Annals of Case Reports
Bouzekri ZAH, et al. Ann Case Rep: 7: 882.
www.doi.org/10.29011/2574-7754.100882
www.gavinpublishers.com

Review Article

Case Review: Primary T-cell CNS Lymphoma in a Pediatric Immunocompetent Patient

Zeinab Al Hawraa Bouzekri, Yara R Neaimeh, Samar Muwakkit , Yara Salameh*

Department of Pediatrics and Adolescent Medicine, American University of Beirut, Beirut, Lebanon

***Corresponding author**: Yara Salameh, Department of Pediatrics and Adolescent Medicine, American University of Beirut, Beirut, Lebanon

Citation: Bouzekri ZAH, Neaimeh YR, Muwakkit S, Salameh Y (2022) Case Review: Primary T-cell CNS Lymphoma in a Pediatric Immunocompetent Patient. Ann Case Report 7: 882. DOI: 10.29011/2574-7754.100882

Received: 25 June 2022; **Accepted:** 29 June 2022; **Published:** 03 July 2022

Abstract

Primary central nervous system lymphoma (PCNSL) is a very rare form of non-Hodgkin lymphoma. In most cases, PCNSL is a B cell lymphoma, making a PCNSL with T cell in origin rare. The latter often occurs in adult immunocompromised patients [1]. There is also no consensus regarding the treatment of CNS T cell lymphoma due to the paucity of confirmed cases. In general, treatment is mainly based on a regimen that includes methotrexate [2]. In this article, we present the challenging case of a 4-year-old boy who was diagnosed with refractory T-cell CNS lymphoma after initial brain biopsy was benign and who presented 2 years after end of treatment with relapsed disease, leading to his mortality.

Introduction

Primary central nervous system lymphoma (PCNSL) is a very rare form of non-Hodgkin lymphoma, occurring in less than 1% of cases. It is limited to the CNS [3,4]. In 90% of cases, PCNSL occur as diffuse large B cell lymphoma. It is rarely a T cell lymphoma [5]. PCNSL occur more often in people with immune dysfunction, which is the only known predisposing factor. If no immune dysfunction is present, PCNSL is extremely rare, making the diagnosis more challenging due to atypical presentation. It is most often seen in elderly patients. Hence, the rarest form of the diseases will be T cell PCNSL occurring in an immunocompetent pediatric patient [3]. In this article we will present a pediatric immunocompetent patient with T cell PCNSL, who was initially misdiagnosed and later presented with relapse. We will also summarize pediatric cases published in the literature with similar diagnosis, including presentation, site of disease and diagnostic tool of each case.

Case Presentation

We present the case of a 4-year-old boy who was diagnosed with refractory T-cell CNS lymphoma after initial brain biopsy was benign and who presented 2 years after end of treatment with relapsed disease, leading to his mortality. Patient initially presented to our institution at the age of 4 years old with 2 months history of progressively worsening headache and ataxia associated with severe neck pain and recurrent episodes of vomiting. Imaging done showed right cerebellar hemisphere and frontal lobe lesions, and the patient was scheduled for sub-occipital craniotomy for debulking of the brain lesion. Pathology results showed no evidence of neoplasm and infectious cause was ruled out with negative tissue biopsy, negative CSF culture and meningitis panel and negative brucella and toxoplasma studies. Patient was suspected to have an inflammatory demyelinating process and was discharged home on tapering steroid schedule. However, few days later, patient presented with nystagmus, ataxia and decreased level of consciousness after steroids were tapered so he was given pulse steroids for 5 days. Good response was noted with clinical improvement, so the patient was discharged home on 6 weeks gradual tapering steroids schedule. After 1 week, patient presented again for weakness and worsening ataxia and brain MRI was repeated showing progression of brainstem lesion which was intensely avid on PET-CT. Given the patient's recurrent symptoms, positive findings on repeat brain MRI and absence of diagnosis on initial biopsy, decision was made to repeat brain biopsy. Atypical T-cell infiltrates were noted on second biopsy with T-cell

