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Comparison of cultures immediately incubated intraoperatively with cultures incubated postoperatively in the laboratory for causes of periprosthetic loosening

Protez gevşemelerinin etyolojik tanısında ameliyathanede hemen ekilen kültürlerin laboratuvarda ekilenler ile karşılaştırılması

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ABSTRACT

Objectives: Our study aimed to show whether cultures that are incubated immediately in the operating room (OR) during surgery are superior to those prepared in the laboratory.

Patients and Methods: The results of bacteriologic cultures of the specimens processed immediately in the OR during surgery or afterwards in the bacteriology laboratory were compared. Thirty two cases were enrolled in this study. C-reactive protein (CRP) levels and erythrocyte sedimentation rates (ESR) levels were detected preoperatively. Liquid, swab, and tissue biopsy specimens were obtained to be processed separately in the OR or in the bacteriology laboratory. Each specimen was also examined by Gram-staining.

Results: Among 32 cases eight were infected. The average level of CRP was significantly higher in the infected group than in the aseptic group (p=0.003). There was no statistical significance for ESR levels between these groups. Of the eight infected patients, only three specimen out of 42 (7.1%) were Gram-positive. For the specimens processed in the operating room the isolation of the bacteria from liquid specimen cultures was found to be significantly higher than the swab, and tissue biopsy cultures (p<0.001).

Conclusion: Statistical analysis showed that the isolation of the bacteria from fluid material was statistically significant (p<0.001). Therefore, we conclude that inoculation of fluid material into the blood culture bottles in the OR may increase the chance of yielding organisms.

Key words: Arthroplasty loosening, Intraoperative culture, Infected loosening, Microbiological diagnosis.

ÖZET

Amaç: Çalışmanın amacı, ameliyat esnasında alınıp, ameliyathanede hemen ekilen materyallerin kültürlerinin, laboratuvarda ekilenlerden üstün olup olmadığını göstermektir.

Hastalar ve Yöntemler: Artroplasti gevşemelerinin etyolojik tanısı hastanın prognozu ve revizyon cerrahisinin sağkalımı için önemlidir. Çalışmamızda, revizyon ameliyatı esnasında alınıp, ameliyathanede hemen ekilen örneklerin kültür sonuçlarını, laboratuvarda ekilen örneklerin kültür sonuçları ile karşılaştırdık. Çalışmaya 32 vaka dahil edildi. Ameliyat öncesinde C-reaktif protein (CRP) ve eritrosit sedimentasyon hızı (ESH) tetkikleri istendi. Hem ameliyathanede hem de sonrasında laboratuvarda ekilmek üzere sıvı, sürüntü ve doku biyopsi örneklerinin her biri ikişer adet olarak alındı. Alınan her örnek Gram boyama ile de incelendi.

Bulgular: Otuz iki vakanın sekizi enfekte olarak değerlendirildi. CRP'nin enfekte grupta aseptik gruba göre anlamlı olarak yüksek olduğu (p=0,003), ESH için ise anlamlı fark olmadığı saptandı. 42 örneğin ancak üçü (%7,1) Gram pozitif boyandı. Ekimi ameliyathanede yapılan materyal grubunda sıvı örneklerde, sürüntü ve doku biyopsisi örneklerine kıyasla daha fazla üreme saptandı. Bu fark istatistiksel olarak anlamlı olarak bulundu (p<0,001).

Sonuç: Sıvı örneklerde üreme, diğer örneklerdeki üreme ile karşılaştırıldığında istatistiksel olarak anlamlı bulundu (p<0,001). Bu neden ile, artroplasti revizyonlarında sıvı örneklerin alınıp kan kültürü şişelerine hemen ameliyathanede ekilmesi mikroorganizma saptama ihtimalini arttırabilir.

Anahtar kelimeler: Artroplasti gevşemesi, Ameliyathanede ekim, Enfekte gevşeme, Mikrobiyolojik tanı

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Introduction

Total joint replacement has recently been giving more successful results due to the developments of new implant materials, surgical techniques and postoperative care and rehabilitation. No matter whether the replacement is aseptic or infected, loosening is a frequent and the most important complication reducing the success of total joint replacement.

Diagnosis and treatment prior to and during the revision surgery are important factors that will affect the life of the patient [1]. Identifying the cause of loosening is difficult, and although the reliability of some methods is quite high, there is no golden stan-

dard test for a definite diagnosis of infection. One of the most reliable diagnostic methods is to show the microorganisms by microbiological culturing processes [2]. However, there are confusing culture results obtained in sterile orthopaedic operations [3]. Even prominent infections with negative culture results have been reported [4]. It has been suggested that the use of fluid aspirate from intraoperative wounds immediately injected into blood culture vials is a highly specific method [5].

