

THE IMPORTANCE OF SONICATION IN THE DIAGNOSIS OF PROSTHETIC JOINT INFECTIONS

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ABSTRACT

Objective: The objective of this study is to investigate the efficacy of sonication method used to determine the cause in the diagnosis of prosthetic joint infections (PJI).

Material and Method: This study included 30 patients who were operated due to prosthesis infection and as a control group 10 patients whose prostheses were removed due to mechanical reasons and who had no sign of infection. Cultures were prepared from these tissue samples through gram staining and conventional methods. The prostheses removed from the patients were put into the sonication device in sterile water with ringer lactate. After sonication, Gram staining, cultures and polymerase chain reaction (PCR) were made.

Results: During the Gram staining done prior to the sonication, microorganisms were found in six patients (20%); after the sonication, microorganisms were seen in

nine patients (30%), but this difference was not statistically significant ($p>0.05$). While agents were found in the cultures of 11 patients (36.7%) that were prepared using the conventional method, agents were found in 20 patients (66.7%) with the sonication method. The rate of detecting the agent in the culture prepared after sonication was statistical significantly higher than in the culture prepared with conventional methods ($p=0.004$). The sensitivity of PCR was found 63.3%.

Conclusion: The sonication method of PJI is basically a procedure performed to increase the detectability of microorganisms. We found in the present study that the sonication method was obviously more precise than conventional methods in the microbiological diagnosis of PJI.

Keywords: Biofilm, prosthetic joint infection, sonication. Nobel Med 2017; 13(2): 22-27

PROSTETİK EKLEM ENFEKSİYONLARININ TANISINDA SONİKASYONUN ÖNEMİ

ÖZET

Amaç: Bu çalışmanın amacı protektik eklem enfeksiyonlarının tanısında, etkeni saptamaya yönelik olarak uygulanan bir yöntem olan sonikasyonun etkinliğinin araştırılmasıdır.

Materyal ve Metot: Bu çalışmaya protektik eklem enfeksiyonu nedeniyle opere edilen 30 hasta ve kontrol grubu olarak protezi mekanik nedenlerle çıkarılan, enfeksiyon bulgusu olmayan 10 hasta dahil edildi. Doku örneklerinden Gram boyama ve konvansiyonel yöntemlerle kültür yapıldı. Hastalardan çıkarılan protezler steril ringer laktatlı su içinde sonikasyon cihazına konuldu. Sonikasyon sonrası Gram boyama, kültür ve polimeraz zincir reaksiyonu (PZR) yapıldı.

Bulgular: Sonikasyon işlemi öncesi yapılan Gram boyamada altı hastada (%20) mikroorganizma

görüldürken, sonikasyon işlemi sonrası dokuz hastada (%30) mikroorganizma saptandı. Ancak bu fark istatistiksel olarak anlamlı değildi ($p>0,05$). Konvansiyonel yöntemle yapılan kültürler ile 11 hastada (%36,7) etken saptanırken, sonikasyon yöntemi ile 20 hastada (%66,7) etken tespit edildi. Sonikasyon sonrası yapılan kültürde etkeni tespit etme oranı konvansiyonel yöntemlerle yapılan kültüre göre istatistiksel olarak anlamlı düzeyde yüksek bulundu ($p=0,004$). PZR'nin duyarlılığı %63,3 tespit edildi.

Sonuç: Protektik eklem enfeksiyonlarında sonikasyon yöntemi temelde mikroorganizmaların saptanabilirliğini artırmaya yönelik olarak yapılan bir işlemdir. Bu çalışmada protektik eklem enfeksiyonlarının mikrobiyolojik tanısında sonikasyon konvansiyonel yöntemlere göre belirgin olarak daha duyarlı bir yöntem olarak saptanmıştır.

Anahtar kelimeler: Biyofilm, protektik eklem enfeksiyonu, sonikasyon. *Nobel Med* 2017; 13(2): 22-27

INTRODUCTION

A prosthetic joint operation is a surgical procedure that is performed at an increasing rate worldwide especially in the elderly population. The rate of these operations is expected to increase even further in the future.^{1,2} Although prosthetic joint procedures improve the quality of life, they can also involve complications such as aseptic necrosis and prosthetic joint infections.³⁻⁵ Infection is a rare complication of this surgical procedure (1-3%), of which diagnosis and treatment is difficult.^{1, 6-8} Although the prevalence of prosthetic joint infections is not known exactly in our country, it is accepted to be around the world average.

