ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

www.surgicalneurologyint.com

Surgical Neurology International

Editor-in-Chief: Nancy E. Epstein, MD, Clinical Professor of Neurological Surgery, School of Medicine, State U. of NY at Stony Brook.

SNI: Neuropathology

Editor
Istvan Bodi, MD
King's College Hospital NHS Foundation Trust; London, United Kingdom

SNI
Open Access

Original Article

Double-hit and double-expressor primary central nervous system lymphoma: Experience from North India of an infrequent but aggressive variant

Mayur Parkhi[1#], Debajyoti Chatterjee[1#], Bishan Dass Radotra[1], Amanjit Bal[1], Budhi Singh Yadav[2], Manjul Tripathi[3]

Departments of [1]Histopathology, [2]Radiation Oncology, [3]Neurosurgery, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

E-mail: Mayur Parkhi - mayur.parkhi@gmail.com; Debajyoti Chatterjee - devchat1984@gmail.com; *Bishan Dass Radotra - bishanradotra@gmail.com; Amanjit Bal - docaman5@hotmail.com; Budhi Singh Yadav - drbudhi@gmail.com; Manjul Tripathi - drmanjultripathi@gmail.com
[#] These authors contributed equally to this manuscript and share the first authorship.

***Corresponding author:**
Bishan Dass Radotra,
Department of Histopathology,
Postgraduate Institute of
Medical Education and
Research, Chandigarh, India.

bishanradotra@gmail.com

Received : 08 April 2023
Accepted : 28 April 2023
Published : 12 May 2023

DOI
10.25259/SNI_307_2023

Quick Response Code:

ABSTRACT

Background: High-grade non-Hodgkin B-cell lymphoma is an aggressive mature B-cell lymphoma that depicts poor treatment response and worse prognosis. The presence of MYC and B-cell lymphoma 2 (BCL2) and/or B-cell lymphoma 6 (BCL6) rearrangements qualifies for triple-hit and double-hit lymphomas (THL/DHL), respectively. We attempted to explore the incidence, distribution, and clinical characteristics of the primary high-grade B-cell lymphoma of the central nervous system (CNS) in our cohort from North India.

Methods: All the histologically confirmed cases of primary CNS diffuse large B-cell lymphoma (PCNS-DLBCL) over a period of 8 years were included. Cases showing MYC and BCL2 and/or BCL6 expression on immunohistochemistry (IHC) (double- or triple-expressor) were further analyzed by fluorescence *in situ* hybridization for *MYC*, *BCL2* and/or *BCL6* rearrangements. The results were correlated with other clinical and pathological parameters, and outcome.

Results: Of total 117 cases of PCNS-DLBCL, there were seven (5.9%) cases of double/triple-expressor lymphomas (DEL/TEL) (six double- and one triple-expressor) with median age of 51 years (age range: 31–77 years) and slight female predilection. All were located supratentorially and were of non-geminal center B-cell phenotype. Only triple-expressor case (MYC+/BCL2+/BCL6+) demonstrated concurrent rearrangements for *MYC* and *BCL6* genes indicating DHL (n = 1, 0.85%), while none of the double-expressors (n = 6) showed *MYC*, *BCL2,* or *BCL6* rearrangements. The mean overall survival of the DEL/TEL was 48.2 days.

Conclusion: DEL/TEL and DHL are uncommon in CNS; mostly located supratentorially and are associated with poor outcome. MYC, BCL2, and BCL6 IHC can be used as an effective screening strategy for ruling out double/triple-expressor PCNS-DLBCLs.

Keywords: Central nervous system, Double-hit lymphoma, Fluorescence *in situ* hybridization, High-grade B-cell non-Hodgkin lymphoma, Immunohistochemistry

INTRODUCTION

Primary central nervous system (CNS) lymphomas are extranodal, malignant non-Hodgkin lymphomas (NHL) that are principally limited to the brain, leptomeninges, eyes or spinal cord, without systemic involvement.[12] Nearly all these cases (90–95%) have diffuse large

