ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Infective Endocarditis Cases in İstanbul-Turkey: Evaluation of Bacteriological, Serological and Molecular Methods on the Microbiological Diagnosis of Infective Endocarditis

İstanbul, Türkiye'de Enfektif Endokardit Olguları: Enfektif Endokarditin Mikrobiyolojik Tanısında Bakteriyolojik, Serolojik ve Moleküler Yöntemlerin Değerlendirilmesi

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ABSTRACT Objective: In spite of being an infectious disease with low incidence, infective endocarditis (IE) has a high mortality risk. In this study, we aimed to evaluate the added values of bacteriological, serological, and molecular methods for the diagnosis of infective endocarditis in Istanbul, Turkey. **Material and Methods:** The study has a multicentre prospective design. Fifty adult patients (age > 14 years), who were hospitalized at the Department of Cardiology of Cerrahpasa Faculty of Medicine in Istanbul University, at Haseki Cardiology Institute in Istanbul University, and at Siyami Ersek Cardiovascular Surgery Hospital with the diagnosis of IE, were included in this study. **Results:** We have gathered 50 IE cases for the study. The mean age of the patients was 47 years. Causative microorganisms were detected in 37 patients (74%), and undefined causative agents were found in 13 (26%) patients. Causative microorganisms were staphylococci (28%), streptococci (12%), enterococci (8%), and *Brucella melitensis* (4%), *Pseudomonas aeruginosa* (2%), *Aggregatibacter actinomycetemcomitans* (2%), *Peptostreptococcus anaerobius* (2%), *Candida parapsilosis* (4%), *Bartonella henselae* (8%) and *Chlamydophila pneumoniae* (4%). **Conclusion:** In this study, among the cases in which the etiological agents were detected, 31 (62%) were culture-positive and 19 (38%) were culture-negative. To reduce the rate of culture-negative cases, samples for blood culture should be collected carefully, and new diagnostic techniques should be used.

Keywords: Endocarditis; infection; diagnosis

ÖZET Amaç:Düşük insidanslı enfeksiyöz bir hastalık olmasına rağmen, enfektif endokardit (IE) yüksek mortalite riskine sahiptir. Bu çalışmada, Türkiye'de enfektif endokardit tanısı için bakteriyolojik, serolojik ve moleküler yöntemlerin tanıdaki yerini değerlendirmeyi amaçladık. **Gereç ve Yöntemler:** Çok merkezli ve prospektif olarak tasarlanmış olan çalışmaya Ocak 2012 - Aralık 2016 tarihleri arasında, İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Kardiyoloji Anabilim Dalı, İstanbul Üniversitesi Haseki Kardiyoloji Enstitüsü ve Siyami Ersek Kalp Damar Cerrahisi Hastanesi'ne yatırılan ve kesin enfektif endokardit tanısı alan 50 (14 yaş üstü) hasta dahil edildi. Enfektif endokarditin mikrobiyolojik tanısı için bakteriyolojik, serolojik ve moleküler yöntemler kullandı. **Bulgular:** Çalışmaya dahil edilen 50 IE olgusunun yaş ortalaması 47 idi. Otuz yedi hastada (%74) olağan mikroorganizmalar saptanrken, 13 (%26) hastada tanımlanamayan nedensel ajanlar tespit edildi. Etken olarak tanımlanan mikroorganizmalar, stafilokoklar (%28), streptokoklar (%12), enterokoklar (%8), *Brucella melitensis* (%4), *Pseudomonas aeruginosa* (%2), *Aggregatibacter actinomycetemcomitans* (%2), *Peptostreptococcus anaerobius* (%2), *Candida parapsilosis* (%4), *Bartonella henselae* (%8) and *Chlamydophila pneumoniae* (%4) olarak tespit edildi. **Sonuç:** Bu çalışmada, tespit edilen etiyolojik ajanların, 31'i (% 62) kültür-pozitif, 6'sı(% 12) kültür-negatif idi. Kültür-negatif vakaların oranını azaltmak için, kan kültürü örnekleri dikkatle toplanmalı ve yeni tanı teknikleri kullanılmalıdır.