The principal problem in the diagnosis and treatment of prosthetic joint infections is that microorganisms form biofilms to protect themselves from environmental influences.^{1,3,5,9-16} In conventional cultures taken from these infections for diagnostic purposes, the sensitivity and specificity rate of reproducing microorganisms is rather low.^{17,18} The major factors affecting growth in a culture include previous antibiotic use, manner of taking specimens, sampling errors, concentration of bacteria, and ability of some microorganisms to form biofilms. In cases where the microorganism cannot be identified, the treatment is selected empirically, taking the possible factors into consideration. This situation negatively affects the success of a treatment.^{1,3,9-11}

Sonication is an increasingly more widely used method to enhance the detectability of the causative microorganisms in prosthetic joint infections. The purpose of this method is to disintegrate the biofilm layer in the infected prosthesis by using sound waves, thus increasing the reproduction rate of the infecting microorganism in a culture.^{1,10,11,14}

The objective of this study is to investigate the effectiveness of sonication to detect the cause of prosthetic joint infections.

MATERIAL AND METHOD

Study Protocol and Patients

This study was conducted with patients who presented with a prosthetic joint infection to the Infectious Diseases and Clinical Microbiology and the Orthopedics and Traumatology Outpatient Clinics of Selcuk University Medical School Hospital, and to the Orthopedics and Traumatology Outpatient Clinic of Farabi Hospital, between January 2012 and December 2013. The study included 30 patients who were operated due to a prosthetic joint infection (15 patients with an infected total hip prosthesis and 15 patients with an infected total knee prosthesis). In addition, there was a control group of 10 patients (five patients with a total hip prosthesis and five patients with a total knee prosthesis) whose prostheses were removed due to mechanical reasons, who had no signs of infection and whose laboratory values were normal

(leukocyte count, C-reactive protein level, erythrocyte sedimentation rate). There was no reproduction in the conventional culture method of the control group patients specimens.

The diagnosis of prosthetic joint infection was made in the patients with relevant clinical findings when one or more of the following criteria were met: presence of signs of infection in the aspirate taken before the operation; presence of synovial fluid or visible pus around the prosthesis; acute inflammation seen histopathologically in periprosthetic tissues; presence of prosthesis-related sinus tract; leukocyte count in joint fluid being above 1700/mm³ or more than 65% of them being granulocytes; and causative agent growth in joint fluid and tissue cultures. When none of these criteria was met, aseptic problems such as aseptic loosening and instability were explored. The inclusion criteria were being over 18 years of age, meeting the diagnostic criteria for a prosthetic joint infection, and not having used antibiotics within 14 days of the prosthesis removal. The patients diagnosed with a prosthetic joint infection by the Orthopedics and Traumatology Clinic underwent the first stage of a two-stage revision operation.

This study was performed in line with the World Medical Association Declaration of Helsinki (2000). An ethics committee approval (2011/09) was obtained for the study from the Ethics Committee of Selcuk University Medical School. An informed consent was obtained from each patient participating in the study. The study was conducted with the support of the Scientific Research Projects Coordination Office of Selcuk University Medical School.

Microbiological Examination and Sonication Method

Tissue specimens were collected from the patients during this operation to be used for cultures. Cultures were prepared from the tissue specimens through Gram stain and conventional methods. Each of the removed prostheses was placed in a sterile container and 400 mL of ringer lactate solution was added to it before being submitted to the microbiology laboratory. The container with the prosthesis and ringer lactate solution was vortexed for 30 seconds and 100 mL of the liquid was taken for cytocentrifuge. The preparation was stained using the Gram stain method. For the culture, 100 µL was taken from the liquid and inoculated into 5% sheep blood agar (Becton Dickinson Diagnostic Systems, Sparks, MD) and eosin methylene blue (EMB) agar (Becton Dickinson Diagnostic Systems, Sparks, MD). Then the specimen container was placed in the sonication device (model

