“Neurobrucellosis – A clinical masquerade in an Indian scenario; Review of 12 cases by correlative diagnostic approach”

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Neurobrucellosis is a most serious complication of brucellosis which has neither a typical clinical picture nor specific cerebrospinal fluid (CSF) findings and mimics other neurological disorders leading to clinical diagnostic dilemmas. Accurate diagnosis is a great challenge for physicians, neurologists and researchers. A retrospective study was conducted to highlight the importance of the integrated diagnostics and clinical approaches to describe and categorize different clinical pictures of patients with neurobrucellosis in Indian scenario. We reviewed the medical records of twelve patients who were diagnosed as cases of neurobrucellosis from January 2010 to September 2013. Clinical details, associated risk factors, image findings were recorded. The serum and CSF Brucellosis work up by Rose Bengal Plate Test (RBPT) Indirect Enzyme Linked Immunosorbent Assay (iELISA), polymerase chain reaction (PCR), was performed and results analysed. Chronic meningitis (33.3%) was the most common form of presentation, followed by infective cerebro venous thrombosis (CVT) (25%), demyelination (16.6%), myelitis (16.6%) and myeloradiculopathy (8.3%). Epidemiological risk factor was present in 59% of the cases. All the twelve cases were positive for serum IgG antibrucella antibodies by ELISA. Two cases had brucella antibodies in the CSF as well. Brucella genus specific PCR was positive in four cases. Neurobrucellosis may be considered as one of the differential diagnoses in unusual cases of neurologic disorders and in cases of neurological dysfunction in absence of any other suitable alternative diagnosis. Multimodal differential diagnostic approaches are essential for accurate diagnosis, effective treatment and to prevent morbidity and mortality associated with neurobrucellosis.
Neurobrucellosis has neither a typical clinical picture nor specific CSF findings [18]. It is well known that examination of the CSF will be better option in diagnosis in cases of nervous system infectious diseases. CSF examination may reveal an elevated protein, depressed glucose concentration, and a moderate leucocytosis composed mainly of lymphocytes, though these parameters are not specific for brucellosis. Culturing the organism from the CSF may have problem with the false negativity because of the low organism load, [19] antibody detection, and PCR have got its own limitations.

Therefore multipronged laboratory diagnosis along with detail clinical examination is the prerequisite for disease confirmation. In view of this, in our study in addition to culture, we have subjected CSF and serum to antibody tests like RBPT, ELISA and PCR and correlated the laboratory results with the clinical and radiological findings.

The aim of this retrospective study was to highlight the importance of the integrated diagnostic and clinical approach to describe and categorize different clinical pictures of 12 patients with neurobrucellosis, in a tertiary care neuro-centre.

**MATERIALS AND METHODS:**

This retrospective study was conducted in the department of Neuromicrobiology, National Institute of Mental Health Neurosciences (NIMHANS), a tertiary neuro-centre, located in South India where on an average 2,500 CSF samples from suspected cases of neurological problems are received every year. We reviewed the medical records of all patients who were diagnosed as cases of Neurobrucellosis over a period of 3 years between January 2010 to September 2013. The demographic, clinical and laboratory information were analyzed. All patients were evaluated for central and peripheral nervous system involvement. The duration of clinical symptoms at the time of admission, occupational and epidemiological risks, history of ingestion of raw milk or milk products or infected animal exposure and laboratory results were recorded for each patient.

In all these cases CSF samples were analyzed for appearance, cytology, protein and glucose levels. In addition, Grams and Ziehl Neelsen smear, aerobic and mycobacterial culture, antitymocobacterial immunology was carried out as per the algorithm. From clinically suspected cases, blood (5ml) was collected in vaccutainers with and without anticoagulant, centrifuged at 2000xg for 5-10 minutes, clear serum and buffy coat samples were separated and stored separately at -20°C. Serum and CSF samples were tested for anti-brucella antibodies by RBPT (IAH&VB, Bangalore), and iELISA was carried out to determine the presence of both IgM and IgG anti brucella antibodies [20]. DNA was extracted from serum, buffy coat and CSF samples using DNeasy blood and tissue kit protocol (QiAgen, USA). The genus specific PCR was carried out using primers as described by Baily GC et al [21]. CT head and/or MRI were performed on all patients.

Twelve cases which were positive either by antibrucella antibody in serum or CSF /PCR positive were included in the study. (Table 1) None of them was culture positive. All cases positive only by RBPT in serum were excluded from the study.

The following diagnostic criteria were considered for Neurobrucellosis [18].

- a) Signs and symptoms of neurological dysfunction with CSF pleocytosis, normal/ low sugar, elevated protein and not having any other suitable alternative diagnosis
- b) Brain imaging suggestive of brucellosis as a differential diagnosis
- c) Serum or CSF positive for anti-brucella antibodies by RBPT and/or iELISA or PCR

**RESULTS:**

Clinical demography of the patients is tabulated in Table. Twelve cases fulfilled the above criteria of whom 7 (58.3%) were males and 5 (41.6%) were females with age ranging from 7 -57 years, mean age of 32. All patients were considered for above mentioned diagnostic criteria after ruling out tuberculous meningitis, neurocysticercosis, neurophilis and cryptococcal infection.

