

## Endophthalmitis patients seen in a tertiary eye care centre in Odisha: A clinico-microbiological analysis

Savitri Sharma, Tapas R. Padhi\*, Soumyava Basu\*, Sarita Kar, Arvind Roy\* & Taraprasad Das\*

*Ocular Microbiology Service & \*Retina-Vitreous Service, L.V. Prasad Eye Institute, Bhubaneswar, India*

Received April 4, 2012

**Background & objectives:** Geographical variations are known to influence different aspects of endophthalmitis. We report the epidemiological, clinical and microbiological profile of patients with infectious endophthalmitis presented to a tertiary eye care centre in Odisha, India, and compare the results with published reports from other parts of India.

**Methods:** Retrospective review of medical records of 107 patients, seen between December 2006 and January 2009 was done. All patients had undergone parsplana vitrectomy with intraocular antibiotics and the management was based on microbiological analysis of the vitreous fluid.

**Results:** Forty six (43.0%) patients had post-operative (PO), 43 had post-traumatic (PT) and 18 (16.8%) had endogenous (EG) endophthalmitis. Males were predominant in all three types of endophthalmitis. Significantly younger individuals constituted PT group. While culture established microbial diagnosis in 45 patients (42%), direct microscopy was positive in 38 patients (35.5%). Fungal aetiology was found in 13 patients (PO-7, PT-4, EG-2) and bacteria accounted for 32. Similar to studies from north, central and south India, fungi and Gram-negative bacteria accounted for a large number of PO endophthalmitis cases. Two PT patients had polymicrobial infection. All Gram-positive bacteria were susceptible to vancomycin. Susceptibility to ceftazidime was variable among the Gram-negative bacteria. Best corrected visual acuity (BCVA) at presentation was less than 20/200 in majority (93%) of the patients. While the treatment outcome was variable in fungal and sterile endophthalmitis, the BCVA was either unchanged or improved in 100 per cent of bacterial endophthalmitis patients.

**Interpretation & conclusions:** The spectrum of infection and outcome of infectious endophthalmitis in Odisha was similar to other parts of the country. Fungi and bacteria were involved in all three types of endophthalmitis. Empirical use of standard intravitreal therapy is recommended while emphasizing on vitreous biopsy for culture and sensitivity whenever possible.

**Key words** Diagnosis - endogenous - endophthalmitis - Odisha - post-operative - post-traumatic - treatment

The exogenous infective endophthalmitis occurs either after intraocular surgery or trauma and the endogenous endophthalmitis is usually the result of haematogenous spread of organisms to the eye from

a site of infection elsewhere in the body or from contaminated catheters or needles. Almost all aspects of the disease have been dealt with in thousands of studies published including epidemiology, type

of organisms, antibiotic susceptibility, diagnosis, prevention, treatment options, treatment outcome, etc. While majority of the reports have been from the USA, there are many reports from other countries that highlight the variation in epidemiology, organism type, their antibiotic susceptibility and treatment outcome in diverse settings<sup>1-5</sup>.

Several studies have been published from the premier ophthalmology institutes of India, reporting different aspects of endophthalmitis in the last two decades, most of which deal with post-operative endophthalmitis<sup>3,6-8</sup>. There are three reports that describe post-traumatic endophthalmitis in great detail<sup>4,9,10</sup>. These reports provide considerable insight into the clinical, epidemiological and microbiological aspects of post-operative and post-traumatic endophthalmitis, however, none of these deals with endogenous endophthalmitis. We present here the data of the first 107 patients of endophthalmitis who were referred to our tertiary eye care centre located in the State of Odisha in India. The aim was to analyze the clinical and microbiological data of patients who were clinically diagnosed as endophthalmitis on presentation. Apart from demographic features and predisposing factors, the treatment profile and outcome were described in patients with culture positive (bacterial, fungal) or negative post-operative, post-traumatic and endogenous endophthalmitis.