Anahtar Kelimeler: Endokarditler; enfeksiyon; tanı

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Infective endocarditis (IE) is a diagnostic and therapeutic challenge for clinicians. Despite major advances in cardiac imaging technology, in antimicrobial treatment and surgical techniques, the morbidity and mortality associated with infective endocarditis remain high. The profile of IE differs between developed and developing countries. In industrialized countries, a decrease in rheumatic heart disease and an increase in degenerative heart disease have led to an increase in patient age. Due to the increased frequency of comorbidities and the increased incidence of nosocomial *Staphylococcus aureus* infections, IE has still a high mortality. In developing countries, patient age, the location of acquisition of the infection, and causal microorganisms may be different due to the ongoing high rate of chronic rheumatic heart disease.[1-3]

The identification of causative microorganisms is crucial for the management of IE cases. Although the rate of identification of causative microorganisms is reported to be very high in the developed world, it is lower in developing countries.[3-6] In developed countries, the epidemiological features of IE are changing with the increase in longevity, with new predisposing factors, and with a higher frequency of nosocomial cases.[7-10]

The epidemiologic profile of IE has changed substantially over the past few years, especially in industrialized countries. The risk factors, the causative microorganisms, and the mean age of the patients with IE have also changed in developed countries.[10]

The aim of this study is to investigate the microbial pathogens in patients diagnosed with IE at different hospitals in Istanbul.

MATERIAL AND METHODS

PATIENT POPULATION

The study has a multicentre prospective design. Fifty adult patients (age>14 years), who were hospitalized between January 2012 and December 2016 at the Department of Cardiology of Cerrahpaşa Faculty of Medicine in Istanbul University, at Haseki Cardiology Institute of Istanbul University, and at Siyami Ersek Cardiovascular Surgery Hospital with the diagnosis of IE, were included in this study. The study was approved by the Clinical Research Ethics Board of Istanbul University, Cerrahpasa Faculty of Medicine (Ethical approval no.: 12831/05.04.2011) and recognized the standards of the Declaration of Helsinki. All patients gave informed consent to participate in the study. Written informed consent was obtained from all patients, as required by the institutional ethics committee. The following variables were recorded for each patient: age, sex, routine laboratory tests, echocardiography, underlying cardiac preposition, surgical interventions, blood culture results, and the results of serologic tests. The patients were diagnosed with definite infective endocarditis according to the modified Duke criteria.[1,9,10] All patients who were discharged from the hospital within 6 months before the onset of symptoms were accepted as having hospital-acquired IE.[9,10]

CONVENTIONAL MICROBIOLOGICAL DIAGNOSIS OF ENDOCARDITIS

Blood Cultures

Three sets of two blood samples of 8-10 mL each (BACTEC Plus an aerobic and an anaerobic vial [BD]) were taken 30 minutes apart. Blood cultures were studied by the BACTEC 9120 (Becton-Dickinson Diagnostic Instrument Systems, USA) automated system. Blood culture vials were incubated at 37°C for 5 days. Culture-negative bottles were incubated for 30 more days, especially for *Brucella* and the HACEK group bacteria to reproduce. Anaerobic culture bottles were incubated for 10 days, and the incubation period was extended when required. Fungal culture bottles were incubated for 14 days. Blood cultures, in which no reproduction was detected at the end of the specified periods, were evaluated as culture-negative endocarditis (CNE). The isolated aerobic bacteria were identified using standard clinical microbiology methods and API Staph and Vitek-2 (Bio-Merieux, FR), while anaerobic bacteria were identified using standard clinical microbiology methods and API 20A. Fungal agents were identified by standard clinical microbiology methods and API ID 32 C.[10,11]

VALVE CULTURE

Heart valve samples resected by surgical operations were moved to a microbiology laboratory in a sterile

container. They were then aseptically disintegrated in a sterile mortar in the biological safety cabinet. Valve samples were systematically cultured according to standard procedures.[12]

SEROLOGY

In this study, *Brucella spp.* and *Chlamydophila pneumoniae* were investigated by serological methods in the serum samples of the patients.[13-16] The Wright agglutination test, as well as blood cultures, were performed for the diagnosis of *Brucella spp.* Furthermore, *C. pneumoniae* IgM and IgG antibodies were investigated by the microimmunofluorescence (MIF-Euroimmun Labordiagnostica, Germany) method as a diagnostic marker for that pathogen.

MOLECULAR DIAGNOSTICS

In the serum samples and resected heart valve samples of the patient group with infective endocarditis, *Aggregatibacter actinomycetemcomitans* was investigated by 16S ribosomal RNA analysis, and *Bartonella henselae* and *Coxiella burnetii* DNA were investigated by the nested PCR method.[15,16]

Primers selected from the gltA gene region of *B. henselae* were used to reveal the *B. henselae* DNA, and the nested PCR method was applied as described previously by Norman et al. (Table 1).[17] The amplification was performed under the described conditions in a thermal cycler (MJ Research, Inc., MA, USA): The *B. henselae* control strain coded ATCC 49882 obtained from Refik Saydam Hygiene Institute was used as a positive control in the study. Furthermore, a negative control was also used to exclude cross-contamination.

