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A clinicopathological study of primary central nervous system lymphomas & their association with Epstein-Barr virus

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***Background & objectives*: Primary central nervous system lymphomas (PCNSLs) are relatively uncommon, accounting for 2-3 per cent of primary brain tumours. Majority of these are diffuse large B cell lymphomas (DLBCL) occurring both in immunocompromised and immunocompetent patients. We undertook this study to classify PCNSL into germinal centre (GC) and non-germinal centre (NGC) type based on Hans classification and to find the role of Epstein-Barr virus (EBV) in pathogenesis both by conventional immunohistochemistry (IHC) and chromogenic *in situ* hybridization (CISH).**

***Methods*: The consecutive cases of PCNSL during a 10 years period were analysed by IHC for CD45, CD20, CD3, B-cell lymphoma 2 and 6 (Bcl-2 and Bcl-6), B-cell specific octamer binding protein-1 (BOB-1), multiple myeloma oncogene-1 (MUM-1), EBV latent-membrane protein 1 (LMP-1), cyclin-D1, CD10, CD5 and CD23, as well as by CISH for EBV.**

***Results*: During a period of 10 years, 65 PCNSL were diagnosed which comprised 0.69 per cent (65/9476) of all intracranial tumours. The mean age of presentation was 49 yr with sex ratio (M:F) of 1.4:1. Most common location was supratentorial region with predominant involvement of frontal lobe. Single lesions were seen in 38 (58.4%) and multifocal lesions in 27 (41.5%) patients. None of the patients were immunocompromised. All cases were B cell immunophenotype and were DLBCL except one case of follicular lymphoma. According to Hans classification, majority of them were NGC (n=51, 79.6%) and 13 (20.3%) were GC type. Bcl-2 expression was noted in 34 (52.3%) tumours. EBV was positive in three (4.6%) cases; two were detected both by IHC and CISH and one case by CISH only.**

***Interpretation & conclusions*: In Indian population, PCNSL occurs mainly in immunocompetent patients, and a decade earlier than in western population. Immunophenotyping revealed that all cases were DLBCL with predominance of NGC type. No prognostic difference was seen between GC and NGC DLBCL. Association of EBV was rare and this virus was possibly not involved in the pathogenesis of PCNSL in immunocompetent individuals. CISH was an easy, economical and less cumbersome method for detection of EBV in PCNSL.**

Key words CISH - CNS - DLBCL - EBV - GC - IHC - Hans classification - lymphoma - NGC - PCNSL

Primary central nervous system lymphomas (PCNSLs) are the rare brain tumours accounting for 2-3 per cent of all primary brain tumours and <1 per cent of all non-Hodgkin's lymphomas[1]. World Health Organization (WHO) classification of haematopoietic and lymphoid tissues describes these as diffuse large B-cell lymphoma of the central nervous system (CNS DLBCL) representing all primary intracerebral, spinal or intraocular lymphomas[1]. Lymphomas of CNS with evidence of systemic disease at the time of presentation are excluded. In the last few decades, a rise in the number of cases has been observed mainly in association with acquired immunodeficiency syndrome (AIDS) but this has started declining after introduction of highly active anti-retroviral therapy (HAART)[2]. Although immunodeficiency either inherited or acquired is a major risk factor for the development of PCNSL, but this increase in incidence has been reported in immunocompetent patients also[2,3]. Usual age of onset reported is between 6th to 7th decades of life, but these occurred earlier in immunocompromised individuals[4]. A few studies published from India found that age of presentation is a decade earlier than western population[5-13]. Epstein-Barr-virus (EBV) is demonstrated to be associated with PCNSL in most of the immunocompromised patients, while rarely in immunocompetent patients[14]. Although a few studies of PCNSL are published from India but none of these classified CNS lymphoma according to Hans classification[15], and role of EBV was not studied by chromogenic *in situ* hybridization (CISH) which is a sensitive and specific method for detection of this virus.

We undertook this study with an aim to subtype PCNSL according to Hans classification for DLBCL and to find out role of EBV in PCNSL pathogenesis both by conventional immunohistochemistry (IHC) for latent membrane protein-1 (LMP-1) and CISH.

