

## RESEARCH ARTICLE

# Primary Central Nervous System Lymphoma: A Clinicopathological and Cytomorphological Study from A Tertiary Care Centre in Chennai, India

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### Abstract

**Background:** The aim of this study was to analyze the clinicopathological and immunohistochemical features of primary central nervous system lymphoma (PCNSL) cases occurring in Indian patients and also study the utility of the crush smear preparation in intraoperative diagnosis. **Materials and Methods:** The immune status, clinical, radiological details, immunohistochemical profile, histopathological findings and cytological features in smear preparation of 32 cases of PCNSL were analyzed. Patients with systemic NHL and skull-base lymphomas were excluded. **Results:** The mean age of our patients was 52 years with a male: female ratio 1:1. A periventricular location was found in 62.5% of patients. None of our PCNSL cases were associated with AIDS. All cases except one were diffuse large B-cell lymphomas. Intraoperative diagnosis using crush smears allowed correct prediction in 93% of cases. **Conclusions:** Our study shows that PCNSL is seen predominantly in immunocompetent patients in India. The age of presentation is relatively young as compared to the West. Our study also stresses the utility of crush smear preparation in establishing an intraoperative diagnosis.

**Keywords:** Primary central nervous system lymphoma - brain tumor - immunohistochemistry - intraoperative diagnosis

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### Introduction

Primary central nervous system lymphoma (PCNSL) is a form of extra-nodal non-Hodgkin lymphoma (NHL), which is confined to the central nervous system (CNS) in the absence of systemic disease (Fischer et al., 2008). More than 90% of PCNSL tumors are high-grade, CD20-positive, diffuse large B-cell NHLs; despite the fact that B-cells are absent from the normal brain. CNS lymphomas exhibit a distinct gene expression profile compared with nodal lymphomas of the same histological type (Kadoch et al., 2006). In the early 1970s, PCNSL was a rare tumour (Henry et al., 1974). The incidence of PCNSL increased from 2.5 cases per 10 million population in 1973 to 30 in 1991-1992; (Corn et al., 1997) it was increasingly diagnosed in the immunodeficient and immunosuppressed patients as well as in the immunocompetent (Schabet, 1999). The increase in incidence of PCNSL had surpassed the milder increase in systemic lymphomas and could not be attributed solely to technological advances in radiology and stereotactic neurosurgery (Olson et al., 2002). However PCNSL rates have decreased among

young adults compared to the elderly since the mid-1990's because of effective therapies for AIDS (Kadan-Lottick et al., 2002). At present, primary CNS lymphomas constitutes about 2.2% of all brain tumours in the USA (Dolecek et al., 2012). However studies from Asia and India have revealed certain differences in PCNSL features compared to the West (Sarkar et al., 2005; Shibamoto et al., 2008; Zhang et al., 2010). CNS cancers are increasing in India and to understand the etiology of these cancers, in depth, analytic epidemiological studies should be planned in the near future (Yeole, 2008)

In the present study we analyze the clinicopathological features of 32 cases of PCNSL and also assess the utility of the crush smear preparation in the intra-operative diagnosis of PCNSL.

### Materials and Methods

Thirty-two cases of PCNSL diagnosed in the Department of Histopathology, Apollo Speciality Hospital, during the period April 2008 to October 2010 were included in this study. Haematoxylin and Eosin (H and

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E) stained slides and immunohistochemistry slides (which included a minimum of Leucocyte common antigen (LCA), CD20, CD79a, CD3, CD43 and Ki-67) of all these cases were retrieved. Immunophenotyping was performed on formalin-fixed paraffin-embedded tissue using the streptavidin biotin conjugate immunoperoxidase method. Cases diagnosed during the latter half of 2010 were immunostained using the Benchmark Immunohistochemistry system (Ventana Medical Systems, Tucson, AZ, USA). Intraoperative crush smear preparation was available in 30/32 cases.

The histopathology of each case was reviewed with the H and E slides. The immunohistochemical features and diagnosis of all these cases were also ascertained. The intraoperative diagnosis was recorded and the cytological features of PCNSL in crush smears were studied. The immune status, clinical and radiological details of these cases were obtained from case records. Computed Tomography (CT) scans and Magnetic Resonance Imaging (MRI) were the imaging modalities. Contrast study with CT or MRI was done in 24 cases. MR spectroscopy was also used in 5 cases. Bone marrow examination was done in 23 cases. Biopsy material was obtained by stereotactic biopsy in 30 cases and by surgical excision in 2 cases.

