

BRUCELLAR UVEITIS IN EASTERN INDIAANINDITA SEN¹, PARTHAJIT BANERJEE¹, DEVARATI DUTTA², MANAS KUMAR PAL³, ATANU RAY¹, SATADAL DAS^{2*}¹Department of Microbiology, M G M Medical College, Kishanganj, Bihar, India. ²Brucella Research Lab, Peerless Hospital and B K Roy Research Centre, Kolkata, West Bengal, India. ³Department of Ophthalmology, Medical College, Kolkata, West Bengal, India.
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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: Out 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.

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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 (IAHVB, 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). Patients showing positive results with *Brucella* specific tests were also subjected to the following routine investigations - Mantoux test, X-ray chest posteroanterior view, toxoplasma IgM and IgG antibodies, rheumatoid arthritis factor, and treponema antibody rapid plasma reagin tests.

RESULTS

Of the 20 blood samples collected from clinically confirmed cases of uveitis, four samples (20%) were found positive for brucellosis. All other investigations in these four cases were not suggestive of any other associated disease.

Case 1 (Fig. 4)

SP, F, Hindu, 40 years, presented with 1 ½ years history that started with the sudden onset of redness and pain in the right eye, which subsided on routine medication that was subsequently followed by a recurrence of similar symptoms with bilateral involvement of both

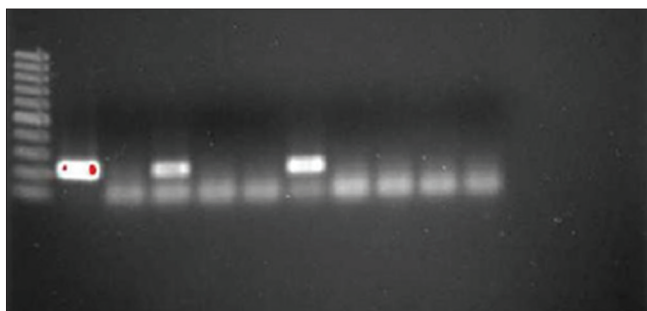


Fig 1: Gel picture represents two positive cases of uveitis with brucellosis in lanes 4 and 7. Lane 1 - Represents 100 bp DNA ladder. Lane 2 - Positive control. Lane 3 - Negative control. Lanes 4 to 11 - Patients' samples

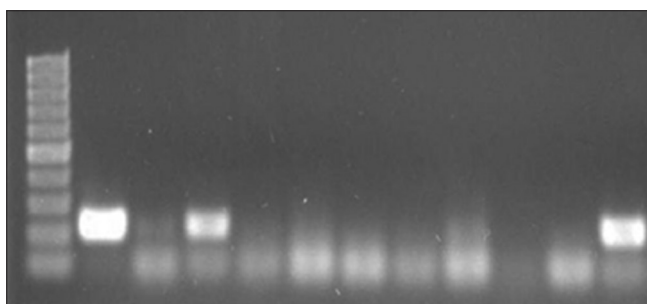


Fig. 2: Gel picture represents two positive cases of uveitis with brucellosis in lanes 4 and 12. Lane 1 - Represents 100 bp DNA ladder. Lane 2 - Positive control. Lane 3 - Negative control. Lanes 4 to 12 - Patient' samples

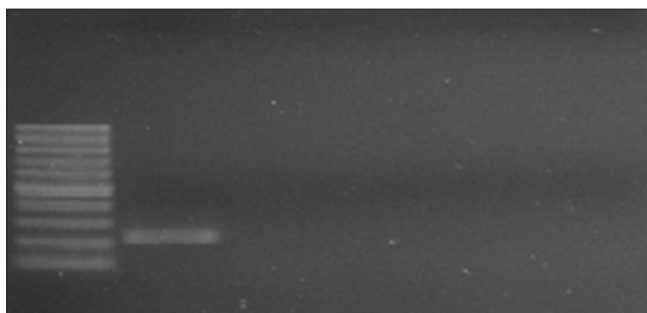


Fig. 3: Gel picture three negative cases. Lane 1 - 100 bp DNA ladder, Lane 2 - Positive control, Lane 3 - Negative control, Lanes 4 to 6: Patient samples

