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ORIGINAL ARTICLE

Comparison of microbiological results of deep tissue biopsy and superficial swab in diabetic foot infections

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ABSTRACT

Objectives: In this study, we aimed to compare superficial swab cultures with deep tissue biopsy cultures and also to evaluate the reliability of superficial swap cultures in diabetic foot infected patients.

Materials and methods: To compare two culture methods, the hospitalized patients with diabetic foot infections were retrospectively evaluated at Dicle University and Diyarbakir Education and Research Hospital, between October 2009 and November 2010. The patients were divided two groups as with osteomyelitis (osteomyelitis group, Wagner ≥3) and with soft tissue infections (soft tissue infection (STI) group, Wagner <3). The cultures of deep tissue biopsy specimens and swab samples were collected from all patients.

Results: In 75 patients with osteomyelitis, the compatibility rate in deep tissue biopsy culture with superficial swab culture was 58.7% whereas in STI group this rate was 89.1% ($p<0.001$). Of 41 superficial swap cultures, 33 of them (81%) had the same microorganisms with the identified microorganisms in deep tissue cultures. *Staphylococcus aureus* was the predominant pathogen isolated from deep tissue biopsy cultures and also from superficial swap cultures. The distributions of microorganisms in deep tissue culture and swap cultures were similar.

Conclusions: This study indicates that superficial swab culture could be valuable to identify the pathogens in infected diabetic wounds without osteomyelitis. The accuracy of swab specimens diminishes when osteomyelitis develops. Deep tissue culture seems more sensitive and reliable in osteomyelitis group. *J Microbiol Infect Dis 2011;1(3):122-127*

Key words: Diabetic foot infection, Wagner Grading System, deep tissue culture, swab culture.

Diyabetik ayak enfeksiyonlarında derin doku biyopsisi ile yüzeyel sürüntü kültürünün mikrobiyolojik karşılaştırılması

ÖZET

Amaç: Bu çalışmanın amacı diyabetik ayak enfeksiyonu olan hastalarda derin doku kültürleri ile sürüntü kültürlerinin sonuçlarını karşılaştırmak ve derin doku kültürü alınmasının gecikeceği veya cerrahi olarak kontrendike olduğu durumlarda sürüntü kültür sonuçlarının derin doku kültür sonuçlarına göre kullanılışlığını test etmek idi.

Gereç ve yöntem: Ekim 2009 ile Kasım 2010 tarihleri arasında, Dicle Üniversitesi Hastanesi ve Diyarbakır Eğitim Araştırma Hastanesi'nde diyabetik ayak enfeksiyonu olan 75 hastanın verileri retrospektif olarak incelendi. Wagner evreleme sistemine göre hastalar osteomyeliti olanlar (osteomyelit grubu, Wagner ≥3) ve yumuşak doku enfeksiyonu olanlar (yumuşak doku enfeksiyonu (YDE) grubu, Wagner <3) şeklinde iki gruba ayrıldı. Hastalardan derin doku ve yüzeyel sürüntü kültürleri alınarak incelendi.

Bulgular: Osteomyelitli hastalarda derin doku kültürleri ile sürüntü kültürleri arasındaki uyum oranı % 58,7 iken, YDE olan hastalarda % 89,1 idi. Osteomyeliti olmayan hastalarda uyum oranı anlamlı derecede yüksekti ($p<0,001$). Ayrıca üremesi olan 41 sürüntü kültüründen 33'ünde (% 81) üreyen mikroorganizmalar derin doku kültüründe üreyen mikroorganizmalar ile aynı idi. Derin doku ile sürüntü kültüründe en sık üreyen patojen *Staphylococcus aureus* olup üreyen mikroorganizma dağılımları benzerdi.

Sonuç: Bu çalışma ile elde edilen veriler sürüntü kültürlerinin osteomyeliti olmayan diyabetik ayak enfeksiyonlarında etken patojeni göstermesi açısından kullanılabileceğini göstermektedir. Ancak osteomyelit gelişen olgularda kullanılabilirliği azalmaktadır. Osteomyelitli olgularda derin doku biyopsi kültürleri daha güvenilir olarak görülmektedir.

Anahtar kelimeler: Diyabetik ayak enfeksiyonu, derin doku kültürü, sürüntü kültürü.

