Brain Abscess with Anaerobic Gram-Negative Bacilli: Case Series

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Abstract:

Brain abscess is usually of polymicrobial etiology involving aerobic and obligate anaerobic bacteria. Anaerobic bacteria are mainly seen in brain abscesses secondary to otogenic and odontogenic source. Investigating for anaerobic etiology is usually neglected considering the tedious and time consuming process of anaerobic culture. It is necessary to perform anaerobic cultures along with routine aerobic cultures as a part of microbiological workup of abscess aspirates in patients with brain abscess which aid in providing appropriate antibiotic therapy. To highlight the significance of anaerobic cultures, we report four cases of brain abscess with anaerobic etiology from a tertiary care hospital in coastal Karnataka.

Key words: Anaerobic Bacteria, Bacteroides fragilis, Brain Abscess, Fusobacterium nucleatum, Polymicrobial.

Introduction

Brain abscess is a localized collection of pus within the brain parenchyma [1]. These life threatening neurosurgical emergencies have an estimated incidence of 1-2% in developed countries; however in developing countries they continue to be a significant health problem with an incidence of 8% [1-3].

Brain abscess is usually of polymicrobial etiology involving aerobic and obligate anaerobic bacteria [2]. The most common source for intracranial abscess include paranasal sinus, dental and middle ear infections. Penetrating brain injury and hematogenous seeding from extra-cranial sites are other sources for intracranial abscesses [4]. Improvement in the microbiological culture methods for anaerobes, neuro-radiological imaging procedures and modern neurosurgical techniques has improved the outcome of brain abscess. Identifying the etiological agent followed by targeted therapy is essential for achieving success in treating these patients [1].

Considering that anaerobes are among the important pathogens causing brain abscess but often neglected, we report our experience with three cases of brain abscess involving anaerobic bacteria. The microbiological work-up included processing the intra-operatively collected pus aspirate for aerobic and anaerobic bacterial; and fungal pathogens

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by microscopy and culture following standard techniques [5-6]. Aerobic culture was done on 5% sheep blood agar and MacConkey agar and the plates were incubated at 37^0C for 24-48 hours. For isolation of anaerobic bacteria, pus was inoculated on to 5% sheep blood agar, neomycin blood agar, and phenyl ethyl alcohol agar with metronidazole (5U, BD) disc and plates were incubated at 37^0C for 5-7 days in anaerobic Gaspak jar (BD) or anaerobic workstation (Whitley A35 Anaerobic workstation, Don Whitley Scientific, Shipley, UK). Identification of anaerobic bacteria was done by gram stain, aerotolerance, fluorescence under ultraviolet light (365 nm), antibiotic identification discs, biochemical tests; and Vitek 2 automated system (bioMerieux Inc.) for species identification. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method for aerobic bacteria and by E-test for anaerobic bacteria (B. fragilis ATCC 25285 was used for quality control) and interpreted following Clinical Laboratory Standards Institute (CLSI) guidelines [7,8].

Case Reports

Case 1

A 10 year old female child presented to the neurosurgery clinic with complaints of headache and dizziness of one month duration. The headache was localized to frontal area with moderate severity with an increased frequency in the previous 15 days and was also associated with on & off vomiting for 2-3 days. She had left ear discharge of six months duration. There was no history of fever; trauma; weakness of limbs; visual abnormalities or bowel and bladder disturbances. On admission, the child was conscious, oriented to time and place and was obeying oral commands. Glasgow Coma Scale score was 15/15. The ophthalmic fundus examination showed bilateral early papilledema. Her other systemic examination was unremarkable.

