Multimodal Diagnostic Approach to Brain Abscess
N Husain, S Sharma, R Verma, N Shukla, R Gupta, K Prasad, M Husain

Citation

Abstract
A tertiary hospital based study comprising of a case series of 25 patients was carried out to evaluate various diagnostic modalities and arrive at a sensitive algorithm for diagnosis of brain abscess. Diagnostic efficacy of MRI scan, MR spectroscopy, PCR for tuberculosis & histological evaluation was assessed against a gold standard of Microbial diagnosis. The study group included 16 cases of pyogenic, 4 tubercular, 4 fungal and one actinomycotic abscess. Additionally, microvessel density and thickness of abscess wall was assessed by histo-morphometry. MRI was diagnostic in 92 % of the cases. MR spectroscopy revealed, lactate, lipids and amino acid metabolites all cases. Acetate and succinate indicated anaerobic etiology. Histological demonstration of microbes was possible in 6/16 cases of pyogenic abscesses, 3/4 cases of tubercular abscesses, in all 4/4 cases of fungal abscess and the 1/1 case had actinomycosis. M. tuberculosis was detected by PCR in all 4 cases of tubercular abscess. Morphometric analysis of the abscess wall showed prominent zone of inflammation in tubercular abscesses, which was significantly wider than in pyogenic abscesses (t=3.987, p= <0.001). This correlated well with the zone of enhancement inT1.weighted images in MR scans. Extent of microvessel proliferation in both groups was the same. Early diagnosis and accurate localization of brain abscess is possible by a combination of MRI & MR spectroscopy. Rapid diagnosis of tuberculous brain abscess can be achieved by PCR allowing initiation of therapy in the immediate postoperative period preventing fulminant infection.

INTRODUCTION
In the past two decades, technologic advancements have facilitated the diagnosis and management of brain abscess. Multiple diagnostic modalities are now available. Early diagnosis of brain abscess and accurate localization by CT and MRI has resulted in significant reduction in mortality. Added to this are the improvements in isolation techniques that have made rapid identification of causative organisms possible, hence increasing cure rates and reducing morbidity significantly.

In the current study we have attempted to assess the efficacy of diagnostic modalities including MRI scan, MR spectroscopy, demonstration of microbial pathogen in pus smears & histological specimen, and PCR for Mycobacterium tuberculosis in the diagnosis of brain abscess. Further morphometric analysis of the abscess wall in terms of the thickness of capsule, inflammatory reaction and microvessel density assessment was done to define variations, if any, between the pyogenic, tubercular and fungal infections and relate them to the MRI appearance.

MATERIALS AND METHODS
A prospective tertiary hospital based study, conducted in a case series with brain abscesses undergoing surgical therapy was done. Cases, which could be categorized on etiological basis by microbial diagnosis, were included. The study group (n=25) comprised of 16 pyogenic (12 aerobic, 4 anaerobic), 4 tubercular, 4 fungal (2 aspergillus and 2 candida) and 1 actinomycotic abscesses.

Microbial examination: Pus was collected at operation in thioglycollate medium with B-lactamase (Whatman,USA ), in sterile screw capped bottles and transported immediately for culture to the laboratory along with the smears prepared simultaneously. Grams, Ziehl Neelson (ZN) and Gomori’s Methenamine Silver (GMS) stained smears were examined for various organisms. The pus was inoculated without delay in 5% sheep blood agar incubated aerobically and anaerobically; 5% sheep blood agar with .01% neomycin; incubated anaerobically; in chocolate agar incubated in 10% CO2; McConkey medium and Lowenstein Jenson medium and Sabouraud's dextrose agar.

Clinical Assessment: included age, sex, presenting symptoms, signs, general condition and predisposing factors.

Radiological assessment included MR Imaging:
MR spectroscopy: Ex-vivo MRS studies involved freezing one portion of exudates with in 5 min of surgical excision. 0.4g of frozen exudate was thawed and directly placed in 5mm nuclear MR tube with 100µl phosphate buffered saline (pH 7) and a capillary containing 100mM 3-trimethylsilyl-2,2,3,3-tetradeuterosodium propionate (TSP) in H2O as a reference for chemical shift and concentration. One dimensional Hahn-spin-echo spectra, modified to saturate the water resonance, were recorded without spinning the sample and the parameters acquired were Total TR 10 seconds, TE 135 msec, Data size 4K, Spectral width 4808 Hz, 64 acquisitions, water presaturation power of 0.2 mW during 1 second. Data were filled zero to 8 K and exponential multiplication (LB=0.2Hz) was applied before Fourier transformation and manual phasing.