Material & Methods

All consecutive cases diagnosed as PCNSL during a period of 10 years (2004 -2013) in the department of Pathology, All India Institute of Medical Sciences, New Delhi, India, were retrospectively included in this study. The slides and blocks were retrieved from archives of the department. Hematoxylin and Eosin (H&E) stained sections were reviewed by three independent neuropathologists, and consensus diagnoses were made according to the WHO classification of tumors of the central nervous system[4]. Three cases were excluded from the study one of which was a case of Hodgkin's lymphoma and two were anaplastic large cell lymphomas involving sphenoid sinuses. Finally, 65 cases with sufficient material in tissue blocks and diagnostic concurrence were included in the study.

Tumour samples received in neutral buffered formalin were routinely processed and paraffin embedded. Five micron thick sections were cut for H&E staining and for IHC. Reticulin staining was done to highlight the perivascular framework of tumour cells. For immunohistochemical staining, streptavidin biotin conjugate immunoperoxidase method[16] was used, after appropriate antigen retrieval wherever required. Immunohistochemical studies were performed on 5µ thick formalin fixed, paraffin-embedded tumour sections using antibodies directed against CD45 (Dako, Denmark, dilution 1:100), CD20 (Neomarker, USA, dilution, 1:200), CD3 (Dako, dilution, 1:100), B-cell lymphoma 2 (Bcl-2) (Neomarker, dilution, 1:50) and multiple myeloma oncogene 1 (MUM-1) (dilution, 1 : 200), B-cell specific octamer binding protein-1 (BOB-1) (dilution, 1:200), Epstein-Barr virus latent membrane protein 1 (EBV LMP-1) (dilution 1:200), cyclin-D1 (dilution 1:100), CD10 (dilution 1:50), CD5 (dilution 1:25), CD23 (dilution 1:100), CD15 (dilution 1:200), CD30 (dilution 1:100) and B-cell lymphoma 6 (Bcl-6) (dilution 1:100) all from Diagnostic BioSystems, USA. Labelled streptavidin biotin kit (Universal) was used as a detection system (Dako, Denmark). Antigen retrieval was performed in a microwave oven using citrate buffer at *p*H 6.0 for all antibodies except Bcl-6, for which ethylenediamine tetra acetic acid (EDTA) was used. For each batch, appropriate positive controls were taken and for negative controls primary antibodies were omitted.

CISH technique: CISH study was performed on 5µ thick formalin fixed paraffin-embedded tumour sections using Zytofast PLUS CISH implementation kit HRP-DAB (Zyto Vision GmBH, Germany). Standard deparaffinization technique as used for IHC was followed by 10 min incubation in 3 per cent H_2O_2. Following rinsing in deionized water (DW), target retrieval was achieved using pepsin digestion in humidity chamber for 15 min. Slides were incubated in EDTA solution at 95°C in a boiling water bath for 15 min after washing in DW. Slides were washed in DW and drained off; 10 µl of CISH probe was poured over each slide, and covered with a cover slip using glue. Denaturation at 75°C for 5 min was subsequently followed by hybridization at 37°C for 60 min in the Thermobrite TM hybridization chamber (Vysis Inc.,

USA). Tris-buffered-saline (TBS) washing, at 55°C and room temperature, each for five min was done concurrently after removal of cover slip. Mouse-anti-DIG (Zyto Vision GmBH, Germany) was poured drop-wise over each slide, and incubated in a humidity chamber at 37°C for 30 min. Three washings, each for a minute with TBS was done, before and after incubating slides in anti-mouse-HRP-polymer for 30 min at room temperature. 3,3'-diaminobenzidine (DAB) solution was prepared as per guidelines (Zytofast PLUS CISH) and poured 3-4 drops in each slide for 10 min at room temperature, and washed in running tap water for two min. Haematoxylin was used for counterstaining. Slides were dehydrated in graded alcohol solutions, air dried and mounted with DPX. Slides were examined under light microscope for evaluation. Only nuclear positivity was taken into account.

Classification of tumours as GC and NGC type: Tumours were sub-classified according to immunoprofile expression using Hans classification system[15]. In the Hans classification a decision tree is devised based on three markers: CD10, Bcl-6, and MUM-1 to distinguish DLBCL into two subgroups. $CD10^+$ only, both $Bcl\text{-}6^+$ and $CD10^+$ and $CD10^-$, $Bcl\text{-}6^+$ and $MUM\text{-}1^-$ positive cases were assigned to the GC group. NGC group included cases with $CD10^-$ and one of either combinations $Bcl\text{-}6^+$ and $MUM\text{-}1^+$ or $Bcl\text{-}6^-$ and $MUM\text{-}1^+$. The cases with all three negative markers were assigned to NGC group.