More than half of our cases (59%) had epidemiological risk factors. Three patients were from endemic area, one patient had the habit of raw milk consumption and three of them had direct exposure to animals as animal handlers; occupational risk was seen in one case. There was no obvious source in 41% of the patients.

Of the 12 cases, 9 were positive for IgG antibrucella antibodies- 8 serum and one CSF sample. One case exhibited both IgG and IgM antibodies in serum and one case was positive for IgG antibodies both serum and CSF and one positive for serum IgM alone by ELISA. Four cases were positive for Brucella genus specific PCR of which two were positive for serum only, one was positive for CSF only and one was positive for both serum and CSF (Figure).

There was one (8.33%) case of myeloradiculopathy, 2 (16.6%) cases each of demyelination and myelitis, 3 (25%) with infective CVT, and 4 (33.3%) cases of chronic meningitis by the multipronged laboratory diagnostics assisted with clinical and radiological data.

All the patients were treated with doxycycline and rifampicin and/or TMP/SMX. However, six cases showed improvement and the rest were lost to follow up.
Table: Showing clinical and laboratory profile of Neurobrucellosis

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Chief clinical presentations</th>
<th>Diagnosis</th>
<th>Risk factor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>M</td>
<td>Double vision, unsteady gait,</td>
<td>Demyelination</td>
<td>Endemic area, consumption of raw milk.</td>
<td>Serum IgG positive by ELISA</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>Fever, headache, vomiting</td>
<td>Chronic meningitis</td>
<td>History of contact with animals.</td>
<td>Serum IgG positive by ELISA, Serum positive for PCR</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>Giddiness, bilateral hearing impairment</td>
<td>Chronic meningitis</td>
<td>Endemic area</td>
<td>Serum IgG positive by ELISA, CSF positive for PCR</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>Headache since 4 years, Seizure</td>
<td>Myelitis</td>
<td>History of handling cattle placenta.</td>
<td>Serum IgG positive by ELISA</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>Weakness of lower limbs since 1 month</td>
<td>Demyelination</td>
<td></td>
<td>Serum IgG positive by ELISA</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>Progressive difficulty in walking, ascending paraesthesia</td>
<td>Myeloradiculopathy</td>
<td></td>
<td>CSF IgG positive by ELISA</td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>Headache since 2 months, vomiting and transient diplopia</td>
<td>Chronic meningitis</td>
<td></td>
<td>Serum IgG positive by ELISA</td>
</tr>
<tr>
<td>40</td>
<td>F</td>
<td>Intermittent visual blurring</td>
<td>CVT</td>
<td>History of contact with animals.</td>
<td>Serum IgG positive by ELISA</td>
</tr>
<tr>
<td>57</td>
<td>M</td>
<td>Headache since 2 months, low grade fever</td>
<td>Chronic meningitis</td>
<td>Occupational history.</td>
<td>Serum IgG positive by ELISA</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>Difficulty in walking, speaking and hearing</td>
<td>Myelitis</td>
<td>Endemic area of North Karnataka</td>
<td>Serum and CSF IgG positive by ELISA</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>Headache, vomiting, since 2 months and pain in ears, Visual disturbance</td>
<td>CVT</td>
<td></td>
<td>Serum IgG positive by ELISA, Serum and CSF positive for PCR</td>
</tr>
<tr>
<td>46</td>
<td>M</td>
<td>Lower limb weakness</td>
<td>CVT</td>
<td></td>
<td>Serum IgG &amp; IgM, positive &amp; CSF IgG positive by ELISA, Serum positive for PCR</td>
</tr>
</tbody>
</table>

DISCUSSION:

Even though neurobrucellosis is a treatable disease, the accurate early diagnosis and treatment is a great challenge for physicians, neurologists and researchers. One patient presented with progressive difficulty in walking, a predominant feature of myeloradiculopathy, in whom anti-brucella antibody was found in CSF alone. Symptoms of the myeloradiculopathy can be because of inflammatory response attributing to infection [22].

Two patients mimicked demyelination disorders comparable to 16.6% as reported in neuro-brucellosis [23]. These two patients had serum positive for anti-brucella antibody and PCR (serum and CSF) negative of whom one presented with double vision, unsteady gait belonging to middle east country which is endemic for brucellosis and raw milk consumption. However, serology negativity in CSF might be due to the usage of antibiotics during the course of diagnosis. Treatment with doxycycline improved the condition. Demyelination in neurobrucellosis can be due to the host pathogen interaction, leading to the triggering of immune system [23, 24, 25].

