Original Article

Review of 17 Cases of Neurobrucellosis: Clinical Manifestations, Diagnosis, and Management

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Abstract

Background: Neurobrucellosis (NB) is a rare, but important complication of brucellosis. The clinical features vary greatly and, in general, tend to be chronic. Many laboratory procedures are usually employed in the diagnosis of NB. Even though the culture method is the gold standard, growth rate is low and time consuming. Thus the rate of sequelae and mortality increase in case of a delay in treatment. Therefore it is necessary to perform serological tests in both serum and cerebrospinal fluid (CSF) in suspected patients. In this study we aim to evaluate clinical features, diagnosis, and treatment of patients with NB.

Method: We enrolled 17 patients diagnosed with NB. Clinical features, cultures, serological tests, additional laboratory findings, and CSF analyses were recorded for all patients.

Results: There were 14 female and 3 male patients. Ten patients presented with neuropsychiatric symptoms and signs (aphasia, diplopia, hemiparesis, facial paralysis, tremor, ataxia, depression, personality disorder, and hallucinations). Serum standard agglutination test (SAT) was negative in 4 (23.5%) patients and serum Coombs' test was negative in 2 (11.7%). CSF SAT was negative in 4 (23.5%) patients and CSF Coombs was negative in 3 (17.6%) patients. *B. melitensis* grew in the blood of 6 (35.2%) patients and in the CSF of 3 (17.6%). Treatment protocol for 11 patients consisted of ceftriaxone, rifampicin, and doxycyline for a period of four weeks, followed by rifampicin and doxycyline for an additional four weeks. The remaining patients were given different treatment combinations. One patient died, mild sequelae was present in another patient and the remaining patients recovered without any sequelae.

Conclusion: NB should be considered in the differential diagnosis of neurological and psychiatric cases that are encountered in endemic areas for brucellosis. In order to prevent overlooking this diagnosis, Coombs' test should be performed in both CSF and serum.

Keywords: Clinic, diagnosis, neurobrucellosis, treatment

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Introduction

Brucellosis is endemic in many countries throughout the world, including Turkey. Particularly in rural areas, this disease is mainly associated with consumption of unpasteurized milk and cheese.[1] In Turkey around 18000 new cases of brucellosis are diagnosed each year. The prevalence of serologically positive individuals in the Turkish population varies from 2.6% to 14.4%.[2,3] Brucellosis is a multisystem disease, which may present with a broad spectrum of clinical manifestations and complications. Neurologic manifestations of brucellosis occur in 0%–25% of patients.[4] The clinical spectrum of neurobrucellosis (NB) may be classified as central and peripheral.[5] Clinical presentation includes meningitis, meningoencephalitis, meningovascular involvement, parenchymatous dysfunction, peripheral neuropathy, radiculopathy, and various degrees of behavioral abnormalities. Among clinical manifestations, meningitis has been the most frequent presentation in a clinical series.[5–7] Central nervous system (CNS) involvement occurs in less than 5% of patients, the majority being around 2%–5%.[1,8] In this study, we describe 17 cases of CNS brucellosis and evaluate the clinical features, diagnosis, and treatment of this disease.

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Materials and Methods

In this retrospective study conducted between 2005–2010, we enrolled 17 patients with NB who were admitted to the Department of Infectious Diseases and Clinical Microbiology of Harran University School of Medicine Sanliurfa, Turkey. Diagnosis of NB requires satisfaction of the following criteria: 1) compatible clinical picture; 2) cerebrospinal fluid (CSF) analysis with lymphocytic pleocytosis (> 20/mm^3), elevated protein content (> 45 mg/dL) and reduced CSF/plasma glucose rate (< 0.50); and 3) the presence of one of the following laboratory findings: isolation of brucella from blood or CSF, or positive standard agglutination (SAT) and/or Coombs' tests (titers ≥ 1/160) in serum, or the presence of brucella antibodies in CSF at any titer obtained by the SAT or Coombs' tests.

We used a commercial kit (Cromatest, Knickerbocker Laboratories, Barcelona, Spain) for the SAT. The Coombs' test used anti-human gammaglobulin sera (Ortho Diagnostic Systems, Madrid, Spain) to detect blocking antibodies. In cases of positive sera and CSF, we performed serial tube dilutions that ranged from 1:10 to

Table 1. Patient symptoms.