B-cell lymphoma (DLBCL) histology and carry a more dismal prognosis than systemic DLBCL.[12] Using Hans' algorithm, nodal DLBCLs are classified into two subgroups named germinal center B-cell (GCB) and non-GCB phenotypes where the former carries better prognosis.[4] However, this classification does not show any prognostic significance in primary CNS DLBCL.[9,14,15] The revised World Health Organization classification of hemato-lymphoid neoplasms (4th edition; 2016) has reclassified high grade B-cell lymphomas based on *MYC*, *BCL2,* and *BCL6* gene rearrangements. A small subset of B-cell NHL resembles DLBCL or Burkitt lymphoma or harbors a blastoid morphology and show *MYC* and *BCL2* and/or *BCL6* gene rearrangements. These are now known as "high-grade B-cell, double- or triple-hit lymphoma (DHL/THL)".[12] This subset of lymphoma shows aggressive behavior and low response to R-CHOP regime, thus carry poor prognosis.[10] The frequency and prognosis of DHL/THL in CNS has been investigated only by a handful of authors who have reported a very low incidence of DHL/THL in CNS.[2,3,5,7] In this study, we explored the incidence, distribution, and clinical characteristics of the primary double/triple-expressor and double/THL in the CNS among our cohort in North India.

MATERIALS AND METHODS

All histologically diagnosed cases of primary CNS (PCNS-DLBCL) over a period of 8 years (July 2013–June 2021) in our department were evaluated. Approval from the Institute Ethic Committee was obtained (reference no. NK/8003/Study/864). Majority of the cases were already worked-up for Han's algorithm as a part of two earlier published studies.[8,9]

Immunohistochemistry (IHC)

For diagnosis, subtyping and double/triple expressor status, the following antibodies were used: Glial fibrillary acidic protein (clone EP672Y, Cell Marque, dilution 1:100), leukocyte common antigen (clone 2B11 + PD7/26, Dako, dilution 1:100), CD3 (Rabbit polyclonal, Cell Marque, dilution 1:500), CD20 (clone L26, Dako, dilution 1:300), CD10 (clone 56C6, Cell Marque, dilution 1:20), B-cell lymphoma 6 (BCL6; cone G1191E/A8, Cell Marque, dilution 1:300), Multiple Myeloma 1 (clone MRQ-43, Cell Marque, dilution 1:300), B-cell lymphoma 2 (BCL2; clone 124, Dako, dilution 1:50), MYC (clone EP121, Cell Marque, dilution 1:50), and Ki-67 (clone SP6, Cell Marque, dilution 1:300). These were performed on Ventana, Biotek automated system with appropriate positive and negative controls run concurrently. Like the previous study, the standard universal cutoffs for positivity were considered.[8,9]

The IHC slides were independently evaluated by two pathologists, who were blinded to the clinical data. The cutoff for MYC, BCL6, and BCL2 positivity were taken as 40%, 50%, and 50%, respectively. Cases showing MYC and BCL2 and/or BCL6 expression (double- or triple-expressor lymphoma, DEL/TEL) were further analyzed by fluorescence *in situ* hybridization (FISH) for *MYC*, *BCL2,* and *BCL6* rearrangements.

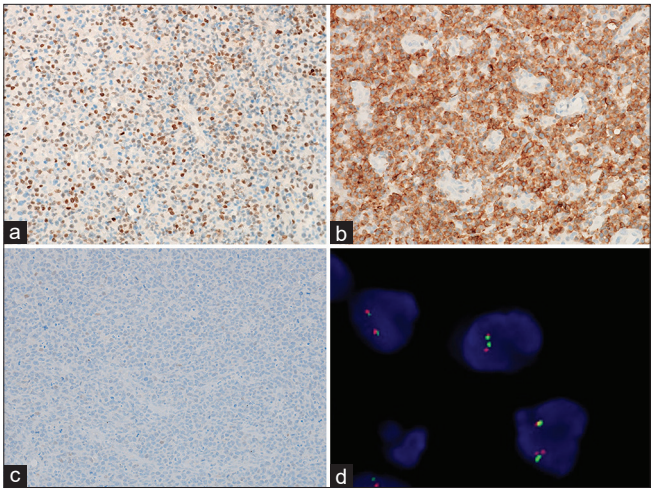

Figure 1: Primary double-expressor central nervous system lymphoma (Case 1). (a) Strong nuclear positivity for MYC (>40%; peroxidase; ×200). (b) B-cell lymphoma 2 (BCL2) showing diffuse cytoplasmic expression (>50%; peroxidase; ×200). (c) B-cell lymphoma 6 (BCL6) showing no positivity (peroxidase; ×200). (d) No *MYC* rearrangement on fluorescence *in situ* hybridization. Furthermore, no rearrangements for *BCL2* and *BCL6* genes (images not provided).

