#### MICROBIAL ENDOPTHALMITIS: INCIDENCE SPECTRUM AND ANTIBIOTIC SENSITIVITY PATTERN OF BACTERIAL ISOLATES

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#### HOW TO CITE THIS ARTICLE:

Vaibhavi Subhedar, Neelam Bharihoke, Praveen Singh, Poonam Gupta, Sudhir Kumar Jain, Kiran Chaturvedi. "Microbial Endopthalmitis: Incidence Spectrum and Antibiotic Sensitivity Pattern of Bacterial Isolates". Journal of Evidence based Medicine and Healthcare; Volume 1, Issue 12, November 24, 2014; Page: 1534-1541.

ABSTRACT: CONTEXT: Endopthalmitis is a microbial infection of the anterior and posterior segments of the eye following a surgical procedure traumatic penetrating injury or of endogenous origin. The visual prognosis depends widely on the timely identification of the infectious agent and the appropriate treatment. Here we present a study of 63 patients in which the incidence of endopthalmitis, spectrum of microorganisms and antibiotic susceptibility of bacterial isolates. Aims: To study the incidence, spectrum and antibiotic sensitivity pattern of bacterial isolates, to study the outcome of visual acuity post treatment and importance of microbiological diagnosis in treatment of microbial endopthalmitis. SETTINGS AND DESIGN: This study was carried out in the Dept of Microbiology Bombay hospital Indore. Aqueous and vitreous samples were collected aseptically by the retina specialist before injections of intravitreous antibiotics and were immediately transported to our lab for microbiological diagnosis. A total of 63 cases of endopthalmitis were studied from January 2013 to May 2014. METHODS AND MATERIAL: The samples were immediately cultured on BA, MA and SDA with chloramphenicol. Microscopy was done with centrifuged deposit. Antibiotic sensitivity test was performed for bacterial isolates by Kirby-Baurer's disc diffusion method. Clinical history of the patients was collected from the retina speciality centre. Statistical analysis used: Standard method for prevalence. RESULTS: Out of the total 63 cases of endopthalmitis 44(69.8%) developed infection post operatively, 13(20.63%)developed due to trauma and 6(9.5%) developed endogenously. Maximum patients 34(53.96%) presented symptoms within one week of exposure. The Grams stain of 59 out of 63 showed either pus cells or organisms or both of which 39 were culture positive 4 cases of which were positive for fungus. Out of the total 39 specimens showing growth 12(30.7%) were Staphylococcus aureus, 10(25.64%) were Streptococcus pneumoniae 4(10.25%) each were Klebsiella pneumoniae and Pseudomonas and 3(7.6%) were Actinomyces, Branhamella and a member of non-fermenter 1 each (2.56%). 4 fungi included, 1candida albicans, 1 Aspergillus niger and 2 cases of Fusarium sp. CONCLUSIONS: In our study out of total 63 specimens 35 showed bacterial growth, while 4 showed fungal growth. Preliminary report of microscopy which was informed to the treating ophthalmic surgeon was very helpful for prompt treatment of the infected cases. 20 patients had good improvement in visual acuity post treatment. **KEYWORDS:** endopthalmitis, visual acuity, etiological agents.

**INTRODUCTION:** Endopthalmitis is an infection of the anterior and posterior segments of the eye resulting from the introduction of microorganisms following a surgical procedure (postoperative), traumatic penetrating injury (posttraumatic) or metastasis from an infection of a distant site in the body (endogenous).<sup>(1, 2, 3)</sup> The vast majority of cases of endopthalmitis result from intraocular surgical procedures, in particular cataract surgery.<sup>(4, 5, 6)</sup> The etiological agents responsible include Gram positive bacteria, Gram negative bacteria as well as fungi.<sup>(1, 4, 5)</sup>

Over the past several decades, the number of postoperative endopthalmitis cases has risen steadily, owing to the increase in the number of invasive ocular surgeries performed.<sup>(7)</sup>

The etiological agents responsible for endopthalmitis include both Gram positive and Gram negative bacteria and fungi.<sup>(8, 9)</sup> Cases of post-surgical endopthalmitis are usually a result of the introduction of members of the normal microbiota of the eyelid and skin surrounding the eye, the most common cause being coagulase-negative staphylococci (CONS).<sup>(2, 10, 11)</sup> Post traumatic endopthalmitis are more frequently caused by environmental bacteria like Staphylococcus aureus and, while endogenous endopthalmitis include S.aureus, Streptococcal species and Gram negative bacteria including Klebsiella pneumonia, E.coli and fungal agents like Candida albicans.<sup>(12, 13, 14)</sup>

The clinical hallmarks of endopthalmitis are acute vision loss, severe ocular pain, periorbital swelling, hypopyon, proptosis and the presence of white cells and flare in the anterior chamber and vitreous.<sup>(15)</sup>

The visual prognosis can vary widely depending on the infectious agent, ranging from mild inflammation and full resolution to devastating blindness and loss of eye. <sup>(16)</sup> With good microbiological evaluation including drug sensitivity and timely intervention with appropriate medication results better outcome, as final visual outcome is heavily dependent on timely recognition and treatment.

