DOI: 10.7860/JCDR/2023/63127.18193

Original Article

Pathology Section

# Analysis of Diffuse Large B-cell Lymphoma using Immunohistochemical Algorithm: A Cross-sectional Study

JASMIN SCARIA[1], ER JYOTHI RANI[2], PP SATHI[3]




## ABSTRACT

**Introduction:** Diffuse Large B-cell Lymphoma (DLBCL) is the most common Non-Hodgkin's Lymphoma (NHL). Using Gene Expression Profiling (GEP) DLBCL has been subtyped into two groups of prognostic importance, Germinal Center B-cell (GCB) like and activated B-cell like. GCB DLBCL has a better survival and can be identified using Hans algorithm with three immunohistochemical markers Cluster Differentiation (CD10), B-cell lymphoma 6 (BCL6) and Multiple Myeloma Oncogene-1 (MUM1).

**Aim:** To analyse DLBCL using Hans algorithm as both GCB lymphoma and non GCB lymphoma have different treatment and prognosis.

**Materials and Methods:** This was a cross-sectional study conducted in the Department of Pathology, Government Medical College, Kozhikode, Kerala, India, from January 2019 to December 2020. A total of 97 DLBCL cases received in the Department of Pathology, from January 2016 to December 2019 were included in the study were subtyped using Hans algorithm. CD10, BCL6 and MUM1 were considered positive, if more than 30% of the tumour cells showed staining by the respective antibodies. The relation between DLBCL subtypes and the age, gender, symptoms, site of initial involvement, organomegaly, Ann Arbor stage, treatment response and overall survival. Findings in the patients were analysed using Chi-square test. Statistical Package for Social Sciences (SPSS) software version 18.0. Overall survival was estimated using Kaplan-Meier method.

**Results:** The median age of study population was 60 years (age range: 31-85 years) and there were 55 (56.7%) males and 42 (43.3%) females. Out of the 97 DLBCL cases 47 (48.5%) were GCB and 50 (51.5%) were non GCB subtype. Statistical analysis was done only in 88 patients (excluded nine recurrent lymphoma patients, which may have a different outcome). There was significant association (p-value=0.003) between stage and subtypes as majority of the non GCB cases presented in an advanced stage. The rate of complete remission with Rituximab Cyclophosphamid Hydroxydaunorubicin Oncovin Prednisone (RCHOP) chemotherapy was higher in GCB (58.75%) compared to non GCB (15.25%) subtypes (p-value=0.001). Overall survival rate of GCB was 74.4% and non GCB was 31% with a p-value of 0.001. There was no statistically significant relation between DLBCL subtypes and other clinicopathological factors.

**Conclusion:** In the present study, the patients within the GCB subtype had better treatment response and overall survival rate compared to non GCB subtype. Non germinal center subtype presented in advanced stage and had a worse prognosis. Therefore, it is essential to subtype DLBCL in all cases to identify non GCB subtype, which may need additional treatment after RCHOP chemotherapy.


**Keywords:** Algorithm, Germinal center, Hans algorithm, Survival rate

## INTRODUCTION

The DLBCL is the most common type of NHL, with several morphologic and clinicopathologic variants. DLBCL comprises about 30-40% of all NHL cases [1]. Distinctive molecular and genetic abnormalities have been identified in DLBCL, and patients with this disease exhibit a wide range of clinical presentations and variable outcome [2]. The Cell of Origin (COO) classification has been the most significant development in the understanding of DLBCL biology. The gold standard test to identify COO is GEP [3]. Recent GEP has subtyped DLBCL into two main prognostically important subgroups GCB type and non GCB type with the germinal center type showing overall better survival [4]. Determination of gene expression profiles requires fresh or frozen tissue for extraction of Ribonucleic Acid (RNA), which is costly and difficult to implement in regular clinical practice. To subtype DLBCL for guiding the treatment and predicting the prognosis many studies have proposed the use of Immunohistochemistry (IHC) with antibodies that represent different B-cell differentiation stages which are more easier and economical [5]. Hans CP et al., postulated an IHC algorithm to classify DLBCL into GCB and non GCB subtypes using three markers CD10, BCL6 and MUM1 [3,6].