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INTRODUCTION

Despite all preventive measures, it is well known that patients with diabetes mellitus (DM) complicating with foot ulcerations and infections create potentially a serious problem. Therefore, it is very important to isolate the causative microorganism for appropriate treatment of the infected diabetic foot ulcers (DFU).[1-6] However, identifying the responsible pathogen is complicated by the presence of both pathogens and colonizers in most foot wounds. Therefore, the culturing technique may be crucial in identifying the true pathogens. Four techniques may be used to obtain material for culture from a wound: swab, aspiration, curettage, and tissue biopsy.[7-13]

Several studies claimed that deep tissue biopsy is the gold standard in culturing technique.[7-11] Swab cultures are regarded as the least reliable because they were reported to contain high numbers of colonizers and too often lack the true pathogens. However, collection of a tissue specimen may not always be advisable due to concern for spreading the infection, ischemia, or damaging adjacent structures. Furthermore, swabs may be collected by any member of the health-care team, from every kind of wound except those completely covered by a dry eschar.[13] Therefore, results from swab cultures are still commonly used in identifying pathogens and selecting antibiotics.[7-11]

Although researches claim that swabs do not give an accurate picture of organisms in the deep tissue biopsy, these studies, except Slater et al., were limited to the most severe infections. Furthermore, these studies show great difference in their results, which shows relation between swab and tissue specimens varied from 9% to 62%.[7-11] This study aimed to reappraise the reliability of swabs according to the depth and severity of the wound.

MATERIALS AND METHODS

This retrospective study was carried out among 75 patients hospitalized with the diagnosis of diabetic foot infections at Dicle University and Diyarbakir Teaching Hospital between October 2009 and November 2010. The diagnosis of infection have been made clinically by existence of inflammation signs (i.e. erythema, warmth, edema, and pain) as well as pus, fetid odor, devitalized tissue, purulent drainage and sinus tracts with undermined borders.

The Wagner Grading System was used for the classification of diabetic foot infections. Patients were grouped according to their age, sex, duration of diabetes, glycated hemoglobin levels, the vascular status of the foot and duration of ulcer. In the examination findings, presence or absence of dorsal-pedal and posterior-tibial pulses were recorded in the patient notes. The exclusion criteria's for the study were having surgical debridement, gangrenous wounds, those with a dry eschar, and antibiotic use before hospitalization. According the localization of the wounds, which were divided into four groups; finger, metatarsal, midfoot and heel. Wound formation occurred less than a month was evaluated as an acute infection whereas infections lasting more than a month evaluated as chronic infections. The wounds were separated into two groups as osteomyelitis (osteomyelitis group) and soft tissue infection (STI group) (ulcer or abscess without osteomyelitis). In the assessment of osteomyelitis, probe to bone test were used in the presence of sinus tract, otherwise bone scintigraphy and MRI used in the evaluation of non-sinus tract infections.

Two cultures were simultaneously taken from each patient in the lack of systemic antibiotic therapy for at least 4 weeks before swabbing and deep tissue culture (DTC). Superficial swab cultures (SC) were taken by rotating directly from the base of the ulcer, after the wound was cleaned with sterile saline. DTC samples were taken from the junction of non-viable and viable tissue by using a new set of sterile instruments: curette and forceps, after the surrounding area of the wound were cleaned with povidone iodine solution, at operating conditions. All non-viable tissue removed from the wounds and/or the furthest extension of sinus tract or abscess was performed in the deep tissue debridement. Samples were inserted into a transport tube containing solid media suitable for both aerobic and anaerobic microorganisms and delivered to the laboratory, for immediate processing, within 15 min after collection. Only one site was sampled from each patient. Culturing of aerobic and anaerobes species were inoculated on to blood agar, EMB (Eosin Metilen Blue) agar, Sabouraud agar and

Wilkins- Chagren anaerobe agar at 35-37°C for 24-48 hours. The hemolysis reaction, catalase test, optochin, bacitracin and co-trimoxazole susceptibility testing was performed for Gram-positive bacteria, while oxidase test were applied for gram-negative bacteria. Resistance to methicillin in Staphylococcus was evaluated with oxacillin disk diffusion method. Identification was done by BD Phoenix 100 (Becton-Dickinson, Maryland, USA). Disc diffusion sensitivity testing was performed according to NCCLS M100-S16.