Results

During a period of 10 years (2004-2013), a total of 9476 intracranial tumours including 4573 (48.2%) of glial tumours were diagnosed in the department of Pathology. Of these, 65 cases were of PCNSL which comprised 0.69 per cent (65/9476) of all intracranial tumours. There was no absolute increase in the incidence of PCNSL in the last decade (Fig. 1).

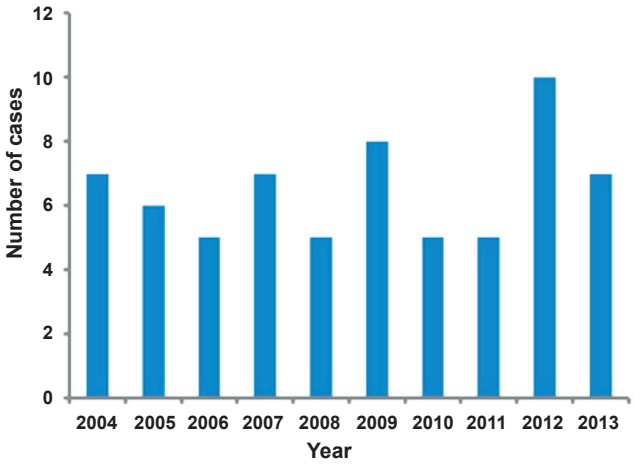

Fig. 1. Year wise incidence of primary central nervous system lymphoma (PCNSL).

Clinicopathological parameters (Table): PCNSL showed a wide age distribution range varying from 14 to 80 yr with a mean age of 49 yr at presentation. Ten patients were under 30 yr age group. A male predominance with a male to female ratio of 1.4:1 was noted. A vast majority of cases were supratentorial (ST) in location (56/65; 85%), followed by infratentorial region (6/65; 9%), and in 4.61 per cent (3 cases) both ST and IT were involved. Within the ST region the most common location was frontal lobes (45%) followed by parieto-occipital (11%), temporo-parietal (7%), basal ganglia (7%), all cortical lobes (7%), thalamus (5%), parietal (5%), occipital (5%) and one case each in selllar-suprasellar, temporal and fronto-temporal region. In the infratentorial region three tumours were in the cerebellum, two in fourth ventricles and one case occurred in the brainstem. Meningeal infiltration was noted in two cases (3.0%). Single lesions were seen in 38/65 (58.46%) of the patients and multiple lesions in 27/65 (41.53%). All cases were non reactive for HIV by ELISA test.

Pathologic findings and imunophenotyping: Tumours were composed of large cells with vesicular nuclei and prominent nucleoli admixed with some mature looking lymphoid cells. Perivascular cuffing and prominent network of reticulin was evident in majority of cases (Fig. 2). Necrosis was observed in 50 per cent of the cases. In five cases diagnoses were missed and were diagnosed as viral encephalitis. These patients were on steroids. A repeat biopsy clinched the diagnosis.

All the tumours were immunopositive for CD45 and CD20 and were B immunophenotype. DLBCL comprised 98.4 per cent (64/65) of the cases and a single case was of follicular lymphoma (FL). The FL was immunopositive for CD10, Bcl-2 besides CD20 and CD45. Of the 64 cases of DLBCL, 79.68 per cent (51/64) were non-germinal centre type (NGC) and 20.31 per cent (13/64) were of germinal centre type. Of the 51 NGC-DLBCL, 41.1 per cent (21/51) showed reactivity to both Bcl-6 and MUM-1, 43.3 per cent (23/51) to MUM-1 only and 13.7 per cent (7/51) were non-reactive to both these markers (Figs 2, 3). Among GC-DLBCL, 30.7 per cent (4/13) were immunopositive for bcl-6 and CD10 while the rest 61.5 per cent (8/13) and 7.6 per cent (1/13) were immunoreactive only to

Table. Clinicopathological features of cases of primary central nervous system lymphomas (PCNSLs)(n=65)