Serological details were not available for five cases. Only Indian patients were included in this study. Patients with systemic NHL and those who showed evidence of extracranial involvement at the time of diagnosis were excluded. Skull-base lymphomas were also excluded in our study. The ethics committee of our Hospital approved this study.

**Results**

*Clinical features*

The mean age of the patients was 52.3 years and the median was 50 years (range 30-72 years). The male: female ratio in our study is 1:1 (Table 1). Of the 32 cases, one was a known case of sarcoidosis (in remission). The only T-cell lymphoma case in our series had a prior history of treatment for ulcerative colitis (in remission). None of the patients were positive for HIV. Only one case was a recurrent PCNSL. The most common clinical features at presentation were symptoms of raised intra-cranial tension (58%), mental status changes (38%), focal neurological deficits (40%), seizures (9%) and ocular symptoms (6%).

**Table 1. Characteristics of 32 PCNSL Patients at Diagnosis**

		No. (%)
Sex	Male	16 (50.0)
	Female	16 (50.0)
Age (in Years)	>65	5 (15.6)
	45-65	20 (62.5)
	<45	7 (21.9)
Localization	Monofocal	21 (65.6)
	Multifocal	11 (34.4)
Nature of involvement	Unilateral	23 (71.9)
	Bilateral	9 (28.1)
Site of involvement	Supratentorial	29 (90.6)
	Supra- and Infratentorial	3 (9.4)

The duration of symptoms varied from 4 days to 5 months.

A periventricular location (basal ganglia, corpus callosum or periventricular white matter) was found in 20 (62.5%) patients. Corpus callosal lesions were seen in 10 (31%) cases. All the lesions were contrast enhancing. Alternative diagnoses (based on the clinical and radiological features) considered before biopsy in our cases included gliomas, metastasis, tuberculoma, abscess and demyelinating disorder. Eight patients had a history of corticosteroid administration, for 1-12 days prior to biopsy.

*Histopathological features*

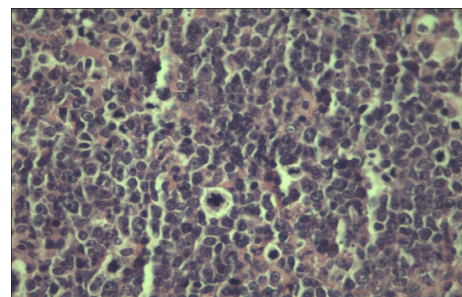
Histopathology showed sheets of medium to large sized lymphoid cells (Figure 1). Their nuclei showed clumped chromatin and prominent nucleoli. Numerous mitoses including atypical forms were evident in almost all the cases. The angio-centric pattern was observed in 13 (41%) cases. Necrosis and reactive gliosis were observed in 10 (31%) and 15 (47%) cases respectively. The cytological features of the tumor cells were better appreciated in crush smears.

*Immunohistochemical features*

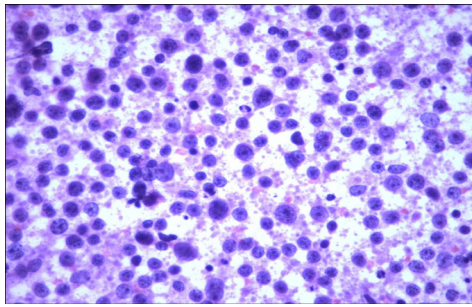
Of the 32 cases, 31 (97%) were diagnosed as diffuse –large B-cell lymphoma (DLBCL) and only 1 (3%) case was labeled as T-cell lymphoma. LCA was positive in all the 32 cases. All the DLBCL cases were positive for CD20 and CD79a. Admixed reactive T-lymphocytes were observed in 4/31 cases of DLBCL. The T-cell lymphoma case showed immuno-positivity for CD2, CD3, CD4 and CD43 and was negative for CD8. It was negative for B-cell markers CD20 and CD 79a.

*The salient cytological features in crush –smear preparation*

The majority of the smears showed moderate to marked cellularity (Figure 2). At higher magnification, the tumor cells showed ill-defined scant cytoplasm, round nuclei with coarse chromatin and 1-3 prominent nucleoli. In almost all the cases, some of the tumor cells showed nuclear indentation and convoluted nuclear borders. Lymphoglandular bodies and apoptotic bodies possessing condensed chromatin were seen in almost all the smears. Mitoses were easily identified (at least 1/10 HPF) in majority of the cases. Reactive astrocytes were evident in 13 (41%) cases. In a few cases the reactive astrocytes showed mild cytological atypia.



