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ORIGINAL PAPER

Ocular culture-proven endogenous endophthalmitis: a 5-year retrospective study of the microorganism spectrum at a tertiary referral center in Turkey

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Abstract
Purpose The aim of this study was to review the clinical profile of endogenous endophthalmitis (EE), including predisposing systemic conditions, responsible microorganisms, clinical presentations, and outcomes.
Methods We reviewed data from 21 eyes of 15 patients diagnosed with EE and compared their clinical characteristics over a 5-year period. All patients were ocular fluid cultures proven. Generalized estimating equations (GEE) were used to analyze the effects of vitrectomy, diabetes, malignity, and clinical presentation condition on VA.
Results Diabetes was the most common illness of EE patients (40.0%). In this geographical region, *Pseudomonas aeruginosa* (4.8%), *Methicillin-sensitive Staphylococcus aureus* (4.8%), *Methicillin-resistant Staphylococcus aureus* (4.8%), and *Mycobacterium tuberculosis* (4.8%) were identified as causative bacterial microorganisms ($n = 5$) in patients with EE, and *Candida Species* (71.4%) and *Aspergillus* (4.8%) were identified as causative fungal microorganisms ($n = 16$) in patients with EE in the vitreous specimens. Fungemia (76.2%) (especially yeasts) was the most common extraocular infection source among patients with EE. Fourteen eyes (66.7%) were managed with intravitreal injections of antimicrobial medicines, and seven eyes (33.3%) also underwent vitrectomy. GEE models revealed that logMAR final VA values were found as lower than initial VA assessments.
Conclusion Depending on the different regions of the world, the characteristics of disease have been declared invariable. This study provides information about the clinical and microbiological profile of ocular culture-proven EE patients in a region of straddling the Asia and European continents. Aggressive medical and surgical treatment may result in favorable outcomes.

Keywords Bacterial endophthalmitis · Candida · Endogenous endophthalmitis · Fungal endophthalmitis · Treatment

Introduction

Endogenous endophthalmitis (EE) is a rare, rapidly progressive and potentially blinding, intraocular infection which is caused by the hematogenous spread of bacteria or fungi from distant foci. The visual prognosis in these cases is often poor, which frequently progresses to severe loss of vision, phthisis bulbi, and evisceration. The pathogens access the eye through the

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posterior vasculature of the globe. The organisms spread into the surrounding tissues causing microbial proliferation and inflammatory reactions within these tissues [1]. Infection then extends from the retina and the choroid into the vitreous cavity and subsequently to the anterior chamber of the eye. Once the organism enters the eye, it rapidly destroys ocular tissues [1]. Incidence rates are reported to be 0.04–0.4% with associated numerous predisposing risk factors, including diabetes mellitus (DM), malignancies, renal failure, indwelling catheters, liver disease, intravenous drug abuse (IVDA), acquired immune deficiency syndrome (AIDS), organ transplantation, urinary tract infections (UTI), and recent major surgeries [2–5]. The etiology of EE is multifactorial, and the list of potential causative organisms is extremely variable, especially in different geographical locations of the world. Gram-positive organisms are more common in Western countries (and *Candida*, followed by *Aspergillosis*, as fungal causes), whereas Gram-negative bacteria species were more prevalent in East Asian countries [6–9]. Studies aimed to describe clinical presentations, sources of infections, causative organisms, predisposing conditions, the effectiveness of different treatment modalities, and prognosis by comparisons between different regions of the world [8, 10–18]. In this study, we aimed to identify these features of the disease and evaluate the clinical course of culture-proven EE patients in a tertiary clinic in Turkey.