The IHC algorithmic subtyping of DLBCL is helpful for clinicians to differentiate which patient requires further treatment after RCHOP based chemotherapy, as non GCB type requires it to improve their outcome. At present there is RCHOP plus treatment with Lenalidomide, Bortezomib in many centres for non germinal centre type DLBCL [7]. The goal of the current study was to subtype DLBCL using Hans algorithm into GCB lymphoma and non GCB lymphoma and to assess their overall survival and treatment response.

## MATERIALS AND METHODS

This was a cross-sectional study conducted in the in the Department of Pathology, Government Medical College, Kozhikode, Kerala, India, from January 2019 to December 2020. A total of 97 patients with histopathological diagnosis of DLBCL received from January 2016 to December 2019 were included in the study. After obtaining approval from Institutional Ethics Committee (Ref.No.GMCKKD/RP 2019/IEC/55 dated 19-1-2019).

**Inclusion criteria:** All patients with confirmed histopathological diagnosis of DLBCL from both nodal and extranodal sites and whose tissue blocks are available in the Department of Pathology were included in the study.

**Exclusion criteria:** Patients with past history of any lymphoma were excluded from the study.

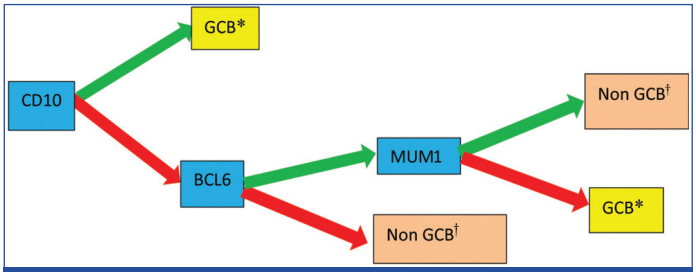
## Study Procedure

The patients were retrospectively selected and analysed after subtyping immunohistochemically into GCB and non GCB subtypes. Treatment response status of patients was assessed after a minimum period of six months following the treatment. The clinical records were reviewed in all patients with particular reference to age at diagnosis, gender, presence of B symptoms, site of involvement, organomegaly, bone marrow infiltration, Ann Arbor stage [8], response to treatment and overall survival.

Haematoxylin and Eosin (H&E) stained sections were examined to identify the morphology. Formalin-fixed paraffin sections of 3 µm thick were used for Immunohistochemistry (IHC) staining. The staining of CD10 (Dako clone 56C6), BCL6 (Dakoclone PG-B6p) and MUM1 (PathnSitu clone EP-190) were done manually. Specifications of the antibodies used in the present study are shown in the table [Table/Fig-1]. These antibodies were considered positive when more than 30% cells were stained with the respective antibody. For each case, the foci with the highest percentage of tumour cells stained were used for analysis. Hans's algorithm was used for the classification of subjects into two subgroups [Table/Fig-2] [3]. According to the algorithm, cases were subtyped to the GCB subgroup, if CD10 alone was positive. CD10 negative cases were further stained with BCL6. If BCL6 was negative, then the case was subtyped to the non GCB subgroup. Cases with BCL6 positivity were further stained with MUM1; if MUM1 was positive, then the case was assigned to the non GCB subgroup and negative cases were grouped under the GCB subgroup.

| Antibody | Clone | Source |
|---|---|---|
| CD10 | 56C6 | Dako |
| BCL6 | PG-B6p | Dako |
| MUM1 | EP-190 | PathnSitu |