### Material & Methods

A retrospective chart review of 107 patients who had presented in the outpatient clinic of the L.V. Prasad Eye Institute, Bhubaneswar, Odisha, India, between December 2006 and January 2009 with a clinical diagnosis of endophthalmitis was done. The study protocol was approved by the institutional review board and written informed consent was obtained from all patients. All patients had undergone complete eye examination under slit lamp biomicroscopy following collection of history and demographic details. Ultrasound B scan was done in eyes when media opacity (corneal oedema, anterior chamber exudates, vitreous exudates) precluded fundus view with indirect ophthalmoscope. Following complete pre-operative evaluation all patients were subjected to pars plana vitrectomy with intraocular antibiotics with or without corticosteroids injection. At the time of vitrectomy, undiluted vitreous sample (0.2-0.5 ml) was collected from all cases prior to intraocular injection, using a syringe attached to the vitrectomy canula and the sample was sent immediately to the microbiology laboratory. Additional surgical procedures were

performed whenever indicated. The vitreous sample was processed within 30 min in the laboratory as per institutional protocol published earlier<sup>11</sup>. Drops of vitreous were used to make three smears (stained with Gram, Giemsa and Calcofluor white stain) for microscopic examination and inoculation of six culture media [5% sheep blood agar, 5% sheep blood chocolate agar, brain heart infusion (BHI) broth, thioglycollate broth, Robertson's cooked meat broth and Sabouraud dextrose agar (SDA), Hi media, Mumbai]. All media were incubated at 37°C except Sabouraud dextrose agar that was held at 27°C in BOD incubator. Chocolate agar was incubated in 5 per cent CO<sub>2</sub>. Any bacterial growth was identified using API system (bioMerieux, France) and fungal identification was based on colony characteristics and microscopic features. All culture media were held for two weeks in case of no growth before declaring the sample as sterile. Only unequivocal or significant culture results were considered<sup>11</sup>. The criteria to determine significance of a culture included (i) confluent growth in any solid media; and/or (ii) growth in more than one medium; and/or (iii) growth in one medium with presence of organism in direct microscopy. All bacterial isolates were tested for their susceptibility to a battery of antibiotics by Kirby-Bauer disc diffusion method<sup>12</sup>. Susceptibility for fungal isolates was not performed.

After collection of vitreous sample all patients received intravitreal vancomycin 1.0 mg/0.1 ml and ceftazidime 2.25 mg/0.1 ml, as per the institutional antibiotic policy. Post-operative treatment of the patients consisted of systemic and topical antibiotics. Patients with fungal infection were given intravitreal amphotericin B (5 µg/0.1 ml). The patients were examined post-operatively on days 1, 3, 7 and weekly thereafter for one month or more based on the response to therapy. Final visual acuity at not less than one month follow up was included in the analysis.

Geographical location of the patients, prior history, presenting visual acuity, type of intervention, length of follow up, microscopy and culture results of the vitreous sample, antibiotic susceptibility of the bacterial isolates and final outcome were analysed. Chi square test for proportions was applied for all comparisons and a  $P < 0.05$  was considered significant.

### Results

Of the 107 patients, 43 (40.2%) were post-traumatic, 46 (43.0%) post-operative and 18 (16.8%) were endogenous. Of the 46 post-operative cases, eight (17.3%) patients had undergone cataract surgery

**Table I.** Demographic details of 107 patients included in the study

	Bacterial endophthalmitis n=32			Fungal endophthalmitis n=12			Culture negative endophthalmitis n=62			Total
	PO	PT	EG	PO	PT	EG	PO	PT	EG	
Males	6	13	6	4	3	2	14	16	6	70
Females	3	3	1	3	0	0	16	7	3	36
Mean age $\pm$ SD (yr)	45.8 $\pm$ 18.91	18.38 $\pm$ 17.11	44 $\pm$ 15.51	55.67 $\pm$ 12.36	24.67 $\pm$ 6.43	28.5 $\pm$ 26.16	54.13 $\pm$ 20.21	23.35 $\pm$ 22.06	20.22 $\pm$ 16.04	
Age range (yr)	8-72	2-48	25-70	32-65	20-32	10-47	9-85	3-72	5-45	

PO, post-operative; PT, post-traumatic; EG, endogenous; Excluded one mixed (*Candida* sp.+*Bacillus* sp.) infection case of a 61 year old male in post-traumatic group

in-house while the remaining were referred from outside. All cases of endogenous and post-traumatic endophthalmitis were referred from outside. The patients came from all over Odisha State, especially in and around Bhubaneswar situated in Khurda district. Six patients (5.6%) came from the neighbouring States of West Bengal, Assam and Andhra Pradesh.