Informed consent was obtained from all patients who participated to our study. This study was approved by the Ethics Committee of the Dicle University. The study protocol conforms to the ethical guidelines of the declaration of Helsinki.

Statistical analysis

Statistical analyses were performed by using SPSS 17.0 for Windows Version program. P <0.05 values were considered statistically significant. Student's t test was used in the evaluation of age, duration of diabetes and HbA1c, Chi-square test was used for gender in each group. Kappa analysis was used in the evaluation of compliance rates, as the deep tissue culture accepted for reference; chi-square test was used in the comparison of the groups.

RESULTS

Seventy-five diabetic patients were included in the study. Of 75 diabetic foot infected patients, 35 (47 %) were male and 40 (53%) were female (mean age 57.3 ± 11.5 years). Mean ages of osteomyelitis group and STI group were 59 (SD ±11.8) years and 55.9 (SD±11.2) years, respectively. Mean durations of diabetes in osteomyelitis and STI group were 8.3 (SD ±5.8) and 9.2 (SD ±7) years, respectively. HbA1c (%) mean in osteomyelitis group and STI group were 10 (SD ±3) and 9.4 (SD ±7). Age, gender, HbA1c and duration of DM were found similar in the both groups (p>0.05).

Of 75 patients, 71 had type 2 and 4 had type 1 diabetes mellitus. Of 75 patients, 33 were diabetic patients with osteomyelitis (44%), 42 (56%) were diabetic patients with STIs. Readmission to the hospital in the first month after the formation of foot ulcers was observed in the 56 (75%) of the patients (changing with a week to 4 months).

According to food ulcer stages; 48 were acute but 27 were in chronic stage. 49 (65%) patients admitted to the hospital for the first time due to diabetic foot infections. Clinical and demographic characteristics of the patients were given in Table 1.

Table 1. The clinical and demographic characteristics of diabetic foot wound

Patients	Osteomyelitis group (n=33)	Soft tissue infection group (n=42)
Anti-diabetic treatment n (%)		
Oral anti-diabetic	20 (61)	30 (71)
Insulin	13 (39)	12 (29)
Duration of wound formation, n (%)		
Acute (<30 days)	12 (36	36 (86)
Chronic (>30 days)	21 (64)	6 (14)
Wagner stage n (%)		
Grade 1	0 (0)	22 (52)
Grade 2	0 (0)	12 (29)
Grade 3	23 (70)	8 (19)
Grade 4	10 (30)	0 (0)
Wound type n (%)		
Ulcer	0 (0)	34 (81)
Osteomyelitis	33 (100)	0 (0)
Abscess (non-osteomyelitis)	0 (0)	8 (19)

Fifty-one microorganisms were isolated from superficial swab specimens of the patients with osteomyelitis (Wagner ≥3). In total, 39 microorganisms were isolated from deep tissue biopsy specimens. *Staphylococcus aureus* was the most commonly isolated microorganism in the deep tissue culture. Three of four *Staphylococcus aureus* was found methicillin-resistant (MRSA). In the STI group (Wagner ≥3), 51 microorganisms were isolated from superficial swab specimens but 47 microorganisms were isolated from the deep tissue cultures. *Staphylococcus aureus* was the most commonly isolated microorganism in the deep tissue culture. Two (12%) of them was MRSA. Isolated microorganisms and their characteristics were given in Table 2.

Table 2. Isolated microorganisms and their characteristics

Patient Type	Osteomyelitis group (n=33)		Soft tissue infection group (n=42)	
Samples	Swab samples, n (%)	Deep tissue samples n (%)	Swab samples, n (%)	Deep tissue samples, n (%)
Mean number of isolates per sample	1.54	1.18	1.21	1.11
MSSA	4 (8)	1 (2)	17 (33)	15 (32)
MRSA	6 (11)	3 (8)	2 (4)	2 (4)
CNS	4 (8)	5 (13)	4 (8)	3 (7)
Enterococci	4 (8)	3 (8)	5 (10)	3 (7)
Group A strept.	4 (8)	-	9 (18)	8 (16)
Bacillus cereus	1 (2)	1 (2)	-	-
Escherichia coli	6 (12)	4 (10)	4 (8)	4 (9)
Klebsiella species	7 (14)	4 (10)	4 (8)	6 (13)
Proteus species	3 (6)	3 (8)	3 (6)	2 (4)
Enterobacter cloacae	6 (12)	6 (15)	2 (4)	2 (4)
Pseudomonas aeruginosa	1 (1)	7 (18)	1 (1)	2 (4)
Citrobacter freundii	3 (6)	-	-	
Bacteroides fragilis	2 (4)	2 (5)	-	-
Total	51	39	51	47