**[Table/Fig-1]:** Specifications of antibody used.



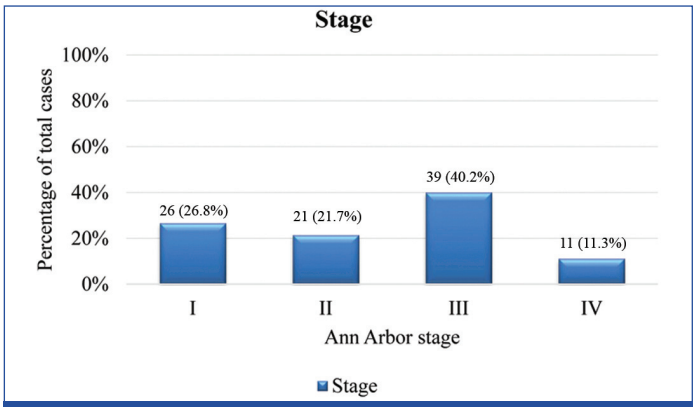

**[Table/Fig-2]:** Hans algorithm: Green arrow indicates positive and red arrow indicates negative.
GCB*: Germinal center B-cell type; Non GCB†: Non Germinal center type [3]; CD10: Cluster of differentiation 10; BCL6: B-Cell Lymphoma 6; MUM1: Multiple Myeloma Oncogene-1

## STATISTICAL ANALYSIS

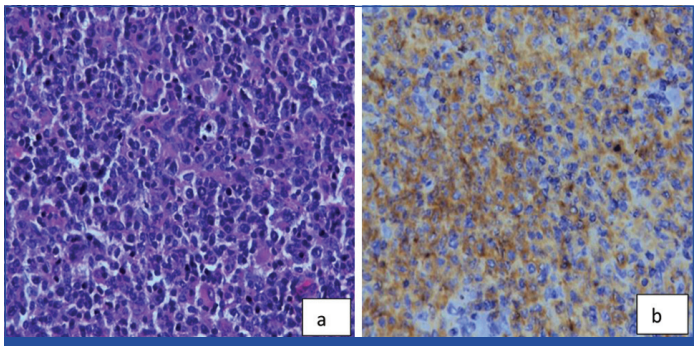
The present study was carried out to analyse DLBCL cases using immunohistochemical algorithm and to assess their treatment response. Findings in the patients were studied and tabulated using Microsoft Excel and statistical analyses were done using SPSS software version 18.0 in windows. The Kaplan-Meier method was used to estimate the overall survival rate and log rank test was used to compare the survival distribution. A p-value of <0.05 was considered statistically significant by applying Chi-square test.

## RESULTS

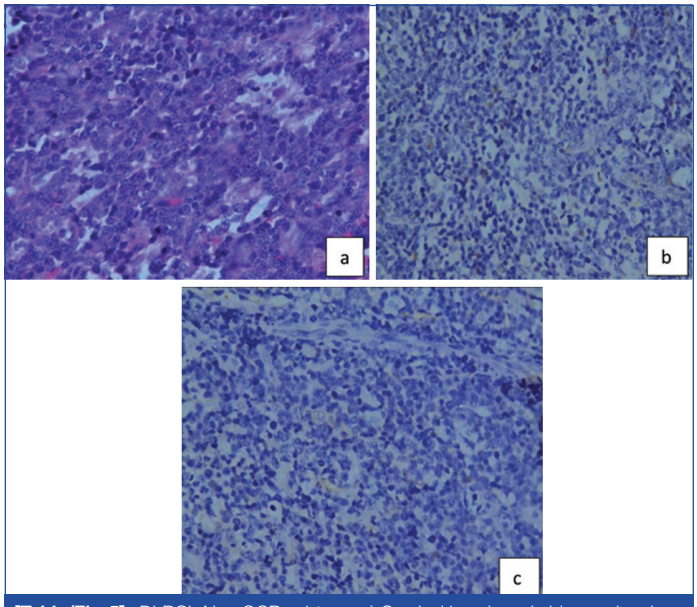
A total of 97 patients were analysed which includes 55 (56.7%) males and 42 (43.3%) females with a median age of 60 years (31-85 years). Among this 38 (39.2%) were in the age group of 55-64 years. Out of the 97 DLBCL cases 47 (48.5%) were GCB and 50 (51.5%) were non GCB subtype. Among the total study group, 62 (63.9%) had presented with lymph node involvement while extranodal presentation was seen in 35 (36.1%) cases. Out of the 35 (36.1%) extranodal DLBCL, the major sites involved were head and neck in 13 (13.4%) and Gastrointestinal Tract (GIT) in 12 (12.3%) patients. Among the total 97 patients, 30 (30.9%) had associated B symptoms, while rest were free of these symptoms. In the present study, 5 (5.20%) were with organ involvement, 1 (1%) with peripheral smear involvement and 9 (9.3%) with bone marrow involvement. With regard to Ann Arbor staging, out of the 97 cases studied, majority presented in stage-III [Table/Fig-3]. Out of the total patients, 47 (48.5%) had limited disease (Ann Arbor stage-I and II), while 50 (51.5%) had advanced disease (Ann Arbor stage-III and IV) by using Hans algorithm, GCB type was found in 47 (48.5%) cases, and non GCB type in 50 (51.5%) cases. CD10 positive GCB comprise 47 (48.5%) [Table/Fig-4a,b]. CD10 and BCL6 negative non GCB comprise 48 (49.45%) [Table/Fig-5a-c]. BCL6 and MUM1 positive and CD10 negative cases constitute 2 (2.1%) cases [Table/Fig-6a-d].