Our study aimed to show whether the cultures that are incubated immediately in the operating room (OR) during surgery are superior to those prepared in the laboratory.

Patients and Methods

Patients, who had been previously operated for either hemiarthroplasty, total hip or knee replacement and were planned for revision surgery due to loosening were enrolled in the study. Totally 32 cases (29 patients) were included. Informed consent of the patients was obtained and the study was conducted according to the Helsinki declaration.

Prior to surgery, C-reactive protein (CRP) levels and erythrocyte sedimentation rates (ESR) were determined for each patient. No other interventions than the routine pre-surgical preparations were carried out. The site of the skin incision was covered with iodophor impregnated incise drapes. Following the induction of anaesthesia, 1 gram of cefazolin was administered intravenously for prophylaxis. The lancet which was used for the incision of the skin was disposed of and new lancets were used for the incision of the subdermal and deep tissue regions. Specimens were taken from the fluid emerging at the opening of the joint capsule, the incised pseudocapsule, and the bed of the implant and especially from tissues where the most inflammation was observed.

All incubations in the OR and in the laboratory, the transport of materials and culture follow-ups were managed by the same doctor from the microbiology department. Duplicate samples of fluid, swab and tissue biopsy were obtained for immediate processing in the OR and afterwards in the laboratory. The joint fluid was inoculated into aerobic and anaerobic blood culture bottles (BACTEC®, Beckton Dickinson, United States of America). The blood culture bottles were incubated for seven days. At the same time, inoculations were also processed on chocolate and blood agar plates. Chocolate and blood agar plates were incubated in a 5% CO2 environment and in anerobic jars for aerobic and anaerobic cultures respectively for 24-48 hours at 37ºC. At the end of this incubation period, plates were inspected for the growth of bacteria. The swab and tissue samples were incubated only on chocolate and blood agar plates. For processing in the laboratory no special transportation method was used; the fluid material was transferred in the syringe with which it was withdrawn, the tissue specimen was transferred in a sterile container and the swab was transferred in its own container by the assigned doctor following the processing of the materials in the OR. Only aerobic culture studies using the same methods were repeated in the laboratory. Growth was designated by classical methods and the antibiotic sensitivities were determined according to the criteria of the Clinical and Laboratory Standards Institute (CLSI). Each specimen was also examined microscopically by Gram-staining. Levels of CRP at 10 mg/L and above and ESR at 30mm/h and above were accepted as positive.

Table I: Information about the patients with infected loosening.

No	Op	CRP	ESR	Material	OR incubated		Lab incubated		Organism
		mg/L	mm/h		Growth	Gram	Growth	Gram	
1.	Knee	90.9	55	Fluid	1	1	0	1	MS CNS
				Swab	0	0	0	0	
				Tissue	0	0	0	0	
2.	Knee	9.2	50	Fluid	1	0	1	0	MS CPS
				Swab	0	0	0	0	
				Tissue	0	0	0	0	
3.	Hip	36.9	35	Fluid	1	0	1	0	MS CNS
				Swab	0	0	0	0	
				Tissue	0	0	0	0	
4.	Hip	39.3	80	Fluid	NA	NA	NA	NA	MS CPS
				Swab	0	0	1	0	
				Tissue	1	1	0	0	
5.	Hip	40.9	40	Fluid	1	0	0	0	MS CNS
				Swab	0	0	0	0	
				Tissue	0	0	0	0	
6.	Hip	90.3	104	Fluid	1	0	1	0	MS CPS
				Swab	0	0	0	0	
				Tissue	0	0	0	0	
7.	Hip	61.3	82	Fluid	1	0	1	0	MR CNS
				Swab	0	0	0	0	
				Tissue	0	0	0	0	
8.	Hip	24	30	Fluid	NA	NA	NA	NA	MS CNS
				Swab	1	0	1	0	
				Tissue	1	0	1	0	

No=Patient number, Op=Operation, CRP=C-reactive protein, ESR=Erythrocyte sedimentation rate, OR=Operating room, Lab=Laboratory, 1=positive result, 0=negative result, NA=not available, MS=Methicillin sensitive, MR= Methicillin resistant, CNS=Coagulase negative staphilococci, CPS=Coagulase positive staphilococci

Windows SPSS program, Mann-Whitney U and X2 tests were used for the statistical analysis.

Results

In our study we evaluated the results of 32 cases (29 patients). Eight of the cases were male (25%), 24 were female (75%) and the average age was 60.15 (range: 27-78).