The demographic details of the patients in different groups are given in Table I. The mean age of patients with post-operative endophthalmitis at 51.8 yr was higher than that of patients with post-traumatic (22.1 yr) and endogenous endophthalmitis (30.9 yr), however, the difference was not significant. Table II provides the analysis of microscopy and culture findings of the vitreous from all patients. Distribution of types of bacteria and fungi causing endophthalmitis is given in Table III. Culture was positive in 45 patients (42%), and microscopy was positive in 38 patients (35.5%). The distribution of infecting organisms was as follows: 31 patients had monobacterial infection, 12 patients had monofungal infection and two patients had polymicrobial infection (bacteria and fungus-1, two bacterial species-1). Of the 34 bacterial isolates, 13 (38.2%) were Gram-negative and 21 (61.8%) were Gram-positive. The results of antibiotic susceptibility testing are shown in Table IV for Gram-negative and Gram-positive bacteria.

Best corrected visual acuity (BCVA) at presentation was less than 20/200 in 94 out of 101 (93%, data missing-6) patients. The presenting BCVA of the patients in various groups was compared with the final BCVA after treatment. All patients had a minimum follow up of at least one month. Thirteen patients (12.1%) were lost to follow up. Fig. 1A shows the comparison of presenting and final BCVA of 32 patients with bacterial endophthalmitis (single bacterial isolate - 31, two bacterial isolates - 1), and 12 patients with

fungal endophthalmitis. One patient with mixed fungal and bacterial infection was excluded from this analysis. Although the mean presenting BCVA of patients with bacterial endophthalmitis (LogMAR 2.75) was less than the eyes with fungal endophthalmitis (LogMAR 2.35), the final visual recovery was better in bacterial than fungal (LogMAR 1.78 and 1.93, respectively) endophthalmitis. The difference was, however, not significant. Presenting and final BCVA in patients with Gram-positive (n=19) and Gram-negative (n=13) bacterial infection is shown in Fig. 1B. The pre- and post-intervention BCVA of bacterial, fungal and culture negative endophthalmitis cases for the three types of endophthalmitis are shown in Figs 2-4. In the endogenous endophthalmitis group, data on pre- and post-treatment visual acuity were available for 6 bacterial, 2 fungal and 7 culture endophthalmitis patients (Fig. 2). Post-intervention BCVA remained unchanged or improved by one line or more for 6 of 6 bacterial, 1 of 2 fungal

**Table II.** Results of microscopy and culture of vitreous samples from all patients with endophthalmitis

Type of endophthalmitis	Only smear positive (%)	Only culture positive* (%)	Smear and culture positive (%)
Post-operative (n=46)	3 (6.5)	4 (8.7)	12 (26)
Post-traumatic (n=43)	5 (11.6)	7 (16.3)	13 (28.3)
Endogenous (n=18)	1(5.6)	4 (22.2)	5 (27.8)
Total (n=107)	9 (8.4)	15 (14)	30 (28)

\*Includes cases with significant growth in culture based on either confluent growth on solid media and/or growth in two media

**Table III.** Microbiological findings of vitreous samples from 45 patients with post-operative, post-traumatic and endogenous endophthalmitis yielding significant growth of bacteria or fungus in culture