receptor gene rearrangement showing the diagnosis of T-cell CNS lymphoma. After diagnosis on 12/09/2019, patient was started on chemotherapy as per the Burkitt lymphoma protocol COPAD M8 and completed maintenance chemotherapy on 23/1/20 as per the ANHL1131 group C3 protocol. He was admitted to the hospital for episodes of febrile neutropenia requiring intravenous antibiotics during the above-mentioned course. Repeat brain MRI after end of chemotherapy showed no change in the overall size, enhancement and extent of brainstem lesion and decision was made to proceed with radiation therapy given the refractory tumor. He received whole brain radiotherapy 24 Gy followed by focal boost of the primary tumor in the brainstem to 36 Gy. Repeat imaging 2 months later showed stable brainstem lesion with decreased enhancement on brain MRI along with decrease in uptake and size of the lesion on PET CT. Patient continued to follow-up with oncology team in outpatient clinics for post-treatment surveillance for disease recurrence.

After end of treatment, patient had residual left-sided weakness and spasticity but was otherwise normal on physical exam. As part of his assessment, MRI done on 08/12/21 showed stable examination of the brain. On 25/01/21, patient presented to the outpatient clinics for regular follow-up and parents complained of one episode of headache associated with diplopia, dizziness and low-grade fever 3 days prior to presentation. Symptoms were attributed to possible viral infection given that his father tested positive for COVID-19 infection. However, symptoms persisted, and patient presented again to the clinics complaining of worsening nystagmus, ataxic gait, lethargy and decreased activity. Urgent brain CT was done and was negative for new lesions or bleed. MRI was scheduled few days later and showed new enhancing lesions adjacent to the surgical bed with multiple enhancing lesions in the left cerebellar hemisphere, left pons and cerebellar peduncles and along the cerebellar leptomeninges, associated with disease recurrence. PET CT confirmed disease recurrence with new FDG uptake in midbrain, cerebral peduncles and faint uptake in the pons. Given that the patient was hemodynamically stable with normal physical exam except for the chronic left-sided weakness, patient was discharged home on dexamethasone 4 mg every 6 hours and was scheduled for central line insertion for chemotherapy (ifosfamide, carboplatin and etoposide) for CNS T-cell lymphoma relapse. Unfortunately, patient presented earlier to the emergency department for seizure and somnolence. He was hemodynamically stable and lab workup within normal range. Urgent CT brain was repeated and showed diffuse supratentorial hypodensity with poor grey-white matter differentiation along with effacement of the sulci suggestive of brain edema (Figure 1). No bleed, infarct or dural venous sinus thrombosis was noted. CT brain also showed re-demonstration of the enhancement along the anterior medial aspect of the surgical cavity and right cerebellar peduncle related to the known disease recurrence (Figure 2). Patient was started on levetiracetam in addition to management of elevated intracranial pressure with head of bed elevation, intravenous hypertonic saline and dexamethasone. Neurosurgery team consulted but edema was attributed to disease recurrence, thus no intervention was indicated at that time. Patient remained somnolent overnight and decision was made to intubate the patient in order to protect his airways. He was admitted to the pediatric intensive care unit for further management.

In the intensive care unit, parents were informed about the patient's condition and poor prognosis, and they decided to proceed with low/intermediate intensity chemotherapy to avoid severe side effects so patient was started on etoposide 100 mg/m2 for 3 days. No improvement was noted after which parents decided to proceed with high dose cytarabine followed by peg asparaginase after oncology team explained its side effects and life-threatening complications. Cytarabine 2000 mg/m2 was started and after the patient received the first dose, physical exam showed left dilated pupil not reactive to light and patient had no response to pain. Urgent brain CT showed worsening diffuse cerebral edema with complete loss of with complete loss of gray-white matter differentiation and diffuse sulci and gyral effacement and white cerebellar sign (Figure 3). Multidisciplinary meeting was held with the family and parents decided to stop chemotherapy. Patient's code was changed to do not resuscitate and he passed away few days later after cardiac arrest.