Revision surgeries were performed due to loosening of hip hemiarthroplasty in four, total hip replacements in 20, total knee replacements in eight of the patients. Patients were classified in two groups as infected loosening or as aseptic loosening according to the isolation of microorganisms in the cultures or on the demonstration of microorganisms on Gram stained smears. There were 24 (75%) aseptic and 8 (25%) infected cases of loosening. Of the materials processed in the OR, bacteria were isolated from fluid samples of six patients, from the tissue material of one patient, and no bacteria were isolated from the swab samples of any patient in infected group (Table I). Statistical analysis showed

that isolation of bacteria from fluid material was statistically significant (p< 0.001).

Of the materials processed in the laboratory, bacteria were isolated from fluid materials of four patients, swab materials of two patients, and tissue material of one patient (Table I). No statistically significant difference was found among the specimens in these groups.

Each type of specimen was compared within itself according to the processing environment. In the tissue and swab group no differences due to the incubation environment were identified. For the fluid groups no statistically significant differences were found although growth was observed in 6 cases incubated in the OR and in 4 cases for the laboratory group. In two patients there was not enough joint fluid to inoculate the BACTEC® bottles but according to the other specimens yielding bacteria these patients were classified as infected.

In the aseptic group, the average level of CRP was 18.06 mg/L (range:0-119) and of ESR 38.73 mm/h (range:5-110). The CRP level was positive in 26.08%, and the ESR was positive in 47.82% of the cases in this group.

In the infected group, the average level of CRP was 49.1 mg/L (range: 9.2-90.9) and of ESR was 59.5 mm/h (range: 30-104). The upper limits for CRP and ESR were exceeded in 87.5% of the infected cases.

The average level of CRP was significantly higher in the infected group than the aseptic group (p=0.003). There was no statistically significant difference for the ESR between the groups. Seven of 13 (53.8%) cases with high CRP levels and 8 of 19 (42.1%) cases with high ESR levels were found to be infected. On the other hand, 17 of 18 (94.4%) patients with normal CRP and 12 (100%) patients with normal ESR were found to be aseptic.

Bacteria were isolated in 7 of the total 12 (58.3%) patients whose CRP and ESR were both high. However, bacteria were not isolated in any of the 11 (100%) patients whose CRP and ESR were both low. It was established that the sensitivity and specificity of the CRP levels were 53.8% and 94.4% and of ESR levels were 42.1% and 100% respectively. The sensitivity and specificity of CRP and ESR together were found to be 58.3% and 100% respectively. The difference was significant when the CRP and ESR levels were evaluated together. It was normal than to be high (p=0.005). The sensitivity and specificity of CRP and ESR together were found to be 58.3% and 100% respectively. It was significant for CRP and ESR together to be normal than to be high (p=0.005).

We have examined the Gram-staining of all samples. In the infected group Gram-stained bacteria were observed in 2 out of 20 (10%) specimens processed in the operating room and in 1 out of 22 (4.54%) specimens incubated in the laboratory. From a total of 8 infected cases, only 3 specimens out of 42 (7.1%) were Gram-positive.

Discussion

Concepts of infected and aseptic loosening show differences in the diagnosis, treatment and prognosis for the patient. The treatment of a patient with aseptic loosening is usually limited by a somewhat simple revision surgery, but in patients with infectious loosening, multiple operations, long periods of hospitalisation and antibiotic use are needed. Also, problems such as a limitation of activities awaits these patients. Besides this, there are patients who have been considered to have an infection despite having no infection and therefore have needlessly experienced the above mentioned problems. There are also patients where infection is overlooked and the adequate and appropriate treatment is not applied; reimplantation in an infected bed would be a disaster for both the patient and the surgeon [6, 7].

Arthroplasties will increase as the population ages so that there is a need for appropriate diagnostic methods to reduce the cost and to decrease the adverse effects of the interventions performed due to infections that cannot be demonstrated. The diagnosis of an infected arthroplasty is quite difficult. There are a number of invasive or non-invasive, pre-operative or intra-operative diagnostic tests but the sensitivity and specifity of none of these is 100% [8, 9].

ESR and CRP are the most useful biochemical laboratory tests in the diagnosis of infections of total joint arthroplasties [7]. ESR levels of 30 or 35 mm/h are generally accepted to be abnormal and levels above these are a sign of infection until proven otherwise [5, 7, 10, 11]. CRP levels above 10 mg/L are accepted as signs of infection by many investigators [5, 7, 10, 12]. Levine, in a study evaluating 34 cases to whom revision surgery was applied due to infected total joint replacement, found that in 21 cases of 25 (84%) with the ESR above 30 mm/h coincided with clinical diagnosis and found this rate as 80% for CRP (12 out of 15 patients) [5].