In the patients with osteomyelitis, SCs and DTCs were found compatible in seven patients whereas found incompatible in 13 patients. In 12 wounds, the microorganisms isolated from superficial swab cultures were similar with the microorganisms isolated from deep tissue cultures but there were also additional microorganisms. Of 32 wounds, 19 (59%) superficial swap cultures were revealed the same microorganisms with the microorganisms isolated from DTCs.

In the STI group, swab and deep tissue specimens were compatible in seven patients, but there was difference in 13 patients. In 30 wounds, similar microorganisms were grown in superficial swap and deep tissue cultures whereas there was incompatibility in six cultures. In two wounds, swab cultures were positive whereas deep tissue cultures were negative. In three wounds, the swab specimen identified all microorganisms isolated from the deep tissue specimen, but also contained additional microorganisms. Thus, of 41 superficial swap cultures, 33 (80%) had the same microorganisms with the identified microorganisms in deep tissue cultures.

As the superficial swab specimen identified all pathogens found in the deep tissue in 41 STI patients, 33 antibiotic coverage based on swab specimen alone would had been adequate in at least 80% of all the wounds. In eight of the remaining wounds, two swab cultures were positive, while all the deep tissue cultures were negative. Consequently, 27 (84%) patients with osteomy-

elitis and 41 (90%) STI patients were treated adequately by using swab specimen results alone.

According to isolated microorganisms, the highest compliance in each culture technique in patients with osteomyelitis was detected in *Bacteroides fragilis* (50%) and Enterococcus (43%), whereas the lowest compliance was seen in *Pseudomonas aeruginosa* (13%). In the STI group, the highest compliance was seen in *Enterobacter cloacae* (50%), and Enterococcus (43%), whereas the lowest compliance was in Klebsiella spp. (19%). Comparison of compliance between SC and DTC of the patients, the reference point was DTC, according to kappa analyses, compliance rate was 89.1% in osteomyelitis group and 58.7% in non-osteomyelitis group ($p<0.001$). In non-osteomyelitic patients, high compliance rate was remarkable.

DISCUSSION

The comparison between the two sampling techniques showed that swap cultures and deep tissue cultures in diabetic patients with STI have similar compatibility. Superficial culture by swabbing was found reliable in STIs. Slater et al. reported that swabs identified all micro-organisms isolated from the deep tissue specimens in 36/40 wounds (90%) that did not extend to bone as opposed to 13/20 wounds (65%) that extended to bone.[13] Our results are similar Slater et al findings. Similarly, Pellizzer et al. found the mean number of isolates per patient as 2.34 by swabbing and 2.07 by tissue biopsy sampling.[14] They observed no statistical differences between the two procedures in terms of isolated microorganisms and/or their frequencies.

On the other hand, Kessler et al. found the mean number of microorganisms isolated by needle puncture significantly lower compared with that obtained by superficial swabbing: 1.09 vs. 2.04 ($P<0.02$).[15] However, they also observed that the swab specimen identified 13 microorganisms (62%) isolated from the needle puncture culture. The present study results are consistent with the results of this study.

Our results are not compatible with those of Sharp et al. and Sapico et al. study reports.[8-11] They reported that swabs do not accurately identify bacterial pathogens in diabetic foot wounds. However, these studies were restricted to patients who underwent amputation. Therefore, the poor performance of swabs in these studies might have been due to the excessive growth of colonizers at the site of the wound after the foot or limb had lost its viability. In contrast, our protocol excluded specimens from infectious gangrene and amputations.

According to our study, in STIs the treatment rate with using superficial swap culture was more frequent when compared with osteomyelitis group. Slater et al. also preferred swab specimen in Grade 1 and 2 wounds in patients with STI.[13] Furthermore, their data indicate that a serious risk of under-treatment was present in only 8% of the wounds, while a genuine risk of over-treatment was present in only 13%. Senneville et al. found the highest compliance of S. aureus (43%) while lowest compliance CNS (3%) at their study between 1996-2004 years.[16,17] They found the highest compliance of S. aureus (82%) at their study during 2006-2008 years also, when they examined the individual compliance rates of the Microorganisms microorganism breeding in tissue with superficial swab cultures. In our study, we found the highest compliance rates at different bacteria, since the culture technique taken as a reference and reproductive rates were the differences. S. aureus is the most common type of bacteria that grew from tissue and from superficial swab samples, and MRSA rates were (7-50%), so high as to be negligible in terms of treatment. Therefore, in the treatment of diabetic foot infected patients, MRSA should be taken into account. Our study is consistent with the above studies in terms of isolated microorganisms and we were happy to observe lower rates of MRSA strains. The major reason may be due to the fact that, the majority of the patients admitted to the hospital for the first time so they were infected with community acquired isolates. Our study was different in terms of to be the first study that compares microbiological compliance in diabetic foot infected patients with osteomyelitis and/or STI.

In conclusion, our data shows that swabs are valuable in identifying pathogens in patients with soft tissue infections. In patients with osteomyelitis, deep tissue cultures are still indispensable. Superficial swap cultures can be used in the initial treatment of diabetic foot infected patients especially in case of contradiction of surgical debridement or in the necessity of immediate care.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to declare regarding funding or conflict of interest with respect to this manuscript.

REFERENCES

1. Joseph WS, Lipsky BA. Medical therapy of diabetic foot infections. J Am Podiatr Med Assoc 2010;100:395-400.
2. Lipsky BA, Berendt AR, Deery HG et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2004; 39:885-910.
3. Cavanagh PR, Lipsky BA, Bradbury AW, Botek G. Treatment for diabetic foot ulcers. Lancet 2005; 366:1725-1735.
4. Levin ME. Management of Diabetic Foot: Preventing Amputation. South Med J 2002; 95 :10-20.
5. Lipsky BA, Berendt AR, Embil J, De Lalla F. Diagnosing and treating diabetic foot infections. Diabetes Metab Res Rev 2004; 20:56-64.
6. Ertugrul B, Baktiroglu S. Diyabetik ayak infeksiyonu. Klimik Dergisi 2005; 18; 6-13.
7. Mackowiak PA, Jones SR, Smith JW. Diagnostic value of sinus-tract cultures in chronic osteomyelitis. J Am Med Assoc 1978; 239: 2772- 2775.
8. Sharp CS, Bessman AN, Wagner FW Jr, Garland D. Microbiology of deep tissue in diabetic gangrene. Diabetes Care 1978; 1:289- 292.
9. Sharp GS, Bessman AN, Wagner FW Jr, Garland D, Reece E. Microbiology of superficial and deep tissues in infected diabetic gangrene. Surg Gynecol Obstet 1979; 149: 217-219.
10. Sapico FL, Canawati HN, Witte JL, Montogomerie JZ, Wagner FW Jr, Bessman AN. Quantitative aerobic and anaerobic bacteriology of infected diabetic feet. J Clin Microbiol 1980; 12: 413-420.
11. Sapico FL, WitteJL, Canawati HN, Montgomerie JZ, Bessman AN. The infected foot of the diabetic patient: quantitative microbiology and analysis of clinical features. Rev Infect Dis 1984; 6: 5171 -5176.
12. Lipsky BA, Pecoraro RE, Larson SA, Hanley ME, Ahroni JH. Outpatient management of uncomplicated lower-extremity infections in diabetic patients. Arch Intern Med 1990; 150: 790-797.
13. Slater R.A, Lazarovitch T, Boldur I, et al. Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. Diabet Med 2004; 21: 705-709.
14. Pellizzer G, Strazzabosco M, Presi S, et al. Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb- threatening diabetic foot infection. Diabet Med 2001;18, 822-827.
15. Kessler L, Piemont Y, Ortega F, et al. Comparison of microbiological results of needle puncture vs. superficial swab infected diabetic foot ulcer with osteomyelitis. Diabet Med 2005; 23, 99-102.
16. Senneville E. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: Concordance with ulcer swab cultures. Clin Infect Dis 2006; 42; 57-62.
17. Senneville E. Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. Clin Infect Dis 2009; 48; 888-893.