Patient no.	Microscopic findings	Culture findings
Post-operative endophthalmitis: n=16, Bacterial - 9, Fungal - 7		
1	Gram variable coccobacilli	<i>Pseudomonas</i> sp.
2	Gram positive cocci	<i>Staphylococcus</i> sp.
3	Thick beaded, long & short bacilli / coccobacilli	<i>Pseudomonas</i> sp.
4	Gram positive cocci	<i>Streptococcus pneumoniae</i>
5	Gram negative bacilli	<i>Pseudomonas</i> sp.
6	Gram positive cocci	<i>Staphylococcus</i> sp.
7	Gram positive cocci	<i>Streptococcus pneumoniae</i>
8	Gram negative bacilli	<i>Pseudomonas aeruginosa</i>
9	No organisms	<i>Bacillus licheniformis</i>
10	No organisms	<i>Acremonium</i> sp.
11	Septate hyaline fungal filaments	<i>Aspergillus flavus</i>
12	No organisms	<i>Aspergillus terreus</i>
13	Budding yeast cells	<i>Candida</i> sp.
14	No organisms	<i>Aspergillus flavipes</i>
15	Septate hyaline fungal filaments	<i>Candida albicans</i>
16	Septate hyaline fungal filaments	<i>Acremonium</i> sp.
*Post-traumatic endophthalmitis: n=20, Bacterial -16, Fungal - 4		
17	Gram negative bacilli	<i>Escherichia coli</i>
18	No organisms	<i>Aeromonas</i> sp.
19	No organisms	<i>Escherichia coli</i>
20	Gram positive cocci	<i>Streptococcus bovis</i>
21	No organisms	<i>Moraxella</i> sp.
22	No organisms	<i>Alpha haemolytic Streptococci</i>
23	Gram variable bacilli with spores	<i>Clostridium perfringens</i>
24	Gram positive cocci	<i>Staphylococcus aureus</i>
25	Gram negative coccobacilli	<i>Enterobacter cloacae</i>
26	Gram positive cocci	<i>Micrococcus</i> sp.
27	No organisms	<i>Bacillus subtilis</i>
28	Gram variable curved bacilli	<i>Cellulosimicrobium cellulans</i>
29	Gram negative bacilli/Gram positive cocci	<i>Klebsiella pneumoniae</i>
30	No organisms	<i>Micrococcus</i> sp.
31	Gram positive cocci/Gram variable coccobacilli	1. <i>Bacillus megaterium</i> , 2. $\alpha$ haemolytic streptococci
32	Gram positive bacilli	<i>Propionibacterium acnes</i>
33	Septate fungal filaments	<i>Aspergillus fumigatus</i>
34	Septate fungal filaments/ budding yeast cells	1. <i>Candida</i> sp., 2. <i>Bacillus</i> sp.
35	No organisms	<i>Scedosporium apiospermum</i>

Contd...

Patient no.	Microscopic findings	Culture findings
36	Septate fungal filaments	Unidentified dematiaceous fungus
Endogenous endophthalmitis: n=9, Bacterial - 7, Fungal - 2		
37	No organisms	<i>Staphylococcus</i> sp.
38	No organisms	<i>Pseudomonas stutzeri</i>
39	No organisms	<i>Acremonium</i> sp.
40	Gram positive cocci	<i>Streptococcus pneumoniae</i>
41	Gram variable beaded bacilli	<i>Escherichia coli</i>
42	Gram positive beaded branching bacilli	<i>Cellulosimicrobium cellulans</i>
43	Gram positive cocci	<i>Streptococcus pyogenes</i>
44	Gram negative bacilli	<i>Pseudomonas</i> sp.
45	No organisms	<i>Candida albicans</i>
*Patient no. 31 had two bacterial isolates and 34 had one bacterial and one fungal isolate Total bacterial isolates = 34, Total fungal isolates = 13		

and 6 of 7 culture negative endophthalmitis patients. Analysis of visual acuity before and after treatment, available for 43 post-operative endophthalmitis (Fig. 3) patients (bacterial-8, fungal-7, culture negative-28) showed unchanged or improved BCVA after treatment in 8 of 8 bacterial, 5 of 7 fungal and 26 of 28 culture negative endophthalmitis patients. Post-intervention BCVA data of post-traumatic endophthalmitis (Fig. 4) available in 35 cases (bacterial-9, fungal-3, culture negative- 23) showed unchanged or improved BCVA in 9 of 9 bacterial, 2 of 3 fungal and 20 of 23 culture negative endophthalmitis patients. Infection was not

controlled in two patients with fungal infection and the eyes needed evisceration (PO-1, *Aspergillus flavipes*, PT-1, *Aspergillus fumigatus*). Data of two patients with polymicrobial infection in post-traumatic group are not included in the figures. The presenting BCVA in both these cases was PL PR inaccurate and remained same after treatment.

