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Concomitant invasive mycoses and bacterial infection as causes of encephalitis

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Running Title: Concomitant invasive mycoses and bacterial encephalitis

Abstract

Invasive mycoses is fungal infection in the blood, normal sterile body fluids, deep tissue, and organ. Concomitant invasive mycoses and bacterial infection of the central nervous system are relatively rare. We describe a case of a 32-year-old female patient who presented seizure and decreased consciousness. Diagnosis of encephalitis was made base on the fulfillment of major (required) and minor criteria. From the clinical examination, there was no sign of neck stiffness. Laboratory results were suggested Cryptococcus sp based on cerebrospinal fluid analysis, and Candida tropicalis, and Streptococcus sp α-hemolytic group based on cerebrospinal fluid culture. Whereas CT-scan showed brain edema.

Keywords: Candida infection, Cryptococcus infection, encephalitis, invasive mycoses, Streptococcus infection

Introduction

Encephalitis is a brain inflammation that occurs due to an infection such as a virus or bacteria, medication, or immune system malfunction. Encephalitis is a rare, often serious condition that requires timely care [1]. Although pathologic examination and testing of brain tissue is considered to be the "gold standard" diagnostic test for this syndrome, encephalitis has previously been defined based on selected clinical, laboratory, electroencephalographic, and neuroimaging features [2].

There are >100 pathogens known to cause encephalitis [3]. The most common causes of encephalitis are viral and autoimmune infections. Infections from herpes, enteroviruses, human immunodeficiency virus (HIV), West Nile, and tick-borne viruses lead to viral encephalitis. This is the

most common cause. The immune system can mistakenly attack the brain, causing autoimmune encephalitis. On rare occasions, these germs can cause bacterial encephalitis [2]. Of the causes of infection, consecutively were tuberculosis (23%), HSV (14%), Varicella-Zoster Virus (VZV, 5%), Listeria sp (7%), and other causes (4%) [3].

Invasive mycoses is a fungal infection in the blood, normal sterile body fluids, deep tissue, and organ [4]. Central nervous system mycoses are relatively rare. With the increasing number of the immunocompromised or hospitalized patients with serious underlying diseases, they have become more common [4,5]. Central nervous system fungal infections present many diagnostic and therapeutic challenges and are associated with a high mortality rate [5].

Case report

Cerebrospinal fluid (CSF) specimen of 32 years old female patient, was sent to the laboratory for routine analysis and aerobic resistance culture. Previously, the patient was treated at the district hospital with a headache and vomiting, which suggested meningitis. It was no abnormalities on CT scan. To date, the patient has *idiopathic thrombocytopenic purpura* and being treated with methylprednisolone. The patient was treated with meropenem 1 g every 8 hours, methylprednisolone 125 mg every 8 hours, paracetamol 1 g every 8 hours, omeprazole 40 mg twice daily, and azathioprine 50 mg twice daily. Because of seizures and decreased consciousness, the patient was referred to our tertiary hospital.

Upon initial physical examination in our hospital, the following conditions were found: compos mentis (Glasgow Coma Scale E4M6V5), weight 60.3 kg, height 167 cm, body mass index 21.62 kg/m^2, blood pressure 154/82 mmHg, heart rate 72 x/minute, respiratory rate 18 x/minute, body temperature 36.8^0 C, and no sign of neck stiffness.

Her laboratory examinations were within normal limits. His hematology parameters showed hemoglobin 14.9 g/dL, leukocyte 26,900/uL, and thrombocyte 153,000/uL. Differential counts showed 1% stab neutrophils, 94% segmented neutrophils, 4% lymphocytes, and 1% monocytes. CD45+ lymphocyte 92/uL, CD3+ T cell 9/uL, and CD4+ T cell 3/uL.

Glucose, protein, cell count by type count, Gram stain, and culture were evaluated in CSF analysis. Glucose, protein, and chloride was remained normal, with elevated leukocyte count and a predominance of mononuclear cells in CSF. *Cryptococcus sp* was found in the India ink examination. The results of the CSF analysis were suggested *Cryptococcus sp* infection. The results of CSF analysis were detailed in Table 1.