All samples were studied by the nested PCR method for the determination of *C. burnetii* DNA, in which high-sensitivity and 4 double primers were used according to the nucleotide sequences of the com1 gene encoding 27-kDa OMP.[15,18] Positive control DNA was provided by Vircell Company. The primer sequences used in the PCR stage are presented in Table 2.

TABLE 1: Primer sequences used in the PCR stage.

Primer name		Sequence (5'-3')
Bart-1F	First stage forward	5' ggt ccc aac tct tgc cgc tat g 3'
Bart-1R	First stage reverse	5' cag ccgaca ctg cgt gct aat g 3'
Bart-2F	Second stage forward	5' atg cct aaa aat gtt aca aga 3'
Bart-2R	Second stage reverse	5' cgt gct aat gca aaa aga ac 3'

TABLE 2: Primer sequences used in the PCR stage.

Primer name		Sequence (5'-3')
OMP1	First stage forward	5'-agt aga agc atc cca agc att g-3'
OMP2	First stage reverse	5'-tgc ctg cta gct gta acg att g-3'
OMP3	Second stage forward	5'-gaa gcg caa caa gaa gaa cac-3'
OMP4	Second stage reverse	5'-ttg gaa gtt atc acg cag ttg-3'

TABLE 3: Baseline characteristics of 50 patients with infective endocarditis.

Valve type	Valve number (%)
Native aortic valve	9 (18 %)
Native mitral valve	11 (22 %)
Native mitral-aortic valve	1 (2 %)
Native tricuspid valve	1 (2 %)
Native mitral-aortic tricuspid valve	1 (2 %)
Prosthetic aortic valve	9 (18 %)
Prosthetic mitral valve	7 (14 %)
Prosthetic mitral-aortic valve	8 (16 %)
Pacemaker	3 (6 %)
Total	50

STATISTICAL ANALYSIS

Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). The chi-square test and Student's t-test were used for the univariate analysis of categorical and continuous variables of patients' characteristics, respectively.

RESULTS

CLINICAL CHARACTERISTICS ON ADMISSION

The baseline clinical features of the 50 infective endocarditis patients are shown in Table 3. The mean age of the patients was 47±31 years (range 15-78), and 21 patients (42%) were males, and 29 patients (58%) were females. Of the 50 cases in the study group, 23 (46%) had a native valve, while 27 (54%) had a prosthetic heart valve (3 had a pacing wire).

BLOOD CULTURES AND VALVE CULTURES

In this study, etiological agents were identified from the blood samples of 37 (74%) out of 50 cases with infective endocarditis, while etiological agents were not detected in 13 patients (26%) (Table 4, Table 5). *A. actinomycetemcomitans* was isolated in one sample of the valve cultures.

SEROLOGY

As a result of the investigation of *C. pneumoniae* IgM and IgG antibodies in the blood serum of the patient group and control group including 17 healthy individuals by the MIF method, IgM 1/16 (+) and IgG 1/256 (+) antibodies were detected in 2 patients at a significant level. Although IgM antibodies were negative in 7 patients, IgG antibodies were determined to be (+) at the titer of 1/512. Compared to the healthy control group, there was a statistically significant difference in the results ($p<0.05$). Serological results were negative in terms of *Brucella spp.* in these patients.

TABLE 4: Total evaluation in this study.

Culture	Number (%)
Culture-positive etiological agents	31 (62 %)
Culture-negative etiological agents	6 (12 %)
Undefined causative agents	13 (26 %)
Total	50 (100)

TABLE 5: Etiological agents of infective endocarditis in this study.