### Discussion

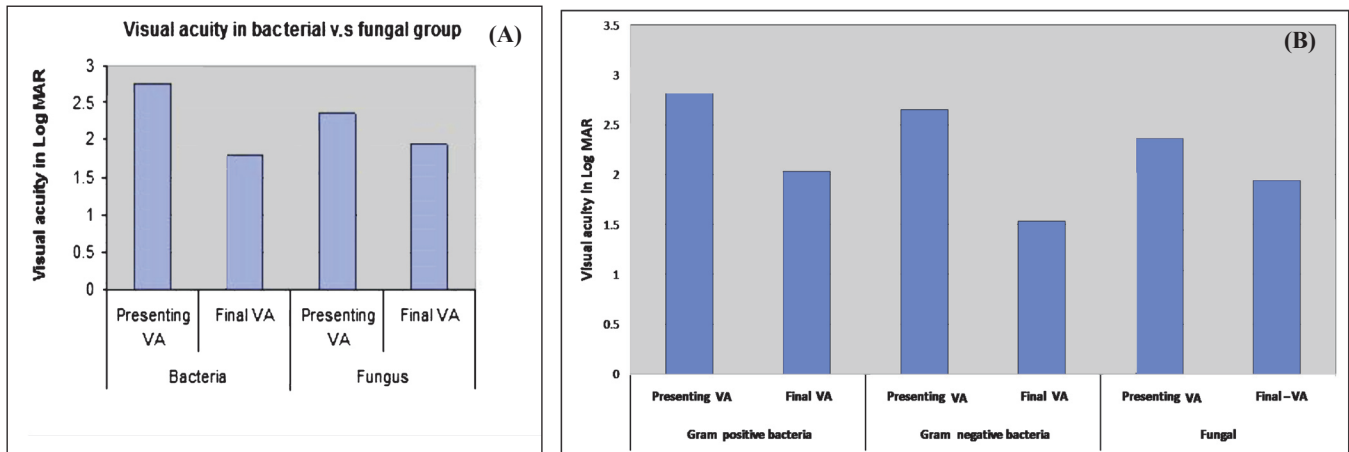
Published in 1975, Forster *et al*<sup>13</sup> described 54 cases of suspected endophthalmitis seen between July 1969 through March 1975. Several reports have

**Table IV.** Antibiotic susceptibility profile of bacterial isolates from different types of endophthalmitis (n=34)

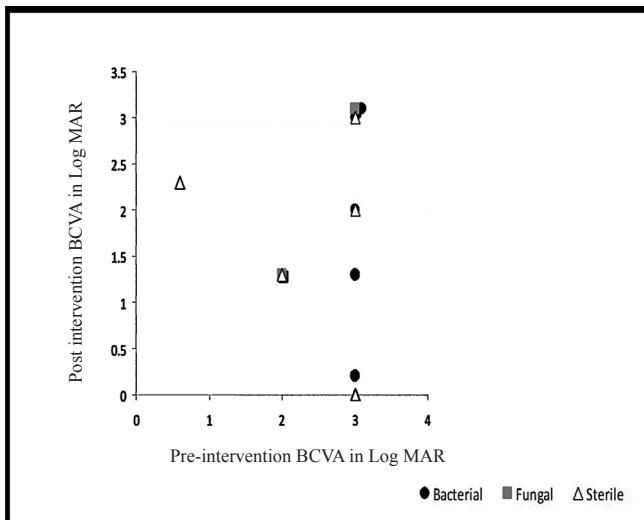
Type of endophthalmitis		Susceptible isolates/Total no. of isolates							
		Ak	Ca	Gf	Cf	Of	Mo	Va	Cz
PO	GN (n=4)	1/4	0/3	1/3	2/4	1/3	1/3	–	–
	GP (n=5)	–	–	3/5	3/5	4/5	0/1	5/5	3/4
PT	GN (n=6)	3/5	5/5	5/6	4/6	5/6	2/2	–	–
	GP (n=12)	–	–	10/12	10/12	9/11	1/3	12/12	11/11
EG	GN (n=3)	2/3	2/3	3/3	3/3	3/3	1/1	–	–
	GP (n=4)	–	–	3/4	2/3	1/2	2/3	4/4	3/4

GP, Gram positive bacteria, GN, Gram negative bacteria, Intermediately susceptible and resistant isolates are not shown  
PO, post-operative; PT, post-traumatic; EG, endogenous; Ak, amilacin; Ca, ceftazidime; Gf, gatifloxacin; Cf, ciprofloxacin; Of, ofloxacin; Mo, moxifloxacin; Va, vancomycin; Cz, ceftazolin