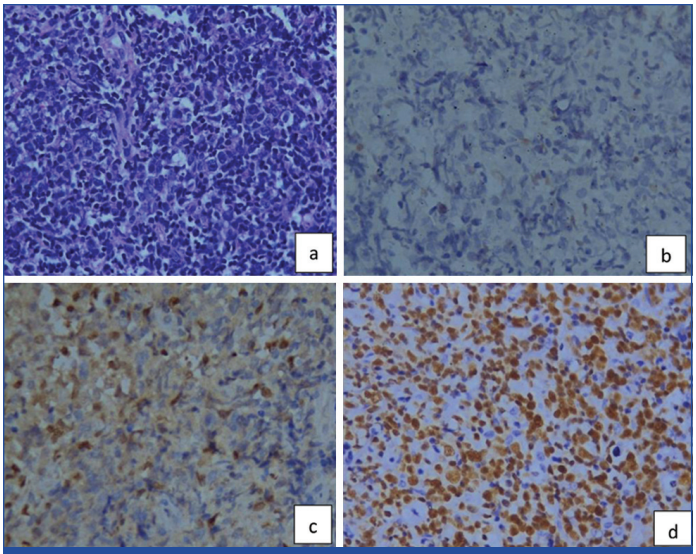

**[Table/Fig-3]:** Ann Arbor staging of all cases as n (%).




**[Table/Fig-4]:** DLBCL GCB subtype: a) Cervical lymph node biopsy showing Diffuse Large B-cell Lymphoma (DLBCL) (H&E, 40X); b) CD10 (IHC, 40X) stain showing strong membrane staining in tumour cells.




**[Table/Fig-5]:** DLBCL Non GCB subtype: a) Cervical lymph node biopsy showing Diffuse Large B-cell Lymphoma (DLBCL) (H&E, 40X); b) CD10 (IHC, 40X) negative; c) BCL6 (IHC, 40X) negative.




**[Table/Fig-6]:** DLBCL Non GCB subtype: a) Cervical lymph node biopsy showing Diffuse Large B-cell Lymphoma (DLBCL) (H&E, 40X); b) CD10 (IHC, 40X) negative; c) BCL6 (IHC, 40X) showing nuclear positivity; d) MUM1 (IHC, 40X) showing nuclear positivity.