Lachiewicz reported that infection was identified in 19 of 150 patients who had undergone revision total hip arthroplasty, and that the preoperative average ESR was 80.8 mm/h in 17 of these cases. On the other hand, high ESR levels were found in 58 of 116 patients (50%) who were not infected [13]. Sanzen accepted the upper limit of CRP as 20 mg/L for a diagnosis of infection and found that in 18 of 23 patients with infected total hip arthroplasties the value exceeded this level [11]. This author recommends that infection should be considered and investigated in patients who had received a total hip replacement and who present with pain and an increased CRP value. Rorabeck emphasized that high CRP levels, as well as high ESR levels, were very frequent in late chronic infections of total knee replacements but that neither of these two tests were adequate to make a diagnosis [14].

CRP has been stated to be superior to ESR in diagnosis of infected loosening [11, 12, 15]. We also found that the average CRP level was significantly high relative to ESR (p=0.003) in our study, whereas no significant difference was established for ESR.

Although Gram-staining, which is one of the methods applied during surgery and which looks like a method for rapid and direct observation and identification of bacteria, has a sensitivity reported in the literature as near 0%. Chimento, obtained no positive results from Gram-staining of materials taken from 32 infected total arthroplasty cases, and hence has reported the sensitivity of the method to be 0% [16]. He has concluded that an inability to detect the bacteria by Gram-staining does not necessarily exclude infection and a decision made during revision surgery should not rely on the findings of Gram-staining. Similar comments have been made in another study, which states that Gram-staining is not a reliable method in the diagnosis of infection in revision arthroplasty and thus could not be a determinant in the choice of treatment [17].

In the light of our Gram-stain results, we share the view of other authors and emphasize the fact that a negative Gram-stain cannot exclude the presence of infection and that this is not a reliable method to determine the course of treatment.

Although, intraoperative cultures are used as the golden standard method in the diagnosis of arthroplasty infections, their results are not always accurate. Culture results obtained even in sterile orthopaedic operations can sometimes be confusing. Fitzgerald, collected specimens of 658 cases of total hip replacements for culture during the operation [3]. Specimens were sent to the laboratory for processing. From the patients who were divided into two groups, positive culture results were obtained from 111 out of 437 (25%) patients who had no previous hip surgery and from 84 out of 221 (38%) patients who had a previous hip surgery. The author found the difference to be significant, but accepted the results generally as contamination. We would like to point out that in Fitzgerald's study only biopsy samples were taken and these were sent to the laboratory for processing.

Spangehl recommends that, at least three tissue samples should be sent to the laboratory and that the procedure should begin immediately [10]. He has also reported that the culture results should not be accepted as negative before final results are obtained. Even late growth and growth in the liquid media only are accepted as contamination; final decisions should be reached after the interpretation of all tests carried out before and during the operation.

Although it can be assumed that the cultures from the periprosthetic membrane should be superior to the cultures from the synovium or the pseudocapsule, it has been found in a recent study that these cultures are not superior one to the other [18]. In 31 patients who were not suspected of infection before or during the revision surgery Tsukayama accepted infection according to positive intraoperative cultures and applied intravenous antibiotic treatment for six weeks [19]. However, three of these patients had later to undergo exchange arthroplasty. He has stated that, preoperative evaluation was not helpful in the differentiation of infected and aseptic loosening of hip replacements and that, if growth is encountered in the culture specimens obtained during revision, the patient should be accepted to be infected and appropriate treatment should be applied.

Buchholz reported negative culture results from 12% of the patients with prominent infection and mixed organism culture results from 15% of patients, however, he did not give the details of the method of obtaining the cultures [4].

The routine procedure of obtaining material for intraoperative culture investigations is to swab or take a tissue biopsy from the suspected location and to send them to the laboratory.

However, there are a number of limitations like specimen transportation time, specimen storage media, and plating problems of these two techniques that can cause confusing microbiology results [5].

The intraoperative culture technique in our study is the extraction of fluid with a syringe from the joint after arthrotomy and the immediate inoculation into a standard blood culture bottle in the operating room. Levine outlined some of the advantages of this technique, such as; the minimalisation of the contamination potential and the enhancement of the growth of facultative organisms due to the direct incubation in the media [5]. In our study, from the materials group processed in the operating room, we have found a significant difference in the fluid compared to the swab and biopsy specimens. This difference shows the importance of the particular use of the fluid specimen in order to obtain a more definite result.

Conclusion

In cases where clinical and preoperative tests cannot definitely exclude infection, even one culture yielding bacteria – especially those inoculated in a blood culture bottle in the operating room should be accepted in favour of infection. In the present study, bacteria were isolated from fluid materials processed in the operating room in six cases in contrast to four in the laboratory. We believe that in a study that includes more infected cases, the difference will be more significant.

In the light of our recent findings, we suggest that inoculation of fluid material into the blood culture bottles in the operating room will increase the chance of yielding organism and will be a reliable diagnostic method in the differential diagnosis of arthroplasty loosening.

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