Materials and methods

We conducted retrospective analyses of case records of 21 eyes of 15 patients with EE at our tertiary referral clinic from October 2012 to April 2017. The study protocol was approved by the local institutional ethics board and conducted according to the tenets of the Declaration of Helsinki. The data collected from the medical records included features such as age, gender, clinical presentations, predisposing factors, causative pathogens, treatment modalities, and visual outcomes. The diagnosis of EE was attributed to the following standards: unilateral or bilateral anterior uveitis and/or vitritis, the presence of retinitis foci, existence of an extraocular source of infection, and positive microbiologic cultures of any specimens such as blood, urine, and indwelling catheter. Precise inclusion criteria were that ocular cultures have to be positive for fungus and/or bacteria. Despite the presence of EE examination findings, if ocular culture was negative such patients were excluded in this study, even if one of the extraocular sample cultures was positive. The patient who had a history of eye trauma or intraocular surgery was also excluded. A history of predisposing factors, including chronic illnesses, DM, cancer or chemotherapy history, radiation therapy, renal failure, indwelling catheters, liver disease, IVDA, alcoholism, organ transplantation, total parenteral nutrition, blood transfusion, UTI, and recent surgeries, and any infection in the whole body was noted. Examination included visual acuity testing (VA), slit-lamp biomicroscopy, tonometry, and fundoscopy performed during the ophthalmic visits. In cases with the absence of red reflex, ultrasonography was done to exclude retinal detachment and to determine the status of vitreous inflammation. If a patient was in the intensive care unit (ICU), ophthalmic examination of both eyes was performed at the bedside of the patient. All cases underwent vitreous tap and had empirical intravitreal injection of antimicrobial agents before the culture results were known before performing PPV (pars plana vitrectomy) surgery. PPV surgery inclusion criteria were described as precise lack of response to intravitreal injections or the systemic therapy. When the general health condition of a patient was appropriate, PPV was performed. Ocular specimens were obtained for cultures via PPV. During the PPV, to avoid a diluted vitreous sample, the aspiration line was connected to 5-ml disposable syringe. While the surgeon was cutting and aspirating the vitreous, the assistant implemented delicate suction with a syringe. During this process, the infusion line was kept enclosed as much as possible. Three samples were acquired with this procedure. At the end of the surgery, the container of the vitrectomy machine was also sent to the microbiology department in sterile condition for the microbiological assessment. The samples were studied with Gram stain and used various microbiological methods for bacteria and fungi detection. Of the all four vitreous samples were seeded in seven different culture mediums. For the growth of fungi, the laboratory used two mediums at 30 and 37 °C temperatures: both Sabouraud's dextrose agar with chloramphenicol and Sabouraud's dextrose agar without chloramphenicol. For the growth of bacteria instead, the laboratory used five mediums at 37 °C temperature: blood agar, anaerob blood agar chocolate agar, MacConkey agar, and thioglycollate liquid medium. *Mycobacterium tuberculosis* was detected with polymerase chain reaction (PCR). Systemic and intravitreal antimicrobial therapies were noted. Intravitreal amphotericin B (5 µg/0.1 ml), vancomycin (1 mg/0.1 ml), cefazolin (2.25 mg/0.1 ml), and ceftazidime (2.25 mg/0.1 ml) were administered according to indicated causative microorganism.

VA was assessed in European decimals (with Snellen chart) and then converted to the logarithm of the minimum angle of resolution (logMAR) for computing. NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Kaysville Utah, USA) program was used for the statistical analysis. The following scales were applied to logMAR values for low vision states: counting fingers (CF), 1.9; hand motion (HM), 2.3; and light perception (LP), 2.7. Generalized estimating equations (GEE) were conducted to analyze the effects of vitrectomy, DM, malignity, and clinical presentation conditions on VA. Significance was defined as $p < 0.05$.