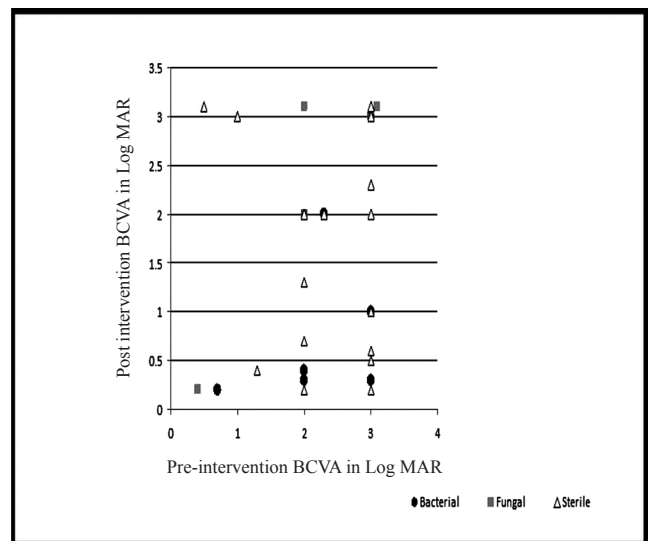




**Fig. 1.** Comparison of presenting visual acuity and final visual acuity between (A) patients with bacterial (n=32), fungal (n=12), (B) Gram-negative (n=19) and Gram-positive (n=13) bacterial endophthalmitis.



**Fig. 2.** Comparison of pre- and post-intervention BCVA (LogMAR) in endogenous endophthalmitis (n=15, bacterial-6, fungal-2, sterile-7, missing data=3).



**Fig. 3.** Comparison of pre- and post-intervention BCVA (LogMAR) in post-operative endophthalmitis (n=43, bacterial-8, fungal-7, sterile-28, missing data=3).

been published from north, south and central India on post-operative endophthalmitis<sup>3,6-8</sup>, and one on post-traumatic endophthalmitis<sup>9</sup>. We have earlier reported on the independent risk factors for poor visual outcome in patients with post-operative and post-traumatic endophthalmitis in a multivariate analysis<sup>10</sup>. Of these, only two have analyzed the visual outcome of the patients after treatment<sup>7,8</sup>. The data presented here nearly represent whole of the eastern State of Odisha because all patients had been referred from various parts of Odisha except six who came from the neighbouring States. Eight patients who developed post-operative endophthalmitis after cataract surgery in this hospital also belonged to Odisha.

There were more males than females in all the groups except culture negative post-operative group. While the male predominance in post-traumatic group could be due to greater chances of exposure of males to trauma due to outdoor activities, the reason for male preponderance in other two groups could possibly be due to more males willing to travel when referred or could be socio-economic. For reasons that young males are more actively involved in outdoor activities, the mean age of patients in post-traumatic group was lower than other two groups.

The vitreous culture was positive in 42 per cent cases and direct microscopy was positive in 35.5 per



might have been sterile, those infective were probably of bacterial origin.

In conclusion, this study in the Odisha State in eastern India demonstrates that the spectrum of infection and outcome following the standard of care in all forms of endophthalmitis is no different from other parts of India. The State has a peculiar climatic condition of extreme dry weather in north Odisha (similar to north India) and extreme humid weather in south coastal Odisha (similar to south coastal India). This does not seem to have affected the endophthalmitis infection spectrum unlike some of the other eye infections such as microsporidial keratoconjunctivitis unique to this region<sup>21</sup>. Hence, based on our results empirical use of standard intravitreal therapy (vancomycin and ceftazidime in bacterial endophthalmitis; amphotericin B in fungal endophthalmitis) may be recommended though vitreous biopsy for culture and sensitivity should always be insisted on.

#### Acknowledgment

Authors acknowledge the Hyderabad Eye Research Foundation, Hyderabad for financial assistance.