FISH

FISH was performed on formalin-fixed, paraffin-embedded tissue for *MYC, BCL2,* and *BCL6* rearrangements using Dual Color Break Apart Probe (Abbott/Vysis). The signals were analyzed on 3–4 μm sections of all respective blocks by fluorescent microscope (Olympus BX53) using appropriate filters and images were assessed by Genesis software (version 8.1.0.47741). Overlapped nuclei, ill-defined borders, or degenerated nuclei were excluded from evaluation. Minimum 100 interphase nuclei were scored. A positivity for rearrangement was defined as >15% of tumor cells with a split or single red signal.

RESULTS

A total of 117 histopathologically confirmed PCNS-DLBCL cases were included in this study. The median age of presentation was 55 years (age range: 19–85 years) with male-to-female ratio of 1.65:1. Ninety-five cases were located in the supratentorial compartment (81.19%). Using Hans' algorithm, the cases were divided into non-GCB (n = 96; 82.05%), GCB (n = 14; 11.96%), and unclassified (n = 7; 5.98%) categories.

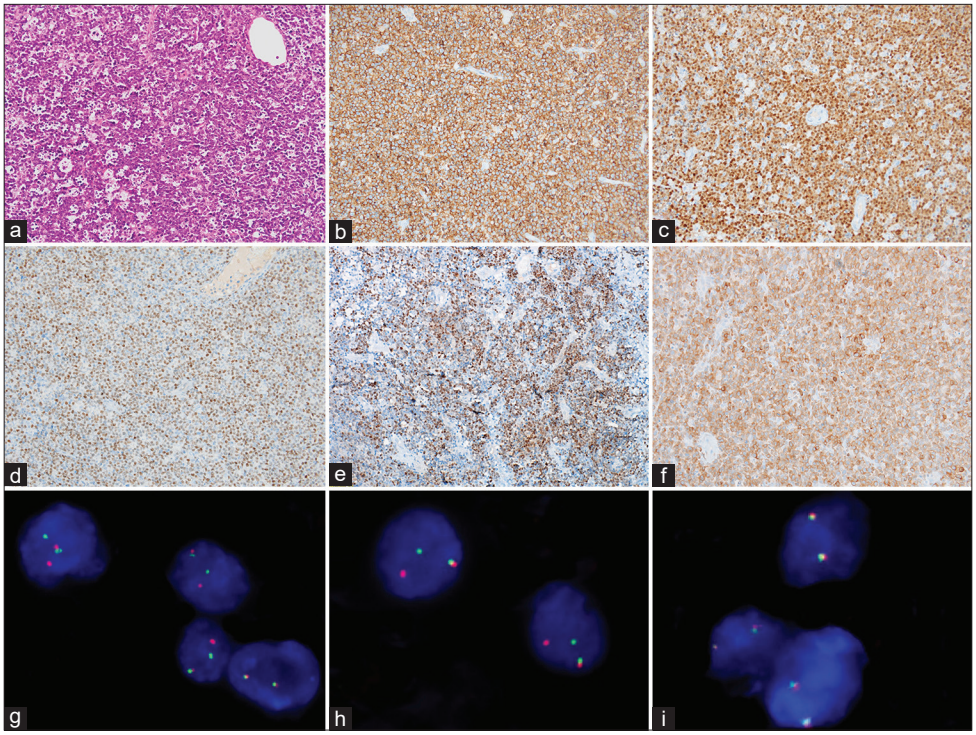

Figure 2: Primary double-hit central nervous system lymphoma (Case 4). (a) High-grade B-cell lymphoma with starry-sky growth pattern and centroblastic morphology (hematoxyline and eosin; ×200). (b) CD20 showing diffuse membranous positivity (peroxidase; ×200). (c) Diffuse nuclear positivity for multiple myeloma 1 (>30%; peroxidase; ×200). (d) Diffuse nuclear positivity for MYC (>40%; peroxidase; ×200). (e) Variable nuclear positivity for B-cell lymphoma 6 (BCL6) (>50%; peroxidase; ×200). (f) Diffuse cytoplasmic positivity for B-cell lymphoma 2 (BCL2) (>50%; peroxidase; ×200). (g-i) Fluorescence *in situ* hybridization using dual color break apart probe shows concurrent rearrangements for *MYC* (g) and *BCL6* (h) genes but no *BCL2* gene rearrangement (i).