S. N.	Age (yr)	Sex	Site	No. of lesions	GC/NGC	LCA	CD 20	CD3	Bcl-2	Bcl-6	BOB1	MUM1	Cyclin D1	CD 10	CD 5	CD 23	EBV LMP-1	CISH
1	63	M	Parieto-occipital	Multiple	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
2	49	F	Basal ganglia	Single	DLBCL, NGC	+	+	-	-	+	+	+	-	-	-	-	-	-
3	42	M	Frontal	Single	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	-	-
4	45	F	Cerebellum	Single	DLBCL, NGC	+	+	-	+	-	-	-	-	-	-	-	-	-
5	35	F	Temporal	Multiple	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
6	51	M	Occipital	Single	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	-	-
7	39	M	Frontal	Single	DLBCL, NGC	+	+	-	-	-	-	+	-	-	-	-	-	-
8	62	F	Temporo-parietal	Multiple	DLBCL, NGC	+	+	-	-	-	-	+	-	-	-	-	-	-
9	44	F	Frontal	Single	DLBCL, NGC	+	+	-	-	-	-	+	-	-	-	-	-	-
10	68	M	Basal ganglion	Single	DLBCL, NGC	+	+	-	+	+	+	+	-	-	-	-	-	-
11	55	M	Basal ganglion	Single	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
12	60	M	Parieto-occipital	Single	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	-	-
13	28	M	Thalamic	Single	DLBCL, GC	+	+	-	-	+	+	-	-	+	-	-	-	-
14	60	M	Occipital	Multiple	DLBCL, NGC	+	+	-	+	-	-	-	-	-	-	-	-	-
15	61	F	Frontal	Single	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
16	80	M	Frontal	Multiple	DLBCL, GC	+	+	-	+	+	-	-	-	-	-	-	-	-
17	36	F	All cortical lobes	Multiple	DLBCL, NGC	+	+	-	-	-	-	+	+	-	-	-	-	-

Contd...

S. N.	Age (yr)	Sex	Site	No. of lesions	GC/NGC	LCA	CD 20	CD3	Bcl-2	Bcl-6	BOB1	MUM1	Cyclin D1	CD 10	CD 5	CD 23	EBV LMP-1	CISH
18	73	F	Parieto-occipital	Multiple	DLBCL, NGC	+	+	-	-	-	-	-	-	-	-	-	-	-
19	70	F	Bifrontal	Multiple	DLBCL, GC	+	+	-	-	+	-	-	-	-	-	-	-	-
20	67	M	Frontal	Single	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
21	69	F	Basal ganglion	Single	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	-	-
22*	63	M	Frontal	Single	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	+	+
23	65	M	Temporo- parietal	Multiple	DLBCL, GC	+	+	-	-	+	+		-	-	-	-	-	-
24	56	F	Fronto-temporal	Multiple	FL	+	+	-	+	-	-	-	-	+	-	-	-	-
25	30	M	Frontal	Single	DLBCL, NGC	+	+	-	-	-	-	-	-	-	-	-	-	-
26	62	F	Frontal	Single	DLBCL, NGC	+	+	-	+	-	+	-	-	-	-	-	-	-
27	62	M	Both supra & infratentorial	Multiple	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	-	-
28*	54	M	Parietal	Single	DLBCL, NGC	+	+	-	-	-	-	+	-	-	-	-	+	+
29	40	M	4th Ventricle	Multiple	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
30	48	F	Frontal	Single	DLBCL, NGC	+	+	-	+	+	+	+	-	-	-	-	-	-
31	48	M	Frontal	Single	DLBCL, NGC	+	+	-	-	-	-	+	-	-	-	-	-	-
32	40	M	Temporo-parietal SOL	Multiple	DLBCL, NGC	+	+	-	+	+	+	+	-	-	-	-	-	-
33	55	F	Frontal	Single	DLBCL, GC	+	+	-	-	+	-	+	-	+	-	-	-	-
34	35	M	Multiple ICSOL	Multiple	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
35	70	F	4th Ventricle	Single	DLBCL, NGC	+	+	-	+	+	+	+	-	-	-	-	-	-

Contd...