At admission, hemoglobin was 11.4 g/dL; total leukocyte count: 14.2×10^3/ µL and erythrocyte sedimentation rate (ESR): 36 mm/hr. The patient was started on empiric ceftriaxone, gentamicin and tinidazole. CT scan of brain showed left temporal ring enhancing well defined hypodense lesion and midline shift to right side with mass effect. Basal cisterns were partially effaced on the left side. A diagnosis of left temporal lobe abscess secondary to otogenic etiology was made. Her course in the hospital was complicated by right upper motor neuron facial palsy with right hemiparesis for which emergency left temporal craniotomy and evacuation of brain abscess was done. Gram stain of the pus showed plenty of gram negative bacilli and gram positive cocci in small clusters. Staining for acid fast bacilli (AFB) was negative. *K. oxytoca* and *E. avium* were grown in aerobic culture and were found susceptible to commonly tested antimicrobials. Anaerobic culture showed the growth of *Bacteroides fragilis* subsp. *vulgatus* which was susceptible to metronidazole with a MIC of 0.25 µg/mL. Antibiotics were changed to crystalline penicillin and chloramphenicol. No fungal growth was observed after 10 days of incubation on Sabouraud's dextrose agar. At discharge, she was put on oral amoxicillin and chloramphenicol. The patient recovered well with no focal neurological deficits. For the ear discharge, a diagnosis of left chronic suppurative otitis media (CSOM), attico-antral disease was made for which she underwent left modified radical mastoidectomy and conchomeatoplasty at a later date.

Case 2

A 18 year old female presented with persistent right sided mucopurulent ear discharge of 4 years duration. She complained of hearing deficit in the right ear for one year and intermittent earache of 6 months duration. She had tinnitus and loss of balance while walking. There was no history of headache, vomiting, neck stiffness or seizure. At

the time of admission, she was conscious and alert. On otoscopic examination, central perforation was observed on right tympanic membrane. Laboratory parameters showed hemoglobin of 10.8 g/dL and total leukocytes count of 13.9 x10^3/µL with 84.8% neutrophils with mild left shift and toxic changes. She was started empirically on crystalline penicillin and metronidazole. CT head showed a well-defined intra-axial lesion measuring 40x36x22 mm with thin enhancing peripheral rim and multiple intra lesional loculi with air fluid levels. The report showed features of right cerebellar abscess secondary to chronic right oto-mastoiditis with intracranial extension. Right sub-occipital retro mastoid craniotomy and evacuation of abscess was performed. The gram stain from intra-operatively collected pus showed numerous pus cells but no bacteria. Staining for AFB was negative. No aerobic bacterial pathogens were isolated. *Bacteroides fragilis* subsp. *fragilis* was grown in anaerobic culture which was susceptible to metronidazole with an MIC of 0.75 µg/mL. Culture of ear swab showed the growth of coagulase negative *Staphylococcus* spp. suggestive to be a colonizing flora. Findings of histopathogical examination of the grey white tissue bits were consistent with cerebellar abscess. Post-operatively she was put on chloramphenicol and metronidazole. Repeat HRCT (high resolution CT scan) showed right CSOM with cholesteatoma destroying posterior wall of mastoid cavity and tegmen tympani with intracranial extension and extensive destruction of ossicles. Right modified radical mastoidectomy was performed for cholesteatoma. Four days after mastoidectomy, repeat CT scan showed regression of the size of right cerebellar abscess. Patient showed clinical improvement and was discharged with oral metronidazole and oral chloramphenicol. The patient was lost for follow-up.

Case 3

A 30 year old male was admitted with complaints of headache and drowsiness of six weeks duration. He had fever and weakness of left upper and lower limbs, gradually progressive in the previous one month. There was no history of vomiting, ear discharge, neck rigidity, seizure disorder, sinusitis or any external injuries. On examination he was conscious, oriented to time and place, afebrile with Glasgow coma scale of 11/15. On motor examination there was left lateral rectus palsy with left hemiparesis; with decreased tone in left lower limb power (2/5).