MYC, BCL2, and BCL6 expression by IHC

There were six double-expressor and one TEL. Among the double-expressor cases (n = 6), the overexpression for both MYC and BCL2 protein (MYC+/BCL2+) was seen in five cases [Figures 1a-c], whereas single case exhibited MYC and BCL6 overexpression (MYC+/BCL6+). None of these double-expressors showed any MYC, BCL2, or BCL6 rearrangements on FISH analysis [Figure 1d]. Only one case demonstrated co-expression of all these three markers (MYC+/BCL2+/BCL6+) and thus qualified for TEL [Figures 2a-f]. The median age of presentation of DEL/TEL was 51 years (age range: 31–77 years), and there were four female and three male patients [Table 1]. All the seven patients were HIV negative and immunocompetent. All were supratentorial in location and belonged to non-GCB phenotype (based on Hans' algorithm).

MYC, BCL2, and BCL6 rearrangements by FISH

FISH for rearrangement was performed on these seven cases of DEL/TEL. The triple-expressor case (MYC+/BCL2+/BCL6+) demonstrated concurrent rearrangement for *MYC* and *BCL6* indicating DHL [Figures 2g-h]; however, there was no *BCL2* rearrangement [Figure 2i]. Briefly, this was a young female, who presented with left frontal mass. The biopsy showed features of DLBCL with centroblastic morphology and was of non-GCB phenotype. She received chemotherapy regime (high-dose methotrexate) but succumbed to her illness after 90 days.

Treatment and follow-up

One case of DEL was lost to follow-up. Remaining six patients received standard chemotherapy (high-dose methotrexate [poly-/monochemotherapy] + rituximab). Five patients (including the DHL) died, and one was alive at last follow-up [Table 1]. The mean overall survival of DEL/TEL was 48.2 days.

DISCUSSION

DHL is a subtype of high-grade B-cell NHL that shows *MYC* rearrangement and additional rearrangement of known oncogenes. *BCL2* acts as the most often co-rearranged

Table 1: The clinicopathological features and outcomes of double- and/or triple-expresser primary central nervous system lymphoma of this study.

Cases	Age	Sex	Site	Type of biopsy	Phenotype	IHC			FISH			Treatment	Follow-up
						c-MYC	BCL2	BCL6	MYC	BCL2	BCL6		
1	42	F	Periventricular area of left parietal, left thalamus, splenium of corpus callosum with extension to opposite side	Open	NGCB	+	-	+	N	-	N	CT	Dead; 36d
2	51	M	Multiple ICSOL with left occipital with intraventricular extension	Open	NGCB	+	+	-	N	N	-	CT	Alive; 66 d
3	48	F	Left thalamus	Stereo	NGCB	+	+	-	N	N	-	CT	Dead; 18d
4	31	F	Left frontal	Open	NGCB	+	+	+	P	N	P	CT	Dead; 90d
5	63	F	Left temporoparietal	Open	NGCB	+	+	-	N	N	-	CT	Dead; 45d
6	77	M	Right thalamus	Stereo	NGCB	+	+	-	N	N	-	CT	LTFU
7	66	M	Left frontal	Open	NGCB	+	+	-	N	N	-	CT	Dead; 34d

M: Male; F: Female, IHC: Immunohistochemistry, FISH: Fluorescence *in situ* hybridization, ICSOL: Intracranial space occupying lesion, stereo: Stereotactic, NGCB: Non-germinal center B-cell, N: Negative; P: Positive, CT: Chemotherapy, LTFU: Lost to follow-up; d: days, BCL2: B-cell lymphoma 2, BCL6: B-cell lymphoma 6

Table 2: Clinicopathological features of double hit primary central nervous system high grade B cell lymphoma reported in the literature.