DH-WUC- D10H; Daihan Science, Inc., South Korea) to be sonicated at 40 kHz for five minutes. Following the sonication, the specimen container was vortexed again for 30 minutes. To have the preparation ready, 100 µL was taken from the liquid through cytocentrifuging and was Gram-stained. For the culture, 100 µL was taken from the same liquid and inoculated into 5% sheep blood agar (Becton Dickinson Diagnostic Systems, Sparks, MD), EMB agar (Becton Dickinson Diagnostic Systems, Sparks, MD), and aerobic and anaerobic blood culture bottles (Becton Dickinson Diagnostic Systems, Sparks, MD). The specimens inoculated into blood culture bottles were monitored in the BACTEC 9120 fully-automated blood culture device (Becton Dickinson Diagnostic Systems, Sparks, MD). Dissociating inoculations were made from the bottles giving signals in the automated blood culture device into 5% sheep blood agar and EMB agar.¹⁷

Inoculations were made from the tissue biopsy specimens into the 5% sheep blood agar (Becton Dickinson Diagnostic Systems, Sparks, MD) and EMB agar (Becton Dickinson Diagnostic Systems, Sparks, MD). The remaining specimen was spread onto a slide and Gram-stained. The Gram stain preparations from the ringer lactate solution and tissue specimens were examined under a light microscope before and after the sonication, and the presence of any microorganisms was recorded. The identification and antibiotic sensitivity tests of the bacteria growing in the culture were carried out using the VITEK 2 Compact (bioMérieux, France) automated system.¹⁷

Examination of Polymerase Chain Reaction (PCR)

Deoxyribonucleic acids (DNAs) were isolated from the specimens using the MagNa Pure Compact Automatic Isolation System. Before the isolation, numbers were written on Sepsis beads (SeptiFast Lysis Kit cat no: 04 404 432 001) and 1.5 mL of specimens were distributed into tubes. They were lysed in lysis kit tubes at 7000 rpm for 70 seconds using the MagNa Lyser device. As soon as the device stopped, the tubes were taken to a MagNa Lyser cooling block and incubated for five to ten minutes to wait for the formation of three phases. Leaving foam at the topmost phase and beads at the bottom phase in the tube, this study's target material at the middle phase was taken to a separate tube with a pipette. The specimen tubes were numbered and five µL internal controls were distributed first. Four hundred µL of the middle phases of the bloods was disintegrated in Sepsis bead tubes and cooled, after which they were put into the tubes to which internal controls were added. For negative control, 400 µL

of apyrogenic water instead of specimen was put into a tube with an internal control. The specimen tubes were loaded into the MagNa Pure Compact Nucleic Acid Isolation System (MagNa Pure Compact Nucleic Acid Isolation Kit cat no: 03 730 964 00) and the protocol was started. The DNAs isolated after approximately 30 minutes (200 µL) were numbered and stored at -20°C.¹⁹

The RM 1a, RM 1b, DM G+, DM G- and DM F vials coming out of the SeptiFast Kit were thawed and vortexed. Six hundred µL of RM 1b was put into RM 1a with a pipette and was gently stirred. Similarly, 200 µL of Reactive Mixture was put into DM G+, DM G- and DM F vials with pipettes and gently stirred to form the Master mixes of MM G(+), MM G(-) and MM F. The required number of capillaries; that is, three capillaries for RCs (positions 1, 2 and 3), three capillaries for NC eluate (positions 4, 5 and 6) and three capillaries for each specimen eluate (position 7 and above), were placed on the SeptiFast Cooling Block. Using pipettes, 50µL of MM G(+) was put into each capillary in the upper line, 50µL of MM G(-) into each capillary in the middle line, and 50µL of MM F into each capillary in the lower line. 50µL of specimen eluate was put into each of the three capillaries in the upper line that contained MM G(+), MM G(-) and MM F and their mouths were plugged. The NC eluate was put into the capillaries in positions 4, 5 and 6. Similarly, RC G+, RC G- and RC F were put into capillary positions 2 and 3, respectively. All the capillaries were transferred based on their numbers in the LightCycler® Specimen Carousel. The LightCycler® Specimen Carousel was centrifuged with an LC Carousel Centrifuge 2.0. The LightCycler® Specimen Carousel was placed in to a LightCycler® 2.0 device. When the system finished running, the analysis stage began.¹⁹

Statistical Analysis

The data was analyzed using the SPSS 16.0 package program. The chi-square and Mc-Nemar tests were used in analyzing the categorical data. $p < 0.05$ was accepted as statistically significant.

RESULTS

Of the 30 patients included in the study, 17 were female and 13 male. The mean age of the patients was 68.9 ± 9.2 (45-88). The 10 patients in the control group consisted of six females and four males. The mean age of the control group was 73.1 ± 5.5 (64-84).