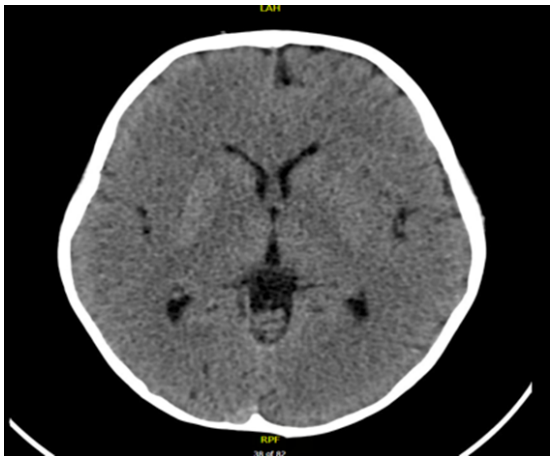

Figure 1: CT brain showing brain edema.

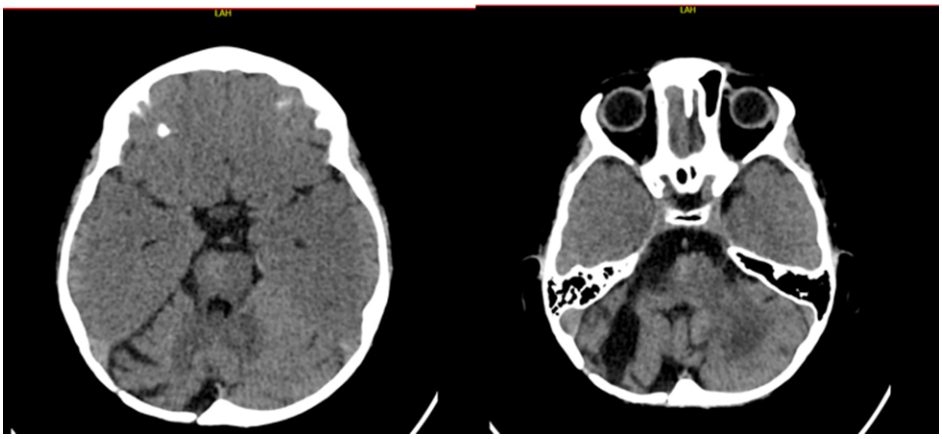

Figure 2: CT brain showing re-demonstration of disease recurrence.

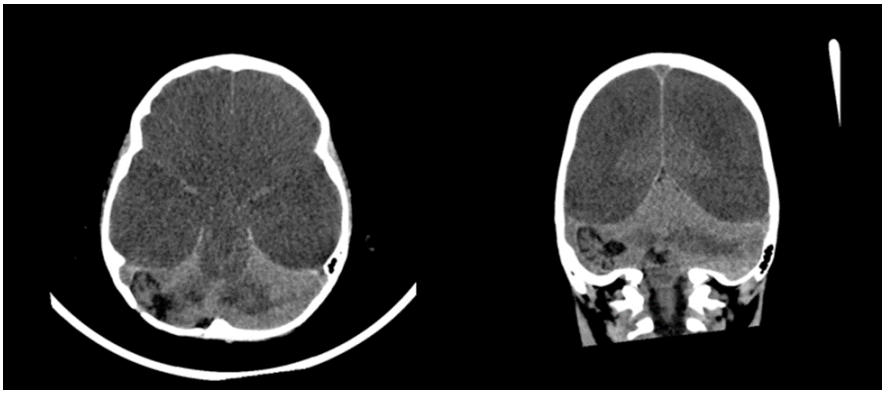

Figure 3: CT brain showing white cerebellar sign and worsening brain edema.

Discussion

All the cases below were collected through a thorough search of the literature. We were able to find 21 cases of T cell PCNSL in immunocompetent pediatric patients. Table 1 summarizes the cases. Among these cases 12 were males. The age group of our patients ranged from 23 months to 17 years with a mean age of 10.05 years [5-19] (Table 1).