S. N.	Age (yr)	Sex	Site	No. of lesions	GC/NGC	LCA	CD 20	CD3	Bcl-2	Bcl-6	BOB1	MUM1	Cyclin D1	CD 10	CD 5	CD 23	EBV LMP-1	CISH
36	43	M	Thalamic	Single	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
37	54	M	Thalamic	Single	DLBCL, GC	+	+	-	-	+	+	-	-	-	-	-	-	-
38	47	M	Frontal	Single	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
39	59	M	Occipital	Single	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
40*	21	M	Frontal	Single	DLBCL, GC	+	+	-	+	+	-	-	-	-	-	-	-	+
41	32	M	All cortical lobes	Multiple	DLBCL, NGC	+	+	-	-	-	+	-	-	-	-	-	-	-
42	50	M	Frontal	Multiple	DLBCL, NGC	+	+	-	-	-	+	+	-	-	-	-	-	-
43	70	M	All cortical lobes	Multiple	DLBCL, NGC	+	+	-	+	+	-	+	-	-	-	-	-	-
44	51	F	Temporo-parietal	Multiple	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
45	24	M	Fontal	Single	DLBCL, NGC	+	+	-	+	-	-	+	-	-	-	-	-	-
46	40	M	Parieto-occipital	Multiple	DLBCL, GC	+	+	-	-	-	+	-	-	+	-	-	-	-
47	45	F	Fronal	Single	DLBCL, NGC	+	+	-	+	+	-	+	-	-	-	-	-	-
48	30	F	Brainstem	Multiple	DLBCL, GC	+	+	-	-	+	-	-	-	-	-	-	-	-
49	60	F	Frontal	Single	DLBCL, GC	+	+	-	+	+	+	+	-	+	-	-	-	-
50	72	F	Frontal	Single	DLBCL, NGC	+	+	-	+	+	-	+	-	-	-	-	-	-
51	30	F	All cortical lobes	Multiple	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
52	43	M	Frontal	Single	DLBCL, NGC	+	+	-	+	+	+	+	-	-	-	-	-	-

Contd...

S. N.	Age (yr)	Sex	Site	No. of lesions	GC/NGC	LCA	CD 20	CD3	Bcl-2	Bcl-6	BOB1	MUM1	Cyclin D1	CD 10	CD 5	CD 23	EBV LMP-1	CISH
53	56	F	Frontal	Single	DLBCL, NGC	+	+	-	+	-	-	+	-	-	+	-	-	-
54	40	M	Cerebellum	Single	DLBCL, NGC	+	+	-	-	-	-	+	-	-	-	-	-	-
55	64	F	Cerebellum	Multiple	DLBCL, NGC	+	+	-	+	+	-	+	-	-	-	-	-	-
56	14	M	Frontal	Single	DLBCL, NGC	+	+	-	-	+	+	+	-	-	-	-	-	-
57	59	M	Parieto-occipital	Multiple	DLBCL, NGC	+	+	-	-	-	-	-	-	-	-	-	-	-
58	28	M	Parietal	Single	DLBCL, GC	+	+	-	-	+	-	-	-	-	-	-	-	-
59	38	F	Frontal	Multiple	DLBCL, GC	+	+	-	+	+	-	-	-	+	-	-	-	-
60	49	M	Both supra & infratentorial lobes	Multiple	DLBCL, NGC	+	+	-	+	+	-	+	-	-	-	-	-	-
61	45	F	Parieto-occipital	Multiple	DLBCL, NGC	+	+	-	+	-	+	+	-	-	-	-	-	-
62	53	F	Parietal	Single	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
63	20	M	Sellar suprasellar	Multiple	DLBCL, NGC	+	+	-	-	+	-	+	-	-	-	-	-	-
64	27	M	Frontal	Single	DLBCL, NGC	+	+	-	+	+	-	+	-	-	-	-	-	-
65	33	F	Frontal	Single	DLBCL, GC	+	+	-	-	+	-	-	-	-	-	-	-	-

M, male; F, female; LMP, latent membrane protein; GC, germinal centre; NGC, non germinal centre; DLBCL, diffuse large B cell lymphoma; BOB1, B cell specific Octamer Binding protein-1; LCA, leukocyte common antigen; BCL2, B cell lymphoma-2; MUM1, multiple myeloma oncogene-1; CISH, chromogenic *in situ* hybridization; ICSOL, intracranial space occupying lesion; FL, follicular lymphoma

