The Profile of P63 Expression and Epstein-Barr-Encoded RNA (EBER) Distribution in Primary Central Nervous System Lymphoma: A Retrospective Bi-Center Study

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Abstract

Background : Primary central nervous system lymphoma (PCNSL) is a type of lymphoma occurring around 0.5 – 1.2% of all intracranial neoplasms. However, recent epidemiological research shows a threefold increase in the number of cases. The Epstein-Barr virus (EBV) and PCNSL are both associated with the condition of immunosuppression or immunodeficiency, which often found to have a significant relationship with each other. Moreover, the TP63 mutation is associated with a poor prognosis.

Methods : This is a descriptive study to assess the expression of TP63 and EBER on PCNSL, and present the characteristics of the disease. The study was conducted on 25 cases from two health centers with the most cases of brain tumors in Indonesia, Siloam Hospital Lippo Village and Cipto Mangunkusumo Hospital (CMH) from 2014 to 2018, the P63 expression and EBER-1 examinations were done by 4 pathologists. A total of 25 patients, 13 (52%) patients were male, ranging from age 30 – 79, with average 57,6 years old, located mostly in the frontal lobe in 8 patients (30.9%).

Result : From the research results obtained positive P63 results in 20 cases (80%), while the EBER test was negative for all 25 patients. Further analysis with software SPSS 25 proving that P63 expression is not associated with germinal center B-cell type (GCB) or non-GCB type (p-value 0,87). Neither, P63 have any association with Ki67 with p-value of 1.00.

Conclusions : This study concludes that there is a possibility that PCNSL cases in Indonesia are not associated with Epstein-Barr virus infection, but most of the cases will have a poor prognosis as indicated by P63 expression.

Introduction

Primary Central Nervous System Lymphoma (PCNSL) is an extra-nodal Non-Hodgkin Lymphoma.[1] It is a rare type of brain tumor, comprising only about 0.5 – 1.2% cases of intracranial neoplasms and <1% of extra-nodal non-Hodgkin's lymphoma.[2] Recent epidemiological research shows a threefold increase in the number of cases in the population.[3] PCNSL has a poorer prognosis compared to systemic lymphomas of the same subtype.[1]

The main risk factor for lymphoma of the central nervous system, both primary and secondary, is immunosuppression or immunodeficiency. HIV patients have a 3600-fold risk for PCNSL compared to the normal population.[2,4] PCNSL related to AIDS is also mainly associated with low CD4 cell counts (<50 cells/ L) and Epstein-Barr virus (EBV) infection.[2,4]

Other causes of lymphoid malignancies are genetic mutations, one of which is TP63. TP63 mutation and one of its loci, TBL1XR1, are also found in both B cell lymphoma and PCNSL.[5] The presence of TP63 and EBER on PCNSL might be the cause for poor prognosis. This is a descriptive study to assess the expression of TP63 and EBER on PCNSL and present the characteristics of the disease.

Material And Methods

Research Sample

This study was conducted with a cross-sectional study design. There was originally a total of 49 identified cases, however due to the specification of the data required, only 26 fulfilled the criteria. All 26 cases of PCNSL patients were taken from two health centers Siloam Hospital Lippo Village (SHLV) and Cipto Mangunkusumo Hospital (CMH) from the year of 2014 to 2018. The diagnostic criteria were reviewed by two pathologists (EK and SM).

PCNSL Patient Specimens

TP63 mutation examination was done with immunohistochemistry staining with P63 (Mouse Monoclonal Antibody, Biocare Medical, ready to use, catalogue number PM 163 AA, H). Positive control was done with prostate tissue while negative control was made by the absence of primary antibody. EBER-1 RNA examination was done with in situ hybridization (ISH) technique.

Immunohistochemistry staining of P63 were evaluated by two pathologists (EK and SM), with positive results with a value of more than 50% is declared as P63 positive, while ISH examinations were evaluated by 4 pathologists (EK, SM, AH and MH) altogether.

Data Analysis

The data was shown in descriptive model.

Result

Basic Characteristics of the Sample

Based on data from SHLV and CMH Anatomical Pathology Department Archives, the total number of samples that fulfilled the inclusion and exclusion criteria from 2014 to 2018 were 26 cases. Out of the 26 cases 16 cases were retrieved from SHLV, and 10 cases from CMH. From all samples, the mean age was 57 years old, with the youngest being 37 years old and the oldest 73 years old. The ratio between male and female patients was 1:1 (13 male and 13 female). The most common tumor location in this study was in the frontal region. (30.8%) (Table 1).

Table 1. Characteristics of the sample.

Characteristic	Category	Count	Percentage (%)
Gender	Male	13	50
	Female	13	50
Age	30-39	1	3.8
	40-49	5	19.2
	50-59	9	34.6
	60-69	7	27
	70-79	4	15.4
Location	Cerebrum	8	30.9
	Frontal	2	7.7
	Temporal	5	19.2
	Parietal	1	3.8
	Thalamic	1	3.8
	Corpus Callosum	3	11.5
	Cerebellum	6	23.1

Reassessment of PCNSL diagnosis includes morphologic and immunophenotypic features re-evaluation according to operational definitions. Morphological picture obtained showed a diffuse malignant lymphoid cell with large nuclei and prominent nucleoli, with plenty mitotic figures (Figure 1).

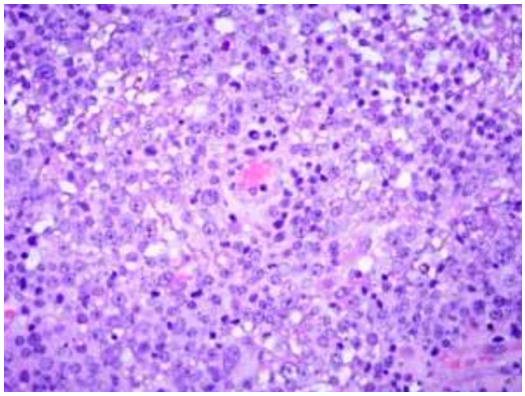

Figure 1. Primary Central Nervous Systems Lymphoma (PCNSL) Hematoxillin Eosin 400x.

After reassessing Hematoxylin and Eosin preparations, the study continued with reassessment of immunohistochemistry (IHC) results to confirm the diagnosis. During the IHC examination, a result of CD 20 positive was observed in all the cases. (Figure 2) From the IHC outline, a result of CD10 positive was found in 3 cases (11,5%) (Figure 3), MUM-1 positive in 24 cases (92.3%) (Figure 4), Bcl6 positive in 21 cases (80.8%) (Figure 5), and Ki- 67 positive on average of 74.1%. (Table 2) Based on the lymphoma molecular subtype by Han's criteria, there were 3 cases of the GCB group and 23 cases in the non-GCB group involved in this study.

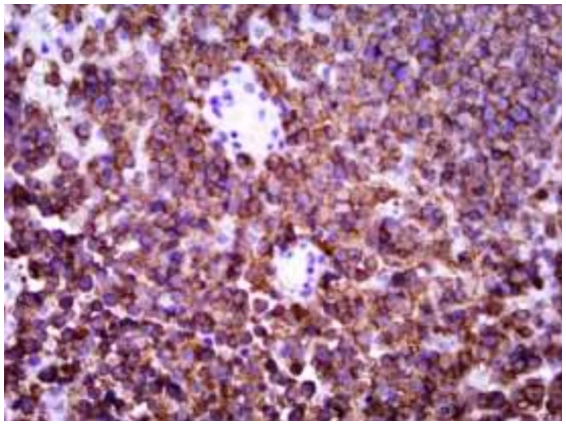

Figure 2. CD20 positive.

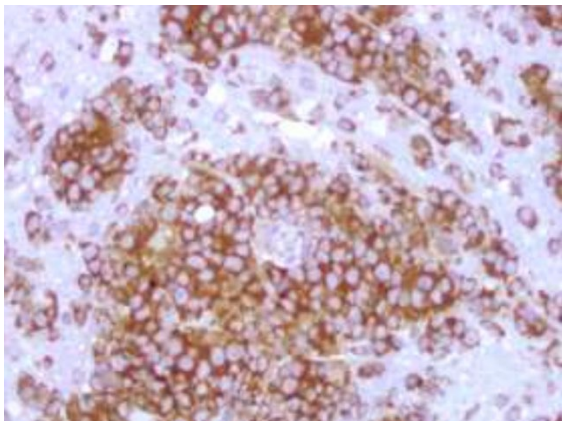

Figure 3. CD10 positive.

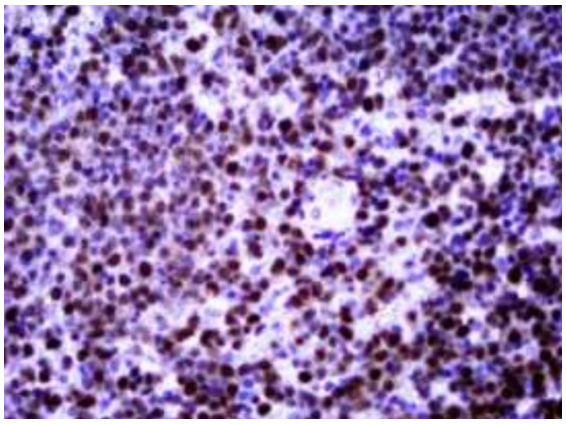

Figure 4. MUM1 positive.