#### References

- Han DP, Wisniewski SR, Wilson LA, Barza M, Vine AK, Doft BH, et al. Spectrum and susceptibilities of microbiologic isolates in the endophthalmitis vitrectomy study. *Am J Ophthalmol* 1996; 122 : 1-17.
- Fisch A, Salvaret A, Prazuck T, Forestier F, Gerband L, Cosca G, et al. Epidemic of infective endophthalmitis in France. *Lancet* 1991; 338 : 1373-6.
- Kunimoto DY, Das T, Sharma S, Jalali S, Majji A, Gopinathan U, et al. Microbiologic spectrum and susceptibility of isolates. Part I. Postoperative endophthalmitis. *Am J Ophthalmol* 1999; 128 : 240-2.
- Kunimoto DY, Das T, Sharma S, Jalali S, Majji A, Gopinathan U, et al. Microbiologic spectrum and susceptibility of isolates. Part II. Posttraumatic endophthalmitis. *Am J Ophthalmol* 1999; 128 : 242-4.
- Zhang YQ, Wang WJ. Treatment outcomes after pars plana vitrectomy for endogenous endophthalmitis. *Retina* 2005; 25 : 746-50.
- Anand AR, Therese L, Madhavan HN. Spectrum of etiological agent of postoperative endophthalmitis and antibiotic susceptibility of bacterial isolates. *Indian J Ophthalmol* 2000; 48 : 123-8.
- Gupta A, Gupta V, Gupta A, Dogra MR, Panday SS, Ray P, et al. Spectrum and clinical profile of post cataract surgery endophthalmitis in north India. *Indian J Ophthalmol* 2003; 51 : 139-45.
- Lalitha P, Rajagopalan J, Prakash K, Ramasamy K, Prajna NV, Srinivasan M. Postcataract endophthalmitis in south India: Incidence and outcome. *Ophthalmology* 2005; 112 : 1884-9.
- Vasumathy V, Nirmalan PK, Ramasamy K, Prakash K, Namperumalsamy P. Clinicomicrobiological profile and visual outcome of posttraumatic endophthalmitis at a tertiary eye care centre in south India. *Indian J Ophthalmol* 2006; 54 : 5-10.
- Das T, Kunimoto DY, Sharma S, Jalali S, Majji AB, Rao TN, et al. Relationship between clinical presentation and visual outcome in postoperative and posttraumatic endophthalmitis in south central India. *Indian J Ophthalmol* 2005; 53 : 5-16.
- Sharma S, Jalali S, Adiraju MV, Gopinathan U, Das T. Sensitivity and predictability of vitreous cytology, biopsy, and membrane filter culture in endophthalmitis. *Retina* 1996; 16 : 525-9.
- Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by standard single disk method. *Am J Pathol* 1964; 45 : 493-6.
- Forster RK, Zachary IG, Cottingham AJ, Norton EWD. Further observations on the diagnosis, etiology and treatment of endophthalmitis. *Tr Am Ophthalmol Soc* 1975; 73 : 221-30.
- Therese KL, Anand AR, Madhavan HN. Polymerase chain reaction in the diagnosis of bacterial endophthalmitis. *Br J Ophthalmol* 1998; 82 : 1078-82.
- Okhravi N, Adamson P, Caroll N, Dunlop A, Matheson MM, Towler HM. PCR-based evidence of bacterial involvement in eyes with suspected intraocular infection. *Invest Ophthalmol Vis Sci* 2000; 41 : 3474-9.
- Results of Endophthalmitis Vitrectomy Study. A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis. Endophthalmitis Vitrectomy Study Group. *Arch Ophthalmol* 1995; 113 : 1479-96.
- Marangon FB, Miller D, Giaconi JA, Alfonso EC. *In vitro* investigation of voriconazole susceptibility for keratitis and endophthalmitis fungal pathogens. *Am J Ophthalmol* 2004; 137 : 820-5.
- Benz MS, Scott IU, Flynn HW Jr, Unonius N, Miller D. Endophthalmitis isolates and antibiotic sensitivities: A six year review of culture proven cases. *Am J Ophthalmol* 2004; 137 : 38-42.
- Gupta A, Gupta V, Dogra MR, Chakrabarti A, Ray P, Ram J, et al. Fungal endophthalmitis after a single intravenous administration of presumably contaminated dextrose infusion fluid. *Retina* 2000; 20 : 262-8.
- Jalali S, Das T, Gupta S. Presumed non-infectious endophthalmitis after cataract surgery. *J Cat Refract Surg* 1996; 22 : 1492-7.
- Das S, Sharma S, Sahu SK, Nayak SS, Kar S. New antimicrobial spectrum of epidemic keratoconjunctivitis: clinical and laboratory aspects of an outbreak. *Br J Ophthalmol* 2008; 92 : 861-2.