	Age	Sex	Presentation	Protein in CSF	Site of disease	Initial LDH (IU/L)	Definitive diagnosis
Uetsuka S et al. [6]	12 years	M	Left sided weakness, vomiting, headache, blurred vision, and diplopia	NA	Right parietal	NA	CNS T cell lymphoma
Lueth, M et al. [7]	6 years, 11 months	M	Right-sided Jackson attacks	None	Left precentral region	None	After resection

Shah, A et al. [8]	23 months	M	Motor function loss, left hemiparesis, and complex partial seizures	NA	Right - hemispheric Leptomeningeal Uncal herniation	NA	After resection
Gualco, G. et al. [3]	10 years	M	3 months history of headache, vomiting, ataxia, and right amaurosis	NA	Right parietal Optic chiasm Cerebellum Luschka-Magendie foramina	876	Pathologic diagnosis
Abla, O. et al. [9]	6 years	F	NA	NA	NA	899	Anaplastic large T - cell
Abla, O. et al. [9]	7 years	M	NA	positive	NA	519	Anaplastic large T - cell
Bassuk AG et al. [10]	10 years	M	Ataxia and fatigue	196 mg/dL	basal ganglia, left caudate head internal capsule	944	Pathology
Bogdahn U et al. [11]	2 years	F	NA	NA	Superior vermis	NA	Lymphoblastic convoluted T cell
O'Neill BP et al. [12]	17 years	M	NA	NA	Right frontoparietal Left cerebellum	NA	NA
Ng HK et al. [13]	16 years	M	Right-sided headache, fever , cough, left hemiparesis	0.64g/l	Right parietal lobe	NA	Large cell T cell
Buxton N et al. [14]	10 years	F	Left-sided sensory disturbance and 8 days of headaches and drowsiness	NA	Right parietal lobe	NA	Ki-1 positive
Abdulkader I et al. [15]	13 years	M	Headache, vomiting	NA	Right parietal Frontal	NA	Primary leptomeningeal ALCL with ALK positivity
Al-Ghamdi H et al. [5]	13 years	F	N/A	NA	Cerebellum		T cell lymphoma
Al-Ghamdi H et al. [5]	10 years	F	Ataxia, nystagmus	NA	Cerebellum	NA	CNS T cell lymphoma
George DH et al. [16]	17 years	M	NA	NA	Right parietal dura	NA	Anaplastic large cell

George DH et al. [16]	2 years	M	Motor dysfunction	NA	Right cerebral leptomeninges	NA	Large cell lymphoma
Choi, J. S. et al. [17]	17 years	F	N/A	NA	Left temporal + dura	NA	Anaplastic large T-cell
Choi, J. S. et al. [17]	4 years	M	N/A	NA	Multilocal brain Brainstem Spinal cord	NA	Anaplastic large T-cell
Choi, J. S. et al. [17]	12 years	F	N/A	NA	Left occipital	NA	Anaplastic large T-cell
Choi, J. S. et al. [17]	17 years	F	N/A	NA	Right parietal-occipital	NA	Anaplastic large T-cell
Singh, G. et al. [18]	9 years	M	Fever for 20 days Swelling over face and eyes for 16 days Deviation of mouth for 16 days	NA	Soft tissue enhancing mass involving skull base centered in basisphenoid nasopharynx and left frontal region	Normal	PCNSL

Table 1: The age group of our patients ranged from 23 months to 17 years with a mean age of 10.05 years.

Epidemiology

T cell PCNSL is a very rare disease. It was thought to only occur in immunosuppressed patients, and thus its occurrence in immunocompetent makes it far down the list of the differential diagnosis. It occurs in less than 2% of all the malignant lymphomas, and less than 1.5% of the intracranial tumors in general. 5 When PCNSL is diagnosed, it is usually B cell in origin. Previous studies of large series looking at PCNSL done in France and Japan showed that T cell lymphomas were only 3.6% in France and 8.5% in Japan [20,21]. Another large case series done in Western countries showed that the proportion of T cell origin PCNSL was only 2% of the cases, which accounted for 8 patients out of 370 [22]. This makes its occurrence not precise. In addition, T cell PCNSL is usually a disease that occur in older patients. In a large retrospective series study that was done in 12 cancer centers in 7 different countries to describe the demographics and tumor characteristics of 45 patients, their median age was 60 years old [23].