Causative Microorganisms	Number of cases (%)
Staphylococcus spp.	
Methicillin-resistant S. aureus-MRSA	2 (4)
Methicillin-susceptible S. aureus-MSSA	7 (14)
S. epidermidis	3 (6)
S. lugdunensis	2 (4)
Streptococcus spp.	
S. mutans	1 (2)
S. sanguis	1 (2)
S. salivarus	1 (2)
S. bovis	1 (2)
Nutritional variant streptococci (NVS)	
Granulicatella adiacens	1 (2)
Granulicatella elegans	1 (2)
Enterococcus spp.	4 (8)
Brucella melitensis	2 (4)
Pseudomonas aeruginosa	1 (2)
HACEK	
Aggregatibacter actinomycetemcomitans	1 (2)
Anaerobic bacteria	
Peptostreptococcus anaerobius	1 (2)
Candida parapsilosis	2 (4)
Bartonella henselae	4 (8)
Chlamydophila pneumoniae	2 (4)
Undefined causative agents	13 (26)
Total	50 (100)

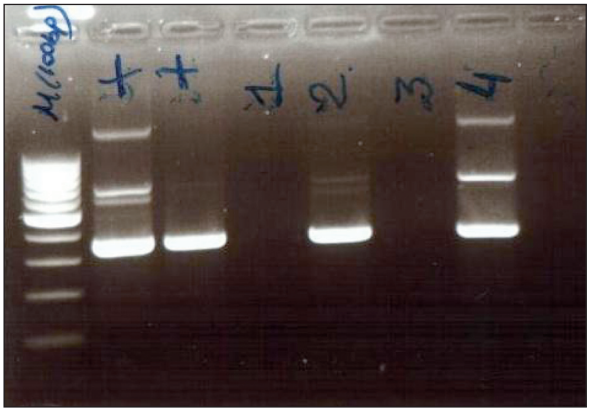

FIGURE 1: *Bartonella henselae* DNA by the nested PCR method.

MOLECULAR DIAGNOSTICS

The bacteria, which reproduced on the resected heart valve during the surgery of a case with infective endocarditis and were considered to belong to the HACEK group, were identified as *A. actinomycetemcomitans* by 16S ribosomal RNA gene analysis. *B. henselae* DNA was detected by the nested PCR method in four cases (in serum + heart valve samples) (Figure 1). No *C. burnetii* was detected in the samples.

DISCUSSION

IE is a rare disease. Recent studies conducted in the developed countries have reported the age of patients with IE to be >60 years.[18] In the studies conducted in Turkey, it has been reported that the average age varies between 36 and 51 years.[2,19] The average age of the patients in the present study is 47 years, and this is observed to be lower than the average age in developed countries. The most important reason for the low average age in Turkey is the high incidence of acute rheumatic fever (ARF) and congenital heart valve diseases. Accordingly, heart valve disease develops at an early age.[2,20,21] While the intravenous

drug use is reported to be a predisposing factor in 10% of all IE cases in developed countries, it is still a very rare condition in Turkey and is held responsible for less than 1% of the cases.[2]

Identifying etiological agents and making a microbiological diagnosis in IE are very important in terms of directing the antimicrobial treatment. In addition to blood cultures, serological methods, and, if necessary, molecular methods should be used to identify the agents for IE diagnosis. In developed countries, the rate of identification of causative microorganisms in IE cases is above 90%.[2,22] In the studies conducted in Turkey, the rate of identification of causative microorganisms varies between 50-84%.[2,21] In this study, as a result of the analyses performed with aerobic, anaerobic, and fungal cultures as well as serological and molecular methods, the detection rate of causative microorganisms in IE cases was found to be 74%; of these microorganisms, 62% were determined by culture methods and 12% by serological and molecular methods. The rate of IE without a causative agent was determined to be 26%.

Coagulase-negative *Staphylococcus* species (CNS) are among the factors that are frequently responsible for prosthetic valve endocarditis cases, and a native valve causes IE, especially in patients to whom an intravascular catheter has been applied.[10,23] *S. lugdunensis*, among the rare IE factors, has begun to be reported at increasing rates as an IE factor. Unlike the other CNS species, *S. lugdunensis*, which leads to IE especially in elderly individuals, causes infections similar to *S. aureus* infections in terms of tissue damage and clinical course, and therefore, its diagnosis and treatment are important. *S. lugdunensis* predominantly causes native valve involvement, and mitral, aortic, and both mitral and aortic valves are involved.[23,24] The CNS species identified in this study are 6% *S. epidermidis* and 2% *S. lugdunensis*. There were prosthetic valves (mitral and aortic valve) in 3 patients with *S. epidermidis*. *S. lugdunensis* was isolated as an IE factor from a 73-year-old female patient with a pacing wire, and the patient to whom antibiotic treatment was applied was discharged with full recovery.