Case	Age	Sex	Symptoms
1	16	Female	Fever, headache
2	17	Female	Fever, headache, neck stiffness, depression, unconsciousness
3	23	Female	Headache, nausea-vomiting
4	23	Female	Headache, nausea-vomiting
5	24	Female	Fever, headache, vomiting, neck stiffness, unconsciousness
6	25	Female	Headache, aphasia, blurred vision, neuritis, neck stiffness, diplopia
7	26	Female	Fever, headache, unconsciousness
8	26	Female	Fever, headache, nausea-vomiting
9	28	Male	Headache, hemiparesis, aphasia, ataxia, facial paralysis, neck stiffness
10	30	Female	Fever, headache, personality disorder, neck stiffness
11	32	Male	Fever, headache
12	34	Female	Fever, headache, tremor in hands
13	41	Female	Fever, headache, dizziness, ataxi, diplopia, neck stiffness
14	40	Female	Headache, nausea-vomiting
15	44	Female	Fever, headache, nausea-vomiting
16	47	Female	Fever, headache, depression
17	68	Female	Fever, headache, polyarthritis

Table 2. SAT, Coombs' test and culture results of serum and CSF.

Cases	Serum SAT	Serum Coombs' test	CSF SAT	CSF Coombs' test	Blood Culture	CSF Culture
1	1/320	1/640	1/40	1/160		
2	N	N	N	N		P
3	1/1280	1/1280	1/80	1/80	P	
4	1/640	1/1280	1/80	1/160		
5	1/1280	1/2560	1/20	1/40		
6	1/5120	1/5120	1/80	1/80	P	
7	1/320	1/320	N	N		
8	1/2560	1/2560	1/40	1/80	P	
9	1/320	1/1280	1/80	1/160		
10	N	N	1/40	1/40		
11	N	1/640	N	1/320		
12	1/1280	1/1280	N	N	P	P
13	1/640	1/640	1/640	1/640		
14	1/2560	1/5120	1/160	1/160	P	
15	1/1280	1/1280	1/80	1/80		
16	1/2560	1/2560	1/160	1/160	P	P
17	N	1/2560	1/40	1/80		

SAT = standard agglutination test; P = positive; N = negative.

1:10240. The BACTEC 9050 blood culture system (Becton, Dickinson and Company, USA) was used to culture brucella. Gram, India ink and Ziehl-Neelsen stains were routinely carried out on the CSF. In addition, blood and CSF were cultured for conventional bacteria, tuberculosis, and fungi. CSF was also analyzed for cells, glucose, and protein content. Tests for typhoid fever, toxoplasmosis, infectious mononucleosis, herpes virus antibodies, blood films for malaria and mantoux reaction test were performed in all cases. All patients underwent chest radiograph, cranial CT and/or MR scans.

Results

During a five-year period, 218 patients with brucellosis were treated in our clinic. Of these, 17 (7.7%) had NB, of which 14 were female and 3 were male. Mean age was 34 (range: 16–68) years. Demographic characteristics of patients are shown in Table 1.

All patients had positive histories of consumption of fresh (unpasteurized) cheese and/or stock farming. In 12 female patients, a history of milking, producing cheese from fresh milk and/or stock farming was noted. Meningoencephalitis was determined by CT/MR. As seen in Table 1, involvement of the peripheral nervous system (retrobulber neuritis, facial paralysis, tremor in the hands) was present in some patients in addition to meningitis. One patient had a history of insufficient, irregular treatment for discitis and infective endocarditis due to systemic brucellosis during the previous year. also initial signs and symptoms of patients are shown in same table. Serum SAT was negative in 4 (23.5%) patients while serum Coombs' test was negative in 2 (11.7%) patients. CSF SAT was negative in 4 (23.5%) patients while Coombs' was negative in 3 (17.6%) patients. In one patient, all tests were negative except for growth in the CSF culture. *B. melitensis* grew in blood from 6 (35.2%) patients and in the CSF of 3 (17.6%) patients. Table 2 shows the results of the SAT, Coombs' test and cultures. CSF biochemical analysis, and leucocyte counts of patients are presented in Table 3.

One patient was diagnosed with a tuberculosis meningitis-NB co-infection; another case was diagnosed with post-treatment NB. The remainder of patients were considered classic NB cases.

