ABSTRACT

Objective: Brucellosis, one of the major zoonotic diseases, still remains an uncontrolled problem, in regions of high endemicity. Ophthalmic brucellosis is not studied and overlooked in most developing countries. Considering the severe outcome of undiagnosed ophthalmic brucellosis, in this paper, we made attempts to find out whether this disease still remains a health problem in a South East Asian developing country, where the study of this disease is largely neglected.

Methods: This study was carried out over a 1-year period from January 2015 to December 2015. Blood samples were collected from clinically confirmed cases of uveitis, and they were subjected to five serological and one genus specific molecular investigations for the detection of Brucella infection.

Results: Of 20 uveitis cases, 4 (20%) cases confirmed as brucellar uveitis, by serological tests followed by polymerase chain reaction confirmation. After treatment of brucellosis, all the four patients were recovered uneventfully.

Conclusion: Brucella infection involving the eye is still a significant problem in South East Asian countries; hence in all uveitis cases in this reason brucellosis should be excluded by available laboratory tests.

Keywords: Brucellosis, Uveitis, Zoonotic diseases, Standard tube agglutination test, Polymerase chain reaction.

INTRODUCTION

Brucellosis is a zoonotic disease found worldwide. Although it has been eradicated in most developed countries, it still represents an important health problem in many parts of the world including Western part of Asia, Middle East, the Mediterranean, Central and South America [1-3]. In some countries such as Peru, Kuwait, and Saudi Arabia, brucellosis is endemic [3,4]. The rationale of this study is that in South East Asian countries problem of brucellosis is largely unknown, and reports of brucellar uveitis are practically missing; thus, this study has been undertaken to reveal the real scenario of brucellar uveitis in India.

METHODS

The study was carried out over a 1-year period from January 2015 to December 2015, in a tertiary care teaching hospital in Eastern India. After getting permission from Institutional Ethical Committee, 20 clinically confirmed patients with uveitis were included in this study, attaining informed consents from them. Blood samples were collected from them for serological and polymerase chain reaction (PCR) tests. Conjunctival swabs were also collected as a routine procedure to know the presence of any pathogenic microbial flora on the eye surface including Brucella. After collection, the swabs were inoculated immediately onto nutrient agar, blood agar, Sabouraud dextrose agar with chloramphenicol (SDC) slants, and in biphasic Castaneda medium. Cultures on nutrient agar and blood agar were observed after overnight incubation at 37°C to find presence of any common pathogenic bacteria; cultures on SDC were incubated at 25°C in BOD incubator and observed up to 21 days, while cultures in Castaneda media were observed for any growth of Brucella up to 21 days post-inoculation, and in subcultures on Brucella selective agar. All isolates were identified by routine diagnostic procedures. Serum samples were kept in separate aliquots and stored at −20°C before further processing. Each sample of serum was subjected to a panel of Brucella tests - Rose Bengal Plate Agglutination Test (IABV, Bengaluru), standard tube agglutination test (SAT; Tulip Diagnostics Pvt. Ltd.), ELISA (Immunolab GmbH, Germany) for the detection of IgM and IgG antibodies and genus-specific PCR (prime).

For PCR tests, the serum samples were first subjected to DNA extraction using QIamp DNA Mini Kit (Qiagen). The extracted DNAs were then subjected to PCR. The PCR was carried out in 50 µL reaction mixture in each PCR tube that contained 5 µL PCR buffer, 1 µL dNTP, 0.2 µL Taq Polymerase, 5 µL template DNA, 1 µL each of forward (F) and reverse (R) primers, and 36.8 µL nuclease free water. The primer sequences used were as follows [5]-BCSP-B4 (F) TGG CTC GGT TGC CAA TAT CAA; BCSP-B5 (R) CGC GCT TGC CTT TCA GGT CTG; amplicon size was 223 bp. The steps in PCR were followed as described by Baily et al. [5]. The amplified products were then subjected to agarose gel electrophoresis using a 100 bp DNA ladder and a positive and a negative control and the bands formed (Figs. 1-3) were seen in a gel doc (Biorad). The amplified products were then subjected to a panel of Brucella tests - Rose Bengal Plate Agglutination Test (IABV, Bengaluru), standard tube agglutination test (SAT; Tulip Diagnostics Pvt. Ltd.), ELISA (Immunolab GmbH, Germany) for the detection of IgM and IgG antibodies and genus-specific PCR (prime).
eyes. Associated with eye involvement, the patient also gave a history of fever that subsided on medication and was of remittent type. The patient was treated for ocular manifestations with oral and topical steroid, and other supportive treatments, which were discontinued following remission of ocular symptoms. The patient used to remain asymptomatic for 3/4 months at a time. Ophthalmological findings are given in Table 1. The patient’s serum was subjected to serological tests and showed positive Brucella SAT with a titer of 1:320 along with a positive Brucella IgM ELISA test. PCR result showed a positive band for Brucella. The culture was negative.