Results

Patient characteristics

A total of 21 eyes of 15 patients with EE were included in the study. One of the patients was unconscious and was examined in the ICU. This patient passed away due to cardiopulmonary complications at the end of the first month. In the initial ophthalmic examination, anterior chamber inflammation, vitreous cells, and haze were observed in all cases. Corneal edema, pupillary distortion secondary to synechiae formation, and cataractous lens changes were seen in 7 (33.3%), 5 (23.8%), and 4 (19.0%) eyes, respectively. Retina could be visualized in 5 (23.8%) eyes. Fluffy white retinal lesions extending into the vitreous, retinal hemorrhages, focal, or diffuse yellowish retinal lesions could be observed in these cases. The patients general characteristics are summarized in Table 1. The leading and most common predisposing systemic ailments in EE were evaluated. The leading and most common predisposing systemic ailments in EE were diabetes mellitus (DM) ($n = 6$, 40.0%), malignancy ($n = 5$, 33.3%), urinary tract infection ($n = 2$, 13.3%), and other medical conditions ($n = 7$, 46.7%), such as, pneumonia, long-term use of corticosteroids, jaw infection, alcohol abuse, and gastrointestinal surgery. All patients were given systemic antimicrobial drugs. Predisposing factors and systemic treatments are summarized in Table 2.

Microbiological results and treatments

A positive culture (only one revealed on PCR) from ocular or other body fluids were obtained in all patients ($n = 15$, 100%). Vitreous specimens were positive in all cases ($n = 15$, 100%). In three patients (20.0%), the causative microorganisms were identified only in vitreous samples. Other samples with positive cultures were blood ($n = 9$, 60%) and urine ($n = 1$, 6.7%). In two cases (13.3%), all samples (vitreous, blood, and urine) were positive. In one case, *Mycobacterium tuberculosis* was identified on PCR.

In 15 causative microorganism-proven cases, *Candida albicans* was the most frequently isolated microorganism in vitreous and all body samples. Among the 21 eyes of the study, 16 (76.2%) had fungal, 5 (23.8%) had bacterial isolates (*one Methicillin-resistant Staphylococcus aureus (MRSA),* one *Pseudomonas aeruginosa,* one *Methicillin-sensitive Staphylococcus Aureus,* one *Stenotrophomonas maltophilia, and* one *Mycobacterium tuberculosis*). Among fungal isolates, 15 (93.75%) were yeast (eleven *Candida albicans,* two *Candida glabrata,* one *Candida krusei, and* one *Candida parapsilosis*) and one was mold (*Aspergillosis*) (6.25%). Identified microorganisms are summarized in Table 3 and Fig. 1.

All cases were managed medically with intravenous and intravitreal injections. Overall, 14 eyes (66.7%) were solely treated with the medical approach; ten eyes received intravitreal antifungal agent (47.6%), one eye received the intravitreal antibiotic (4.8%), and three eyes received the intravitreal antifungal agent with antibiotic (14.3%). Seven eyes (33.3%) underwent vitrectomy (Table 3). During the follow-up period, two eyes (28.6%) needed a repeat PPV due to the persistence of recurrent intraocular infection. One of them underwent epiretinal membrane peeling due to accompanying epiretinal membrane. Five (71.4%) eyes had no complications. At the final examination, all eyes had clear vitreous cavity and flat retina.

Table 1 General characteristics of patients with endogenous endophthalmitis

Age (*years*)	Min–Max (Median)	19–77 (51.0)
	Mean ± SD	51.73 ± 18.11
Gender *n* (%)	Woman	8 (53.3)
	Man	7 (46.7)
Laterality *n* (%)	Unilateral	9 (60.0)
	Bilateral	6 (40.0)
Initial complaint* *n* (%)	Loss of vision	14 (93.3)
	Pain	9 (60.0)
	Redness	4 (26.7)
	Unconscious	1 (6.7)
Presentation *n* (%)	Outpatient	7 (46.7)
	ICU	8 (53.3)
Follow-up time (*month*)	Min–Max (Median)	1–55 (20.0)
	Mean ± SD	27.00 ± 18.47