Treatment regimens and results are shown in Table 4. Doses of antibiotics were as follows: ceftriaxone (2 g, Bid/IV), doxycyline (100 mg, Bid/oral), rifampicin (600 mg, Qd/oral), sulfamethoxazole-trimethoprim (960 mg, Bid/oral), and ciprofloxacin (500 mg, Bid/oral). All 11 patients who received the combination of ceftri-

Table 3. Cerebrospinal fluid analysis.

Laboratory parameter	Results
CSF protein (mean ± SD)	118.40 ±18.30 mg/dL (normal: 15–45 mg/dL)
CSF/serum glucose rate (mean ± SD)	0.42 ± 0.14 (normal: 0.50–0.66)
CSF leucocyte count (mean ± SD)	286.60 ± 30.50/mm³
CSF leucocyte type	Mononuclear (> 60%)
Pressure height of CSF	14 cases
CSF color	3 mildly blurred, 14 clear

CSF = cerebrospinal fluid.

Table 4. Treatment protocols and patient outcomes.

Cases	Therapy	Outcome
1	C+D+R (4W) followed by D+R (4W)	Recovered
2	C+D+R (4W) followed by D+R (4W)	Recovered
3	C+D+R (4W) followed by D+R (4W)	Recovered
4	C+D+R (4W) followed by D+R (4W)	Recovered
5	Cip+D+R (5 M)	Recovered
6	C+D+SXT (6 M)	Decrease in vision
7	C+D+R (4W) followed by D+R (4W)	Recovered
8	C+D+R (4W) followed by D+R (4W)	Recovered
9	C+D+SXT (6 M)	Recovered
10	C+D+S (3W) followed by D+R (3M)	Recovered
11	C+D+R (4W) followed by D+R (4W)	Recovered
12	C+D+S (3W) followed by D+R (3M)	Recovered
13	C+D+SXT (6 M)	Recovered
14	C+D+R (4W) followed by D+R (4W)	Recovered
15	C+D+R (4W) followed by D+R (4W)	Recovered
16	C+D+R (4W) followed by D+R (4W)	Recovered
17	C+D+R (2 W)	Exitus (after 2 week)

C = ceftriaxone, R = rifampicin, D = doxycyline, SXT = sulfamethoxazole-trimethoprim, Cip = ciprofloxacin, S = streptomycin, W = week, M = month.

axone, doxycyline and rifampicin had normal CSF findings from lumbar puncture (LP) at the end of week four. Therefore, these patients underwent doxycyline and rifampicin treatment for an additional four weeks, then treatment was terminated. No complaints or pathological findings were noted during the one-year follow-up visits.

Discussion

CNS involvement of brucellosis is a rare, but important complication. In a number of studies, the rate of NB has been reported at < 5%.[4,8–10] In our study, this rate was 7.7%. This increased rate has been attributed to the referral of all NB patients to our center since our hospital is a third-level medical center. Some of the non-NB brucellosis cases are treated in second-level hospitals and do not refer to third-level medical centers.

In various studies, the male:female ratio has been reported as 2:1, 3:2, and 1:2.[8,11,12] In our study, the female:male ratio is considerably high (5:1). Our region is the center of agriculture and stock farming in Turkey and women are actively involved in the care of animals, milking, and producing cheese from fresh milk, which might possibly explain our elevated findings.

NB may present in various forms but the most frequent clinical presentations are meningitis and meningoencephalitis.[7,8] Similarly in our study, 3 cases were diagnosed with meningoencephalitis and 13 were diagnosed as meningitis.

As seen in Table 1, NB may present with neurological (aphasia, dizziness, diplopia, hemiparesis, facial paralysis, tremor, and ataxia) and psychiatric (depression, personality disorder, and hallucinations) symptoms. Therefore, some patients are referred to neurology or psychiatry departments. Clinicians should be cautious in this regard. Yetkin et al. have reported symptoms of headache (85%), fever (70%), and nausea-vomiting (30%) in their study.[6] In our study, headache was seen in all patients, while fever was present in 38% and nausea-vomiting in 33%. We associated low grade fever with frequent use of antipyretics; we believe that headache was due to elevation of CSF pressure. Meningeal irritation findings in NB have been reported at < 50%.[13] A rate of 33% in our study was compliant with data from the literature.

Diagnosis of NB is usually confirmed by detection of specific antibodies in CSF; cultures of CSF being positive in less than one-half of cases.[14] In our study, growth in blood culture was observed in 6 (35.2%) cases and growth in CSF was seen in 3 (17.6%). The low rate of growth in cultures was due to frequent use of antibiotics before referring to the hospital.