Case 2 (Fig. 5)
PM, F, Hindu, 45 years, a known case of uveitis, presented with a 1-year history of recurrent bilateral redness, pain and watering from eyes and blurring of vision. The patient had 3-4 recurrences during the year. Symptoms subsided temporarily following routine treatment. On presentation, her acute inflammatory stage had subsided to some extent with previous conservative medication. The patient was from rural background and gave history of rearing cattle at home and consumption of unpasteurized milk. The patient had two episodes of fever during this year that subsided with medications. Ophthalmological findings are given in Table 1. The patient’s serum showed a positive Brucella SAT with a titer of 1:160 and positive Brucella IgM ELISA test. PCR showed a positive band for brucellosis. Culture showed negative result.

Case 3 (Fig. 6)
HA, F, Muslim, 45 years, attended outpatient department with redness, watering, and pain of left eye 2-3 times over the last 1 year. The patient gave history of similar episodes in the left eye 4-5 times over the last 3 years. Each time, symptoms subsided with the use of medications. The patient also gave history of occasional fever that subsided with treatment after consulting physician. Ocular symptoms subsided with the use of oral and topical steroids, and topical antibiotics (moxifloxacin), topical timolol maleate, and atropine. Ophthalmological findings are given in Table 1. The patient’s serum showed a positive Brucella SAT with a titer of 1:80 and a positive Brucella IgM ELISA test. PCR showed a positive band for brucellosis. Culture showed negative result.

Case 4 (Fig. 7)
AM, M, Muslim, 36 years, was a known case of bilateral panuveitis. He responded well to treatment (subtenon triamcinolone acetonide injection). He gave a history of recurrent attack of redness of both eyes and dimness of vision for the last 2 years and also the history of 5-6 episodes of recurrences during that period. The patient also gave a history of occasional fever of remittent type, when these symptoms started 2 years back. Ocular symptoms subsided well each time with oral and topical steroids, topical cycloplegic, topical antibiotic, and subtenon injection of triamcinolone acetonide. Ophthalmological findings are given in Table 1. The patient’s serum showed a positive Brucella SAT with a titer of 1:80 and a positive Brucella IgM ELISA test. PCR showed a positive band for brucellosis. Culture showed negative result.

Considering the positive serological and molecular biological tests (Table 2) and excluding other causes of uveitis, these patients were diagnosed as having brucellosis. Thus in our study, we found four confirmed cases of brucellosis with the ocular presentation as chronic anterior and intermediate uveitis.
Table 1: Clinical ophthalmological findings of the four *Brucella* positive uveitis cases

|                  | SP, F, Hindu, 40 years | PM, F, Hindu, 45 years | HA, F, Muslim, 46 years | AM, M, Muslim, 36 years |
|------------------|------------------------|------------------------|------------------------|------------------------|
| **Right eye**    | **Left eye**           | **Right eye**          | **Left eye**           | **Right eye**          | **Left eye**           |
| V/A (visual acuity) | 6/36                   | 6/12                   | 6/60                   | Finger count 2 feet    | 6/9                    | 6/60                   | 6/60                   |
| Slit lamp examination | Normal                  | Normal                  | Normal                  | Normal                  | Normal                  | Normal                  | Normal                  |
| Lid              | Normal                 | Normal                 | Normal                 | Normal                 | Normal                 | Normal                 | Mild congestion         |
| Conjunctiva      | Normal                 | No congestion          | Normal                 | Congestion subsided    | Normal                 | Congestion subsided    | No congestion           |
| Cornea           | Few KPs on the endothelium | Very old KPs on the corneal endothelium | No oedema | few old KPs | Clear | Mild oedema | present | KPs seen over endothelium | No oedema, KPs present | No oedema | KPs present |
| Anterior Chamber | Cells+2, Flair+1, Iris visible | Absence of posterior synechia | Normal, no flair | No cell no flair | Normal, no cell, no flair | No cell no flair | Few cells | Few cells |
| Pupil            | Pharmacologically dilated, irregular due to posterior synechia | Absence of posterior synechia | Posterior synechia | Normal, circular | Pharmacologically mid dilated and irregular due to posterior synechia | Circular (pharmacologically dilated) | Circular (pharmacologically dilated) |
| Lens             | Pigment deposition on anterior lens capsule, early cataractous changes | Early cataractous change, pigmentation over lens capsule | Pseudo phakia | Cataractous changes | Early cataractous change | Cataractous change present, pigment deposition over anterior capsule | No cataractous change, pigment deposition over anterior lens capsule | 18 mmHg | 18 mmHg |
| IOP              | Normal                 | Normal                 | Normal                 | Normal                 | 18 mmHg | 30 mmHg on the day of examination | 18 mmHg | 18 mmHg |
| Fundus examination | Normal                 | Disc, retina appears normal | Vitreous haze, disc, retina appears normal | Normal | Vitreous haze present, disc and macula just visible | Hazy media, disc just visible, margin blurred, 2nd order vessels visible | Hazy media, disc just visible, margin blurred, 2nd order vessels visible |
| Lacrimal Sac     | No regurgitation        | No regurgitation        | No regurgitation        | No abnormality detected | No abnormality detected | No Abnormality detected | No abnormality detected |