Histopathological evaluation: Paraffin embedded sections was done using Hematoxylin and eosin stain and special stains including Gram's, Ziehl Neelson, and Gomori's Methanamine Silver (GMS) stains.

Morphometric analysis: Quantification of angiogenesis was done in sections of the abscess wall stained immunohistochemically using antibody to CD 34 antigen (Dakopatts, Denmark). Streptavidin-biotin method for detection (LSAB2 kit, Dakopatts Denmark) was used for detection. Fields showing highest vascularity (i.e. hot spots) were chosen to determine the microvessel density excluding vessels with muscular wall. In each case 10 microscopic fields were photographed with a Canon digital camera mounted on Axiolab microscope (Zeiss, Germany) at 400 X magnification Morphometric analysis included thickness measurements of the capsule (fibrosis and gliosis) & inflammatory reaction in 10 random areas of the wall calculated in digitalized photographs automatically using Biovis Image Plus Software (Expert Vision, Mumbai). Microvessel density (number of vessels/high power field) and differential count of the inflammatory infiltrate was assessed manually in 10 random fields.

PCR for Mycobacterium tuberculosis: DNA from fresh and paraffin embedded tissue was extracted. 3 x 20 um-thick sections from paraffin blocks were de-paraffinized with xylene and cleaned with ethanol. The ethanol was evaporated and tissue pellet resuspended in ATL buffer (QIAamp DNA Mini Kit (Qiagen, Germany). For fresh tissue 25 mg of biopsy material was frozen in liquid nitrogen, crushed and suspended in ATL buffer. DNA was extracted in spin columns by the kit protocol. The amplification reaction was performed in a final volume of 50 µg and the reaction mixture consisted of 10mM Tris HCL (pH9.0) 10X, 1.5mM MgCl2, 50mM KCl, 0.01%gelatin, 50 µlTaq polymerase buffer (supplied with enzyme), 12.5 µl of 10mM dNTP's, 3.3l units of Taq polymerase, 5 µg lyophilized primers, 5 µl of sample DNA extracted from the clinical specimens. The oligonucleotide primers used were T4 (5’CCT GCG AGC GTA GGC GTC GG-3’) and TS (5’CTC GTC CAG CGC CGC TIC TG-3’) (Bangalore Genei, India). These primers amplified a target fragment of 123 base pairs (bp) from the insertion-like M. tuberculosis sequence element IS6110. The amplification was done in a Techne Progene, Syngene, UK DNA thermal cycler with an initial denaturation at 94°C for 7min, followed by a 3 step profile of denaturation at 94°C for 2 min, annealing at 68°C for 2min and extension at 72°C for 2 min, for a total of 35 cycles followed by final cycle of 72°C for 10 min. Each reaction set was accompanied by positive and negative controls. The products were separated by electrophoresis on 2% agarose gel in TAE buffer, stained with ethidium bromide and sized with a gel documentation system [13].

Statistical analysis: Mean of morphometric parameters, p values, sensitivity and specificity of the various diagnostic modules using microbial diagnosis as gold standard. 

RESULTS

Clinical Assessment: Brain abscess was most common in young adults. The age ranged between 2 to 50 years with 60 % of cases falling between 11 to 30 years of age. The mean age of the cases was 19.8 ±11.9 years. Male to female ratio was 3:2. The abscesses were more frequently located in the posterior fossa (32%), followed by tempo-parietal location (20%). In three cases of pyogenic, two of tubercular, one of fungal, multiple abscesses were present and one case of fungal abscess showed multiloculation. Headache was the most common symptom (n=24) accompanied by fever, nausea and vomiting (n=23). Detailed clinical examination of the cases revealed papilledema (n= 15), altered sensorium (n=12) and cerebellar signs (n=7) as the most common signs of brain abscess. Limb weakness and cranial nerve palsies were evident in six cases. Sensory disturbances were not found in any of the cases. Head injury (n=10) and CSOM (n=8) were the commonest predisposing factors; cyanotic heart disease was present in 2 cases. In five cases no predisposing factors were evident. None of our cases were HIV-positive. The solitary case of actinomycosis presented initially with multiple abscesses in the brain. He responded partially to therapy after surgery and diagnosis. The abscesses recurred after 6 months in the brain with a sinus
extending to the scalp.