At admission, his hemoglobin was 15.7 g/dL, total leukocytes count: 12x10^3/µL with neutrophils: 76.7% and ESR: 8 mm/hr. Magnetic resonance scan of brain (Gd-DTPA enhanced) showed lesion in the right frontal lobe measuring 4.4x5.0x4.9 cm with smooth peripheral enhancement and midline shift, with subfalcine and uncal herniation. Features were suggestive of right frontal lobe abscess. Frontal burr hole and evacuation of abscess was done. Thick brown pus of approximately 50 mL was drained. On microscopy, gram stain showed numerous pus cells but no organisms. Aerobic culture grew *Streptococcus* spp. Anaerobic culture showed the growth of *Fusobacterium nucleatum* and *Parvimonas micra*. Both the anaerobic isolates were susceptible to metronidazole with an MIC of 0.064 µg/mL. Blood cultures on automated blood culture system (Bact/Alert, Biomerieux Inc.) were sterile. During his hospital stay, he was treated with vancomycin, metronidazole and amikacin. Repeat CT scan before discharge showed regression in the size of the abscess and reduction in mass effect of the lesion. The patient improved clinically during his stay in the hospital and was discharged on request. The patient was lost to follow-up.

Case 4

A 72 year old male presented to the neurosurgery clinic with complaints of high grade fever with chills of three weeks duration and severe headache for 15 days. He also complained of projectile, non-

bilious vomiting of 3-4 episodes per day for 10 days with increased frequency in mornings. There was no history of seizures, head trauma or sensory symptoms/motor deficits. On admission, he was conscious, disoriented to time and place but was obeying oral commands. Glasgow coma scale score was 13/15. On motor examination, tone was normal with at least 3/5 power in all 4 limbs.

At admission, hemoglobin was 11.0 g/dL, total leukocyte count: $8.3 \times 10^3/\mu L$ with 65.5% neutrophils. The patient was started on empiric amikacin and metronidazole. CT scan of brain showed three well defined hypodense lesions in left frontal and basifrontal lobe, largest measuring 3.3x3.2x3.9 cm in left frontal lobe with significant perilesional edema predominantly involving white matter of left frontal and temporal lobe. A diagnosis of left frontal abscess was made, for which he underwent left frontal craniotomy with evacuation of abscess. During the procedure around 35 ml of foul smelling purulent pus was aspirated. Gram stain of the pus showed plenty of pus cells with gram negative coccobacilli. Staining for AFB was negative and aerobic cultures showed no growth of pathogenic bacteria. While, anaerobic culture showed the growth of *Prevotella buccae* and *Fusobacterium nucleatum* which were susceptible to metronidazole with an MIC of 2 µg/mL & 0.094 µg/mL respectively. Post-operatively, he developed gradual dysphasia and weakness of right upper and lower limbs which showed improvement with steroids. Amikacin and metronidazole were continued for three weeks. He was discharged with stable condition, and was put on oral ciprofloxacin and metronidazole.

Discussion

Brain abscess which is considered as a microbiological and neurosurgical emergency is a serious and life threatening infection and can be a diagnostic challenge [9]. Following infection with pyogenic bacteria, localized areas of inflammatory changes in brain parenchyma referred to as cerebritis are seen initially which progress to a suppurative lesion encapsulated in a vascularized membrane [10]. Brain abscess accounts for a significant number of patients in the neurosurgical clinic. V Lakshmi *et al*. have reported an incidence of 25% among patients with space occupying lesions [9]. At our tertiary care center, 47 cases of brain abscess are recorded between 2012-15, with an average of 12 cases per year.

A male predominance for brain abscess has been reported in earlier literature [1-3,9,11-14]. Brain abscess is reported across all age groups from infancy to elderly. However, the mean age of patients affected by brain abscess as reported in the earlier studies shows a significant variation [1-3,9,11-14]. The signs and symptoms at presentation depend on the number, site and size of abscess, the etiological agent and underlying medical/surgical condition [4]. A high index of clinical suspicion is needed as a large majority of patients present with non-specific signs and symptoms [11]. The common presentations include headache, altered mental status, focal neurological findings (hemiparesis, hemisensory deficits, aphasia, ataxia etc.), nausea, vomiting, and fever. Seizures are less commonly noted. The classic triad of fever, headache and focal neurological deficits is seen in a minority of patients with brain abscess [1,4,11].