KPs: Keratic precipitates

Fig. 6: Picture of the affected eye of case 3

Before diagnosis of brucellosis, the patients were initially given symptomatic management for uveitis, with topical steroid eye drop prednisone acetate 1% and topical antibiotic eye drop tobramycin 0.3% and mydriatic cum cycloplegic eye drop atropine 1%, (one patient needed timolol maleate eye drop so as to prevent rise of intraocular pressure, sometimes they needed oral steroid and periocular steroid injection) and responded well. Subsequently following the diagnosis of brucellosis, they were given specific treatment with oral doses of doxycycline 100 mg along with rifampicin 300 mg both twice daily continued for 6 weeks. The patients were followed up at intervals of 2-3 weeks and showed signs of improvement.

**DISCUSSION**

A high index of clinical suspicion coupled with appropriate diagnostic tests can detect ophthalmic brucellosis at an early stage. Brucellosis may...
manifest as an acute or chronic disease. It has a wide range of clinical manifestations, which makes it diagnostically challenging. Sometimes, it is easy to identify the classical symptoms of brucellosis. However, some manifestations such as ocular brucellosis are difficult to identify. Ocular manifestations of brucellosis may be in the form of dacryoadenitis, conjunctivitis, episcleritis, keratitis, iritis, iridocyclitis, neuroretinitis, retinitis, choroiditis, panuveitis, pars planitis, and hyalitis. The clinical manifestations and signs may include injection, blurred vision, eye pain, watering, diplopia, foreign body sensation, cotton wool lesions, exudative retinal detachment, and retinal hemorrhage [6–10]. It can only be detected if it is kept in the differential diagnosis along with other diseases. This will lead to early diagnosis and treatment and will, in turn, reduce the number of complications arising out of delayed diagnosis of the disease [6]. Lemaire in 1924, made the first diagnosis of ophthalmic brucellosis [7].

Two explanations may be there for the pathogenesis of ophthalmic brucellosis-direct invasion of Brucella and immune complex dependent pathogenesis [6,8,11]. In 2008, Rolando et al. reported about two different manifestations of brucellosis, ophthalmologic and neuro-ophthalmologic types [7]. During the long period from January 1980 to December 2005, 1551 brucellosis patients were studied by them, and 52 patients were diagnosed as having ocular brucellosis [7]. In 1953, Puig Solanes et al. identified 60 patients with ophthalmic complications of brucellosis and in them 48 patients had neuro-ophthalmic involvement [8]. In most of the studies, it has been found that uveitis is the most common ocular manifestation of brucellosis and that posterior uveitis is the most common form of uveitis [7,8,11,12]. Ocular manifestations are mainly seen during the chronic phase of the disease [7,8,13–16]. Several studies on brucellosis from Eastern India [17–21] indicated that it is prevalent.