A comparison of the Gram stain before and after the sonication procedure revealed that six (20%) of the

30 patients had the causative agent in their Gram stain before the sonication procedure. These causative agents were Gram (+) cocci in four patients, Gram (-) bacilli in one patient and both Gram (+) cocci and Gram (-) bacilli in one patient. Causative agent was found in nine (30%) of the 30 patients after the sonication procedure. These causative agents were Gram (+) cocci in six patients, Gram (-) bacilli in one patient, and both Gram (+) cocci and Gram (-) bacilli in two patients. This difference was not statistically significant ($p > 0.05$) (Table).

When the conventional method and the sonication method were compared in detecting the causative microorganism, a causative agent was found in 11 (36.7%) of the 30 patients with the cultures prepared using the conventional method. These causative agents were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia*. A causative agent was found in 20 patients (66.7%) with the sonication method. These causative agents were *S.aureus*, *S.epidermidis*, *K.pneumoniae*, *K.oxytoca*, *E.faecium*, *E.faecalis*, *E.coli*, *P.aeruginosa*, and *B.cepacia*. Growth of more than one microorganism was found in two patients in both of the methods. The rate of detecting the causative agent in the cultures prepared after a sonication was found to be higher than in the cultures prepared with conventional methods, and this was statistically significant ($p = 0.004$) (Table). A causative agent was detected in 19 (63.3%) of the 30 patients in the examination of the sonication fluid with PCR.

DISCUSSION

Although infections are rare complication of prosthetic joint surgery, they are considered as an important problem due to the difficulty of diagnosis and treatment and also recurring need for surgical interventions, and the related expense.^{1,20-22}

The microbiological tests for detecting the causative agent in prosthetic joint infections include Gram stain, culture methods and PCR. In these infections, Gram stain is considered a test with low sensitivity but high specificity.^{5,10,23} The sensitivity of Gram stain may be affected by parameters such as manner of taking the material, contamination risk, dye used, intensity of microorganisms in the environment, and personnel assessing the case. In the study by Trampuz *et al.*³ which assessed prosthetic joint infections, the sensitivity of Gram stain was found to be 44.7% before the sonication, but the rate increased to 100% after the sonication, and these

Table. Microorganisms detected in Gram stain and grown in cultures using the conventional method and sonication method in patients with a prosthetic joint infection.								
Age / Gender	Location of prosthesis	Gram stain before sonication	Gram stain after sonication	p value	Conventional method	Sonication method	PCR results	p value
45/M	TKP	-	Gram (+) cocci Gram (-) bacilli	>0.05	-	<i>S. epidermidis</i>	+	0.004
68/F	TKP	-	-		-	<i>S. epidermidis</i>	+	
57/M	TKP	-	-		-	-	-	
65/F	TKP	-	-		-	-	-	
68/F	TKP	-	-		-	-	-	
73/F	TKP	Gram (+) cocci	Gram (+) cocci		<i>S. epidermidis</i>	<i>S. epidermidis</i>	+	
59/F	TKP	-	-		-	<i>S. epidermidis</i>	+	
69/M	TKP	-	-		-	-	-	
81/F	TKP	Gram (+) cocci	Gram (+) cocci		<i>S. epidermidis</i>	<i>S. epidermidis</i>	+	
73/M	TKP	-	-		<i>K. oxytoca</i>	<i>K. oxytoca</i>	+	
73/F	TKP	Gram (+) cocci	Gram (+) cocci		<i>S. epidermidis</i>	<i>S. epidermidis</i>	+	
73/F	TKP	Gram (+) cocci Gram (-) bacilli	Gram (+) cocci Gram (-) bacilli			<i>E. faecium</i> <i>E. coli</i>	+	
66/F	TKP	-	-		-	<i>S. epidermidis</i>	+	
64/M	TKP	-	-		-	<i>S. epidermidis</i>	+	
69/M	TKP	-	-		-	<i>S. epidermidis</i>	+	
75/F	THP	-	Gram (+) cocci		<i>E. faecalis</i>	<i>E. faecalis</i>	+	
60/M	THP	-	-		-	<i>K. pneumoniae</i>	+	
72/F	THP	-	-		<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	+	
62/M	THP	Gram (+) cocci	Gram (+) cocci		<i>B. cepacia</i> <i>S. epidermidis</i>	<i>B. cepacia</i> <i>S. epidermidis</i>	+	
71/F	THP	Gram (-) bacilli	Gram (-) bacilli		<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	+	
59/M	THP	-	-		<i>S. epidermidis</i>	<i>S. epidermidis</i>	+	
75/M	THP	-	-		-	-	-	
84/F	THP	-	-		-	-	-	
65/M	THP	-	-		<i>S.aureus</i>	<i>S.aureus</i>	+	
88/M	THP	-	Gram (+) cocci		-	-	-	
69/F	THP	-	-	-	-	-		
56/F	THP	-	-	-	-	-		
71/F	THP	-	-	-	<i>S. epidermidis</i>	-		
73/F	THP	-	-	-	-	-		
85/M	THP	-	-		<i>E.coli</i>	+		