Radiological Examination: CT scan revealed that in 50% of the cases abscess size ranged between 2.5-5cms. 75% cases showed capsular enhancement (64% regular, 12% irregular and only 12% loculated) and peri-lesional edema. The edema was severe in 6 cases where it was seen involving more than half of the hemisphere and or extending to the opposite side. Mass effect was moderate to severe in majority of the cases (defined by midline shift and bilateral diffuse effacement of sulci; tentorial herniation and hydrocephalus respectively).

MR imaging was done in 17 cases. In T1 weighted images showed a central zone of hypointensity surrounded by a thin rim of isointense to hyperintense tissue and an outer zone of hyperintensity, while T2 weighted images showed hypointense center, well-defined hypointense capsule and hyperintense surrounding edema.

Microbial Studies: Microbial cultures showed 16 cases of pyogenic abscess. Aerobic bacteria were found in 12 cases. These included Streptococcus in 4, Staphylococcus aureus in 4, Escherichia coli in 2, Enterococcus in 1 & Proteus species in 1. In four cases anaerobic bacteria were isolated including Streptococcus in 1 and Bacteroides in 1. In two cases mixed anaerobes viz. Peptostreptococcus and Streptococcus in one and Bacteroides and Streptococcus in another were seen. In grams stain of direct pus smears organisms could be seen in only in 8 of 16 cases. Four cases had tubercular abscesses. Three of these cases could be diagnosed in Ziehl Neelson stained smears. Lymphocytes along with plasma cells and histiocytes were common in tubercular abscess while the fungal abscesses showed predominantly neutrophils. The thickness of zone of fibrosis was greater in pyogenic abscess (223.72µ ± 61.253). There was no significant difference in the thickness of the abscess wall in aerobic (12) vs. anaerobic (4) abscesses. The zone of inflammation was greatest in cases with a tubercular etiology (224.87µ+±49.809). The extent of neovascularization was similar in all the cases (Table 1). Vasculitis was prominent in the fungal abscess.

H1MR Spectroscopy was performed in all cases of pyogenic and tubercular abscesses. Pyogenic abscesses showed lipid and lactate levels of 1.3 ppm and aminoacids mostly leucine, isoleucine and valine, in addition succinate 2.41 ppm (n=6), acetate 1.92 ppm (n=8), alanine 1.48 ppm (n=9) and glycine 3.56 ppm (n=12) was seen. Tubercular abscesses showed lipid & lactate (n= 3) with or without glycine and alanine (n=1). In three of four patients we observed only lipids and lactate in MR spectra. There was no evidence of amino acids at 0.9 ppm.

The sensitivity and specificity of PCR for Mycobacterium tuberculosis in fresh tissue and pus was 100% in tubercular abscesses, while in DNA extracted from formalin fixed paraffin embedded tissue sections was only 50%.
Multimodal Diagnostic Approach to Brain Abscess

**Figure 1**

Table 1: Direct Demonstration Of Microbes And Morphometric Analysis In Brain Abscesses

<table>
<thead>
<tr>
<th>Category</th>
<th>Pus Smear Features</th>
<th>Positive Culture Method</th>
<th>Morphometry</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Cephalosporin resistance</td>
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<td></td>
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<td>Inflammatory zone (mm)</td>
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<td>Bacterial</td>
<td>100% (Gram +)</td>
<td>100%</td>
<td>223.72 ± 61.25</td>
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<td></td>
<td></td>
<td>31.25</td>
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<td>6.8 ± 1.9</td>
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<td></td>
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<td></td>
<td>49.31</td>
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<tr>
<td>Tuberculous</td>
<td>75% (AFB)</td>
<td>100%</td>
<td>100.05 ± 59.03</td>
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<td></td>
<td></td>
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<td>49.03</td>
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<td></td>
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<td>49.31</td>
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<tr>
<td>Fungal</td>
<td>100% (GMS)</td>
<td>100%</td>
<td>246.66 ± 73.38</td>
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<td>48.41</td>
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<td>48.41</td>
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<tr>
<td>Actinomycotic</td>
<td>100% (GMS)</td>
<td>100%</td>
<td>191.25 ± 69.25</td>
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<td>219.48</td>
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**Figure 2**

Pyogenic brain abscess in a 35-year-old female. Post-contrast T1-weighted image shows well-defined ring enhancing lesion in the right parietal region. Pus was drained from the lesion grew Staphylococcus aureus.

Tuberculous brain abscess in a 15-year-old female. Post-contrast T1-weighted axial MR image shows well-defined lesion in the left occipital lobe with rim enhancement. The culture from the pus showed M. tuberculosis. The rim of the lesion is thicker in (B) compared to (A).

**Figure 3**

Abscess wall showing the defined zones of inflammation and fibrosis (H&E x 125)

Actinomycotic abscess showing hematoxyphilic filamentous organisms with surrounding Splendor-Hopplei phenomenon. (H&E x 1250)

Fungal abscess with granulomatous reaction (H&E x 500), inset: silver impregnated hyphae (Gomori’s stain x 1250)

Microvessels in the brain abscess wall stained with anti-CD34 antibody (LSAB x 500)

**DISCUSSION**

Our cases include an uncommon case of multiple actinomycotic abscesses, four cases of fungal abscess caused by Candida (2) and Aspergillus (2) as well as four rare cases of tuberculous abscesses. Clinical features were common to the varied etiology of the abscesses. The classic triad of headache, fever and focal deficit occurs in less than 50% of patients with brain abscesses. CT appearance of brain abscess may be confused with neoplastic lesions and other infectious and non-infectious diseases, especially in the stage of early cerebritis. If the CT findings are not clear, MRI should be performed [1]. In our study, CT scan revealed, that both pyogenic and tuberculous brain abscesses had a rim of greater than normal density, surrounding an elliptical or round center of low density and the whole lesion encircled by a low-density area of brain edema. We have correlated the enhancement with the measured thickness of the zone of inflammation in tuberculous, pyogenic and fungal abscesses. Vascular nature of the abscess wall is reflected by its marked enhancement after infusion of contrast medium. MRI in T1 weighted images revealed a hypo-intense core surrounded by
Multimodal Diagnostic Approach to Brain Abscess

a thin rim of iso-intensity and an outer zone, which was hyper-intense. T2-images showed a hyper-intense center and well defined hypo-intense capsule and hyper-intense surrounding edema.

Anaerobic bacteria were isolated in 4 cases and aerobic bacteria in 12. There was no significant difference in the clinical presentation or treatment outcome in these groups. Studies have reported that anaerobic brain abscesses have better outcome and more frequent association with ENT abscesses as the source of infection [3]. Three of the four cases with anaerobes had chronic suppurative otitis media in our series. Anaerobes should be considered either alone or in combination with aerobic organisms in patients presenting with foci of sepsis in ENT region, so that appropriate antimicrobial cover and timely surgical excision of these foci could prevent metastatic brain abscesses. The incidence of positive cultures is lower in the post antibiotic era. Samples collected in liquid anaerobic culture media containing antibiotic inactivators give a better yield. In the current study we have included only 16 of the 38 pyogenic abscesses screened, which yielded a positive culture and required surgical excision.

Tuberculosis of the CNS has a large spectrum of manifestations. Tuberculous meningitis is commonest followed by tuberculoma, tubercular abscess and other forms such as cerebral miliary tuberculosis, tuberculous encephalopathy, tuberculous encephalitis, and tuberculous arteritis [38]. Tubercular brain abscess (TBA) is a rare manifestation of CNS tuberculosis [38]. A history of pulmonary tuberculosis may be present; however, in our cases we could not demonstrate any extra-cerebral clinical manifestations of tuberculosis. A relatively long clinical history and an enhancing capsule with thick wall are suggestive of TBA. Pyogenic abscesses have a thinner rim on contrast CT. On morphometry we have analyzed the abscess wall in terms of the granulation tissue and the capsule comprising of the region of gliosis and fibrosis. We have observed that the capsule, comprising of fibro-collagenous tissue, was greater in pyogenic abscesses while the zone of inflammation was wider in tubercular abscesses. The capsule of TBA was formed of vascular glial tissue with some fibrosis, while the granulation tissue consisted of a mixture of acute and chronic inflammatory cells, particularly polymorphs, indistinguishable from pyogenic abscess. Diagnosis could be established on positive staining for acid-fast bacilli. In one case, a few granulomas were also present along with the mixed inflammatory infiltrate. This case could have been a case of cerebral tuberculoma containing purulent focus Culture for aerobic and anaerobic bacteria was negative in this case. Direct smear for AFB was also negative. PCR and culture yielded positive results for Mycobacterium tuberculosis. Morphometric analysis of the abscess wall also showed prominent zone of inflammation in tubercular abscesses, which was significantly wider than in pyogenic abscesses (t=3.987, p= <0.001).

Tuberculous brain abscesses (TBA) are rarely encountered, even in countries where CNS tuberculosis is relatively common. Nishimoto et al [38] have reported a case of TBA in a patient with occult multiple myeloma. Immuno-compromised status [39] and immune related disorders like multiple myeloma [38] and hyper-IgE syndrome [38] have been demonstrated as predisposing factors in patients with TBA as well as fungal abscesses [3], especially in HIV-infected, AIDS and transplant recipient patients. It has also been found, that combination of HAART-therapy with anti-tubercular medication can significantly decrease mortality rates [3]. None of our cases were HIV positive.

Aspergillus brain abscess is a rare and frequently fatal disease. Despite the scarcity of reported survivors, a combination of medication and surgical treatment might be effective even in cases of multiple abscesses [3]. In our study, the aspergillus abscess was solitary and the patient is doing well with surgical extirpation and Fluconazole medication. The cause of fungal colonization in the brain in this case could not be determined despite thorough work up.

Actinomycosis is a subacute or chronic bacterial infection, which can affect immunocompetent or immunodeficient subjects. It most often occurs in cervico-facial or thoraco-abdominal locations. Central nervous system infection is rare but of severe prognosis [3]. A solitary case of multiple actinomycotic brain abscesses in the brain as well as systemic dissemination was also included in our study. The 45-year-old male presented with altered sensorium, three parenchymal lesions with a large lesion in the posterior fossa. The abscess was biopsied and drained. Partial response to therapy with oral tetracycline and penicillin injections was obtained but the disease relapsed and the patient developed a large frontal lesion with sinuses in the scalp due to uncontrolled growth involving the skull. The underlying granulomas in the frontal lobe were completely excised, the patient survived only for two months on a combination of antibiotics.

MR Spectroscopy is a non-invasive technique to identify the
biochemical characteristics of tissues, particularly for brain studies where routine biopsy is not favored [13]. Our study revealed lactate, lipid and amino acid metabolites in all cases. The lipid component probably resulted from a high bleed and contamination from adjacent parenchyma. Lactate was derived from fermentation processes of streptococci and the like; and from the enhanced glycolysis in necrotic tissue [14]. Succinate, one of the end products of propionic acid fermentation in anaerobic metabolism, can be used as a marker of anaerobic infection [15], as found in one of the cases in our study. This feature can also be exploited to differentiate between degenerating cysticerci and anaerobic abscesses [1]. There is no metabolic pathway in mammalian cells to produce acetate [16, 17]. Thus, it has never been reported in tumors. Hence, it appears to be the ideal marker for brain abscesses. In addition to this, it has also been demonstrated that Restricted diffusion on diffusion-weighted imaging (DWI) with reduced Apparent Diffusion Coefficient values (ADC) is highly suggestive of brain abscess; however, in the absence of restriction, Proton magnetic resonance spectroscopy (PMRS) is mandatory to distinguish brain abscesses form non-abscess intracranial mass lesions. [18, 19]

Brain abscesses show the characteristic resonance on MRS for aminoacids like leucine, isoleucine and valine along with lactate, alanine and acetate. This is accompanied by absence of N-acetyl aspartate, choline and creatinine which differentiate it from brain neoplasia [20, 21, 22]. Pyogenic abscesses are composed of aminoacids, lipid and lactate with or without acetate and succinate and are detected by MR spectroscopy. Despite antibiotic treatment, aminoacids are always found in such cases [23, 24, 25]. The amino acids are conspicuous by their absence in tubercular abscesses since they contain large numbers of tubercle bacilli and lack proteolytic enzymes usually found in abundance in pyogenic abscesses. Tubercular abscesses have abundant lipid.