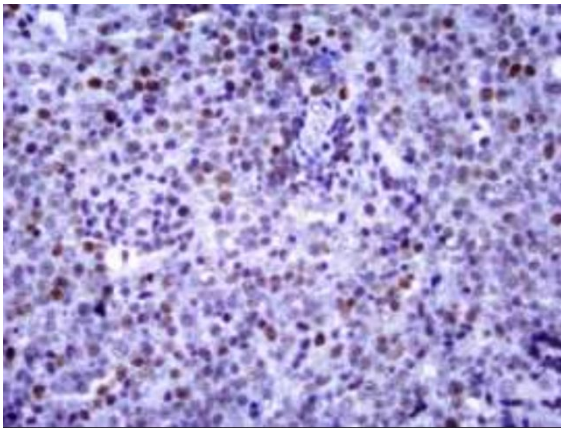

Figure 5. BCL6 positive.

Table 2. Antibody expression

Antibody Expression	Number of Cases Positivity	Percentage (%)
CD20	26.0	100
MUM-1	24.0	92.3
BCL6	21.0	80.8
P63	21.0	80.8
EBER-1 RNA (ISH)	0.0	0

Analysis of P63 and EBER expressions

P63 and EBER expressions assessment was done qualitatively. The test results were assessed by 2 researchers simultaneously. The results obtained showed a positive P63 expression in 21 cases (80.8%) (Figure 5), and EBER negative results were obtained from all cases (Figure 6). Because one of the variables obtained negative results in all cases, the correlation analysis test cannot be performed.

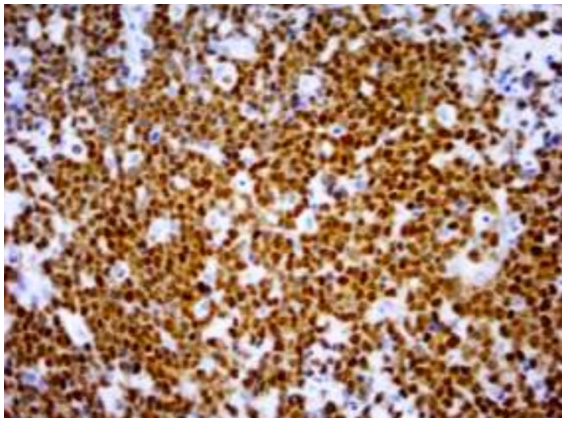

Figure 6. p63 positive.

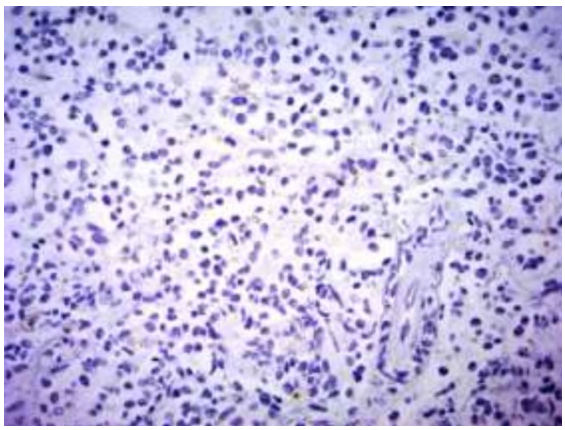

Figure 7. EBER negative.

Discussion

Basic Characteristics of Samples

The nature and findings of the study are placed in context of other relevant published data. Caveats to the study should be discussed. Avoid undue extrapolation from the study topic.

Primary central nervous system lymphoma (PCNSL) is an aggressive type of lymphoma that arises in the brain parenchyma, spinal cord, eyes, cranial nerves, and meninges. PCNSL patients involved in this study consisted of an equal ratio between men and women. This is consistent with the study conducted by Shiels et al, which shows the similarity in predominance between men and women in PCNSL cases in America.[6]

Primary central nervous system lymphoma (PCNSL) can occur in immune-competent individuals or immune-compromised individuals, including HIV-infected individuals and post-transplant patients. PCNSL is the main cause of morbidity and mortality in HIV-infected individuals before the advent of antiretroviral therapy. However, with the effective combination of antiretroviral therapy, the incidence of this disease begins to decrease.[2] Moreover, the incidence of PCNSL is increasing in HIV-negative individuals, where PCNSL is currently <1% of all non-Hodgkin's lymphoma (LNH) and around 2-3% of all brain tumors.[3]

About 90-95% of PCNSL is histologically classified as diffuse large B-cell lymphoma (DLBCL) which consistently expresses B cell antigens and has a generally positive MUM-1 and BCL-6, and only less than 10% express CD10.[3] Overall, PCNSL does not possess a molecular profile as in systemic DLBCL and B cell subtypes, but has a unique transcriptional description of gene expression profiling.[4] That is consistent with our study which showed that only 3 of the cases were CD10 positive.