Presenting symptoms

The presentation of isolated CNS lymphoma varies according to tumor site and patient characteristics. Generally, patients with isolated CNS lymphoma present with cranial and peripheral nerve involvement. In some cases, uveitis is the initial manifestation. Patients can also present with symptoms due to leptomeningeal involvement with neuronal lymphomatosis as the sole manifestation, such as headache, difficulty speaking, altered mental status, seizures and weakness. Additional presenting symptoms include signs of elevated intracranial pressure, 6th nerve palsy, bilateral 3rd nerve palsy, mononeuritis multiplex, unilateral hearing loss, and bilateral 7th nerve paralysis [24]. A cross-sectional retrospective study conducted in Shohadaye Tajrish Hospital in Tehran, Iran, during a 25-year period (1990 – 2014) showed that symptoms are often widespread and multifocal. Due to the characteristics of the disease, symptoms mainly include cognitive impairment, disorientation, slow psychomotor activity, and personality changes. Seizures is another manifestation and can develop in 2%–33% of patients. It is important to note that the most common presenting symptoms are hemiparesis (56.2%), headache (51.7%), personality disorders and cognitive impairment (26.7%), seizure (12.5%), cranial nerve palsy (10.8%), ataxia (9.7%), aphasia (9.1%), and eye involvement (4%), respectively [25].

Diagnosis and its pitfall

The diagnosis of T cell PCNSL is challenging due to the

variable locations that it can present in. It could be a single tumor or a multifocal disease. The most common site is a space occupying parenchymal mass [24]. On imaging, there is no definitive pattern for diagnosis and reactive processes cannot be ruled out. T cell PCNSL could resemble an abscess, demyelinating lesion, metastatic lesion, infection or inflammatory reaction, thus making it difficult to diagnose based on imaging solely [26]. In the large retrospective study mentioned previously where 45 patients with T cell PCNSL were described, 26 (58%) of them had cerebral hemisphere involvement and 16 (36%) patients had deeper parenchymal involvement [23]. In a study looking at 18 patients with T cell PCNSL, 5 of them had leptomeningeal enhancement, in which one patient had an extensive form. Furthermore, grading of T cell PCNSL by imaging is another challenge because infiltrations in the parenchyma might not always lead to signal abnormalities. Non-specific lab findings might be found sometimes including elevated lactate dehydrogenase level [26]. As for CSF analysis for diagnosis, an elevated protein level was shown to be elevated in 19 out of 24 patients (79%) [22]. A helpful finding for diagnosis includes lymphoma cells in CSF. However, lymphoma cells should be differentiated from reactive lymphocytes. They have larger nuclei, are more irregular and might have vacuoles. These findings could help in diagnosing CNS lymphoma, mainly when leptomeningeal enhancement in present [27]. Unfortunately, this is not always the case, and diagnosis is rarely confirmed from CSF analysis alone. Thus, obtaining a tissue biopsy from the lesions itself is most often a requirement for definitive diagnosis [22]. Furthermore, even after a confirmation of T cell PCNSL, one cannot know if the T cell lymphoma is high grade or has atypical feature, which is contrary to B cell lymphomas. In some instances, a mixed lineage could be seen, including B cell, T cell and macrophages. As for the T cell markers, CD3, CD4 and CD8 are usually found. However, in the literature, cases have been reported that are CD4 positive only, CD8 positive only, both CD 4 and CD8 positive, or both CD 4 and CD 8 negative [28].

Treatment

In the abovementioned retrospective series done in 12 cancer centers, the disease specific survival time was 25 months. These results were similar to the median survival time of B cell PCNSL. The administration of methotrexate as the primary treatment was associated with better survival [23]. In a study looking at 116 patients, a combination of chemotherapy and radiotherapy was superior to radiotherapy alone, and the patients treated with chemotherapy that included high dose methotrexate (HDMTX) had better survival than those with no methotrexate regimen. Furthermore, HDMTX with high dose cytarabine gave better results than HDMTX alone [23]. In general, treatment is divided into 3 parts: surgery, whole-brain irradiation and chemotherapy.

Each part will be discussed separately below:

Surgery

Previously, literature did not recommendation CNS lymphoma resection based on the evidence that aggressive surgery may increase the risk of postoperative deficit and provide no survival benefit compared with biopsy alone. A retrospective analysis of the German PCNSL Study Group-1 (GPSG-1) trial concluded that aggressive resection of CNS lymphoma in the setting of well-circumscribed lesions correlated with improved progression-free survival, immediate relief of mass effect and facilitated rapid tapering of glucocorticoids [22].