The most common sources for intracranial abscess are foci in contiguous structures of paranasal sinuses, middle ear and teeth [4]. Other routes of infection include the hematogenous spread following endocarditis, intra-abdominal infections, urinary tract infections and pulmonary infections like pneumonia, empyema and abscess [4,11]. However, cryptic abscesses with no obvious source of infection can be seen in 20-30% cases [4], also noted in two of our patients. Knowing the potential source of infection and other risk factors will help

in determining the probable microflora causing the abscess and to initiate empirical therapy.

The common microbial etiological agents of brain abscess include *S. aureus, Streptococcus* spp., enterobacteriaceae and obligate anaerobic bacteria like *Bacteroides* spp., *Clostridium* spp., *Peptostreptococcus* spp. and *Propionibacterium acnes* [11,15]. A large number of other organisms including mycobacteria, fungi and protozoan parasites have been reported to cause brain abscess [3,9-11,15]. Infections with new or emerging pathogens and drug resistant bacterial strains are a cause for concern [9,10]. Monomicrobial infection and aerobic bacterial flora are the frequent findings in brain abscesses. Polymicrobial isolation is seen in up to 23% cases. Three of our patients in this case series had infection with polymicrobial flora. The microbial flora of brain abscess is influenced by geographic area, age, sex, immune status, anatomic source of infection and site of abscess [4,14].

The neuro-imaging techniques including, computed tomography (CT) and magnetic resonance imaging (MRI) help in detection and characterization of the abscesses [1]. Diffusion weighted MRI, proton magnetic resonance spectroscopy (MRS) help in differentiating abscess from malignancy. Serial weekly scans also help in assessing the response to therapy and aid in management of individual cases [4,11]. Leukocytosis and elevated ESR may be seen. Blood cultures are recommended, but have low yield (30%) [15]. Cerebrospinal fluid (CSF) analysis may reveal elevated cell counts and protein, but may be normal in significant proportion of patients [4,11]. Etiological diagnosis by microbiological examination of abscess material obtained during neurosurgical drainage helps in providing targeted therapy. The number of organisms in the specimen (10^3 CFU/mL) determines the sensitivity of staining techniques [9]. V Lakshmi *et al*. [9] and Menon S *et al*. [3] have reported smear microscopy positivity rate of 82% (n=41) and 67.3% (n=51) respectively. In our series, gram stain revealed the presence of bacteria in two cases. Use of automated culture systems are reported to enhance the yield of organisms from pus specimens. Inoculation of aspirated pus, soon after collection in to anaerobic bottles of automated blood culture systems will improve the recovery of both obligate anaerobes and facultative anaerobes [9]. Among anaerobes, literature shows *B. fragilis* and *Peptostreptococcus* spp. as the commonly isolated anaerobes from brain abscesses [3,9,11,14]. *B. fragilis* group and *F. nucleatum* were isolated from two cases each in this series. Menon S *et al*. [3] have earlier isolated anaerobes as monomicrobial flora in 12 (16%), polymicrobial flora in 1 (1.33%) and mixed with aerobic bacteria in 3 (4%) patients. V Lakshmi *et al*. have documented an increasing occurrence of polymicrobial anaerobic abscesses [9]. In two of our patients, anaerobes were isolated along with aerobic bacteria. Hseih MJ *et al*. [16] found *Fusobacterium* spp. in 6% (7/98) of monomicrobial bacterial brain abscess and in 17% (4/24) of polymicrobial infections. However, initiation of empirical antibiotic therapy before specimen collection can lead to sterile growth in cultures [11].

Anaerobic bacteria are mainly seen in abscesses secondary to otogenic and odontogenic source, also noted in two of our patients who had CSOM. Anaerobes are also isolated following metastatic spread from lung abscess (*Actinomyces* spp, *Fusobacterium* spp.) and intra-abdominal infections; and following penetrating trauma (*Clostridium* spp.) [15]. Anaerobic bacterial pathogens have been isolated from 13.7% to 17% of patients with brain abscess [2,3,9,13,14]. However, limitations in culturing anaerobic bacteria can influence the pathogen prevalence in studies on brain abscess [14].

With the availability of metronidazole and the tedious, time consuming process of anaerobic culture, investigating for anaerobic etiology is

usually neglected. Of the limited number samples received for anaerobic culture (n=21), significant anaerobic growth was observed in four patients with brain abscess. Use of anaerobic transport techniques, timely inoculation in to appropriate media and incubation in gaspak system or anaerobic workstation can increase the yield of anaerobic bacteria. Also, techniques like gas liquid chromatography for identification of anaerobes directly from specimen provide rapid identification [4,9].

Empirical antibiotic coverage should be provided based on presumptive source of infection and the pus microscopy results, which provide a clue about the probable etiological agent [4]. The antibiotics are chosen based on their ability to penetrate the blood brain barrier and abscess cavity; bactericidal nature; and activity against the suspected agent [4,15,17]. Surgical options include needle aspiration and evacuation depending on the location and type of abscess. cefotaxime/ceftriaxone/ceftazidime, vancomycin and metronidazole are the empirical options which have replaced the traditional choices of penicillin G and chloramphenicol [1]. Definitive therapy is based on the results of culture and susceptibility test results.

The wide spectrum of action against many strict anaerobes, good oral absorption, excellent penetration into CSF and brain abscess and bactericidal activity have made metronidazole the drug of choice for abscesses with suspected anaerobic etiology [4]. However, considering its toxicity profile, reports of metronidazole resistance in anaerobic bacterial isolates and intrinsic resistance seen towards metronidazole among gram-positive anaerobic bacteria, it is necessary to perform both aerobic and anaerobic cultures which will help in initiating appropriate antibiotic therapy.

Table 1: Clinical profile of patients with anaerobic brain abscess

Patient	Age/ Sex	Underlying condition	Clinical manifestation	Leukocyte count	Location of abscess	Organisms isolated	Metronidazole (S/R), MIC	Surgical treatment	Outcome
1	10/F	Lt. chronic suppurative otitis Media	Headache, dizziness, Lt. ear discharge	$14.2 \times 10^3/\mu L$	Temporal	*K .oxytoca* *E. avium*		Lt. temporal craniotomy & evacuation of abscess	Improved
						B. vulgatus	S, 0.25 µg/mL		
2	18/F	Rt. oto - mastoiditis	Rt. Ear discharge, Rt. hearing deficit & tinnitus	$13.9 \times 10^3/\mu L$	Cerebellar	*B. fragilis*	S, 0.75 µg/mL	Rt. sub occipital retro mastoid craniotomy & evacuation of abscess	Improved
3	30/M	-	Headache and drowsiness; fever and weakness of left upper and lower limbs	$12 \times 10^3/\mu L$	Frontal	*F. nucleatum*	S, 0.064 µg/mL	Frontal burr hole and evacuation of abscess	Improved
						P. micros	S, 0.064 µg/mL		
						Streptococcus sp.			
4	72/M	-	Fever, headache & projectile vomiting	$11 \times 10^3/\mu L$	Frontal	*P. buccae*	S, 2 µg/mL	Lt. frontal craniotomy and evacuation of abscess	Improved
						F. nucleatum	S, 0.094 µg/mL		

MIC: Minimum inhibitory concentration; S: susceptible; µg/mL: Microgram per milliliters

It is recommended that parenteral antibiotics are given for a prolonged duration of 6 to 8 weeks followed by 2 to 3 month course of oral antibiotics [15]. The duration of therapy depends on the patient's response to therapy based on clinical improvement and serial radio-imaging studies [1]. Even though the outcome of treated brain abscess has improved considerably over the years, mortality rates vary between 0-30%. Neurologic sequelae including hemiparesis, seizures and cognitive decline are seen in 30-56% patients [15]. With prompt management, all four cases of brain abscess with anaerobic etiology recovered and no mortality was noted in our case series. Table 1 shows the clinical details of all the four cases of brain abscess with anaerobic etiology.

Conclusion

A combination of high index of clinical suspicion followed by appropriate neuro-radiological and microbiological investigations and therapeutic interventions will help in better management of patients with brain abscess. Anaerobic cultures should be performed as part of microbiological workup of specimens from patients with brain abscess.

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