Table 1. Cerebrospinal fluid (CSF) analysis

	Result	Unit	Reference range
Color	Clear		Clear
Clot	Negative		Negative
Leukocyte count	**16**	cell/uL	0 - 5
Polymorphonuclear cells	12	%	
Mononuclear cells	88	%	
India ink	***Cryptococcus sp* positive**		Negative
Nonne	Negative		Negative
Pandy	Negative		Negative
CSF protein	33	mg/dL	< 50
CSF glukosa	54	mg/dL	
Serum glukosa Serum	121	mg/dL	
Chloride	121	mmol/L	118 - 132

CSF: cerebrospinal fluid

The CSF was incubated on blood agar and yielded positive growth after 24 hours of aerobic incubation at 37^0 C. Two different forms of colonies were found on blood agar. One of the colonies was cream and no hemolysis around the colony, and the other has partial hemolysis around the colony and showed Gram-positive. Then, all isolates were examined by Vitex® 2 Compact (bioMérieux). Awaiting results of the antibiotic susceptibility, the patient was treated with antibiotic therapies of levofloxacin 750 mg once daily and fluconazole 600 mg twice daily. Methylprednisolone was continued to be given 4 mg twice daily, whereas paracetamol was given 500 mg every 8 hours.

On the 4^{th} day, the patient was screened for HIV, and the result was negative. The patient was also tested for *M. tuberculosis* using PCR, and the result was negative. CT scan was examined again and showed brain edema.

The isolates from CSF culture were identified as *Candida tropicalis* and *Streptococcus sp α-hemolytic group*. *Streptococcus sp α-hemolytic group* isolates were tested for antibiotics susceptibility. The antibiotics susceptibility results come out on the 5^{th} day. It was susceptible to ampicillin-sulbactam, amoxicillin clavulanic acid, vancomycin, and tigecycline. The result of cultures was detailed in Table 2.

Table 2. Culture and resistance test result

Specimen Isolate	CSF *Streptococcus sp α-hemolytic group*
Susceptibility	
Oxacillin	R
Penicillin G	R
Ampicillin	I
Chloramphenicol	R
Cotrimoxazole	I
Gentamicin	R
Erythromycin	R
Tetracycline	R
Amikacin	R
Sulbactam/Ampicillin	S
Lincomycin	R
Cephalotin	R
Cefotaxime	R
Amoxicillin Clavulanic Acid	S
Ceftriaxone	R
Ceftazidime	R
Ciprofloxacin	R
Piperacillin/Tazobactam	R
Cefoperazone/Sulbactam	I
Doripenem	R
Cefpirome	R
Vancomycin	S
Tigecycline	S
Teicoplanin	I
Meropenem	R
Imipenem	R
Levofloxacin	I
Moxifloxacin	I

CSF: cerebrospinal fluid; R: resistant; I: intermediet; S: sensitive

As sensitivity results come out, antibiotic therapies were changed according to the results. The patient has treated with ampicillin sulbactam 2 g every six hours and fluconazole 600 mg twice daily. Methylprednisolone was continued to be given 8 mg twice daily, whereas paracetamol was given 500 mg every 8 hours and mannitol 75 mL was given every 6 hours.

Discussion

Encephalitis has previously been defined based on selected clinical, laboratory, electroencephalographic, and neuroimaging features [2]. The encephalitis diagnosis is made base on the fulfillment of major (required) and minor criteria. Major criteria are presenting to medical attention with altered mental status (defined as decreased or altered level of consciousness, lethargy, or personality change) lasting ≥24 hours with no alternative cause identified. Minor criteria require 2 criteria for possible encephalitis or ≥3 criteria for probable or confirmed encephalitis: 1) Documented fever >38°C (100.4°F) within the 72 hours before or after presentation; 2) Generalized or partial seizures not fully attributable to a preexisting seizure disorder; 3) New onset of focal neurologic findings; 4) CSF leukocyte count >5/uL; 5) Abnormality of brain parenchyma on neuroimaging suggestive of encephalitis that is either new from prior studies or appears acute in onset; 6) Abnormality on EEG that is consistent with encephalitis and not attributable to another cause [2]. From the patient's history, the disease began with headache and vomiting, worsening with seizures and decreased consciousness. From CSF analysis was found an increase of the cell count in and from CT scan was found brain edema. The patient met the major criteria of encephalitis which was

decreased consciousness, and minor criteria of encephalitis which was seizures, pleiocytosis of brain fluid 16 cells/uL, and brain edema on CT scan.

Consensus group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) published standard definitions for invasive fungal infections. The definitions assigned 3 levels of probability to the diagnosis of invasive fungal infection that develops in immunocompromised patients, namely, "proven," "probable," and "possible" invasive fungal infection [6]. Invasive mycoses in our patient was caused by *Cryptococcus sp* and *Candida tropicalis*. *Cryptococcus sp* was found in CSF analysis with Indian ink staining, while *Candida tropicalis* was found in culture, according to proven criteria for opportunistic mycoses. Clinical symptoms that support *Cryptococcus sp* infection in patients are headache without meningismus, while candidiasis symptoms are found in accordance with encephalitis. The possibility of tuberculosis as one of the most common causes of encephalitis can be ruled out because the results of the PCR examination did not detect *Mycobacterium* tuberculosis and non-tuberculosis *Mycobacterium*.