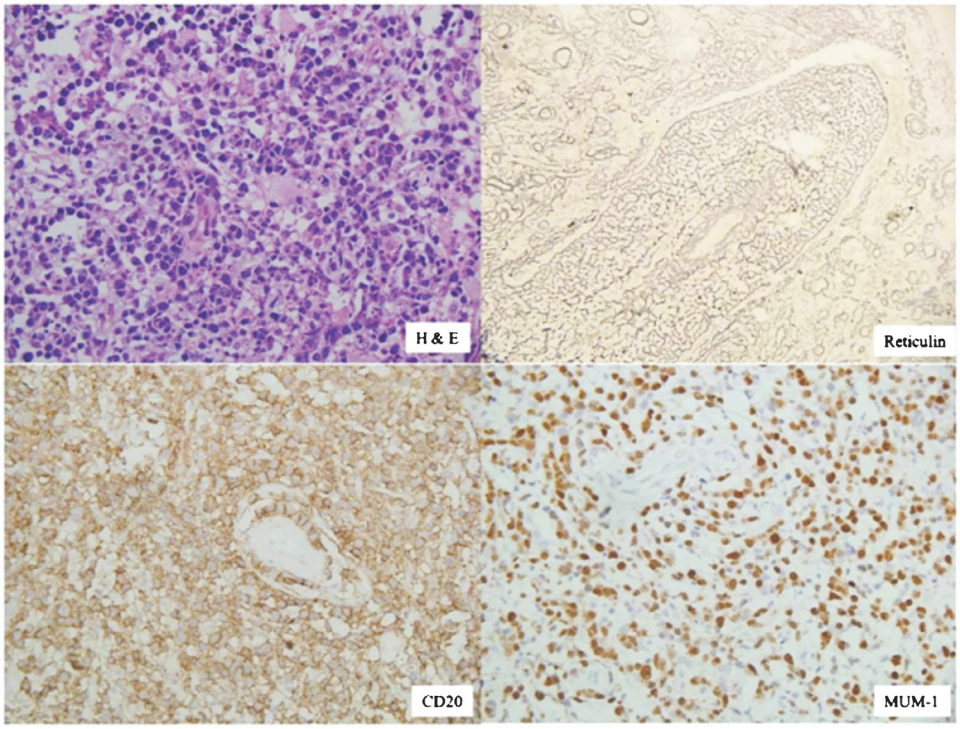

Fig. 2. Showing H&E staining of non-germinal centre (NGC) diffuse large B cell lymphoma (DLBCL) with immunopositivity for CD20, MUM-1 and reticulin rich network (X 200 each). DAB was used as chromogen.

Bcl-6 and CD10, respectively. Bcl-2 expression was noted in 52.30 per cent (34/65) cases. Cyclin-D1, CD5 and CD23 were immunonegative in all cases. LMP-1 immunopositivity was seen in only two cases in the cytoplasm.

CISH study: CISH showed positivity for EBV only in three cases (4.6%), of whom two were immunopositive for LMP by IHC. EBV positivity was seen in two cases of NGC (case nos 22 & 28) and one case of GC type (case 40) (Fig. 4). Only in one case there was discordance between the two methods. EBV positivity by CISH was stronger as compared to LMP immunopositivity. This positivity was observed in a few cells which were neoplastic. None of the mature looking lymphoid cells were positive.

Discussion

PCNSL is relatively a rare but distinct form of extranodal lymphoma commonly seen in the immunocompromised individuals. Initially, with increased incidence of HIV, it was predicted that PCNSL would surpass the glial tumours; however, the incidence decreased possibly with introduction of HAART[2,17]. PCNSL constituted 0.69 per cent of all intracranial tumours and 1.42 per cent of all glial tumours in the present study, similar to that reported earlier[5,6,8]. Manoj *et al*[13] from south India reported an increase in the incidence in PCNSL. In the present study, there was no absolute increase in the incidence of PCNSL and these results were similar to previous study from our centre[5].

The PCNSL occurred over a wide age range with a peak incidence in the sixth or seventh decades in immunocompetent, and at a younger age in immunocompromised individuals[4]. All individuals with congenital as well acquired immunodeficiency are prone to develop PCNSL. Interestingly, the age of onset was a decade earlier in Indian patients who were immunocompetent[5-13]. Immunodeficiency is the major predisposing factor for the development of PCNSL, but none of our patient was immunodeficient. This has been observed earlier also from our centre as well as from other centres from India[5,6,12]. Manoj *et al*[13] reported a large series of 76 patients of PCNSL and found HIV association in three of 35 patients (8.6%) tested. This observation was duplicated in most of the studies from India[8, 10]. In a series of 56 patients Paul *et al*[8] found only one patient to be HIV positive.