ICU: *intensive care unit*

Multiple complaints could be seen in a patient

Table 2 Diagnosis, predisposing factors, and systemic treatments

Culture source *n* (%)	Vitreous	3 (20.0)
	Vitreous + Blood	9 (60.0)
	Vitreous + Urine	1 (6.7)
	Vitreous + Blood + Urine	2 (13.3)
Medical/surgical history *n* (%)	Malignancy	4 (26.7)
	DM	2 (13.3)
	DM + UTI	2 (13.3)
	Trauma	1 (6.7)
	DM + Pneumonia	1 (6.7)
	DM + Malignancy + Urosepsis	1 (6.7)
	Intestinal surgery	1 (6.7)
	Jaw infection	1 (6.7)
	Long-term steroid usage	1 (6.7)
	Alcoholism	1 (6.7)
Systemic treatment *n* (%)	Fluconazole	4 (26.7)
	Amphotericin B	2 (13.3)
	Cefuroxime	1 (6.7)
	Fluconazole + Ceftazidime	2 (13.3)
	Fluconazole + Tazocin	1 (6.7)
	Fluconazole + Moxifloxacin	1 (6.7)
	Fluconazole + Caspofungin + Moxifloxacin	1 (6.7)
	Fluconazole + Caspofungin + Vancomycin	1 (6.7)
	Fluconazole + Vancomycin +Tazocin + Moxifloxacin	1 (6.7)
	Antituberculosis combination treatment	1 (6.7)

DM: *diabetes mellitus*; *UTI*: *urinary tract infection*

Table 3 Treatment and microbiological profiles

Treatment *n* (%)	IVAF	10 (47.6)
	IVAB	1 (4.8)
	IVAF + IVAB	3 (14.3)
	PPV*	7 (33.3)
Identified microorganism *n* (%)	Bacteria	5 (23.8)
	Yeast	15 (71.4)
	Mold	1 (4.8)
Vitreous culture *n* (%)	*C albicans*	11 (52.0)
	C glabrata	2 (9.6)
	C krusei	1 (4.8)
	MRSA	1 (4.8)
	Pseudomonas aeruginosa	1 (4.8)
	MSSA	1 (4.8)
	Stenotrophomonas maltophilia	1 (4.8)
	Mycobacterium tuberculosis	1 (4.8)
	Aspergillus	1 (4.8)
	C parapsilosis	1 (4.8)
Blood and urine culture *n* (%)	*C albicans*	11 (52.0)
	C glabrata	2 (9.6)
	MSSA	1 (4.8)
	Mycobacterium tuberculosis	1 (4.8)
	Aspergillus	1 (4.8)
	C alb + S malth + A baum	1 (4.8)
	C krusei + S ang + F nuc	1 (4.8)
	A baum +P aur + E coli	1 (4.8)
	C para + MRSA + ECC + A baum + K pne	2 (9.6)

More than one causative microorganism was isolated in the same patient

A baum: Acinetobacter baumannii; *C alb: C albicans*; *C para: C parapsilosis*; *E coli: Escherichia coli*; *ECC: Enterobacter cloacae complex*; *F nuc: Fusobacterium nucleatum*; *IVAB: Intravitreal Antibacterial Injection*; *IVAF: Intravitreal Antifungal Injection*; *K pne: Klebsiella pneumoniae*; *MRSA: Methicillin-resistant Staphylococcus aureus*; *MSSA: Methicillin-sensitive Staphylococcus aureus*; *P aur: Pseudomonas aeruginosa*; *PPV: Pars plana vitrectomy*; *S ang: Streptococcus anginosus*; *S Malth: Stenotrophomonas maltophilia*