Even though the ELISA test has a fast, accurate diagnosis; in a number of studies ELISA was reported to have no superiority over the Coombs' test.[14,15] Sanchez Sousa et al. found positive anti-brucella antibodies in CSF by the Coombs' test in one patient whose serum test was negative.[14] Haji-Abdolbagi et al., in their series of 31 cases, found negative SAT results by Coombs' test in the CSF of two patients and negative results in the serum of two patients.[9]

NB is can be seen in the early stages of the disease, or it may develop during the convalescence stage, even months or years after recovery from acute infection.[16] One of our patients was treated for 20 months due to positive serum (1/1280) and CSF SAT (1/80) with no improvement in clinical symptoms or in the CSF serological and biochemical findings. Following the detection of CSF adenosine deaminase (ADA) activity of 23 U/L, tuberculosis treatment was added based on a diagnosis of co-infection with tuberculosis meningitis and NB. Clinical and CSF laboratory findings of this patient totally improved in two months. ADA activity > 15 U/L is considered as a strong indicator for tuberculosis meningitis.[17] In the literature, one case was reported that presented

as a tuberculosis meningitis-NB co-infection.[18] In one of our patients, while CSF biochemical features were compliant with NB, serum and CSF Coombs' SAT results were negative. Diluting the patient's serum to a titer of 1/1280 did not change SAT negative results, however dilution between titers of 1/2560 to 1/10240 produced positive results. Similar false negative results due to prozone phenomenon were reported in the literature.[19]

Various issues are encountered in diagnosis of NB. Even though culture method is the gold standard, the growth rate is low and this method is time consuming. Thus, the rate of sequelae and mortality will increase in case of a delay in treatment. Therefore in patients whose clinical findings are compliant with NB, serological tests should always be performed both in serum and in CSF. The Coombs' test is preferable as it is more sensitive compared to the SAT (Coombs 96.1%, SAT 88.2%).[20] In our cases, we have noted a high sensitivity of the Coombs' test. The dilution titer for this test should be increased to levels over 1:1280 due to prozone phenomenon. In some laboratories, this test is only performed up to titers of 1:320–1:640 in order to save time and antigens, which may lead to false negative results.

Radiology was determined in only three patients with MR and CT, therefore it was apparent that radiology does not assist with the diagnosis of NB.

Also CSF laboratory analysis not considered strictly diagnostic; these can only be used as supplementary diagnostic criteria in addition to other findings.

Issues in the treatment of NB are still valid. Difficulty in treatment is due to intracellular localization of microorganisms and the requirement for a sufficient antibiotic dose level in the CSF. No consensus exists on treatment regimens and duration of treatment.[16,21–23]

The mortality rate of NB in the postantibiotic era is 0%–5.5% but permanent neurologic deficits, particularly deafness, are common.[8,24] Pedro-Pons et al.,[4] in series of 42 NB cases, have reported only two cases of mortality while Haji-Abdolbagi et al.[9] reported one death of unidentified origin in a series of 31 cases. Akdeniz H et al.[15] and Heper et al.[25] did not report any deaths in their NB cases. In the current tial, one of our NB patients who had infective endocarditis due to brucellosis died, however, treatment compliance was not satisfactory in this patient. He was unable to receive appropriate doxycyline and rifampicin treatment and developed sepsis, which progressed to death. In patients who received ceftriaxone, doxycyline, and rifampicin, the second LP after four weeks revealed normal CSF findings (in all cases protein 15–45 mg/dL, CSF/blood glucose rate > 0.50 mg/dL, leukocyte counts < 20). Therefore doxycyline and rifampicin was administered for four additional weeks and treatment was stopped at week eight. No relapse was seen in any patient during follow-up visits. It was evident that ceftriaxone was efficacious in the treatment of NB with a shortened treatment duration. Various studies have used the same treatment protocol for shorter (six weeks total) and longer (months) durations with successful results.[5–9]

In conclusion, NB should always be considered in the differential diagnosis of neurological and psychiatric cases that are encountered in endemic areas for brucellosis. Although culture is the gold standard for diagnosis, this method is time consuming and has a low growth rate. Therefore serum and CSF Coombs' tests should both be performed, taking into consideration rare serological conditions. We believe that treatment with the combination of ceftriaxone, doxycyline and rifampicin is effective in NB cases that are limited to CNS involvement.

Conflict of interest: No conflict of interest to declare.

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