MRI features recognize pyogenic abscesses fairly accurately. A central area of liquefaction gives high signals while the surrounding edematous brain tissue gives low signals on T1 weighted images. On T2 weighted images, the necrosis shows higher signals similar to the grey matter [26]. The maturity of the abscess is indicated by the rim, which is formed probably by the collagen and inflammation due to free radicals and microhemorrhages in the abscess wall [27]. We have observed that the zone of inflammation is significantly thicker in tubercular as compared to pyogenic abscess in morphometric analysis of histologic sections. This correlated well with the thickness of the abscess rim as observed in T1 weighted images. Other than this we did not find any predictor of etiology in T1 and T2 weighted images in MRI. Vascularity of the wall was not significantly different in abscesses of varied etiology. Differential diagnosis of abscesses in MRI is hematomas, metastases and granulomas since a similar low signal rim is obtained on the T2 images in such cases [28, 29, 30].

Brain abscesses are life threatening and detection and identification of the causative pathogens is crucial to substantiate the diagnosis and select the optimal antibiotic regimen. It is known that in approximately 20% of the patients microbiological cultures of abscess material remain sterile for reasons enumerated above. The polymerase chain reaction (PCR) provides a new alternative, but data reporting the specific use of broad-spectrum PCR assays to detect the causative pathogens in brain abscesses are infrequent in literature [31]. PCR is an excellent tool to detect hardy and obligate organisms that require stringent growth conditions like Fusobacterium species. [32] and Aspergillus [33]. We have applied the PCR in our study to tubercular abscess samples. PCR is rapid, sensitive, and does not depend on the viability of tubercle bacilli in the samples. 100% sensitivity and specificity was obtained with frozen tissues and pus aspirates [34]. In formalin fixed paraffin embedded tissue was only 50%. There is need for optimization of the assay in order to use it on formalin fixed, paraffin embedded tissues. Efficiency of the PCR depends upon fixative used (best being 10% buffered formalin), fixation time, DNA extraction procedure, length of PCR target, concentration of target DNA and the procedure itself [35, 36, 37]. The failure of amplification may be due to endogenous inhibitors and those induced by tissue processing. One way to reduce such inhibitors is to reduce target DNA concentration [38, 39]. But this leads to decrease in the sensitivity of PCR and its specificity by formation of primer-dimer artifacts [12], particularly in paucibacillary lesions. The longer the amplified fragment, the higher the likelihood of its degradation and lower is the efficacy of the amplification. Hence we have used PCR with final amplification to 123 bp. Target fragment repetitiveness is another limiting factor in PCR. We have used IS6110, a mobile genetic element usually present in multiple copies in genomes of almost all members of M. tuberculosis complex, which has the best sensitivity [40, 41, 42, 43].

CONCLUSION

We have observed that the thickness of inflammatory
exudates is wider in tuberculous abscesses, which in turn correlated with the zone of enhancement in CT and MRI studies. Radiological imaging including CT scan and MR imaging form the backbone of diagnostic modalities for brain abscesses, and help in localization, sizing and enumeration of brain abscesses. Microbial culture methods are irreplaceable for etiological diagnosis of the causative organism but may not always yield positive results. Multiple or individual PCR for common organisms causing infections along with per operative smear examination for bacteria, fungi and mycobacteria can allow early, rapid and sensitive diagnosis and identification of causative organism. Early initiation of specific therapy for tuberculosis and fungal infections can prevent fulminant disease in the postoperative period. MR Spectroscopy is a non-invasive and fairly accurate prediction of the etiology and differential diagnosis (abscess vs. tumors). Spectroscopy however requires expensive infrastructure and reporting expertise, which may not always be available in clinical set-ups. Correlation with MR imaging is vital.

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