Authors	Year	Inclusion criteria	Sample size	No. of DHL/THL	Age	Sex	Phenotype	DHL	Treatment	Outcome
Cady *et al.*[3]	2008	All PCNS DLBCL	75	1	NA	NA	NA	MYC/BCL6 rearrangements	NA	NA
Brunn *et al.*[2]	2013	All PCNS DLBCL	50	1	NA	NA	NA	MYC/BCL6 rearrangements	NA	NA
Nosrati *et al.*[5]	2018	All PCNS DLBCL	78	1	81	M	NGCB	MYC/BCL2 rearrangements	NA	NA
Pina-Oviedo *et al.* [7]	2020	Double/triple-expressor PCNS DLBCL	12	2	60	M	NGCB	MYC/BCL6 rearrangements	Chemotherapy	Alive; 36 m
					73	F	NGCB	MYC/BCL6 rearrangements	High dose methotrexate	Dead; 3.9m
Present study	2022	Double/triple-expressor PCNS DLBCL	7	1	31	F	NGCB	MYC/BCL6 rearrangements	Chemotherapy	Dead; 3m

N: Number, NA: Not available, M: Male, F: Female, NGCB: Non-germinal center B-cell, DHL: Double-hit lymphoma, PCNSDLBCL: Primary central nervous system diffuse large B cell lymphoma, THL: Triple-hit lymphoma, m: Months

gene (75%), followed by *CCND1 (*13%), *BCL6 (*10%), and *BCL3 (*2%).[1] The incidence of DHL/THL increases with the patient age. Most of the nodal DHL patients are in the advanced stage (III/IV) and have median overall survival of 7–19 months.[6] Thirunavukkarasu *et al.*[13] from our institute studied a large series of 109 systemic high-grade B-cell NHL and observed an incidence rate of 11.9% DHL among the North Indian population where all these cases had *MYC*

and *BCL2* rearrangements. However, we registered a single (0.85%) case of primary CNS DHL in the same population with almost similar number of cases. DEL, although not a distinct subset of DLBCL, accounts for 20–30% of DLBCL cases, and carries poor prognosis.[11] In comparison to the systemic DLBCL, the prognostic significance of DEL/TEL and DHL/THL in the CNS is inconclusive, as the number of these cases are very sparse.

In this study, we came across a single case of DHL (*MYC*/*BCL6* concurrent rearrangement) in a young female, who presented with left frontal mass. The biopsy showed features of DLBCL with centroblastic morphology which was of non-GCB phenotype. She received chemotherapy regime (high dose methotrexate) but succumbed to her illness after 90 days of follow-up. Only five cases of DHL have been documented in the CNS till date, while there is no report of THL [Table 2].[2,3,5,7] In 2008, Cady *et al*.[3] documented the first case of DHL in a case series of 75 PCNS-DLBCL that showed translocations for *MYC* and *BCL6* genes. Five years later, Brunn *et al*.[2] examined 50 cases of PCNS-DLBCL and found single case showing concurrent rearrangements for *MYC* and *BCL6*. In addition to this, Nosrati *et al*.[5] screened 78 cases of PCNS DLBCL and found another case of DHL with *MYC* and *BCL2* rearrangements, that belonged to GCB phenotype. Recently, two more cases of DHL were reported, where both showed concurrent translocations of *MYC* and *BCL6* genes.[7] Here, the authors, like our study, screened only the double or TEL (n = 12) by FISH and detected 2 DHL cases. Likewise, we also discovered single case of DHL with *MYC*/*BCL6* concurrent rearrangement out of seven double/triple-expressor cases. IHC for MYC, BCL2, and BCL6 is a useful screening method to recognize the DHL/THL.[13] Thus, compared to nodal DHLs, where *MYC* and *BCL2* rearrangement is the commonest combination, *MYC* and *BCL6* rearrangement is more common in PCNS DHLs. In nodal DLBCL, almost all cases of *MYC*/*BCL2* rearranged DHL are of GCB phenotype, whereas *MYC*/*BCL6* rearranged DHL mostly belong to non-GCB phenotype. In the CNS also, *MYC*/*BCL6* rearranged DHL mostly belong to the non-GCB type.[5,7] Similar to DHL, the incidence of DEL is also not well described in the CNS. We found 5.9% cases were DEL, which is much less compared to their frequency in nodal DLBCL, which varies from 20% to 30%.[11] The majority of the systemic DEL cases show MYC and BCL2 expression, and we also observed a similar trend in the CNS. The DEL cases in this study had a poor outcome with mean overall survival of 48.2 days, compared to median survival of 675 days in non-GCB PCNS-DLBCL as documented in our previous study.[9] The prognosis and treatment protocol of primary CNS DEL/DHL is difficult to determine due to extremely low number of documented cases.