**PPV patients underwent intravitreal antimicrobial treatment as well*

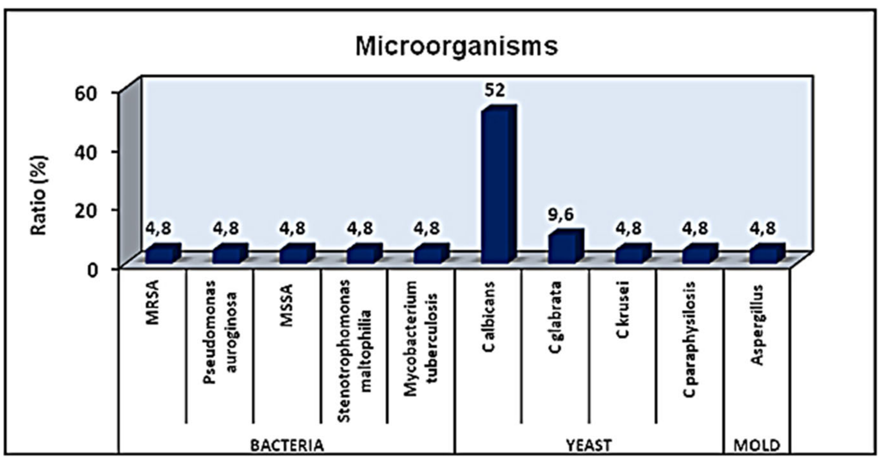

Fig. 1 Distribution of the causative organisms (isolated from vitreous). *MSSA*: *Methicillin-sensitive Staphylococcus aureus*, *MRSA*: *Methicillin-resistant Staphylococcus aureus*

Visual acuity and prognostic factors

Except an unconscious patient, VA of all eyes was available at initial and last follow-up examination. (Last follow-up examination was accepted as between 8 and 12 months of follow-up period for the statistical evaluation.)

For evaluation of effects of vitrectomy, DM, malignity, and clinical presentation condition on VA, five different GEE models were created. VA was assessed as the dependent variable in all GEE models. Time and side of the eye were defined as within-subject effect (Table 4).

Model 0

As an independent factor, only time was included. Effect of the time was found as significant ($p < 0.001$). Final VA values were found as lower at a 0.660 level than initial VA assessments [β (95% CI) − 0.660 (− 1.014, − 0.306)].

Model 1

Main effects of time and vitrectomy variables which were independent factors were investigated. Effects of interactions of time and vitrectomy variables were also assessed. Vitrectomy and effects of interactions of time and vitrectomy variables were found as insignificant; however, effect of time was assessed as significant ($p = 0.367$, $p = 0.454$, $p < 0.001$, respectively). Final VA values were found as lower at a 0.600 level than initial VA assessments [β (95% CI) − 0.600 (− 0.954, − 0.246)].

Table 4 Generalized estimating equations (GEE)

	Source	Wald χ^2	p
Model 0	Intercept	104.095	< 0.001**
	Time	13.318	< 0.001**
Model 1	Intercept	127.040	< 0.001**
	Time	12.618	< 0.001**
	Vitrectomy	0.813	0.367
	Time * Vitrectomy	0.561	0.454
Model 2	Intercept	101.473	< 0.001**
	Time	19.069	< 0.001**
	DM	1.422	0.233
	Time * DM	3.353	0.067
Model 3	Intercept	194.257	< 0.001**
	Time	22.263	< 0.001**
	Malignity	0.442	0.506
	Time * Malignity	2.108	0.147
Model 4	Intercept	110.953	< 0.001**
	Time	9.227	0.002**
	CPC	0.030	0.862
	Time * CPC	0.188	0.664

Summarize of GEE models

CPC: *clinical presentation condition*; *DM*: *diabetes mellitus*

**$p < 0.01$

Model 2

Main effects of time and DM variables which were independent factors were investigated. Effects of interactions of time and DM variables were also assessed. DM and effects of interactions of time and DM variables were found as insignificant; however, effect of time was assessed as significant ($p = 0.233$, $p = 0.067$, $p < 0.001$, respectively). Final VA values were found as lower at a 0.978 level than initial VA assessments [β (95% CI) − 0.978 (− 1.289, − 0.667)].