Of the two (16.6%) cases with myelitis, one of them presented with chronic headache with the history of association with animal placenta. Serum was positive for anti-brucella antibody, however CSF was negative. Based on the history of exposure, clinical picture and adjunctive laboratory evidence, the patient was treated with doxycycline and showed improvement thereafter. The second case had difficulty in walking, speaking with defective hearing. The patient being a native of north Karnataka, the suspected endemic area; both CSF and serum were found to be positive for anti-brucella antibody. Anti-brucella treatment was instituted but was lost to follow up. Myelitis in these cases could be attributed to pathophysiological changes caused by the organism. Similarly Kochar et al and Nurgul et al [25, 24] have reported 16.6% cases of myelitis in their series.

Cerebral venous thrombosis (CVT) in brucellosis has been reported [26] which was seen in three of our cases (25%) with correlative MRI findings suggestive of infectious CVT and all were ruled out for other infectious diseases clinching to neurobrucellosis. Two of them had visual disturbances and one
had animal exposure with associated ear pain. Of the above three, in one case serum and CSF antibody was positive in addition to serum PCR, in another serum antibody and CSF and serum PCR was positive; in the third case antibody was positive serum only.

Neurobrucellosis commonly presents as meningitis [13, 24, 23, 27]. We report 4 (33.3%) cases with headache of long duration, a feature of chronic meningitis (Table). In all, serum was positive for antibrucella antibody. In addition, PCR was positive in two cases (one in serum and another in CSF). MRI brain was suggestive of meningitis in these patients. No detailed data exist in the literature on the accurate diagnosis of chronic brucella meningitis or meningoencephalitis. Headache may be due to the hypertension developed in the CNS, release of immunomodulatory compounds and/or due to the presence of organism itself. Hearing impairment must be associated with malfunctioning of 8th nerve because of infection of the most frequently involved vestibulocochlear nerve [24, 28]. Vision impairment due to affected cranial nerves III, IV, VI may occur in brucella meningoencephalitis. Suspected cases presenting with chronic meningitis like symptoms with any unexplained neurologic symptoms should have neurobrucellosis as differential diagnosis.

The pathogenesis of CNS involvement is not yet fully understood and it has been reported that depressed immune system is expected to be a risk factor transforming brucellosis to neurobrucellosis [29]. Its protean clinical manifestations can mimic other neurological disorders like viral and TB meningitis, meningoencephalitis, cerebrovascular accidents, space occupying lesions, degenerative disk prolapse, multiple sclerosis, etc. Thus a high degree of suspicion and multipronged diagnostic approaches are essential especially in endemic regions like India.

It is well known that bacterial culture is the gold standard for the diagnosis, but in case of neurobrucellosis, it is associated with less sensitivity (<20%) which could be either due to low bacterial load in CSF or due to usage of antibiotics [30, 31] and often been thought to be suboptimal for CSF samples [32]. In a study of a large number of neurobrucellosis cases, Brucella culture was positive only in 28% of blood and 14% of CSF [28]. Thus in present study, diagnosis was based on correlation with clinical history followed by immunological tests and/or PCR along with routine CSF examination and CT/MRI imaging.

ELISA is a sensitive and specific test for the diagnosis of neurobrucellosis [24]. In our study, serum of 12 patients was positive for IgG antibrucella antibodies by IgG ELISA among which one of them was IgM ELISA positive. Two cases were positive for antibrucella antibodies in CSF also. Antibody titers in CSF are usually lower than in serum. In some patients agglutination test is negative at the beginning of the illness, this is a well-known feature in localized brucellosis and sometimes more than one serologic test is necessary to ascertain positivity [24].

Similarly, 2 out of 12 patients were positive by PCR among whom CSF and buffy coat sample PCR was positive in one patient each. It was interesting to note that PCR positive patients were also positive serologically. CSF PCR assay for Brucella is an evolving novel and promising diagnostic method which may prove to be an optimal alternative/supportive tool for immediate and accurate diagnosis [33].

Combination of doxycycline and rifampicin and/or TMP/SMX for six weeks to one year was most often used to achieve high levels in the CSF for effective cure [34]. In most of our patients, combination of doxycycline and rifampicin was recommended for 6-8 weeks and in few cases, triple drug regimen was also advocated. However, 6 cases showed improvement, the rest were lost to follow up and hence therapeutic comparisons were confounded.

The reported incidence of CNS involvement in brucellosis have ranged from 0% to 25% [35, 36] with the average incidence rate of <5% [15, 37]. In this study, we have reviewed 12 cases diagnosed as neurobrucellosis. Incidence of human brucellosis and neurobrucellosis are under reported due to lack of awareness, and non-availability of relevant laboratory facilities.

CONCLUSIONS:

Neurobrucellosis may be diagnosed based on epidemiology, clinical presentations supported by bacterial culture, serological and molecular tests and/or imaging studies, where two or more diagnostic tests need to be correlated with clinical history. Early diagnosis and treatment of neurobrucellosis will be helpful in decreasing the sequelae of the CNS complications. Neurobrucellosis should be considered as a differential diagnosis in patients with any unexplained neurologic symptoms.

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REFERENCES:


