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ORIGINAL ARTICLE

Comparison of Soft Tissue Culture and Bone Tissue Culture in Diabetic Foot Infections with Osteomyelitis

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Abstract

Introduction: This study aimed to compare soft tissue and bone tissue cultures obtained from patients with diabetic foot infections accompanied by osteomyelitis.

Methods: The study included 36 patients aged 18 years and older who underwent bone debridement or amputation due to diabetic foot infection with osteomyelitis between April 1, 2017, and April 1, 2018. The data of 36 patients were analyzed prospectively. Bone tissue cultures were compared with soft tissue cultures taken during debridement or amputation. Statistical analyses were performed using IBM SPSS Statistics 22. The concordance between soft tissue and bone tissue cultures was assessed using the McNemar test and the kappa coefficient.

Results: A total of 36 patients participated in this study, 80.5% (29/36) of whom were male, with a mean age of 64.2±11.6 years (range 43-86). According to the Wagner classification, 14 patients (38.9%) had stage 3, 17 patients (47.2%) had stage 4, and 5 patients (13.9%) had stage 5 diabetic foot wounds. The most common wound localization was the plantar area. Osteomyelitis was diagnosed in 28 patients (68.7%) through clinical evaluation and direct radiographs, in 6 patients (16.6%) by MRI, in 1 patient (2.8%) by CT, and in 1 patient (2.8%) by histopathology. The most frequently isolated microorganisms from bone and soft tissue cultures were *Pseudomonas aeruginosa* (16.6%), coagulase-negative *Staphylococcus* (CNS) (15.1%), and *Escherichia coli* (13.6%). The same microorganism was detected in both bone and soft tissue cultures in 20 (55.5%) of the 36 patients. In five patients (13.9%) with culture-positive soft tissue specimens, bone culture specimens remained sterile. In one patient (2.8%) with a culture-positive bone specimen, the soft tissue specimen remained sterile. One patient (2.8%) had different microorganisms in bone and soft tissue specimens. In nine patients (25%), no bacterial growth was observed in either bone or soft tissue cultures. A total of 29 patients (80.5%) were found to have concordant bone and soft tissue cultures. In the statistical analysis, the kappa coefficient was 0.574, which was considered moderate agreement ($p>0.05$, kappa coefficient=0.574).

Discussion and Conclusion: According to the results of our study, soft tissue cultures may be used instead of bone tissue cultures to predict microorganisms in diabetic foot osteomyelitis. However, our findings need to be validated by studies with larger sample sizes.

Keywords: Bone tissue culture; diabetic foot infection; osteomyelitis.

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Approximately 422 million people worldwide have diabetes, the majority of whom reside in low- and middle-income countries. Diabetes is also responsible for 1.5 million deaths annually[1]. The Centers for Disease Control and Prevention (CDC) estimates that 37.3 million Americans are living with diabetes. Of these, 28.7 million have been diagnosed, while up to 8.5 million individuals are unaware that they have the disease[2]. In Türkiye, an estimated 7 million people have diabetes mellitus (DM), with more than one million affected by diabetic foot ulcers (DFU) and 500,000 suffering from diabetic foot infections (DFI)[3].

DFU is one of the most common complications in diabetic patients and represents a significant issue with major consequences for affected individuals. The lifetime risk of developing a DFU in diabetic patients ranges from 4% to 25%[4-6]. DFUs contribute to lower limb amputations, increased mortality rates, prolonged hospital stays, higher treatment costs, and reduced quality of life[5].

Diabetic foot is responsible for 50–70% of non-traumatic foot amputations[6]. Every 30 seconds, a limb is lost due to diabetic foot wounds. The combined effects of diabetes-related vascular disease and neuropathy are the primary contributors to diabetic foot wound formation. The complex nature of these lesions and the multiple factors involved in their etiopathogenesis necessitate a multidisciplinary approach in patient care[3].

Osteomyelitis is a significant complication in advanced stages of DFI and typically progresses along with infection in the surrounding soft tissue. Osteomyelitis is present in 10-15% of moderate infections and in 50% of severe infections associated with DFI[7].

Despite the fact that DFIs frequently necessitate amputation due to ineffective diagnostic and treatment strategies, they can be effectively treated with appropriate management. Osteomyelitis is particularly challenging to diagnose and requires prolonged treatment[8]. To administer adequate antibiotic therapy, it is essential to identify the causative agents of both osteomyelitis and soft tissue infections. Bone tissue culture is the preferred method for determining the causative pathogen of osteomyelitis[9]. However, bone biopsy is an invasive procedure, making bone tissue sampling a challenging approach. Consequently, osteomyelitis treatment is often based on soft tissue cultures or initiated empirically.

In this study, we aimed to evaluate the effectiveness of soft tissue cultures in guiding treatment and identifying the causative agents of osteomyelitis in the absence of bone tissue sampling. With these findings, we hope to contribute to the existing body of knowledge and improve the causal treatment strategy for diabetic foot osteomyelitis.

Materials and Methods

Patient Characteristics and Inclusion Criteria

This study was conducted at University of Health Sciences, Haydarpaşa Numune Training and Research Hospital. The study included patients aged 18 years or older who were diagnosed with DFI and osteomyelitis, admitted to the diabetic foot board and other outpatient clinics between April 1, 2017, and April 1, 2018, and who underwent bone debridement or amputation. Patients with diabetes who had no osteomyelitis and those younger than 18 years old were excluded. Both prospective and observational analyses were performed on patients whose data met these criteria.

The Haydarpaşa Numune Training and Research Hospital Clinical Research Ethics Committee of Health Sciences University granted ethical approval for the study (HNEAH-KAEK 2017/KK/32). All patients provided written, voluntary informed consent. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, as revised in subsequent amendments.

Study Design and Data Collection

A case report form was created for the study. Patient information was collected from both the patients and the hospital's information management system. The case report forms included details on the patient's date of admission, type and duration of diabetes mellitus, duration of DFI, demographics, underlying diseases (hypertension, renal disease, cardiovascular disease, malignancy, collagen tissue disease), anti-diabetic medications, history of hospitalization and recurrent foot infections, presence of ischemia, and antibiotic use in the three months prior to the procedure. Foot wounds were categorized according to the Wagner, PEDIS, and IDSA classification systems.

Additionally, laboratory parameters such as complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin, HbA1c, and biochemical values at admission were recorded. Osteomyelitis was diagnosed based on clinical signs and confirmed using direct radiography, MRI, or histology.

Under local or general anesthesia, bone and soft tissue samples were collected during the same surgical procedure. Concordance was defined as the presence or absence of

growth in both bone and soft tissue samples for the same microorganism with the same susceptibility pattern.

Soft tissue and bone tissue samples were inoculated onto MacConkey agar, chocolate agar, and 5% sheep blood agar. All plates were incubated at 37°C for 72 hours. Isolate identification was performed using the VITEK system.

Statistical Analysis

The Shapiro-Wilk test was used to assess the normality of data distribution. The Student's t-test was applied to compare normally distributed quantitative parameters between two groups. For qualitative data, the Fisher's exact test, Continuity (Yates) correction, and McNemar tests were used.

Correlations between factors were evaluated using Pearson correlation analysis, while the Kolmogorov-Smirnov test was employed to assess adherence to normal distribution.

Table 1. Demographic characteristics of patients

	Number of patients (n)	%
Gender		
Female	7	19.5
Male	29	80.5
Age		
<65 years	18	50
≥65 years	18	50
Anti-diabetic drugs used		
Insulin	31	86.1
Insulin+ OAD	3	8.3
Those who do not take medications	2	5.6
Use of antibiotics in the last 3 months	23	63.8
DA wounds according to Wagner grades		
Grade 3	14	38.9
Grade 4	17	47.2
Grade 5	5	13.9
DA wounds according to IDSA/PEDIS grades		
Moderate/Grade3	24	66.6
Severe/Grade 4	12	33.3

Parameters that deviated from normal distribution were compared using the Mann-Whitney U test. The kappa coefficient was calculated to determine the degree of concordance, and statistical significance was set at $p<0.05$.

Results

A total of 36 individuals were included in the study, of whom 29 (80.5%) were men and 7 (19.5%) were women. The patients ranged in age from 43 to 86 years, with a mean age of 64.2±11.6 years. Table 1 presents the demographic information of the patients.

The most prevalent comorbidity associated with diabetes in the study population was hypertension (66.6%). Renal failure and cardiovascular disease were identified in 12 (33.3%) and 19 (52.7%) patients, respectively. Table 2 presents the laboratory results of the patients at admission. A total of 23 (63.8%) patients had used antibiotics within the previous three months. Ten (27.7%) patients had received ciprofloxacin either alone or in combination with another antibiotic. The most commonly used antibiotic combination was ciprofloxacin and amoxicillin-clavulanic acid.

Table 1 also presents the distribution of patients according to the Wagner and IDSA/PEDIS classification systems. Eighteen patients (50%) had ulcers on their right extremity, 15 (41.6%) had ulcers on their left, and 3 (8.4%) had ulcers on both extremities. The sole of the foot was the most common wound localization, representing 27.7% of all cases at the time of presentation.

In addition to clinical symptoms, osteomyelitis was diagnosed in 28 patients (77.7%) by direct radiography, 6 patients (16.6%) by MRI, 1 patient (2.8%) by computed tomography, and 1 patient (2.8%) by histology.

A total of 15 patients (41.7%) underwent amputation, while 21 patients (58.3%) underwent debridement. One patient with bilateral DFI had an above-knee amputation on the right leg and debridement on the left foot.

Table 2. Laboratuary findings

Analysis	n	Average±SD	Median value	Smallest to largest value
ESR (mm/h)	31	75.0±24.7	70	32-137
CRP (mg/dl)	35	11.7±8.7	11.6	0.6-32.5
Leukocyte count (/mm^3)	36	13969.7±9631.9	11350	4260-60900
Procalcitonin (ng/m)	22	1.7±3.5	0.39	0.10-16.33
HbA1C (%)	29	8.3±1.9	8.1	5.8-12.1
Hemoglobin (g/dl)	36	10.8±1.6	10.9	7.14-13.2

ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

Surgical procedures included:

- Five toe amputations (33.3%)
- One foot amputation (6.66%)
- One heel amputation (6.66%)
- Three below-knee amputations (20%)
- Five above-knee amputations (33.3%)

Following amputation, 3 patients (8.3%) died due to cardiac causes. Among the deceased patients, 1 had undergone a below-knee amputation, while 2 had undergone above-knee amputations.

According to the Wagner classification system, amputation rates increased as the wound stage advanced. However, there was no statistically significant difference in amputation rates based on the IDSA/PEDIS classification.

Among the 24 patients (66.6%) with moderate (grade 3) DFUs based on the IDSA/PEDIS classification, amputation was performed in 10 patients (41.7%) and debridement in 14 patients (58.3%). Among the 12 patients (33.3%) with severe (grade 4) DFUs, 7 (58.3%) underwent debridement, and 5 (41.7%) underwent amputation ($p>0.05$).

Based on the Wagner classification:

- Among the 14 patients with grade 3 DFUs, 11 (78.5%) underwent debridement and 3 (21.5%) underwent amputation.
- Among the 17 patients with grade 4 DFUs, 10 (58.8%) underwent debridement and 7 (41.2%) underwent amputation.
- All 5 patients with grade 5 DFUs underwent amputation.

Overall, amputations were performed in 15 of 36 patients, yielding a total amputation rate of 41.6% ($p<0.05$).

During the surgical procedure, soft tissue and bone samples were collected for culture. In the bone cultures of 14 patients (38.8%) and the soft tissue cultures of 10 patients (27.7%), no pathogens were identified. Because the antimicrobial drugs used by the patients were not discontinued, the pathogen could not be identified in one-fourth of the patients.

In our study, a total of 30 microorganisms were detected in the bone tissue cultures of 22 patients. Among the 30 isolates from bone tissue cultures, 11 (36.7%) were aerobic gram-positive bacteria, while 19 (63.3%) were aerobic gram-negative bacteria. In soft tissue cultures, 36 microorganisms were detected in 26 patients. Of these 36 isolates, 12 (33.3%) were aerobic gram-positive bacteria, while 24 (66.6%) were gram-negative.

The distribution of the identified pathogens is presented in Table 3. The most prevalent pathogens in all samples were *Pseudomonas aeruginosa* (16.6%), coagulase-negative *Staphylococcus* (CNS) (15.15%), and *Escherichia coli* (13.6%).

Table 3. Microorganisms isolated from bone and soft tissue

Microorganism	Softtissue n=36 (%)	Bone tissue n=30 (%)	Total n=66 (%)
Pseudomonas aeruginosa	7 (19.4)	4 (13.3)	11 (16.6)
Coagulase negative Staph(CNS)	6 (16.6)	4 (13.3)	10 (15.15)
E.coli	5 (13.8)	4 (13.3)	9 (13.6)
Corynebacterium spp.	3 (8.2)	5 (16.8)	8 (12.13)
Enterobacter spp.	2 (5.6)	2 (6.7)	4 (6.07)
Acinetobacter baumanii	2 (5.6)	2 (6.7)	4 (6.07)
Morganella morgani	2 (5.6)	2 (6.7)	4 (6.07)
Serratia marcescens	2 (5.6)	2 (6.7)	4 (6.07)
Proteus spp.	1 (2.8)	1 (3.3)	2 (3.04)
Enterococcus faecalis	1 (2.8)	1 (3.3)	2 (3.04)
Streptococcus	1 (2.8)	1 (3.3)	2 (3.04)
Klebsiella pneumoniae	1 (2.8)	1 (3.3)	2 (3.04)
Sphingomona spaucimobilis	1 (2.8)	1 (3.3)	2 (3.04)
Citrobacterkoseri	1 (2.8)	0	1 (1.52)
Listeria spp.	1 (2.8)	0	1 (1.52)

Among the 36 patients in our study, blood cultures were obtained from 20 patients (55.5%). Blood culture growth was observed in five cases. However, only two of these five patients had the same causative agent as those identified in their bone and soft tissue cultures. In addition to the pathogen detected in blood cultures, other microorganisms were also identified in the tissue cultures of these patients. Table 4 presents the distribution of bacteria in patients with positive blood cultures.

In 20 patients (55.5%), the same bacterium was identified in both bone and soft tissue cultures. In five patients (13.9%), the causative agent was found in the soft tissue culture, but no growth was observed in the bone tissue culture. In contrast, in one patient (2.8%), the causative agent was detected in the bone tissue culture but not in the soft tissue culture. Different pathogens grew in bone and soft tissue cultures in one patient (2.8%). In nine patients (25%), no pathogens were identified in either bone or soft tissue cultures (Table 5).

The nine patients (25%) with negative cultures in both bone and soft tissue and the 20 patients (55.5%) with identical pathogens in both cultures were considered compatible. As a result, bone and soft tissue cultures were found to be compatible in 80.5% of the patients. Statistical analysis showed a moderate level of agreement, with a kappa coefficient of 57.4% (Kappa=0.574, $p>0.05$).

Table 4. Agents isolated from blood cultures and tissue cultures of patients with growth in blood cultures

Blood culture	Soft tissue culture	Bone tissue culture
Klebsiella pneumoniae	*Acinetobacter baumanii*	*Acinetobacter baumanii*
	Klebsiella pneumoniae	*Klebsiella pneumoniae*
Methicillin-resistant CNS	*Citrobacterkoseri*	No causative agent could be
	P. aureginosa	isolated.
Serratia marcescens	*Corynebacterium xerosis*	*Corynebacteriumxerosis*
	Staphylococcus epidermidis	*Staphylococcus epidermidis*
E. coli	*E. coli*	*E. coli*
	Group A beta hemolytic streptococcus	Group A beta hemolytic streptococcus
E. coli	*Proteus spp.*	*Proteus spp.*
	P. aureginosa	*P. aureginosa*

Table 5. Compatibility of bone and soft tissue cultures with each other

Soft tissue culture	Bone tissue culture	N (%)
+*	+*	20 (55.5)
+	-	5 (13.9)
-	+	1 (2.8)
-	-	9 (25)
+**	+**	1* (2.8)

(+) Agent isolated in culture (-) Agent not isolated in culture; *Both bone and soft tissue were found to be causative and the causative agents were the same. ** Both bone and soft tissue agents were detected, but the agents were different from each other.

Discussion

Bone biopsy, bone tissue culture, and histopathological examination of bone are necessary for a definitive diagnosis of osteomyelitis[10]. However, bone biopsy is often not a viable option as it requires a skilled surgeon and is a challenging and invasive procedure[11]. According to a study by Zuluaga et al.,[12] cultures collected from soft tissue surrounding an infected bone were insufficient to identify the causative agent of osteomyelitis. Conversely, Kessler et al.[13] demonstrated that soft tissue samples obtained via needle biopsy from the tissue closest to the bone surface yielded results comparable to bone tissue cultures.

Malone et al.[14] conducted a study comparing the probe-to-bone test with bone biopsy and deep tissue swab culture after debridement in patients with osteomyelitis. In this study, 34 patients were examined, and in nine cases, the causative agent was undetected. Among the 25 patients in whom the agent was identified in both bone and deep tissue swab cultures, the same agent was isolated in 64% of cases. When all patients were considered, the agreement rate was 47%. Swab cultures collected from wounds are generally not recommended, as they are often insufficient to distinguish between colonization and infection. To mitigate this limitation, they obtained swabs following debridement. The sample collection techniques used in our study differed from those employed by Malone et al.[14]

In a study by Ertuğrul et al.,[15] which compared bone and deep soft tissue cultures, the rate of growth of the same agent in both cultures among patients with osteomyelitis was reported to be 49%. In patients with chronic osteomyelitis, excluding those with diabetes, the concordance rate between bone and non-bone specimens was 36% in the study by Lavery et al.[16] and 28% in the study by Zuluaga et al.[12] The exclusion of diabetic foot osteomyelitis from the study by Zuluaga et al.[12] may have contributed to the lower concordance rate.

A study by Xuemei Li et al.[17] comparing swab, soft tissue, and bone tissue cultures from 60 patients with diabetic foot osteomyelitis found a 76.7% concordance rate between soft tissue and bone tissue cultures. In cases where multiple pathogens were detected, an additional 8.3% were classified as partially concordant. The overall concordance rate was reported as 85%.

Unlike diabetic foot infections, which originate in the soft tissue and gradually spread to the bone, chronic osteomyelitis is an infection that primarily affects the bone through hematogenous spread or trauma. Based on this distinction, it is reasonable to expect greater compatibility between pathogens in soft and bone tissues in diabetic foot osteomyelitis compared to other forms of chronic osteomyelitis. In our study, the same causative agent was identified in both cultures in 55.5% of cases, and when the absence of a causative agent in both cultures was

considered, the concordance rate increased to 80.5%. In the study by Ertuğrul et al.,[15] no causative agent was found in either culture in 11% of cases, and when this percentage is taken into account, the concordance rates are comparable to those in our study.

One patient in our study had a positive bone culture but no detectable causative agent in the soft tissue culture. This suggests that antibiotic therapy may reduce the bacterial load in soft tissue earlier than in bone tissue, but it may not have reached an effective level in bone tissue at that stage.

Conclusion

In 80.5% of cases, bone and soft tissue culture results were concordant. Statistical analysis classified this agreement level as moderate. Based on our findings, we believe that soft tissue cultures can be used as an alternative to bone tissue cultures for identifying the causative agent of diabetic foot osteomyelitis.

Most studies in the literature compare soft tissue cultures with swab cultures[18-20]. A key strength of our study is its significant contribution to the literature, as it is one of the few studies to compare both bone and soft tissue cultures directly. However, a limitation of our study is the relatively small patient population, highlighting the need for larger datasets to validate our findings.

Ethics Committee Approval: The study was approved by Haydarpaşa Numune Training and Research Hospital Ethics Committee (No: HNEAH-KAEK 2017/KK/32, Date: 27/03/2017).

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