Model 3

Main effects of time and malignity variables which were independent factors were investigated. Effects of interactions of time and malignity variables were also assessed. Malignity and effects of interactions of time and malignity variables were found as insignificant; however, effect of time was assessed as significant ($p = 0.506$, $p = 0.147$, $p < 0.001$, respectively). Final VA values were found as lower at a 0.540 level than initial VA assessments [β (95% CI) − 0.540 (− 0.971, − 0.109)].

Model 4

Main effects of time and clinical presentation condition variables which were independent factors were investigated. Effects of interactions of time and clinical presentation condition variables were also assessed. Clinical presentation condition and effects of interactions of time and clinical presentation condition variables were found as insignificant; however, effect of time was assessed as significant ($p = 0.862$, $p = 0.664$, $p = 0.002$, respectively). Final VA values were found as lower at a 0.550 level than initial VA assessments [β (95% CI) − 0.550 (− 1.055, − 0.045)].

As a prognostic factor associated with poor functional outcome, causative microorganisms were analyzed. The same seven eyes which had poor VA (worse than counting fingers) at the first and at the last follow-up examination were evaluated in terms of identified microorganisms. In these eyes, the most common causative endophthalmitis pathogens were identified as *Candida* species ($n = 6$, 85.7%) and MRSA ($n = 1$, 14.3%), respectively. Relationship between causative microorganisms, visual outcome, and surgical approach is shown in Table 5.

Discussion

In this report, our aim was to investigate patients with EE restricted to our specific geographical transition zone between the European and Asian continents. All the patients included in the study were Turkish. Patients from a different race and ethnicity did not exist in this study. All the cases are living in a transcontinental zone between Europe and Asia, in this case, in and around Istanbul, Turkey. Given the relative rarity of the condition, in our opinion, case series which are reported from different locations in the world are valuable for the understanding the nature of the causative pathogen microorganisms in EE cases.

Predisposing medical conditions of EE were reported with different ratios from the various locations of the world. Shrader et al. [4] and Binder et al. [9] revealed that diabetes was the most common comorbid diseases. Cho et al. [19] reported that IVDA was the most common predisposing systemic condition of EE, which was similar to Connell et al's studies [16]. Ratra et al. [21] reported that DM was the most common predisposing systemic ailment for EE. In this study, DM (40.0%) was the most common predisposing systemic condition of the EE.

The organisms responsible for EE differ depending on the geographical location [1]. Fungi, especially *Candida* species, and Gram-positive bacteria were the major causative organisms of EE in Western patients, while Gram-negative bacteria were in East Asian patients [2, 7, 9–12, 14]. Cho et al. [19] compared the clinical features of EE in patient populations from the USA and Korea. In American patients, bacteria (46.3%) and fungi (34.3%) showed similar frequency as the causative organism. Gram-positive bacteria and *Candida* species were most frequently identified in EE. In Korean patients, unlike Western cases, bacterial endophthalmitis (65.6%) was more common than fungal (16.4%) as the causative organism. Gram-negative bacteria were more predominant in Korean patients. The results of this study, which compared West and East at a different location of the world, were similar to the previous reports [19]. In the present study, of 21 microorganism-proven vitreous samples, 16 (76.2%) had fungal and 5 (23.8%) had bacterial organisms. The leading cause of EE was found to be

Table 5 Relationship between causative microorganisms, visual outcome, and surgical approach

Microorganism ($n^* = 20$)	LogMAR visual acuity	Initial examination; *n (%)*		Final examination; *n (%)*	
		PPV (−)	PPV (+)	PPV (−)	PPV (+)
Bacteria ($n = 4$)	< 0.4	–	–	–	1 (33.3)
	0.4–1.0	–	–	–	1 (33.3)
	1.1–1.9	–	1 (33.3)	–	1 (33.3)
	> 1.9	1 (100)	2 (66.7)	1 (100)	–
Yeast ($n = 15$)	< 0.4	1 (9.1)	–	2 (18.2)	–
	0.4–1.0	–	–	1 (9.1)	3 (75.0)
	1.1–1.9	1 (9.1)	1 (25.0)	3 (27.3)	–
	> 1.9	9 (81.8)	3 (75.0)	5 (45.5)	1 (25.0)
Mold ($n = 1$)	< 0.4	–	–	–	–
	0.4–1.0	–	–	1 (100)	–
	1.1–1.9	–	–	–	–
	> 1.9	1 (100)	–	–	–