Whole brain radiation

Whole-brain irradiation is highly effective because it generates an immediate response in patients with CNS lymphoma. However, the utility of this type of treatment in CNS lymphoma patients is limited due to 3 factors: (1) insufficient local control of lymphoma; (2) dissemination of lymphoma cells within the CSF circulation and (3) neurological effects of radiation on brain function. The overall response rate of whole brain radiation is 90%. The median survival is 11.6 months.

Chemotherapy

No prospective trials in pediatric patients with T cell PCNSL were conducted. Therefore, there is no consensus regarding the treatment of CNS T cell lymphoma and this is due to the paucity of confirmed cases. Case reports and series have demonstrated that multi-agent chemotherapy including HDMTX and cytarabine is effective in most patients [22]. So far, HDMTX (high dose methotrexate) is the backbone of most of the induction regimens and is the most significant treatment-related prognostic variable related to survival in T cell PCNSL [28]. In the few reported cases of pediatric T cell PCNSL many treatment modalities have been used, including surgical resection, radiation, chemotherapy, and most recently allogeneic stem cell transplant. Stem cell transplant emerged as a treatment option for refractory and relapsed T cell PCNSL with survival rates ranging from 51% to 70% at 5 years [18].

Conclusion

The presenting symptoms of PCNSL are varied and depend on the area of the brain that is involved. The diagnosis is challenging and requires pathology for definitive diagnosis. There is no consensus regarding the treatment of PCNSL due to paucity of cases. Our case demonstrates that PCNSL should always be on the list of the differential diagnoses even if the initial biopsy is negative. Increasing awareness of T cell PCNSL is needed to better understand the molecular biology of the disease and develop better treatment regimens. Finally, classical therapy did not work for our patient. However, other therapies such as Nelarabine or

Bortezomeb or other new targeted therapies should be considered for these patients in the future.

Contributors' Statement

Dr Bouzekri, Dr Salameh and Dr Neaimeh wrote the manuscript. All authors contributed equally.

Dr Muwakkit reviewed the manuscript.

Parental consent to publication was obtained.

All authors were involved in direct patient care and approved the submitted manuscript.

