Correlation of Culture Results and Hematological Parameters with Tissue PCR Test in the Diagnosis of Periprosthetic Joint Infection

Periprostetik Eklem Enfeksiyonu Tanısında Kültür Sonuçları ve Hematolojik Parametrelerin Doku PCR Testi ile Korelasyonu

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ABSTRACT

Objective. Detection of the pathogen and appropriate early treatment are very important in the treatment of periprosthetic joint infection (PJI). In our study, we evaluated the correlation of culture results and hematologic parameters with tissue polymerase chain reaction (PCR) test in the diagnosis of PJI.

Methods. The study included 26 patients whose culture and tissue PCR samples were examined with suspicion of PJI in our clinic. Tissue samples were obtained for PCR and culture during revision surgery. All data were statistically analyzed. Cut-off values of acute phase reactants were tried to be determined according to the culture results. Pathogens were detected in only one patient in culture, whereas pathogens were detected in 5 patients in PCR test.

Results. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values of patients with positive PCR test results were significantly higher than those with negative PCR test results. As a result of the ROC analysis performed to determine the cut-off values, the cut-off value of CRP was 24.5 with 100.0% sensitivity and 90.0% specificity (p<0.05), and the cut-off value of ESR was 64.5 with 80.0% sensitivity and 85.7% specificity (p<0.05).

Conclusions. Diagnosing infection with PCR test reduces the possibility of making mistakes. In addition, we tried to find the cut-off values for CRP and ESR in the diagnosis of PJI in our study. To confirm these cut-off values we need prospective studies with larger number of patients.

Keywords. Periprosthetic joint infection, polymerase chain reaction, arthroplasty, tissue culture, acute phase reactant, pathogenic microorganism.

ÖZET

Giriş: Periprostetik eklem enfeksiyonu (PJI) tedavisinde patojenin tespiti ve uygun erken tedavi çok önemlidir. Çalışmamızda PJI tanısında kültür sonuçları ve hematolojik parametrelerin doku polimeraz zincir reaksiyonu (PCR) testi ile korelasyonunu değerlendirdik.

Gereç ve Yöntem: Çalışmaya kliniğimizde PJI şüphesiyle kültür ve doku PCR örnekleri incelenen 26 hasta dahil edildi. PCR ve kültür için doku örnekleri revizyon cerrahisi sırasında alındı. Tüm veriler istatistiksel olarak analiz edildi. Çalışmada akut faz reaktanlarının sınır (cut-off) değerleri kültür sonuçlarına göre belirlenmeye çalışıldı. Kültürde sadece bir hastada patojen tespit edilirken, PCR testinde 5 hastada patojen tespit edildi.

Bulgular: PCR test sonuçları pozitif olan hastaların C-reaktif protein (CRP) ve eritrosit sedimantasyon hızı (ESR) değerleri PCR test sonuçları negatif olanlara göre anlamlı derecede yüksekti. Sınır değerleri belirlemek için yapılan ROC analizi sonucunda CRP'nin sınır değeri %100,0 duyarlılık ve %90,0 özgüllük ile 24,5 (p<0,05), ESR'nin sınır değeri ise %80,0 duyarlılık ve %85,7 özgüllük ile 64,5 olarak belirlenmiştir (p<0,05).

Sonuç: PCR testi ile enfeksiyon tanısı koymak hata yapma olasılığını azaltmaktadır. Ayrıca çalışmamızda PJI tanısında CRP ve ESR için kesim değerlerini bulmaya çalıştık. Bu eşik değerlerini doğrulamak için daha fazla sayıda hastayı içeren prospektif çalışmalara ihtiyacımız var.

Anahtar Sözcükler: Periprostetik eklem enfeksiyonu, polimeraz zincir reaksiyonu, artroplasti, doku kültürü, akut faz reaktanı, patojen mikroorganizma.

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INTRODUCTION

Total or partial joint arthroplasty surgery is a frequently applied and very effective treatment method in advanced osteoarthritis of the hip and knee joints. More than 95% successful results have been reported in hip and knee arthroplasty in an average follow-up of 10 years (1). Despite the low infection rates after arthroplasty surgery, deep periprosthetic infection after surgery continues to be a serious problem for orthopedic surgeons.