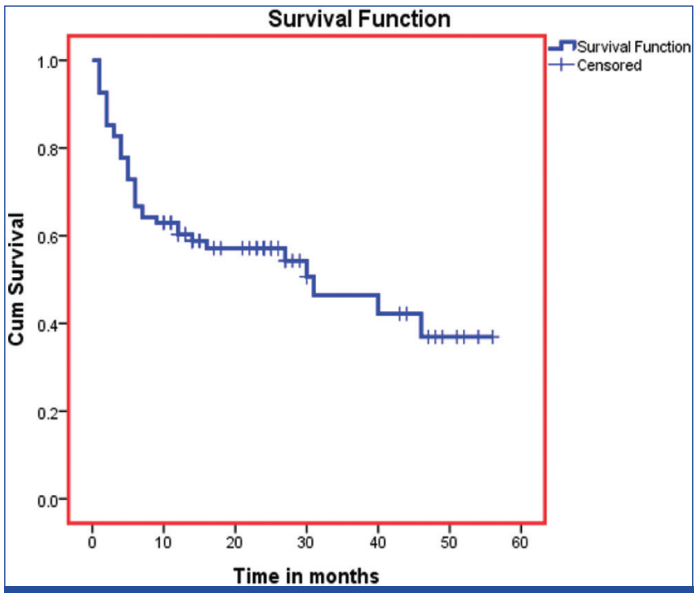
Among the total 97 cases, nine patients with a past history of lymphoma were excluded from the statistical analysis as they would have a different behaviour. Thus, statistical analysis was done in 88 (90.75%) cases, which included 42 (47.7%) GCB subtype and 46 (52.3%) non GCB subtype. There was no significant relation between DLBCL subtypes and other clinicopathological factors. Out of the total 88 DLBCL patients, follow-up regarding the treatment taken was obtained in 81 cases, and it was found that 46 (52.3%) patients could complete RCHOP chemotherapy. Response of the 46 (52.3%) cases, who had completed the whole six cycles of RCHOP chemotherapy was noted and is illustrated in [Table/Fig-7].

| Characteristics | Total patients n (%) | GCB* n (%) | Non GCB n (%) | p-value |
|---|---|---|---|---|
| **Gender (n=88)** | | | | |
| Male | 49 (55.7) | 26 (29.5) | 23 (26.1) | 0.261 |
| Female | 39 (44.3) | 16 (18.2) | 23 (26.1) | |
| **B symptoms† (n=88)** | | | | |
| Present | 28 (31.81) | 10 (11.4) | 18 (20.5) | 0.123 |
| Absent | 60 (68.2) | 32 (36.4) | 28 (31.8) | |
| **Site (n=88)** | | | | |
| Nodal | 54 (61.4) | 26 (29.5) | 28 (31.8) | 0.921 |
| Extranodal | 34 (38.6) | 16 (18.2) | 18 (20.5) | |
| **Organomegaly (n=88)** | | | | |
| Absent | 83 (94.3) | 37 (42) | 46 (52.3) | 0.121 |
| Hepatomegaly | 3 (3.4) | 3 (3.4) | 0 | |
| Splenomegaly | 1 (1.1) | 1 (1.1) | 0 | |
| Both | 1 (1.1) | 1 (1.1) | 0 | |
| **Bone marrow (BM)‡ infiltrate (n=88)** | | | | |
| BM not done | 5 (5.7) | 2 (2.3) | 3 (3.4) | 0.841 |
| Present | 9 (10.2) | 5 (5.7) | 4 (4.5) | |
| Absent | 74 (84.1) | 35 (39.8) | 39 (44.3) | |
| **Ann Arbor stage (n=88)** | | | | |
| Limited disease | 42 (47.7) | 27 (30.7) | 15 (17) | **0.003** |
| Advanced disease | 46 (52.3) | 15 (17) | 31 (35.2) | |
| **Treatment response (n=46)** | | | | |
| Complete response | 34 (73.9) | 27 (58.75) | 7 (15.25) | **0.001** |
| Recurrence | 6 (13.1) | 1 (2.2) | 5 (10.9) | |
| Death | 6 (13.1) | 1 (2.2) | 5 (10.9) | |