References

1. Grommes C, DeAngelis LM (2017) Primary CNS lymphoma. Journal of Clinical Oncology. 35: 2410.
2. Mazur MD, Ravindra VM, Alashari M, Raetz E, Poppe MM, et al (2015) Primary T cell central nervous system lymphoblastic lymphoma in a child: case report and literature review. Child's Nervous System. 31: 977-984.
3. Lukas RV, Stupp R, Gondi V, Raizer JJ (2018) Primary Central Nervous System Lymphoma: PART 1: Epidemiology, Diagnosis, Staging, and Prognosis. Oncology (08909091). 32: 17-22.
4. Menon MP, Nicolae A, Meeker H, Raffeld M, Xi L, et al (2015) Primary CNS T-Cell Lymphomas: A clinical, morphologic, immunophenotypic and molecular analysis. The American journal of surgical pathology. 39: 17-19.
5. Al-Ghamdi H, Sabbah R, Martin J, Patay Z (2000) Primary T-cell lymphoma of the brain in children: a case report and literature review. Pediatric hematology and oncology. 17: 341-343.
6. Uetsuka S, Kajiwara K, Suehiro E, Nishizaki T, Ito H, et al (1999)T cell malignant lymphoma in the central nervous system after acute lymphoblastic leukemia in a child. Child's Nervous System. 15: 486-489.
7. Lueth M, Stein H, Spors B, Henze G, Driever PH (2012) First case report of a peripheral T-cell lymphoma, not otherwise specified, of the central nervous system in a child. Journal of Pediatric Hematology/Oncology. 34: e66-8.
8. Shah AC, Kelly DR, Nabors LB, Oakes WJ, Hilliard LM, et al (2010) Treatment of primary CNS lymphoma with high-dos methotrexate in immunocompetent pediatric patients. Pediatric blood & cancer. 55: 1227-1230.
9. Abla O, Sandlund JT, Sung L, Brock P, Corbett R, et al (2006) A case series of pediatric primary central nervous system lymphoma: favorable outcome without cranial irradiation. Pediatric blood & cancer. 47: 880-885.
10. Bassuk AG, Keating GF, Stumpf DA, Burrowes DM, Stack C (2004) Systemic lymphoma mimicking acute disseminated encephalomyelitis. Pediatric neurology. 30: 129-131.
11. Bogdahn U, Bogdahn S, Mertens HG, Dommasch D, Wodarz R, et al (1986) Primary non-Hodgkin's lymphomas of the CNS. Acta neurologica scandinavica. 73: 602-614.
12. O'Neill BP, Kelly PJ, Earle JD, Scheithauer B, Banks PM (1987) Computer-assisted stereotaxic biopsy for the diagnosis of primary central nervous system lymphoma. Neurology. 37: 1160-1164.
13. Ng HK, Lo ST, Poon CY, Poon WS (1988) Primary cerebral T-cell lymphoma: A case report. British Journal of Neurosurgery. 2: 523-528.
14. Buxton N, Punt J, Hewitt M (1998) Primary Ki-1-positive T-cell lymphoma of the brain in a child. Pediatric neurosurgery. 29: 250-252.
15. Abdulkader I, Cameselle-Teijeiro J, Fraga M, Rodriguez-Núnez A, Allut AG, et al (1999) Primary anaplastic large cell lymphoma of the central nervous system. Human pathology. 30: 978-981.
16. George DH, Scheithauer BW, Aker FV, Kurtin PJ, Burger PC, et al (2003) Primary anaplastic large cell lymphoma of the central nervous system: prognostic effect of ALK-1 expression. The American journal of surgical pathology. 27: 487-493.
17. Choi JS, Nam DH, Ko YH, Seo JW, Choi YL, et al (2003) Primary central nervous system lymphoma in Korea: comparison of B-and T-cell lymphomas. The American journal of surgical pathology. 27: 919-928.
18. Singh G, Saini A, Agrawal S, Rekhi KS. Pediatric Primary Central Nervous System Lymphoma.
19. Hayabuchi N, Shibamoto Y, Onizuka Y, Members JC (1999) Primary central nervous system lymphoma in Japan: a nationwide survey. Int J Radiat Oncol Biol Phys 44: 265-272.
20. Bataille B, Delwail V, Menet E, Vandermarcq P, Ingrand P, et al (2000) Primary intracerebral malignant lymphoma: report of 248 cases. Journal of neurosurgery. 92: 261-266.
21. Ferreri AJ, Reni M, Pasini F, Calderoni A, Tirelli U, et al (2002) A multicenter study of treatment of primary CNS lymphoma. Neurology. 58: 1513-1520.
22. Steer CB, Marx GM, Galani E, Harper PG, Khayat D (2002) Quality of life: it's never too late. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 20: 2915-2917.
23. Levin N, Soffer D, Grissaru S, Aizikovich N, Gomori JM, et al (2008) Primary T-cell CNS lymphoma presenting with leptomeningeal spread and neurolymphomatosis. Journal of neuro-oncology. 90: 77-83.
24. Ashrafi F, Zali A, Amiri M, Shabani F (2016) Clinical presentations of 176 cases of primary central nervous system lymphoma: a case series. Archives of Neuroscience. 3.
25. Johnson BA, Fram EK, Johnson PC, Jacobowitz R (1997) The variable MR appearance of primary lymphoma of the central nervous system: comparison with histopathologic features. American Journal of Neuroradiology. 18: 563-572.
26. Zhao H, Ma M, Zhang L, Zheng G, Lv H, et al (2019) Diagnosis of central nervous system lymphoma via cerebrospinal fluid cytology: a case report. BMC neurology. 19: 1-6.
27. Dulai MS, Park CY, Howell WD, Smyth LT, Desai M, et al (2008) CNS T-cell lymphoma: an under-recognized entity?. Acta neuropathologica. 115: 345-356.
28. Rubenstein JL, Gupta NK, Mannis GN, LaMarre AK, Treseler P (2013) How I treat CNS lymphomas. Blood, The Journal of the American Society of Hematology. 122: 2318-2330.