PCNSL is a unique clinicopathological entity in DLBCL. PCNSL has a centroblastic morphology and originates from late germinal centre or early post germinal centre exit B cells. These cells have MUM-1 and BCL6 co-expression but not CD138. Thus, DLBCL PCNSL is different from systemic DLBCL because it cannot be classified into prognostic groups according to its cell origin. The prognostic effect of molecular changes remains to be determined. In addition, other things to note are the cell derivation and pathogenesis of DLBCL PCNSL.[6]

From a virology point of view, the DLBCL PCNSL contains an Epstein-Barr virus (EBV) infection, and can be detected in immune-compromised individuals.[4] In a study by Mahadevan, 24 patients with DLBCL PCNSL were analyzed in South India. These cases consistently showed centroblastic morphology and overall showed activated B-cell phenotype with MUM-1 expression, but not CD138. That study also showed BCL6 and MUM1 expression in 50% of cases. All cases were also negative for EBV with EBER in situ hybridization and latent membrane protein 1 (LMP1) immunohistochemistry. According to Mahadevan, patients with systemic DLBCL showed predominance of GCB origin in several studies and activated B-cell origin in several other studies.[6]

Because the CNS contains little lymph nodes and lymphatic vessels, there is a hypothesis that PCNSL can originate from B cells derived from systemic lymphoid tissue and normally circulates in and out of the CNS. The exact cell derivation is still unknown, and the pathogenesis of PCNSL is also still not well understood. The evidence currently available is only limited to the role of EBV in PCNSL relating to AIDS. Lymphoma cells in EBV infection often expresses encoded LMP-1 oncoprotein, which, in turn, upregulates the expression of BCL2. This suggests that EBV can act as an oncogenic agent or at least as an oncogenic cofactor.[7]

In contrast, EBV does not play a role in lymphomagenesis in immune-competent PCNSL patients. On genetic examination, immune-competent DLBCL PCNSL patients showed ongoing aberrant somatic hyper-mutation that, besides the IG locus, targets the PAX5, TTF, MYC, and PIM1 genes. Some important pathways are activated such as B-cell receptors (BCR), toll-like receptors, and nuclear factor-κB pathways. Genomic transitions on PCNSL include the loss of chromosome 6p21 which contains the locus of human leukocyte antigen (HLA).[6]

A case report in China reported that out of 9 cases, 2 presented with multiple lesions, while 7 patients exhibited a solitary lesion.[8] In this study, out of 26 cases, 6 cases reported to have multiple lesions, while 20 patients presented with solitary lesion. It is reported that around 34% of

PCNSL patients revealed multiple lesions.[9] These locations contribute to the symptoms occurred in patients.

The p63 gene, a homolog of the tumor suppressor gene TP53, maps to chromosome 3q27-28, a region frequently displaying genomic amplification in squamous cell carcinomas. In a study conducted by Cyrus et al, p63 expression was examined by immunohistochemistry using a monoclonal antibody (clone 4A4), while distinction of p63 isoforms was analyzed by Western blotting and reverse transcription-polymerase chain reaction using isoform-specific primers. The study found that a subset of DLBCL (32% of cases) expressed p63 in the nuclei of neoplastic lymphocytes. In this study, P63 was found in 84% of the cases, in which p63 is associated with high proliferative index, as assessed by Ki-67 immunostaining.[10]

A study in India, evaluating the Epstein-Barr virus as an etiology of PCNSL in immunocompetent individuals, found that ISH for EBER was negative in all 19 patients.[11] Th study concludes that in the region associated with this study, EBV likely has no etiologic role in the development of PCNSL in immunocompetent individuals. Correspondingly, EBER examination was found to be negative in all 26 patients involved in this study. However, a study regarding EBER in elderly patients on immunosuppressive medications by Kleinschmidt-DeMasters et al., found that in 4 patients examined positive for EBER, none of the patients develop symptoms of HIV-AIDS. These patients shown neuroimaging features typical for PCNSLS in immunocompromised patients (i.e. multifocal and ring-enhancing lesions).[12]

Conclusion

From this study, we confirm that most of the PCNSL are DLBCL non-GCB subtype, with p63 staining positive in 84% (21 cases) and all the cases we retrieved were EBER negative. According to the data we have collected most of the patients are 50 – 59 years old, consistent with the typical age associated with the occurrence of PCNSL. In HIV-AIDS patients, PCNSL tends to occur at a younger age and is associated with EBV infections. It is possible that the patients the researchers have gathered are HIV negative and therefore is not associated with EBV infections. Moreover, more data and examinations are needed to confirm the hypothesis. Nevertheless, it can be concluded that the demographic data in Siloam Hospital Lippo Village and Cipto Mangunkusumo Hospital both describing patients with PCNSL with EBER negative and therefore shows that EBV infection rate is low.

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