Here we present a study of 63 cases of endopthalmitis in which the microbiological diagnosis helped the ophthalmologist in timely and appropriately treating the infected patients.

**SUBJECTS AND METHODS:** Aqueous and vitreous samples from 63 clinically confirmed patients of endopthalmitis were collected by the retina specialist and were sent to our Department of Microbiology Bombay Hospital Indore for microbiological investigation during January 2013 to May 2014. All the samples were collected before injection of intravitreous antibiotics.

All the samples were cultured on Blood agar, Mackonkeys agar and Sabouraud's dextrose agar with chloramphenicol for bacterial and fungal identification. Centrifuged smears of the specimens were observed in KOH preparation for observation of fungi and Grams stain smears were observed for identification of bacteria. The positive microscopic reports were immediately informed to the treating retina specialist, which was very helpful for initiating the right and timely treatment.

A few drops of the specimen were expressed through the needle onto the BA, MA and two SDA with chloramphenicol agar plates. The plates were incubated aerobically at 37°c, while the specimen inoculated on Sabourauds dextrose agar were placed at 25°c as well as at 37°c. The fungal cultures were kept for 21 days before reporting them as sterile. After aerobic culture, a drop of specimen was added to Robertson's cooked meat broth for enrichment of anaerobic

bacteria if suspected, which were sub cultured on BA and MA agar and incubated anaerobically for 7 days before reporting them as sterile.

If cultures on Blood agar and Mackonkeys agar were negative at the end of 48hrs, BA were placed at 25°c for another 7 days for the growth of fungus if by chance the specimen was scarce and could not be inoculated on SDA. All the 4 fungi which grew on SDA also grew on BA plates. The isolated bacteria and fungi were then identified by standard methods. The antibiotic sensitivity test was performed for the bacterial isolates by the standard Kirby-Baurer's disc diffusion method and the sensitive zone size were measured and reported as per the CLSI guidelines.

Whenever Gram-positive filamentous thin branching bacilli were seen in Gram stained smears, a modified Ziel Nelson stain (Kinyoun's method) was performed with 1% H<sub>2</sub>SO<sub>4</sub> to identify and distinguish Actinomyces and Nocardia sp.

Detailed clinical history of the patients was noted in the form of age, sex presenting clinical features, anatomical type of endopthalmitis, presenting visual acuity and time taken for the appearance of the symptoms post exposure to differentiate between early and late endopthalmitis.

**RESULTS:** Out of the total 63 cases of endopthalmitis 43(68.25%) were male patients and 20(331.75%) were female. Most cases of endopthalmitis were in the age group of 31-50 years. Out of the 63 cases, 44(69.84) patients were of post-operative endopthalmitis, 13(20.63%) were of post traumatic endopthalmitis and 6(9.52%) were of endogenous endopthalmitis (Table 1). 61 patients presented symptoms within 6 months of exposure (early endopthalmitis) 34 of them presenting symptoms within 1 week. Only 2 patients presented symptoms after 6 months (late endopthalmitis).

The microscopic findings as described in Table 2 suggests gram's stain of specimens of 48 patients with pus cells and bacteria, while 4 specimens showed fungal elements in the KOH preparations, 9 specimens showed only pus cells and 2 specimens showed only bacteria. 4 specimens did not show any pus cells or organisms. Of the total 59 specimens which were Gram's stain positive 35 showed bacterial growth while 4 showed fungal growth. The organism wise distribution as showed in Table 3 indicates that Staphylococcus aureus was the commonest organism isolated in cultures 12(30.7%), followed by Streptococcus pneumoniae 10(25.6%), Klebsiella pneumoniae and Pseudomonas 4 each(10.25%), Actinomyces 3(7.69%) followed by Branhamella sp and a member of nonfermenter 1 each(2.56%). The fungi isolated included Candida albicans 1(2.56%), Aspergillus niger 1(2.56%) and Fusarium sp. 2(5.12%).

The antibiotic sensitivity pattern of the organism indicates that Staphylococcus aureus showed good sensitivity for Vancomycin, Teicoplanin, Linezolide, Levofloxacin and chloramphenicol. Streptococcus pneumoniae showed sensitivity to Vancomycin, Clindamycin, Penicillin G, Erythromycin, Cefepime and Chloramphenicol. Klebsiella pneumoniae showed sensitivity to Piperacillin+tazobacum, Meropenem, Ertapenem and Tigecycline while Pseudomonas showed sensitivity to Colistin, Polymyxin-B, Aztreonam and Amikacin. Out of 63 patients nearly 20 patients had improved visual acuity post treatment (Table 4, 5). It was observed that the bacterial infection had better outcome as compared to fungal endopthalmitis.