**Figure 1. Histopathological Picture of a PCNSL (H&E×400)**



**Figure 2. Smear Illustrating Monolayered Discohesive Sheets of Lymphoma Cells with Scant Cytoplasm and Round Nuclei with Prominent Nucleoli (Rapid H&E x400)**

The cytological features correlated well with histopathological features. Lymphoglandular bodies were not observed in the histological sections. The perivascular cuffing with concentric laminar pattern was better appreciated in histopathological sections. The cytological features of the T-cell PCNSL case were similar to the B-cell PCNSL cases.

The intraoperative diagnosis was correctly predicted (as “NHL” in 23 cases and “Malignant round cell tumor” in 5 cases) in 28 (93%) cases. Of the other two cases, one case was reported as “malignant neoplasm”. There were numerous admixed reactive astrocytes raising the possibility of a high grade glioma. Another case was reported as “Lymphoid aggregates? Exact nature”. Low power examination revealed only marked gliosis with a lymphoid infiltrate, with high power examination showing few atypical cells. Some of the reactive astrocytes in this case showed mild atypia. Histological sections also showed marked gliosis with lymphoid aggregates which were confirmed to be neoplastic by immunohistochemistry. Steroids were administered pre-operatively in this case. The usage of corticosteroid can obscure the diagnosis by causing transient regression of tumor due to its pronounced lymphodepletive action. Another possibility is that this case was probably biopsied from the periphery of the lesion. However the intraoperative diagnosis was correct in all the other 7/8 cases with history of corticosteroid administration. A definite histopathological diagnosis was rendered in all the eight cases and none of them required a re-biopsy. Thus the pre-operative usage of corticosteroids did not significantly affect the intraoperative/final diagnosis of PCNSL in our study.

## Discussion

The median age of our patients was 50 years, which is about a decade younger than that reported in Western literature (Bataille et al., 2000; Bessell et al., 2011). An earlier multi-centric hospital based study from India had also showed that PCNSL occurs a decade earlier in Indian patients and is only very rarely associated with AIDS (Sarkar et al., 2005). A study from Korea also revealed a lower mean age of 51.5 (Zhang et al., 2010). This is quite similar to our study. The typical age at presentation for patients with AIDS is lower, with a mean age of 31-36 years (Fine and Mayer, 1993; Newell et al., 2004).

In the present study, none of the cases were associated with AIDS.

Two autopsy studies from India on AIDS patients have also not documented even a single PCNSL case, (Lanjewar et al., 1998; Satishchandra et al., 2000) although autopsy studies in the west have demonstrated that PCNSL occurs frequently (12-40%) in patients with AIDS (Loureiro et al., 1988; Goplen et al., 1997). These facts appear paradoxical because HIV prevalence is quite high in India. This is possibly due to earlier deaths of Indian patients due to AIDS associated opportunistic infections (Sarkar et al., 2005). Interestingly studies from Japan and Korea have also not shown any association of PCNSL with AIDS (Shibamoto et al., 2008 ; Zhang et al., 2010). A recent study estimated that in USA about 13% of CNS lymphoma cases were associated with AIDS in the period 2001-2007. A definite decline is evident since about 48% of CNS lymphoma cases were associated with AIDS in the period 1990-1995 (Shiels et al., 2011). However studies from Asia have not shown any significant association of PCNSL with AIDS in the past or in the present (Hayabuchi et al., 1999; Sarkar et al., 2005; Shibamoto et al., 2008; Pasricha et al., 2011).

Iatrogenic immunosuppression due to transplant and autoimmune and inflammatory diseases like myasthenia gravis, rheumatoid arthritis, SLE and sarcoidosis are risk factors for PCNSL (Eichler and Batchelor, 2006). In a previous study of non -AIDS associated PCNSL, 5% of patients had received immunosuppressive medications for medical conditions like ulcerative colitis, renal transplantation, rheumatoid arthritis and Wegener granulomatosis (Haldorsen et al., 2009).