We can examine the risk factors in periprostatic joint infections (PJI) under two main headings as patient and surgical factors. Some of the factors belonging to the patient are advanced age, obesity, diabetes, steroid use, malignancy, immunodeficiency, malnutrition, smoking and alcohol use. Surgical factors are the length of the surgical time, inappropriate antibiotic prophylaxis, revision surgery, and lack of sterilization (2-4). The most common pathogenic microorganisms responsible for the etiology of PJIs are bacteria. In addition, fungal and viral pathogens can cause infection (5-7). Timely correct diagnosis and treatment are very important in PJIs, and the criteria used in the diagnosis have been defined in previous studies (8). Commonly used diagnostic methods in PJI are blood erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and blood leukocyte count; the number of leukocytes in the joint fluid, the percentage of neutrophils are checked, and the isolation of the pathogen is examined by culture from joint fluid or joint synovial tissue samples (8,9). Serological tests may vary depending on the patient's age, gender, drugs used, and comorbidities, and the use of reference values from the normal population may lead to misdiagnosis and treatment in a patient with suspected periprosthetic joint infection. (10-12). Although fluid and tissue culture are considered as the gold standard in the diagnosis of periprostatic joint infection, false positive and false negative results have been reported at a rate of 5% to 37% (13-15). The culture result may be affected by antibiotic use and sowing time, and the existing microorganism may not be grown in culture (16,17). In the treatment of PJIs, it is important to identify the correct microorganism and to initiate the pathogen-specific treatment without delay (18).

There are many studies showing that the polymerase chain reaction (PCR) method of detecting bacterial DNA is a more effective method in the diagnosis of periprosthetic joint infection. Advantages such as accurate and short pathogen identification in PJIs have been reported (19,20).

In our study, we retrospectively evaluated the patients who underwent tissue culture and simultaneous PCR test with the suspicion of PJI in clinical and radiological evaluation. The PCR test was accepted as the gold standard in the diagnosis of infection, and the correlation of hematological parameters with PCR results was examined.

MATERIAL and METHOD

After the approval of the ethics committee of our institution (Date: 29.03.2022; No: 331), the patients whose culture and tissue PCR samples were examined with the suspicion of PJI in our clinic were retrospectively found from archive records and included in the study. A total of 26 patients, 19 women and 7 men (21 total knee arthroplasty, 5 total hip arthroplasty performed) with a mean age of 72.19 (56-85), were included in the study.

Before revision surgery, clinical and radiological evaluations of all patients were performed in terms of infection and ESR, CRP and blood leukocyte count, erythrocyte distribution width and neutrophil/lymphocyte ratio in blood count were studied. In the radiology of all patients in the study, there were signs of loosening of the prostheses. Patients who were using drugs that would suppress the immune system, affecting ESR, CRP and blood leukocyte count, urinary tract infection, oral and dental infection, and respiratory tract infection were excluded from the study.

Antibiotics of the patients who were using antibiotics were stopped 2 weeks before the surgery so that they would not affect the culture result. All patients underwent revision surgery. While a one-stage revision was performed in 10 patients, two-stage revision was performed in 16 patients with infection findings. Samples were taken from the patients for PCR testing and tissue culture during surgery. The determination of whether to pursue a one-stage or two-stage revision surgery was predicated upon an extensive evaluation of the clinical and radiological observations, in conjunction with the analysis of pertinent blood parameters. Two-stage revision surgery was undertaken in patients exhibiting elevated blood values of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and leukocyte count, surpassing normal levels. Additionally, patients who displayed clinical manifestations indicative of infection, such as localized temperature elevation and redness, coupled with radiological indications suggestive of abscess formation and prosthetic loosening, were selected as candidates for two-stage revision surgery. Two-stage revision surgery was performed in 16 patients with clinical, radiological and intraoperative evaluation and PJI findings. In the first session, all implants were removed and extensive debridement was performed. An antibiotic spacer was placed. Appropriate samples were taken from the tissues thought to be infected for culture and PCR examination.

The PCR used in our study is the general name of the reactions applied for the enzymatic amplification of a specific region between two known DNA segments. In the study, the samples obtained from the tissues were separated under sterile conditions. DNA and RNA were determined from the obtained tissue. The enzyme Taq polymerase was used for separation and the microorganism was identified by obtaining a sufficient length of chain from a limited number of DNA segments by replication.

A revision was performed in the first session in 10 patients whose periprosthetic joint infection was not considered according to preoperative clinical examination and erythrocyte sedimentation rate, CRP and blood leukocyte results, but who had prosthesis loosening. In order not to miss the subclinical infection in these patients, samples were taken from the tissues and sent to the laboratory for culture and PCR analysis within 30 minutes. No pathogenic microorganism was detected by culture and PCR in any of the aforementioned 10 patients.

Samples were delivered to the appropriate laboratory for culture and PCR study within 30 minutes. The culture results gave results in an average of 3 days (between 2-4 days), and PCR within 24 hours. While culture growth was observed in only one of 16 patients, pathogenic microorganisms were detected in 5 patients by PCR test (Escherichia coli in one patient, Staphylococcus aureus in two patients, Clostridium bacterium in one patient and Staphylococcus epidermidis in one patient). Bacteria (Staphylococcus aureus) were detected both in culture and by PCR in one patient.