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



Fig. 4: Picture of the affected eye of case 1



Fig. 5: Picture of the affected eye of case 2

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 triamcetonolone 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 triamcetonolone 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	Right eye	Left eye
V/A (visual acuity)	6/36	6/12	6/60	Finger count 2 feet	6/9	6/60	6/60	6/60
Slit lamp examination								
1. Lid	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2. Conjunctiva	Circumcorneal congestion	No congestion	Congestion subsided	Congestion subsided	Normal	Congested, circumcorneal congestion present	No congestion	Mild congestion
3. Cornea	Few KPs on the endothelium	Very old KPs on the corneal endothelium	No oedema few old KPs	Few old KPs	Clear	Mild oedema present, KPs seen over endothelium	No oedema, KPs seen over endothelium	No oedema KPs present
4. Anterior Chamber	Cells+2, Flair+1, Iris visible	AC Normal, no cells and flare	No cell no flair	No cell no flair	Normal, no cell, no flair	Cells present (plenty), flair present	Few cells	Few cells
5. Pupil	Pharmacologically dilated, irregular due to posterior synechia	Absence of posterior synechia	Irregular posterior synechia	Posterior synechia	Normal circular	Pharmacologically mid dilated and irregular due to posterior synechia	Circular (pharmacologically dilated)	Circular (pharmacologically dilated)
6. Lens	Pigment deposition on anterior lens capsule, early cataractous changes	Early cataractous changes, 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	No cataractous change, pigment deposition over anterior lens capsule
IOP	Normal	Normal	Normal	Normal	18 mmHg	30 mmHg on the day of examination	18 mmHg	18 mmHg
Fundus examination	Retina, disc and macula visible and normal, mild vitreous haze	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, 2 nd order vessels visible	Hazy media, disc just visible, margin blurred, 2 nd order vessels visible
Lacrimal Sac	No regurgitation	No regurgitation	No regurgitation	Normal	No abnormality detected	No abnormality detected	No abnormality detected	No abnormality detected

KPs: Keratic precipitates

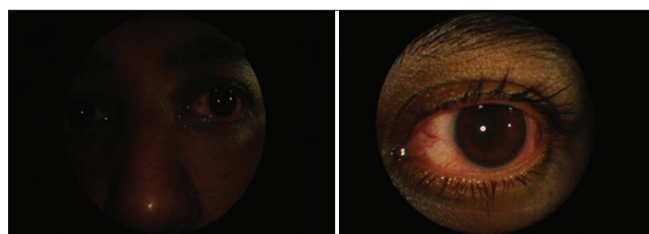


Fig. 6: Picture of the affected eye of case 3

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.



Fig. 7: Picture of the affected eye of case 4

DISCUSSION

A high index of clinical suspicion coupled with appropriate diagnostic tests can detect ophthalmic brucellosis at an early stage. Brucellosis may

Table 2: Diagnostic tests done for 20 patients

S. No.	Age years	Sex	Serological tests				Molecular test	Conjunctival swab culture				
			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	+	-	-	<i>S. aureus</i>	<i>S. aureus</i>	-	-	-
2	30	Male	1/40	1/80	-	-	-	-	-	-	-	-
3	27	Female	1/20	1/40	-	-	-	-	-	-	-	-
4	29	Male	1/80	1/160	+	-	-	<i>S. pyogenes</i>	<i>S. pyogenes</i>	-	-	-
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	-	-	-	<i>S. aureus</i>	<i>S. aureus</i>	-	-	-
10	36	Male	1/80	1/160	-	+IgM	+	-	-	-	-	-
11	44	Female	1/40	1/80	-	-	-	-	-	-	-	-
12	37	Female	1/40	1/80	+	-	-	-	-	-	-	<i>Fusarium</i>
13	72	Male	1/80	1/160	-	-	-	-	-	-	-	-
14	45	Female	1/160	1/640	-	+IgM	+	-	-	-	-	-
15	63	Male	1/80	1/160	-	-	-	<i>S. aureus</i>	<i>S. aureus</i>	-	-	-
16	39	Male	1/20	1/80	-	-	-	-	-	-	-	-
17	57	Male	1/40	1/80	-	-	-	-	-	-	-	<i>A. niger</i>
18	31	Male	1/20	1/40	-	-	-	-	-	-	-	-
19	42	Male	1/40	1/80	-	-	-	<i>S. aureus</i>	<i>S. aureus</i>	-	-	-
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 Kuzhershkaya	1945	1 case of recurrent keratitis, 3 cases of recurrent iritis due to brucellosis	Great Britain	
		1951	1 case of iridocyclitis, 1 case of bilateral optic neuritis, 1 case of bilateral optic atrophy among 101 cases of brucellosis	Great Britain	
	Cited in 8	Pagliarani	1951	4 cases of <i>Brucella melitensis</i> 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
	Puig Solanes <i>et al.</i>	1953	60 brucellosis patients with ocular complication, of them 48 had neuro-ophthalmic involvement	USA	
Cited in 7	Rolando <i>et al.</i>	2008	Among 1551 patients of brucellosis studied, 52 had ocular manifestations	Peru	
Cited in 11	Rolando <i>et al.</i>	2009	Among 12 patients with clinical and laboratory findings suggestive of <i>Brucella</i> uveitis, 4 had negative ocular agglutination and 8 had positive agglutination test, 1 had positive culture for <i>B. Melitensis</i> in subretinal fluid. Goldmann Witmer coefficient was positive in 3 patients, tissue samples showed lymphoplasmacytic infiltrate	Peru	
Cited in 15	Ozlem Gurses <i>et al.</i>	2010	A case of brucellosis in a 28 years female, presented as bilateral optic nerve and right abducent nerve involvement and endocarditis complicated by right premacular hemorrhage	Turkey	
Cited in 16	Lutfi Akyol <i>et al.</i>	2015	Combined presentation of sacroiliitis and uveitis in case of brucellosis in a 28 years old lady	Great Britain	

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, chorioiditis, panuveitis, pars planitis, and hyalitis. The clinical symptoms 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

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 sclera, 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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