It has been observed that peripheral DLBCL do not have uniform behaviour. Alizadeh *et al*[18] and Rosenwald

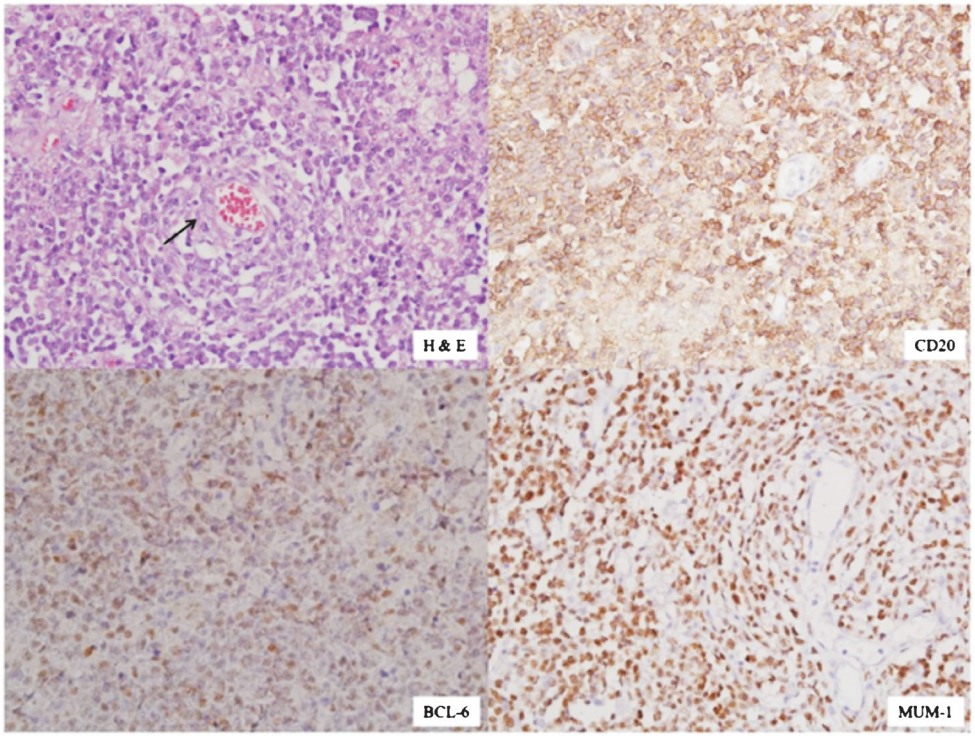

Fig. 3. Non-germinal centre (NGC) diffuse large B cell lymphoma (DLBCL) showing perivascular cuffing of the tumour cells in H&E staining (arrow) with immunopositivity for CD20, Bcl-6 and MUM-1 (X 200 each). DAB was used as chromogen.

