas cervical was the most common site and among extranodal lymphomas, intestinal lymphomas were the most common (including colorectal) [Figure 2].

Relative proportion of GCB among extranodal was 28.78%, whereas in nodal DLBCL it was 16.67% [Figure 3]. After

![Figure 1: Age distribution of germinal center B-cells (GCB) versus non-GCB type diffuse large B-cell lymphoma](image1)

![Figure 2: Site distribution of nodal versus extranodal diffuse large B-cell lymphoma](image2)
In our study, mean age of extranodal DLBCL is higher than nodal DLBCL. Occurrence of DLBCL is more in males. This is in accordance with Newton et al. who opined that the frequency of extranodal lymphomas arises in countries where lymphoma incidence is higher.\textsuperscript{[11]} Khera et al. also inferred that the earlier age of onset, male dominant sex ratio, and higher frequency of B symptoms sets apart DLBCL in the Indian population from that in the developed countries.\textsuperscript{[12]} In our study, among nodal lymphomas cervical was the most common site and among extranodal lymphomas, intestinal lymphomas were commonest (including colorectal). Similar finding was demonstrated by Khera et al. and Sardar et al.\textsuperscript{[12, 13]} Different studies have been carried out in the last decade for the identification of the molecular subtypes of the DLBCL that are of clinical and prognostic significance. Hans et al. found that the molecular subtypes could also be predicted using panel of only three immunostains, that is CD10, BCL-6, and MUM1, and the panel reproduced the gene expression results in 71% of GCB and 88% of non-GCB.\textsuperscript{[6]} The current study showed that the frequency of GCB subtype of DLBCL is significantly lower than the non-GCB subtype, similar to the results of the Kim et al., López-Guillermo et al.\textsuperscript{[14, 15]} In our study we have found that most extranodal DLBCL belongs to the non GCB phenotype which are similar to other studies.\textsuperscript{[12–27]} Whereas, it is reversed in western countries.\textsuperscript{[18–22]} Several studies have examined the immunophenotypes in extranodal cases only (G.I., tests, breast and CNS) and have shown that most extranodal DLBCL belongs to the non-GCB phenotype.\textsuperscript{[22–24]} CD10 plays a critical role for GCB allocation in the Hans method, but it has been found CD10 expression is highly variable among DLBCL.\textsuperscript{[25]} In the present study, CD10 membrane positivity was found in 15% of the cases. This is much lower than the study by Colomo et al. and Dogan et al.\textsuperscript{[26, 27]} It is also to be noted that all these are western data. Sahai et al. though their finding regarding CD10 is 65%,\textsuperscript{[29]} The slightly higher incidence may be because of ethnic reasons or maybe due to the selection of a lower cut-off value. In our study, the expression of Bcl-6 among all the cases was found to be 29%. Hans et al. had found a Bcl-6 positivity of 56%.\textsuperscript{[6]} Dogan et al. found near 80% positivity.\textsuperscript{[27]} Sahai et al. found it to be 20%.\textsuperscript{[28]} However, the study of the Lunenburg lymphoma biomarker consortium emphasizes that Bcl-6 is the most variable and difficult marker to score.\textsuperscript{[29]} About 50% or more of non-GCB cell subtypes are known to express MUM1. Hans et al. and Chang et al. had introduced it as a marker of non GCB cell subtype of DLBCL.\textsuperscript{[6, 21]} Sahani et al. has 43% MUM1 positivity.\textsuperscript{[22]} Falini et al. suggested that DLBCL related to GCB cell subtype do not express the MUM1 protein.\textsuperscript{[30]} In our study, 17% of the cases were seen to express MUM1 which is quite less than the aforesaid studies. Based on the results of the present study, among extranodal mean ki67 was 36 ± 0.29% for nodal DLBCL it was 26 ± 0.29%, thus showing proliferative activity was considerably higher in GCB type and for extranodal DLBCL in our study.
proportions of NHL subtypes among developing countries and between developing countries and the rest of the world presumably arise from differences in environmental and genetic factors that influence lymphomagenesis and strongly suggest that more research in developing countries would provide valuable insights into the pathogenesis of lymphoid neoplasm.

The strength of this study lies in the fact that it proved an Institute with limited infrastructure with only 3 IHC markers is sufficient to categorize morphological diagnosis of DLBCL into prognostic categories like GCB and non-GCB. Literature review showed a lot of overlap and contradictory result regarding the cell of origin classification and its prognostic values. Hence, authors feel that categorization by Hans algorithm remains a basic standardized approach to primary prognostication of a vast complicated entity like DLBCL.

Limitation remains the small sample size, lack of a robust statistical analysis and comparison with molecular and IHC subcategorization by methods other than Hans algorithm.

CONCLUSION

Proportion of extranodal GCB type DLBCL compared to nodal DLBCL is considerably higher in our study population, though it varies greatly among Asian and world data. Uniform meta-analysis and systematic review are necessary to stratify. We must remember that the pathogenesis of DLBCL is not isolated but an intricate interaction between various markers and factors. The best and the most consistent of these have to be identified and authenticated with respect to different subpopulations so that tailor-made; individualized and effective therapy can be instituted. We suggest that much work needs to be done to standardize the Hans and other IHC methods which currently should be considered unreliable surrogates for molecular classification in DLBCL.

REFERENCES

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