Viridans streptococci continue to be the main factor for IE in children. The disease course is usually subacute with numerous non-specific symptoms. More than 80% of the patients have underlying heart diseases. In young females, most of the IE cases with mitral valve involvement consist of viridans streptococci.[2,5,10,25] Four (8%) cases caused by viridans streptococci were determined in this study. *S. mutans*, which is among the oral microbiota members and is identified as the major factor for tooth decay, was isolated from a 44-year-old patient with a native mitral valve. The patient underwent mitral valve replacement, and antibiotic therapy was applied, and the patient was discharged with full recovery. *S. sanguinis* is responsible for 16.4% of the patients with native valve endocarditis.[10] In this study, *S. sanguinis* was isolated from the hemoculture of a 16-year-old male patient with native aortic valve involvement. *S. salivarius* was reported to be the responsible agent in 1.3% of IE cases.[10] In this study, *S. salivarius* was isolated from a 21-year-old male patient with prosthetic aortic valve involvement. All three patients recovered with antibiotic treatment. *S. bovis* is found in the gastrointestinal tract microbiota of humans and animals. In this study, *S. bovis* was isolated from a 42-year-old male patient with prosthetic aortic valve involvement.

Granulicatella species, among nutritional variant streptococci (NVS), are found in the oral, urogenital, and intestinal microbiota of humans. It has been reported that most of the IE cases caused by *Granulicatella* species have their teeth treated in the last 6 months, and aortic and mitral valves are often involved. The number of the reported *G. adiacens* IE cases is over a hundred.[26] However, IE due to *G. elegans,* the most fastidious organism among all *Granulicatella* species, is very rare.[27] In this study, two different *Granulicatella* species were produced from the blood cultures of two patients. The first case, from which *G. adiacens* was isolated, was a 46-year-old male patient with native aortic and mitral valve involvement. The second case, from which *G. elegans* was isolated, was a 15-year-old male patient with prosthetic aortic valve involvement.

Enterococci are responsible for 5-18% of IE cases, and their incidence is observed to be gradually

increasing. The cases of enterococcal IE have a subacute course with non-specific symptoms, and it is reported that more than 40% of patients do not have predisposing factors.[2,10,28] In this study, *E. faecium* was isolated from a 60-year-old male patient with native aortic+mitral valve involvement.

Non-HACEK group Gram-negative bacilli are not among the common pathogens in IE cases. These Gram-negative bacilli are predominantly *Pseudomonas aeruginosa* and coliform bacteria (especially *Enterobacter* species).[10] In this study, *P. aeruginosa* was isolated from the blood culture of a 40-year-old male patient with native aortic+mitral valve involvement.

Brucella species are relatively fastidious bacteria, but they can be isolated from blood cultures in more than 80% of cases if the incubation period is extended to 4-6 weeks. The tube agglutination method is the gold standard in the diagnosis, and antibody titers are over 180 in active *Brucella* endocarditis. Furthermore, the IFA and ELISA tests are frequently used.[29,30] In this study, *B. melitensis* reproduced after the fourth week in a blood culture of a patient with native aortic+mitral valve involvement, and also, the Wright agglutination test was positive.

Gram-negative bacilli, known as the HACEK group, include *Haemophilus, Aggregatibacter* (formerly *Actinobacillus spp.*), *Eikinella,* and *Kingella species* and can be isolated during the standard blood culture incubation period using automated blood culture systems.[2,10] *Aggregatibacter spp.,* among the HACEK group bacteria, rarely causes IE, and the mortality rate in these subacute infections is about 30%. Approximately 40 *A. actinomycetemcomitans* IE cases have been reported to date.[6] IE cases due to these bacteria are very rare in our country.[2] In this study, *A. actinomycetemcomitans* was isolated from the blood culture of a 19-year-old patient with a prosthetic mitral valve, and the DNA of the bacterium was also detected in the serum sample by the nested PCR method.

The prevalence of anaerobic IE cases is lower compared to those caused by non-anaerobic pathogens and is around 2-16%.[22] Anaerobic bacteremia occurs in more than half of the individuals subjected to dental procedures. A late diagnosis causes complications such as valvular destruction, septic emboli, and septic shock at high rates, and death.[10,22] In this study, *Peptostreptococcus anaerobius* was produced from the blood culture of a 56-year-old patient with rheumatic heart disease and prosthetic aortic+mitral valve involvement.The patient has undergone several dental procedures within the last six months.