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**DISCUSSION:** Endopthalmitis is a rare entity which occurs in any age group ranging from 1 week to 85 years. Even our study showed a variation in age group ranging from 1 to 80 years of age. This study attempted to look at the incidence of endopthalmitis, factors responsible and outcome of the patients after microbiological diagnosis and treatment.

Post-operative endopthalmitis poses a significant public health issue as millions of people have cataract surgery each year.<sup>(1, 17, 18)</sup> In our study also the incidence of post-operative endopthalmitis was high as compared to post traumatic and endogenous. Incidence of endopthalmitis has been declining in the past several decades due to improved surgical techniques, sterilization methods and better postoperative care and use of broad-spectrum antibiotics. Source of infection could not be evaluated in our series as the patients were referred from a distant collaborative eye clinic where microbiological facilities were not present.

In our study out of the total 63 samples received 35 specimens showed bacterial growth and 4 specimens showed fungal growth which correlates with the other studies. All the 4 fungi which grew on SDA also grew on BA plates which correlates with other studies.<sup>(19)</sup> As shown in table 4 and 5, final best corrected visual acuity was improved in nearly 20 patients, which correlates with other studies. Outcome of endogenous endopthalmitis is generally worse than exogenous endopthalmitis. However the incidence of endogenous endopthalmitis was very low in our study.

Gram stain techniques are very useful tools in the diagnosis and treatment of endopthalmitis. Even in our study preliminary report informed to the consultant ophthalmologist in the form of Gram's stain and KOH mount was very helpful in starting prompt and appropriate treatment which gave a better outcome.

The most common cause of infective endopthalmitis as reported in many studies is the normal skin flora i.e Coagulase negative Staphylococcus, but in our case Staphylococcus aureus was the commonest organism. The reason being most of the cases of endopthalmitis were postoperative and the infections were either nosocomially or iatrogenically acquired.<sup>(20)</sup> Second commonest organism was Streptococcus pneumoniae.<sup>(21)</sup>

To conclude, with good microbiological evaluation including drug sensitivity and timely intervention with appropriate medication results appear promising as seen in our case. Once the diagnosis has been made or strongly considered, prompt consultation with the ophthalmologist will help in improving the final visual acuity. In absence of microbiological data, clinical "prediction" is not reliable enough to guide antibiotic therapy. Limitation of our study was that the follow up period was short. Long term outcome could not be determined as the patient did not turn up for follow up as they were from distant places. Also we could not evaluate the collaborative eye clinic for the focus of the infection.

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Post Operative 44	No. of Cases		
Cataract Surgery	34		
Lens Implantation	5		
IV Injection	5		
Post Traumatic 13			
Dust exposure	3		
Needle Stick Injury	7		
RTA cases	3		
Endogenous	6		
Total	63		
Table No. 1: CLINICAL CLASSIFICATION OF ENDOPTHALMITIS			

MICROSCOPY	NO. OF PATIENTS	PERCENTAGE
Pus cells	9	14.2
Micro-organism	2	3.1
Pus cells+ micro-organism	48	76.1
No pus cells +no micro-organism	4	6.3
KOH (+ve)	4	6.3
Table no. 2: MICROSCOPIC EVALUATION OF THE SPECIMENS		



ORGANISMS	NO. OF ORGANISMS		
Staphylococcus aureus	12		
Streptococcus pneumoniae	10		
Klebsiella pneumoniae	4		
Pseudomonas spp.	4		
Actinomyces	3		
Member of nonfermenter	1		
Branhamella spp.	1		
Candida albicans	1		
Aspergillus niger	1		
Fusarium spp.	2		
Total-	39		
Table no. 3: ORGANISM WISE DISTRIBUTION			
OF SPECIMENS SHOWING GROWTH			

PRESENTING VISUAL ACUITY	NO. OF PATIENTS	PERCENTAGE
Hand movement	18	28.5
Counting figure	30	47.6
Vision 1/60-3/60	8	12.6
Vision 6/60-6/36	6	9.5
Table no. 4: PRESENTING VISUAL ACUITY BEFORE TREATMENT		

BEST CORRECTED VISUAL ACUITY	<b>NO. OF PATIENTS</b>	PERCENTAGE
Hand movement	12	19.4
Counting figure	18	28.7
Vision 6/6-6/60	20	31.7
Vision 3/60-1/60	13	20.6
Table no. 5: BEST CORRECTED VISUAL ACUITY POST TREATMENT		

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> Date of Submission: 28/10/2014. Date of Peer Review: 29/10/2014. Date of Acceptance: 08/11/2014. Date of Publishing: 21/11/2014.