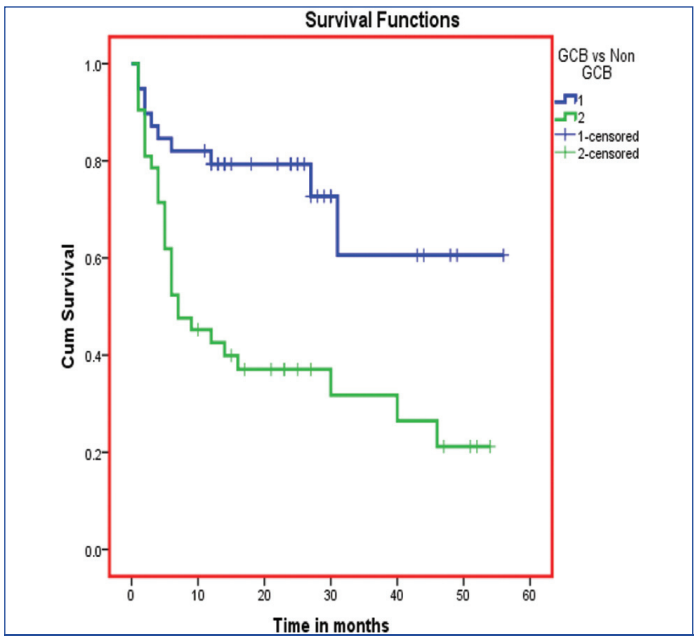
**[Table/Fig-7]:** Clinicopathologic characteristics of patients within DLBCL subtypes. *GCB: Germinal Center B-cell lymphoma; †B symptoms: Fever, night sweats, significant loss of weight; ‡BM: Bone marrow; The p-value in bold font indicates statistically significant values

There was no association between DLBCL subtypes and age as p-value was 0.45. The present study could find a statistically significant association between DLBCL subtypes and stage as majority of the non GCB had advanced disease, whereas GCB had limited disease [Table/Fig-7]. But there was no significant statistical relation between DLBCL subtypes with gender, B symptoms, site of initial involvement, organomegaly and bone marrow infiltration as shown in the table [Table/Fig-7]. Association between DLBCL subtypes and treatment response was statistically significant [Table/Fig-7]. Majority of the GCB cases achieved complete response to chemotherapy compared to non GCB subtype. Disease recurrence and death was more in non GCB subtype compared to GCB.

Overall survival rate was 51.9% and mean survival time was 30.15 months with a standard error of 2.9 [Table/Fig-8]. In this group, 32 (39.5%) had a follow-up period of more than 20 months and mean follow-up period was 17.8 months. Patients who had completed RCHOP chemotherapy had better overall survival rate with a statistically significant p-value of 0.001 (p-value <0.05) mean survival time of GCB patients was 40.286 months with a standard error of 4.368 and non GCB patients was 20.965 months with a standard error of 3.437 [Table/Fig-9]. GCB patients had better overall survival rate (74.4%) than non GCB type (31%) with a statistically significant p-value of 0.001 (p-value <0.05).




**[Table/Fig-8]:** Kaplan-Meier curve showing overall survival of all DLBCL patients.




**[Table/Fig-9]:** Kaplan-Meier curve showing overall survival of DLBCL subtypes. In the figure blue line: (1) indicates GCB; and (2) green line indicates Non GCB.

## DISCUSSION

The DLBCL is the most common type of B-cell lymphoma. Heterogeneity at molecular and clinical level of DLBCL makes it difficult for prognostication and treatment [9]. The intention of the study was to classify DLBCL into two prognostically important subtypes (GCB type and Non GCB type) and to find their relative proportion.

In the present study, all DLBCL cases received during the study period were analysed. Compared to the study done by Hans CP et al., where among 152 patients, non GCB predominated {n=88 (58%)} over GCB {n=64 (42%)}. In the present study out of 97 patients, both were almost of equal proportion with GCB being 47 (48.5%) and non GCB being 50 (51.5%) [6]. But in other Indian studies (Dwivedi A et al., Gogia A et al., and Sahai K et al., they found GCB subtype cases to be more than that of non GCB [10-12]. This may be due to difference in genetic factors of the study group that may influence the lymphoma genesis. In the present study, the cases ranged from a minimum age of 31 years to a maximum of 85 years with a median age of 60 years. Majority (39.20%) of the study population was in the age group 55-64 years. Like in the study done by Hans CP et al., (p-value=0.56) and Ichiki A et al., (p-value=0.06) in the present study too there was no significant relation with age and DLBCL subtypes (p-value=0.45) [6,13]. In the present study out of 88 cases, 56.7% were males and 43.3% were females with a male to female ratio of 1.26:1. Similar to other studies in literature (Dwivedi A et al., Ichiki A et al., and Berglund M et al., the present study also did not find a statistically significant relationship between gender and DLBCL subtypes [9,10,13].