M: Male, F: Female, TKP: Total knee prosthesis, THP: Total hip prosthesis, PCR: Polymerase chain reaction

results were found statistically significant. In this study, the sensitivity of Gram stain was found to be 20% before the sonication and 30% after the sonication, and the difference was not statistically significant ($p>0.05$) (Table). This was linked to the fact that Gram stain is influenced by many parameters.

The standard method used for detecting the causative agent in the diagnosis of prosthetic joint infections is the conventional culture method. The sensitivity and specificity of this method is low. The biofilm formed by microorganisms on the surface of the prosthesis is agreed to be the major cause that lowers the rate of detecting the causative agent in prosthetic joint infections.^{3,10-12,14,19} The rate of reproducing the causative agent increases with the sonication method, which is based on the principle of increasing the density of microorganisms in the environment by breaking apart the biofilm layer. The most important advantage of the sonication method is that it is simple, inexpensive, and easily used by several microbiology laboratories.^{5,9,14} Piper *et al.*¹⁵ found in their study on 33 patients with a prosthetic joint infection that the rate of detecting the causative agent was 54.5% using conventional method, while the rate increased to 66.7% after a sonication, in a 33 patient group with prosthetic joint infection. Trampuz *et al.*³ assessed 79 patients with a prosthetic joint infection, and they found the rate of detecting the causative agent to be 60.8% using conventional method and 78.5% after a sonication. Similarly, Evangelopoulos *et al.* found in their study assessing 34 patients with a prosthetic joint infection that the rate of detecting the causative agent was 47.1% using conventional method and 70.5% after a sonication.⁹ The increase in the rate of isolating the causative agent detected through the sonication method was agreed to be statistically significant in the studies performed on this issue. The results of this study are similar to the above-mentioned findings. In this study, which assessed 30 patients diagnosed with a prosthetic joint infection, the rates of growth in conventional cultures and in post-sonication cultures were 36.7% and 66.7%, respectively, and these results were statistically significant ($p=0.004$) (Table).

PCR is a fast test used to detect the genetic material of a microorganism, and it has varying sensitivity levels depending on the method used. In the study of Cazanave *et al.*²⁴, which assessed 144 prosthetic joint infections, the conventional culture, post-sonication culture, and post-sonication PCR results were reported as 70.1%, 72.9%, and 77.1%, respectively. This study found the conventional culture, post-sonication culture, and post-sonication PCR sensitivities as 36.7%, 66.7%, and 63.3%, respectively. In this study, no PCR positivity was found in the subjects who were negative by conventional culture. PCR positivity was detected in the samples that were applied a sonication procedure.

The causative agents found responsible for prosthetic joint infections most frequently are reported to be Gram-positive cocci (staphylococci and enterococci). Gram-negative microorganisms cause prosthetic joint infections at a rate of approximately 6-10%.^{1,5,25-27} Holinka *et al.*, Achermann *et al.* and Trampuz *et al.* reported Gram-positive microorganisms as the most frequently detected causative agents in their studies.^{11,19,28} Similarly, this study found that Gram-positive microorganisms were the most frequently found causative agents. In cases where the cause cannot be detected, Gram-positive bacteria should be considered first and the empirical

treatment approach should definitely include Gram-positive bacteria.

In conclusion, it has been shown in this study that in prosthetic joint infections, the possibility of detecting the cause increases when the sonication method is used as compared to the Gram stain and culture methods. Sonication is an important method that contributes to diagnosis in these infections which involve high mortality and morbidity rates.

*The authors declare that there are no conflicts of interest.



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