Although fungal endocarditis is still rare, it has been increasingly reported in alcoholics, individuals with a prosthetic heart valve, and hospitalized patients taking antibiotics for a long period. Of fungal endocarditis with a high mortality rate, it is reported that *Candida* species are responsible for only 1%. The most common species among *Candida* species is *C. albicans* (approximately 25% of fungal endocarditis), followed by *C. parapsilosis.*[10,11] In this study, *C. parapsilosis* was produced in two patients (4%) as a fungal endocarditis factor. The first one of the cases was a 41-year-old female patient with native mitral valve involvement. The other one was a 42-year-old male patient with prosthetic aortic and mitral valve involvement.

Blood culture-negative endocarditis is often very severe and difficult to diagnose.[6,8] In this study, while blood culture positivity was determined at the rate of 62%, culture-negative cases were determined at the rate of 38%. However, 12% of the blood culture-negative IE cases were found to have an etiological agent by serological and molecular methods. Thirteen (26%) blood culture-negative cases without a factor were considered to have received antimicrobial therapy, considering the intensity of antibiotic use in our country.

Bartonella species take an important place among the pathogens responsible for CNE.[31,32] The most commonly responsible ones are *B. henselae* and *B. quintana*. The diagnosis of *Bartonella* endocarditis is made by serological (immunofluorescent assays (IFA), PCR/DNA sequencing, and histological examinations in resected heart valve and blood samples.[27,30] In this study, *B. henselae* DNA was detected in five patients with CNE (four serum samples and one heart valve sample), i.e. at the rate of 10%, by the nested PCR method.

C. burnetii is another important bacterial pathogen isolated from CNEs, and Q fever endocarditis is reported to be the factor in 5% of all IE cases worldwide. The majority of patients with Q fever endocarditis have previous valvular heart disease.[28,31,33] In this study, the DNA of *C. burnetii* was investigated by the nested PCR method in patients with CNE, but it was not detected in any of them.

C. pneumoniae is rarely detected in patients with CNE. Immunohistochemical and molecular methods, particularly serological methods, are also used for the identification of these bacteria, obligate intracellular parasites, as IE factors.[26] There are some problems in the serological identification because the prevalence of antibodies against *C. pneumoniae* is considerably high in the community, and it is also reported that false-positive results are obtained due to common antigenicity with Bartonella species.[13] In this study, IgM and IgG antibodies formed against *C. pneumoniae* were investigated by the MIF method, and a statistically significant difference ($p<0.05$) was found between the healthy control group and culture-negative patient group. In the 2 cases in the patient group, IgM 1/16 was determined as (+) and IgG 1/256 as (+). These two IE cases between the ages of 54 and 67 years were considered as *C. pneumoniae* IE due to the fact that they exhibited IE findings clinically, both the IgM and IgG antibodies were detected at a significant level by the MIF method, and also that Bartonella species DNA could not be detected in these patients by the PCR method.

CONCLUSION

In this study, etiological agents were investigated by bacteriological, serological, and molecular methods in IE patients diagnosed according to the evaluations made with the modified Duke criteria. Of the 50 patients with IE, etiological agents were detected in 37 patients (74%) and could not be detected in 13 patients (26%). Among the cases, in which etiological agents were detected, etiological agents were determined in 31 (62%) culture-positive cases, and in 6 (12%) culture-negative cases by serological and molecular methods. *A. actinomycetemcomitans* was isolated from a patient's blood culture, and the bacterial DNA was also detected in the serum sample by the nested PCR method. In this study, any polymicrobial IE cases caused by two or more factors were not detected. To reduce the rate of culture-negative cases, samples for the blood culture should be carefully collected, and new diagnostic techniques such as serological tests and molecular tests should be used.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Sinem Özdemir, Müzeyyen Mamal Torun, Serap Şimsek Yavuz;* ***Design:*** *Müzeyyen Mamal Torun, Serap Şimsek Yavuz;* ***Control/Supervision:*** *Müzeyyen Mamal Torun;* ***Data Collection and/or Processing:*** *Sinem Özdemir;* ***Analysis and/or Interpretation:*** *Müzeyyen Mamal Torun, Serap Şimsek Yavuz;* ***Literature Review:*** *Sinem Özdemir;* ***Writing the Article:*** *Sinem Özdemir, Müzeyyen Mamal Torun;* ***Critical Review:*** *Sinem Özdemir, Müzeyyen Mamal Torun;* ***References and Fundings:*** *Serap Şimsek Yavuz, Sinem Özdemir;* ***Materials:*** *Serap Şimsek Yavuz, Sinem Özdemir.*

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