PPV: Pars plana vitrectomy

**Due to the presence of the unconscious patient, overall visual acuity assessments were performed on 20 eyes* similar to Western countries. *Candida* species dominancy was also similar to the West. Blood cultures were more likely to be positive than vitreous [3, 6, 20]. Ratra et al. [21] reported that the ocular samples tended to give positive culture results in a ratio of 58.6% in their case series. Cho et al. [19] reported that no positive culture result, extraocular or intraocular, was obtained in 18.0% of the eyes. Paulus et al. [22] reported that inclusion criteria of their study were blood culture positivity. The ocular culture was positive in none of the eye specimens in this report [22]. In the present study, we excluded such cases, since we aim to show the microbiological profile. In our opinion, due to this excluding criteria, the number of enrolled patients could be decreased in our study. Nonetheless, in this study, we preferred to include patients who were definitely diagnosed with positive culture in vitreous specimens to achieve clarity in comparison with all parameters, especially because in the ICU patients, generally more than one microorganism is identified in the various body samples. For this reason, in such patients, suspected causative organism of EE may not be precisely assessed. According to our understanding, this is the sole study in the literature which accepted these inclusion criteria in an EE research.

There are no specific treatment guidelines for EE [2, 23]. Because of the lack of a large series of cases, the preparation of specific guidelines for EE may be difficult. Therefore, during the treatment course, the clinicians may benefit from the experiences of the physicians in the case reports. The treatment of EE usually starts with intravitreal antimicrobial injections followed by systemic antibiotic or antifungal treatments, depending on the causative pathogen. However, sometimes EE may occur in inpatients and ICU patients who are given strong antimicrobial drugs. Such agents may not protect the patients from dissemination of the causative organisms to the eyes. This may indicate that intraocular penetration with systemic antibiotics and antifungal agents may be insufficient. Therefore, antimicrobial medications need to be administered via intravitreal injection in order to effectively treat the intraocular component of the infection [24, 25].

Case reports and case series have shown that EE is generally associated with poor VA outcomes [2, 7, 9, 12, 26]. The efficacy of vitrectomy for the treatment of EE is still controversial due to the lack of a large, randomized control study. Nevertheless, many studies have revealed that vitrectomy may be a useful approach for the diagnosis and treatment of EE [1, 19, 27]. We created GEE models for the evaluation of effect of some conditions on VA of patients with EE. The reason for using this is that there were patients with bilateral disease. Two eyes from the same patient would not be expected to behave as independent statistical events since they are linked. The typical manner to control for this is the use of GEE models. Effect of vitrectomy on VA was found as insignificant in GEE Model 1. According to the other GEE models, effects of DM, malignity, and clinical presentation condition on VA were also found as insignificant. However, final VA values were found as significantly improved than initial VA assessments in all cases. At the end of the follow-up period, favorable outcomes were gained in different conditions. Given the results of this study, it may be considered that more studies are needed to investigate the factors affecting prognosis in EE patients.

We expect that this study may improve the existing knowledge about this rare, but potentially harmful disease. According to our knowledge, this is the first report which evaluates those factors in our geographical location. Based on the results, the causative organisms of EE in our series are closer to the western part of the world. We hope that the results of this study contribute to the pathogen geographical map of the world's EE causative agents. These results may help to the clinics close to this geographical area during their practice. We also hope that this study will lead to larger, controlled, multicenter, prospective studies in the future.

Compliance with ethical standards

Conflict of interest None of the authors has conflict of interest with the submission. The authors alone are responsible for the content and writing of the paper

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