Regarding relationship between B symptoms and DLBCL in the present study, there was no statistically significant relation and this matched the results obtained by Ichiki A et al., and Berglund M et al., [9,13]. Unlike the study done by Ichiki A et al., where 47.18% of non GCB had extranodal involvement with a statistically significant p-value of 0.008. In the current study, there was no statistically significant relationship between site of involvement and DLBCL subtypes [13]. In the present study, among the total number of cases, there was only 38.6% with extranodal involvement while the major bulk of the cases studied by Ichiki A et al., had extranodal involvement (66.8%) [13]. This may be the reason why this study did not get a relation between extranodal presentation and DLBCL subtypes. Similarly study done by Ichiki A et al., also could not find any significant relationship between bone marrow infiltrate and DLBCL subtypes [13]. Unlike studies done by Hans CP et al., and Dwivedi A et al., the present study found statistically significant association between stage and DLBCL subtypes [6,10].

Among the 88 cases, follow-up was obtained in 81 cases and of these 46 (52.3%) patients were able to complete RCHOP chemotherapy. The rate of complete remission was higher in GCB phenotype (58.75%) compared to non GCB (15.25%) showing a statistically significant p-value of 0.001. This was in concordance with the study done by Ichiki A et al., where it was showing a complete response of 77.5% for GCB and 52.6% for non GCB type with a p-value=<0.0001. Disease progression was seen in six cases even after RCHOP chemotherapy and among this five cases were of non GCB phenotype [13].

The present study found that overall survival rate of all DLBCL patients excluding those with past history of lymphoma was 51.9% with a mean survival time of 31 months. Compared to the GCB, the non GCB subtype showed higher incidence of disease recurrence and also higher mortality rate even after completion of chemotherapy. Among the patients who expired during chemotherapy, a higher number were of the non GCB subtype. This indicates that it is important to differentiate between these two subtypes. The present study found a significant difference in the overall survival of GCB subtype (74.4%) which is more than twice that of non GCB subtype (31%) with a p-value of 0.001. In the study done by Ichiki A et al., also they had a higher five year overall survival rate among GCB subtype (78%) compared to non GCB subtype (54%) with a p-value of 0.0012 [13]. However, the study done by Dwivedi A et al., could not find any statistically significant correlation between overall survival and these subtypes (p-value=0.51) [10].

### Limitation(s)

The limitations of the present study were LDH level and Karnofsky score were not available for calculating the International Prognostic Index (IPI). The present study could not attribute the exact cause of death of the patients who had expired during chemotherapy or after the completion of chemotherapy, whether it was disease related or chemotherapy related. GEP to compare the subtypes which had been done by other studies in literature was not done in these patients as it was not locally available and affordable. Since the present study duration was from 2016-2019 with a mean follow-up period of 17.81 months, further follow-up of the surviving patients may be needed for calculating five year overall survival rate.

## CONCLUSION(S)

The DLBCL can be subtyped using Hans algorithm and IHC into germinal center and non germinal center subtypes. In the present study, GCB type had better overall survival and treatment response and non GCB subtype presented in an advanced stage compared to GCB subtypes. Therefore, it is essential to subtype DLBCL in all cases to identify non GCB subtype which may need further treatment after RCHOP chemotherapy.

### Acknowledgement

The authors are extremely thankful to Dr. Feroze M, Former Professor, Department of Pathology, Government Medical College, Kozhikode, Kerala, India for his guidance and support. The authors acknowledge the help rendered by Dr. Biju George, Associate Professor, Department of Community Medicine, Government Medical College, Kozhikode in statistical analysis for present study. The authors express their gratitude to Mrs. Sreeja, Mrs. Usha, technicians of Histopathology laboratory, Government Medical College, Kozhikode and Mrs. Silja, technician of Multidisciplinary Research Unit of Government Medical College, Kozhikode, Kerala, India.