Statistical analysis

Statistical analyses were performed using a package program called SPSS (IBM SPSS Statistics 24). Frequency tables and descriptive statistics were used to interpret the findings. "Independent Sample-t" test (t-table value) for comparison of measurement values of two independent groups in data with normal distribution; "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data not having normal distribution. Fisher-Exact test was used to examine the relationships between two qualitative variables. ROC curves were used to determine the cut-off.

RESULTS

As a result of the statistical study, there was no statistically significant relationship between the results of the PCR test and the gender, surgical site and culture results of the patients (p>0,05) (Table 1).

Table 1. Examining the relationships between PCR status and some parameters

PCR status	Negative (n=21)		Positive (n=5)		Statistical analysis*
Variables	n	%	n	%	Probability
Gender					
Female	17	81,0	2	40,0	p=0,101
Male	4	19,0	3	60,0	
Case					
TKA	17	81,0	4	80,0	p=0,961
THA	4	19,0	1	20,0	
Culture result					
Negative	21	100,0	4	80,0	p=0,192
Positive	-	-	1	20,0	

* Fisher-Exact test was used to examine the relationships between two qualitative variables.
TKA- total knee arthroplasty; THA- total hip arthroplasty.
PCR - polymerase chain reaction

While there was no statistically significant difference in terms of age (years), WBC, RDW and N/L according to PCR groups (p>0.05), a statistically significant difference was found in terms of ESR and CRP (p<0.05).

The CRP and ESR values of those with positive PCR results were significantly higher than those with negative results (Table 2).

Table 2. Comparison of some parameters according to PCR status.

PCR	Negative (n=21)		Positive(n=5)		Statistical analysis *
Variable	$\bar{X} \pm S.S.$	Median [Min-Max]	$\bar{X} \pm S.S.$	Median [Min-Max]	Probability
Age (yr)	70,62±8,81	73,0 [56,0-83,0]	76,60±11,15	80,0 [57,0-85,0]	Z=-1,531 p=0,126
CRP	14,32±9,75	12,0 [3,0-48,0]	38,80±20,92	31,0 [26,0-76,0]	Z=-3,127 **p=0,002**
ESR	44,48±17,92	45,0 [16,0-84,0]	70,20±18,66	72,0 [40,0-87,0]	t=-2,865 **p=0,009**
WBC	7065,71±1861,94	6600,0 [3990,0-11000,0]	7940,00±1299,23	8000,0 [6600,0-10000,0]	t=-0,987 p=0,334
RDW	14,12±2,22	14,4 [9,1-18,1]	15,70±2,70	14,6 [13,1-18,6]	t=-1,372 p=0,251
N/L	2,61±0,96	2,6 [1,4-5,3]	3,15±1,07	3,1 [1,8-4,8]	Z=-1,334 p=0,182

* "Independent Sample-t" test (t-table value) for comparison of measurement values of two independent groups in data with normal distribution; "Mann-Whitney U" test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data not having normal distribution.

There was no statistically significant correlation between RDW and N/L in PCR positive patients. (p>0,05) (Table 3).

Table 3. Examination of the relationship between RDW and N/L in PCR positive patients.

PCR (+)	RDW	
Correlation*	R	P
N/L	-0,614	0,270

* Pearson correlation coefficient was used to analyze the relationships of two quantitative variables.

As a result of the ROC analysis to determine the cut-off values; it was determined that the PCR test was significant predictors of differentiating CRP and ESR values from positive patients.

It was detected that the cut-off value of CRP was 24,5 with 100,0% sensitivity and 90,0% specificity (p<0.05), the cut-off value of ESR 64,5 with 80,0% sensitivity and 85,7% specificity (p<0.05) (Graph 1 and table 4).
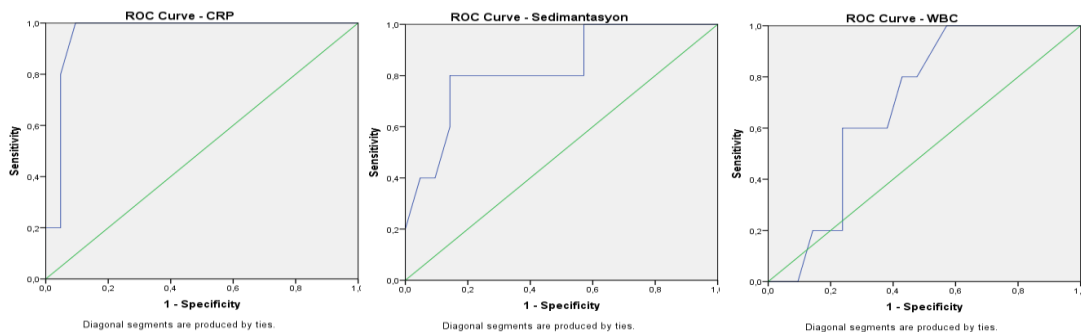
Graph 1. ROC curve according to PCR positive status of CRP, ESR and WBC parameters