The clinical presentation of immunocompetent PCNSL patients includes focal deficits, neuropsychiatric symptoms, headache, nausea, vomiting suggestive of raised intracranial pressure, seizures and ocular symptoms (Fine and Mayer, 1993; Bataille et al., 2000). Patients with AIDS related PCNSL are more likely to present with mental status changes or seizures (Fine and Mayer, 1993; Eichler and Batchelor, 2006).

Computed Tomography (CT) scans and Magnetic Resonance Imaging (MRI) typically show single or multiple periventricular, homogeneously enhancing lesions. These lesions are predominantly supratentorial (Koeller et al., 1997; Buhring et al., 2001). Ring like enhancement is rarely observed in immunocompetent patients, but can be commonly found in immunocompromised patients (Johnson et al., 1997; Haldorsen et al., 2008; 2009; 2011, Sutherland et al., 2012). In our study ring enhancement was observed in only two cases, one of which was labeled as Tuberculoma on radiological assessment. PCNSL can be mimicked by a variety of lesions like malignant gliomas, metastasis, demyelinating disorders, abscess and other infections. Toxoplasmosis is an important differential in immunocompromised patients. CT and MRI scan generally provide results suggestive but not conclusive for PCNSL (Koeller et al., 1997; Herrlinger et al., 1999).

Majority of the cases are of B-cell phenotype. The occurrence of T-cell PCNSL is very rare in Western as well as Asian studies (Bataille et al., 2000; Sarkar et al.,



2005; Zhang et al., 2010; Gelabert-González et al., 2012). A nationwide survey of PCNSL in Japan during the period 2000-2004 revealed that T-cell PCNSL accounted for 1.7% of all cases (Shibamoto et al., 2008), whereas a similar earlier study (1985-1994) from the same country showed a higher figure of 8.5% (Hayabuchi et al., 1999).

PCNSL is a highly radiosensitive and chemosensitive infiltrative tumor. Surgery is restricted only for diagnostic purposes, as PCNSL is often multifocal with a propensity to involve the subarachnoid space. Stereotactic biopsy is the preferred procedure for obtaining tissue for pathological diagnosis (Schultz and Bovi, 2010). Therefore an intraoperative diagnosis is necessary before proceeding with surgical resection if PCNSL is suspected.

Frozen section (FS) of CNS neoplastic lesions is usually accurate. However cytologic preparations are often superior to FS in demonstrating the morphology of malignant lymphoid cells. Diagnostic challenges have been encountered at FS in CNS lymphomas (Plessec and Prayson, 2007). Crush smears have the added advantage of being a cheaper and more rapid tool in establishing the intraoperative diagnosis. Less tissue is used than in frozen sections, allowing conservation of tissue, without artefactual changes, for permanent sections and subsequent confirmatory immunohistochemistry. Frozen section was employed in only one of our 30 cases (The case with partial correlation reported as “malignant neoplasm”).

Crush smears are highly accurate in the intraoperative diagnosis of PCNSL as evident from our study. Tilgner et al found almost 90% complete correlation for the group of primary and secondary lymphomas in their study to validate intraoperative diagnoses using smear preparation from stereotactic brain biopsies (Tilgner et al., 2005). However we have encountered certain diagnostic pitfalls in our experience. PCNSL usually has a central area densely packed with tumor cells. The surrounding brain tissue shows gliosis, inflammation and scattered tumor cells. Hence the site of the biopsy, especially stereotactic biopsy is important. Difficulties can arise when the central hypercellular zone is not sampled adequately. The presence of reactive astrocytes can sometimes raise the possibility of a glial tumor or an inflammatory lesion.

Steroids are generally withheld till the diagnostic procedure in suspected cases of PCNSL. Previous reports have shown that apart from their therapeutic anti-edematous effects, they can cause rapid lymphodepletion. They can also produce reactive gliosis with a variable infiltration of B- or T lymphocytes and macrophages, thus obscuring the diagnosis in some cases (Geppert et al., 1990; Weller, 1999; Choi et al., 2006). However a recent study at the Mayo clinic showed that corticosteroid administration before biopsy did not significantly affect the histopathological diagnosis of PCNSL cases (Porter et al., 2008). Similarly steroid administration did not significantly affect the intraoperative diagnosis/final diagnosis in the present study.

The present study reveals that PCNSL occurs at an earlier age in Indian patients and is almost limited to immunocompetent patients. It also illuminates the fact that intraoperative crush smear technique is a fairly accurate,

rapid, simple and cost effective tool in the management of PCNSL, especially in a country like India.

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