Predisposition for *Cryptococcus sp* infection, which was found in the patient was a history of using methylprednisolone and a decrease in the number of T cells. Long-term steroid use (at least 0.3 mg/kg/day of prednisone or equivalent) for >3 weeks was a probable factor for opportunistic mycoses [6]. Together with the use of meropenem and vascular catheters, a history of using methylprednisolone and decreased T cell counts also predisposes to *Candida tropicalis* infection. The use of meropenem will facilitate fungal colonization and intravascular access, use of methylprednisolone caused immunosuppression, whereas vascular catheters caused direct access to blood vessels [2]. For the occurrence of invasive mycoses, the patient is a high-risk patient due to immunosuppression, and immunodeficiency due to the use of corticosteroid therapy for ITP.

Neutrophilia based on the hematological examination may be caused by encephalitis. In the presence of infection, the ability of neutrophils to adhere to the vascular endothelium decreases. As a result of the release of neutrophils from the marginal pool into the circulation, neutrophilia occurs. The hypothalamic response to inflammation also increases the number of circulating leukocytes. The number of lymphocytes decreased in both CD3+ (9 cells/uL) and CD4+ (3 cells/uL) T cells. This may be due to the use of corticosteroid therapy for ITP. During hospitalization before being referred, the patient was given azathioprine which has the effect of reducing the number of lymphocytes. This may exacerbate the patient's lymphocytopenia. HIV infection as the most common cause of lymphocytopenia can be ruled out because of the non-reactive HIV test result. However, due to a decrease in the number of lymphocytes, HIV antibodies might have been no longer produced in high enough levels to be detectable in an HIV antibody test, so the result of the HIV test was non-reactive.

Fluconazole as antifungal therapy was given as definitive therapy for *Cryptococcus sp*. The first line for severe cryptococcosis in the central nervous system is a combination of amphotericin B and flucytosine. Following the antifungal management guidelines for the combination of cryptococcal meningitis and invasive candidiasis, the patient was given a combination of amphotericin B and flucytosine. Whereas for *Streptococcus sp α-hemolytic group* infection, before the antibiotics susceptibility results come out, the patient was treated with levofloxacin. If there is no meningeal inflammation, levofloxacin has better penetration into the cerebrospinal fluid than other β-lactam

antibiotics. Ampicillin Sulbactam has been given for the management of the patient since the result of the susceptibility test come out.

Conclusion

A 32 years old woman, with encephalitis was caused by *Cryptococcus sp, Streptococcus sp α-hemolytic group,* and *Candida tropicalis*. The diagnosis of encephalitis due to *Cryptococcus sp* was made based on the Indian ink staining on the cerebrospinal fluid analysis, while the diagnosis of encephalitis due to *Streptococcus sp α-hemolytic group* and *Candida tropicalis* was made based on the culture results. The predisposition for *Cryptococcus sp* infection was the history of using methylprednisolone and decreased T cell counts. The use of meropenem, vascular catheters, methylprednisolone, and decreased T cell counts also predisposed to the occurrence of *Candida tropicalis* infection. Patients are at high risk because of immunosuppression, and immunodeficiency due to the use of corticosteroid therapy for ITP. The treatment given for invasive mycoses with a combination of *Cryptococcus sp* and *Candida tropicalis* is amphotericin B and flucytosine. Based on antibiotics susceptibility results, the *Streptococcus sp α-hemolytic group* was sensitive to ampicillin-sulbactam, amoxicillin-clavulanic acid, vancomycin, and tigecycline.

References

1. Enchephalitis. https://my.clevelandclinic.org/health/diseases/6058-encephalitis. Accessed on June 2, 2021.
2. Venkatesan A, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, et al (2013) International encephalitis consortium case definitions, diagnostic algorithms, and priorities in encephalitis: Consensus statement of the International Encephalitis Consortium. Clin Infect Dis 57(8):1114–1128.
3. Khan M, Piquet AL (2017) Differential diagnosis of meningitis and encephalitis. MJH life sci 10. https://www.neurologylive.com/view/differential-diagnosis-meningitis-and-encephalitis.
4. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev in Microbiol. 2010;36(1):1-53.
5. Rodriguez T (2018) Fungal infections of the CNS: Diagnostic and treatment approaches. https://www.neurologyadvisor.com/topics/general-neurology/fungal-infections-of-the-cns-diagnostic-and-treatment-approaches
6. Pauw BD, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al (2008) Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/ invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) consensus group. Clin Infect Dis 46:1813–1821

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