CONCLUSION

Primary DEL and DHL are extremely rare in the CNS. Compared to the systemic DHL where *MYC*/*BCL2* rearrangement is seen in the majority, CNS DHL cases mostly show MYC/BCL6 rearrangement. In this study, DEL and DHL in CNS uniformly showed poor outcome. IHC for MYC, BCL2, and BCL6 is an effective screening strategy to exclude the DEL and DHL cases in PCNS-DLBCL, since such cases may require more aggressive therapy. Thus, we recommend performing this panel of IHC in all cases of PCNS-DLBCL, even if FISH is not available.

Declaration of patient consent

The Institutional Review Board (IRB) permission obtained for the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Aukema SM, Siebert R, Schuuring E, van Imhoff GW, Kluin-Nelemans HC, Boerma EJ, *et al*. Double-hit B-cell lymphomas. Blood 2011;117:2319-31.
2. Brunn A, Nagel I, Montesinos-Rongen M, Klapper W, Vater I, Paulus W, *et al*. Frequent triple-hit expression of MYC, BCL2, and BCL6 in primary lymphoma of the central nervous system and absence of a favorable MYC(low)BCL2 (low) subgroup may underlie the inferior prognosis as compared to systemic diffuse large B cell lymphomas. Acta Neuropathol 2013;126:603-5.
3. Cady FM, O'Neill BP, Law ME, Decker PA, Kurtz DM, Giannini C, *et al*. Del(6)(q22) and BCL6 rearrangements in primary CNS lymphoma are indicators of an aggressive clinical course. J Clin Oncol 2008;26:4814-9.
4. 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;103:275-82.
5. Nosrati A, Monabati A, Sadeghipour A, Radmanesh F, Safaei A, Movahedinia S. MYC, BCL2, and BCL6 rearrangements in primary central nervous system lymphoma of large B cell type. Ann Hematol 2019;98:169-73.
6. Ok CY, Medeiros LJ. High-grade B-cell lymphoma: A term re-purposed in the revised WHO classification. Pathology 2020;52:68-77.
7. Pina-Oviedo S, Bellamy WT, Gokden M. Analysis of primary central nervous system large B-cell lymphoma in the era of high-grade B-cell lymphoma: Detection of two cases with

MYC and BCL6 rearrangements in a cohort of 12 cases. Ann Diagn Pathol 2020;48:151610.
8. Parkhi M, Chatterjee D, Bal A, Vias P, Yadav BS, Prakash G, *et al*. Prognostic implications of the tumor immune microenvironment and immune checkpoint pathway in primary central nervous system diffuse large B-cell lymphoma in the North Indian population. APMIS 2022;130:82-94.
9. Radotra BD, Parkhi M, Chatterjee D, Yadav BS, Ballari NR, Prakash G, *et al*. Clinicopathological features of primary central nervous system diffuse large B cell lymphoma: Experience from a Tertiary Center in North India. Surg Neurol Int 2020;11:424.
10. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: Double hit and triple hit lymphomas and double expressing lymphoma. Blood Rev 2017;31:37-42.
11. Riedell PA, Smith SM. Double hit and double expressors in lymphoma: Definition and treatment. Cancer 2018;124:4622-32.
12. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, *et al*., editors. WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues. Revised. 4th ed. Lyon, France: IARC Press; 2017.
13. Thirunavukkarasu B, Bal A, Prakash G, Malhotra P, Singh H, Das A. Screening strategy for detecting double-hit lymphoma in a resource-limited setting. Appl Immunohistochem Mol Morphol 2022;30:49-55.
14. Villa D, Tan KL, Steidl C, Ben-Neriah S, Al Moosawi M, Shenkier TN, *et al*. Molecular features of a large cohort of primary central nervous system lymphoma using tissue microarray. Blood Adv 2019;3:3953-61.
15. Yuan XG, Huang YR, Yu T, Xu Y, Liang Y, Zhang XH, *et al*. Primary central nervous system lymphoma in China: A single-center retrospective analysis of 167 cases. Ann Hematol 2020;99:93-104.

How to cite this article: Parkhi M, Chatterjee D, Radotra BD, Bal A, Yadav BS, Tripathi M. Double-hit and double-expressor primary central nervous system lymphoma: Experience from North India of an infrequent but aggressive variant. Surg Neurol Int 2023;14:172.