## REFERENCES

[1] Li S, Young KH, Medeiros LJ. Diffuse large B-cell lymphoma. Pathology (Phila). 2018;50(1):74-87.
[2] Iqbal J, Meyer PN, Smith LM, Johnson NA, Vose JM, Greiner TC, et al. BCL2 predicts survival in germinal center B-cell-like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. Clin Cancer Res Off J Am Assoc Cancer Res. 2011;17(24):7785-95.
[3] Choi WWL, Weisenburger DD, Greiner TC, Piris MA, Banham AH, Delabie J, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res Off J Am Assoc Cancer Res. 2009;15(17):5494-502.
[4] Rosenwald A, Staudt LM. Gene expression profiling of diffuse large B-cell lymphoma. Leuk Lymphoma. 2003;44(Suppl 3):S41-47.
[5] Li JM, Hou J, Li L, Wang Y. Immunohistochemical subtypes of diffuse large B-cell lymphoma in the head and neck region. Genet Mol Res GMR. 2015;14(2):3889-96.
[6] Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103(1):275-82.
[7] Wang L, Li LR, Young KH. New agents and regimens for diffuse large B cell lymphoma. J Hematol OncolJ Hematol Oncol. 2020;13(1):175.
[8] Rosenberg SA. Validity of the Ann Arbor staging classification for the non-Hodgkin's lymphomas. Cancer Treat Rep. 1977;61(6):1023-27.
[9] Berglund M, Thunberg U, Amini RM, Book M, Roos G, Erlanson M, et al. Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. Mod Pathol. 2005;18(8):1113-20.
[10] Dwivedi A, Mehta A, Solanki P. Evaluation of immunohistochemical subtypes in diffuse large B-cell lymphoma and its impact on survival. Indian J Pathol Microbiol. 2015;58(4):453-58.
[11] Gogia A, Das CK, Kumar L, Sharma A, Tiwari A, Sharma MC, et al. Diffuse large B-cell lymphoma: An institutional analysis. South Asian J Cancer [Internet]. 2018 [cited 2020 Oct 31];7(3):200-02. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6069326/.

[12] Sahai K, Gangadharan V, Singh H, Mani N. A study of the newer prognostic markers in diffuse Large B Cell Lymphomas. Med J Armed Forces India [Internet]. 2011 Jan [cited 2020 Oct 31];67(1):41-45. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4920616/.

[13] Ichiki A, Carreras J, Miyaoka M, Kikuti YY, Jibiki T, Tazume K, et al. Clinicopathological analysis of 320 cases of diffuse large B-Cell Lymphoma using the Hans classifier. J Clin Exp Hematop JCEH. 2017;57(2):54-63.

**PARTICULARS OF CONTRIBUTORS:**
1. Senior Resident, Department of Pathology, Government Medical College, Kozhikode, Calicut, Kerala, India.
2. Associate Professor, Department of Pathology, Government Medical College, Kozhikode, Calicut, Kerala, India.
3. Former Professor and Head, Department of Pathology, Government Medical College, Kozhikode, Calicut, Kerala, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
ER Jyothi Rani,
Krishna House, Thalayi Street, Edacheri Post, Vadakara,
Kozhikode-673502, Kerala, India.
E-mail: jyothiranier@gmail.com

**PLAGIARISM CHECKING METHODS:** [Jain H et al.]
- Plagiarism X-checker: Feb 09, 2023
- Manual Googling: Mar 24, 2023
- iThenticate Software: Apr 25, 2023 (18%)

**ETYMOLOGY:** Author Origin

**EMENDATIONS:** 6

**AUTHOR DECLARATION:**
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jan 29, 2023**
Date of Peer Review: **Mar 21, 2023**
Date of Acceptance: **May 01, 2023**
Date of Publishing: **Jul 01, 2023**