Table 2: Diagnostic tests done for 20 patients

| S. No. | Age | Sex | SAT titre | SAT titre with 2 ME | RBPT | ELISA | PCR | N agar | Blood agar | Castaneda Brucella selective agar | SDC |
|--------|-----|-----|-----------|---------------------|------|-------|-----|--------|------------|---------------------------------|-----|
| 1      | 50  | Male| 1/80      | 1/160               | -    | -     | -   | S. aureus | S. aureus | -                                | -   |
| 2      | 30  | Male| 1/40      | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 3      | 27  | Female| 1/20     | 1/40                | -    | -     | -   | -       | -          | -                                | -   |
| 4      | 29  | Male| 1/80      | 1/160               | -    | -     | -   | S. pyogenes | S. pyogenes | -                                | -   |
| 5      | 52  | Female| 1/40     | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 6      | 40  | Female| 1/320    | 1/640               | IgM  | -     | -   | -       | -          | -                                | -   |
| 7      | 61  | Male| 1/40      | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 8      | 32  | Female| 1/80     | 1/160               | -    | -     | -   | -       | -          | -                                | -   |
| 9      | 55  | Male| 1/20      | 1/40                | -    | -     | -   | S. aureus | S. aureus | -                                | -   |
| 10     | 36  | Male| 1/80      | 1/160               | IgM  | -     | -   | -       | -          | -                                | -   |
| 11     | 44  | Female| 1/40     | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 12     | 37  | Female| 1/40     | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 13     | 72  | Male| 1/80      | 1/160               | -    | -     | -   | -       | -          | -                                | -   |
| 14     | 45  | Female| 1/160    | 1/640               | IgM  | -     | -   | -       | -          | -                                | -   |
| 15     | 63  | Male| 1/80      | 1/160               | -    | -     | -   | S. aureus | S. aureus | -                                | -   |
| 16     | 39  | Male| 1/20      | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 17     | 57  | Male| 1/40      | 1/80                | -    | -     | -   | -       | -          | -                                | -   |
| 18     | 31  | Male| 1/20      | 1/40                | -    | -     | -   | -       | -          | -                                | -   |
| 19     | 42  | Male| 1/40      | 1/80                | -    | -     | -   | S. aureus | S. aureus | -                                | -   |
| 20     | 45  | Female| 1/80     | 1/320               | IgM  | -     | -   | -       | -          | -                                | -   |

S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, A. niger: Aspergillus niger. SAT: Standard tube agglutination test, RBPT: Rose bengal plate agglutination test, PCR: Polymerase chain reaction, SDC: Sabouraud dextrose agar with chloramphenicol

Table 3: Reported cases of Brucella uveitis

| Reference no. | Author's name | Year | Findings | Country |
|---------------|---------------|------|----------|---------|
| Cited in 14   | Woods & Guyton| 1944 | 15 cases of brucellosis among 200 cases of uveitis, 7 of them had recurrent iritis, 5 had generalized uveitis, 3 had choroiditis | Great Britain |
|              | Harris        | 1945 | 1 case of recurrent keratitis, 3 cases of recurrent iritis due to brucellosis | Great Britain |
|              | Kuzhverskaya  | 1951 | 1 case of iridocyclitis, 1 case of bilateral optic neuritis, 1 case of bilateral optic atrophy among 101 cases of brucellosis | Great Britain |
|              | Pagliarani    | 1951 | 4 cases of Brucella melitensis with ocular symptoms | Great Britain |
|              | Nelson Jones  | 1952 | Described the different clinical presentations of brucellosis | Great Britain |
|              | Barrett & Rickards | 1953 | Gave a detailed description of chronic brucellosis | Great Britain |
| Cited in 8    | Puig Solanes et al. | 1953 | 60 brucellosis patients with ocular complication, of them 48 had neuro-ophthalmic involvement | USA |
| Cited in 7    | Rolando et al. | 2008 | Among 1551 patients of brucellosis studied, 52 had ocular manifestations | Peru |
| Cited in 11   | Rolando et al. | 2009 | Among 12 patients with clinical and laboratory findings suggestive of Brucella uveitis, 4 had negative ocular agglutination and 8 had positive agglutination test, 1 had positive culture for B. Melitensis in subretinal fluid, Goldmann Witmer coefficient was positive in 3 patients, tissue samples showed lymphoplasmacytic infiltrate | Peru |
| Cited in 15   | Ozlem Gurses et al. | 2010 | A case of brucellosis in a 28 years female, presented as bilateral optic nerve and right abducens nerve involvement and endocarditis complicated by right premaular hemorrhage | Turkey |
| Cited in 16   | Lutfi Akyol et al. | 2015 | Combined presentation of sarcoiditis and uveitis in case of brucellosis in a 28 years old lady | Great Britain |
in this part of our country. The previous studies on brucellar uveitis are summarized in Table 3. This study has been undertaken to diagnose the presence of brucellosis among clinically confirmed cases of uveitis. Uveitis strictly means inflammation of uveal tissue only. But clinically, there is always some associated inflammation of adjacent structures such as retina, vitreous, and cornea. The infective causes of uveitis can be bacterial (Mycobacterium, Treponema, Staphylococcus, Streptococcus, Brucella, etc.), viral, fungal, and parasitic [22]. Acute uveitis is one that persists for 6 weeks to 3 months and chronic uveitis persists for more than 3 months to years.

In our study, we found four confirmed cases of brucellosis with the ocular presentation as chronic anterior uveitis. The patients have been given the standard therapy with doxycycline and rifampicin and responded well to treatment.

CONCLUSION
To conclude, every case of systemic brucellosis should undergo routine ophthalmological evaluation and similarly all patients suffering from uveitis should be screened for brucellosis. This could reduce the possibility of blindness associated with brucellosis.

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