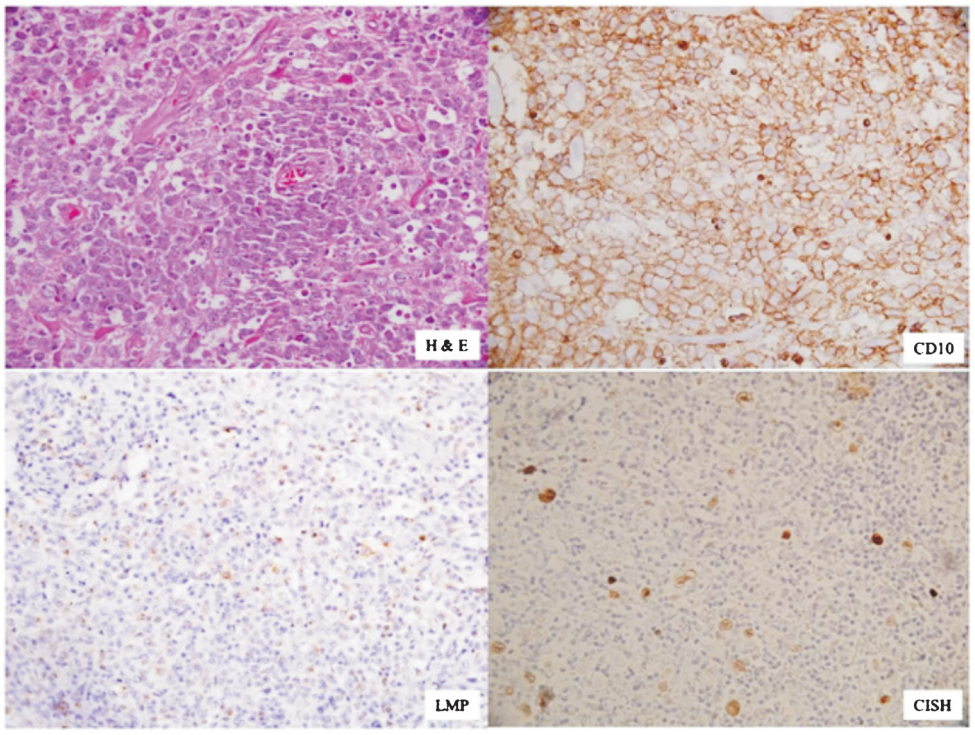

Fig. 4. Showing H&E staining of GC DLBCL with immunopositivity for CD10, LMP-1 (cytoplasmic) and chromogenic *in situ* hybridization (CISH) positivity (nuclear) for EBV(X 400 each). DAB was used as choromogen.

et al[19] have shown that (based on molecular profiling) DLBCL are two different groups. Further, Hans *et al*[15] have shown that these groups can be segregated into two categories by immunohistochemical staining and both subgroups are prognostically different. Systemic DLBCLs have been shown to pose a more heterogenous clinical and immunohistochemical features[20]. Many large studies on systemic DLBCL have shown that NGC DLBCLs behave in worse manner as compared to GC DLBCL. However, PCNSL have been found to be more homogenous and have aggressive behaviour and poor prognosis[21,22]. Camilleri-Broët *et al*[21] reported a series of 83 patients of DLBCL of brain and found 96.3 per cent of them were NGC subtype and attributed poor prognosis to this subtype. Similar results were reported by Bhagavathi *et al*[22] in another study. In the present study of PCNSL, the NGC subtype was the predominant group and comprised 80 per cent of the cases but this was not that dominant as reported in earlier studies[21,22].

EBV is commonly implicated in the aetiopathogenesis of various lymphoproliferative disorders in immunocompromised individuals including PCNSL but less frequently in immunocompetent individuals[23]. Rao *et al*[7] studied 11 cases of PCNSL for EBV by *in situ* hybridization and found only one case to be positive, HIV status of that patient was not known. Tandon *et al*[9] reported 19 patients of PCNSL and all of them were immunopositive for LMP of EBV; however, none was positive by *in situ* hybridization, thus suggesting false positivity by IHC. In the present study of 65 patients of PCNSL, only three were positive for EBV; two by IHC for LMP and CISH and one additional case was detected with CISH technique but was missed by IHC. Therefore, our study showed that EBV was infrequently associated with the development of PCNSL in immunocompetent patients. Further, there is a possibility that EBV may also not be the aetiological agent of PCNSL in HIV patients. These observations are supported by some earlier studies from India[24,25]. Lanjewar *et al*[24] from western part of India, reported central nervous system changes in 84 autopsies conducted on HIV positive individuals and did not find any case of PCNSL. This is further supported by another study on 135 patients by Sharma *et al*[25] who did not find any case of PCNSL except two cases of peripheral non Hodgkin's lymphoma. This low incidence of PCNSL could be due to short survival of AIDS patients as HAART was not available in India earlier. There is a possibility that with increasing awareness, affordability, free availability of treatment from government agencies and longer survival of these patients the number of PCNSL will increase in future.

Several laboratory detection techniques have been advocated for EBV. Southern blotting detection of EBV-DNA requires frozen tissue which can be used to determine the EBV clonality based on the variable number of tandem repeat sequences at the end of each EBV DNA molecule. Detection of EBV related antigens by IHC gives inconsistent results due to variable expression profiles[26]. A PCR based positivity may occur due to EBV-DNA in reactive lymphocytes[26]. Paraffin section DNA *in situ* hybridization using radiolabelled probes is a difficult and time consuming technique due to low concentration of specific nucleotide sequences and masking by the associated proteins. However, CISH is a simple and convenient technique with high sensitivity and specificity which can be incorporated in the routine evaluation of EBV association in PCNSLs.

To conclude, PCNSLs occurred a decade earlier in Indian patients and primarily in immunocompetent patients. There was no absolute increase in the incidence of PCNSL over a period of 10 years, although number of cases diagnosed per year increased. NGC subtypes were the most common and probably attributed to the poor prognosis. EBV was not the aetiological factor for PCNSL in India. CISH is a good and easy technique for the detection of EBV in PCNSL.

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***Conflicts of Interest*:** None.

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