Table 4. Cut-off values of CRP, ESR and WBC

Variable	Area	Standard Error	Probability	AUC %95 G.A.		Cut-off values
				Lower	Upper	
CRP	0,957	0,040	**0,002**	0,878	1,000	24,5
ESR	0,829	0,105	**0,025**	0,622	1,000	64,5
WBC	0,695	0,105	0,182	0,490	0,901	-

DISCUSSION

Although very successful results have been reported in knee and hip arthroplasty surgery, periprosthetic infection due to patient and surgical reasons continues at a rate of 0.3 – 2% (21-23). Although periprostatic joint infection is a rare complication, its treatment is very costly both in terms of health expenditures and seriously affecting the quality of life of the patient (24). Therefore, accurate identification of the pathogen and early initiation of appropriate treatment are very important in PJIs.

In cases where we suspect a periprosthetic joint infection, the appropriate treatment decision is made according to the clinical and radiological evaluations of the patient and the results of laboratory tests of blood and joint fluid/tissue samples (8). Frequently used laboratory tests; white blood cell count, ESR, CRP and joint fluid/tissue culture.

In recent years, PCR test has been used more frequently in the diagnosis of periprosthetic infections due to its advantages such as detecting the pathogen in the diagnosis of bacterial and viral infections and resulting in a shorter time (19,20,25,26). Liu et al. in their study, stated that PCR is a diagnostic method that has an equivalent or better diagnostic value than intraoperative tissue culture and can provide an important perspective in the diagnosis of periprosthetic joint infection (25). In the study of Renz et al. they reported that PCR may be advantageous because it has the same sensitivity rate as the culture, as well as giving results in a short time (26). In addition, Morgensten et al. in their study, compared the success of PCR in diagnosis with culture and stated that the success rate in diagnosis was similar (27). In our study, pathogen was detected by PCR test in 4 patients with culture-negative PJI.

In the study of Toossi et al. 1856 revision cases applied to 1543 patients were examined. Of these cases, periprosthetic infections were detected in 751 joints, including 463 knees and 288 hips. As a result of the study, they concluded that the leukocyte level in the blood has 55% sensitivity and 66% specificity, and the leukocyte level has minimal accuracy in the diagnosis of periprosthetic infections (28). In our study, there was no statistically significant difference in white cell counts in the blood of patients with PJI between those with negative PCR tests and those with positive PCR tests (p=0.334) (Table 2).

Although acute phase reactants such as ESR and CRP are frequently evaluated in the diagnosis of PJI, these parameters increase due to many reasons such as autoimmune diseases, other bacterial, viral and fungal infections, malignancies, and traumas. CRP, which is frequently used in the diagnosis of PJI, increases after surgery and falls to reference values at the end of an average of 3 weeks (10-12). In their meta-analysis study, Chen et al. reported that the sensitivity of the CRP value in diagnosing infection was 78%, the specificity was 81%, and the sensitivity of the ESR value was 68% and the specificity was 83% (29). Lindsay et al. reported that their sensitivity in diagnosing infection after 16 knee and 5 hip surgeries was 50% for CRP and 75% for ESR (30). In our study, we found that ESR and CRP values were statistically significantly higher in patients with positive PCR tests than in patients with negative PCR tests (p=0.009; p=0.002, respectively) (Table 2). In our cut-off study for blood laboratory tests, it was determined that the cut-off value of CRP was 24,5, and the cut-off value of ESR was 64,5, especially at high sensitivity and specificity rates (Graph 1 and Table 4). The limitations of our study are the limited number of patients, the period of not waiting long enough for culture results, and the retrospective nature of our study. The bacterial growth rate could have been higher if we had waited for the ideal 2-week period for culture results. However, based on the very low margin of error of PCR in detecting bacterial infection, we investigated the relationship between PCR testing and acute phase reactants and other blood parameters.

In conclusion, it is obvious that diagnosing infection with PCR test reduces the possibility of making mistakes. In addition, we tried to find the cut-off values for CRP and ESR in the diagnosis of PJI in our study. To confirm these cut-off values we need prospective studies with larger number of patients.

Conflict of interest
No conflict of interest was declared by the author.

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