

Evaluation of ^{99m}Tc -Ubiquicidin 29–41 Scintigraphy in Differentiation of Bacterial Infection from Sterile Inflammation in Diabetic Foot

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ABSTRACT

Introduction: Ubiquicidin (UBI) 29–41 is a synthetic antimicrobial peptide that binds with the microbial cell membrane at the location of infection. This study was conducted to evaluate its probable efficacy as an infection-imaging agent with potential to differentiate bacterial infection from sterile inflammation in humans.

Methods: Fifteen diabetic foot patients (10 males and 5 females) with suspected bacterial infection, prior to starting antibiotic treatment, were selected for this study. First a routine three phase bone scan and later a ^{99m}Tc -UBI scan was performed for all the patients. 555-740 MBq of ^{99m}Tc -UBI was injected intravenously. A 10 minute dynamic study was followed by spot views of the suspected region of infection and corresponding normal areas (liver and kidneys) at 60 and 120 min. Whole-body anterior and posterior images were also acquired. To interpret the studies as positive or negative, visual score (0 –3) was used, with scores of 0 (minimal or no uptake; equivalent to soft tissue) and 1 (mild; less uptake than in liver) being considered negative and scores of 2 (moderate; uptake equal to or greater than that in liver) and 3 (intense uptake equal to or greater than that in kidneys) being considered positive.

Results: Of 15 studies performed with ^{99m}Tc -UBI, all had positive bacterial cultures. The result of bone scan was positive for osteomyelitis in 12 patients (80%). ^{99m}Tc -UBI Scintigraphy was positive in 6 patients, but negative in nine. The sensitivity of ^{99m}Tc -UBI for detection of infection was therefore 40%. From 12 patients who had positive bone scans, only 6 had a positive ^{99m}Tc -UBI (50%) indicating the sensitivity of 50% for ^{99m}Tc -UBI in osteomyelitis cases. ^{99m}Tc -UBI was not positive in any patient who had evidence of soft tissue infection in the bone scan.

Conclusion: Although ^{99m}Tc -UBI 29–41 was well tolerated by all the patients without any side effects, considering low sensitivity of this agent, this radiopharmaceutical is not of great value for diabetic foot infection diagnosis.

Keywords: ^{99m}Tc -UBI 29–41, Ubiquicidin, Diabetic foot, Infection, Radiolabeled peptide

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INTRODUCTION

Patients with diabetes mellitus may develop foot ulcers because of ischemia, neuropathy (sensory, motor, and autonomic deficits), or both. The initiating injury can be due to an acute mechanical or thermal trauma or from repetitively or continuously applied mechanical stress (1). Developing infection in these wounds is common and can be serious. Prevention and treatment of such wounds is of great importance, as they can lead to foot amputation (2-4). Diabetic foot wounds are diverse, and can range from cellulitis of a toe to gangrene of the foot (5, 6). Also early diagnosis of osteomyelitis, a major complication of diabetic foot, is difficult because neuroarthropathy complication with the similar presentation is commonly co-present in these patients. Early subtle changes from osteomyelitis seen on plain film are not easily differentiated from other superimposed changes in feet of diabetic patients (7, 8). Moreover, co-existing vascular insufficiency may cause an atypical presentation making diagnosis even more difficult (8). Differentiation between infectious and noninfectious inflammation is a serious problem in several clinical settings. The available imaging techniques such as ultrasonography, MRI and CT scan have high sensitivity for inflammation, but are not specific for infection (9).

Scintigraphic imaging modalities such as Ga-67 citrate, In-111 and Tc-99m labeled leukocytes and labeled antigranulocyte antibody are used to detect infection. There are however some disadvantages to each procedure, limiting its application. Ga-67 Citrate, the first well-known radiotracer for this purpose, although sensitive for inflammation, is not specific for infection (10).

In-111 labeled leukocyte imaging is associated with high sensitivity and specificity rates for detection of infections (11). However, restrictions such as difficult

and time-consuming procedures with danger of blood borne infections especially HIV, hepatitis B and C, limitation in neutropenic patients and risk of generation of human antimouse antibodies limit its application (11, 12).

Unfortunately we have to face the fact that none of the currently used agents for the scintigraphic detection and localization of infection/inflammation meets all of the ideal criteria in this area.

So, there is the need to develop new and better agents to discriminate bacterial infections from nonbacterial infections and sterile inflammations.

Clearly, radiopharmaceuticals that bind to a variety of bacteria would be better candidates for specific infection imaging. The first proposed agent with this characteristic was ciprofloxacin radiolabeled with Tc-99m. However, it could not distinguish between infections and sterile inflammatory lesions, which implies that its specificity for the detection of bacterial infections is not warranted (13, 14).

A synthetic antimicrobial peptide Ubiquicidin (UBI) 29-41 labeled with Tc-99m (^{99m}Tc -UBI) is recently introduced in nuclear medicine to image the molecular localization of infectious microorganisms. Tc-99m labeled antimicrobial peptides UBI 29-41, UBI 18-35, UBI 31-38, hLF 1-11, and defensins accumulate significantly in tissues infected with gram-positive and gram-negative bacteria and *C. albicans*. Significantly lower ($P < 0.01$) accumulation of these peptides occurs in sterile inflamed tissues. These data indicate that the peptides preferentially tag microorganisms at the site of infection, which is in agreement with their preferential binding to the microorganisms in vitro and in vivo (14). With such a potential, it is even suggested that, in the future, ^{99m}Tc -UBI molecular imaging could become the gold standard to detect infection sites and to discard sterile inflammation (14, 15)

Recently, several studies have shown that this synthetic antimicrobial peptide fragment (Tc-99m labeled ubiquicidin 29-41) is a highly sensitive and specific agent for the scintigraphic detection of bacterial and fungal infections in animals and humans (14, 16, 17).

As diabetic foot present a challenging diagnostic dilemma, this study was designed to evaluate ^{99m}Tc-UBI 29-41 scintigraphy in detection of infection, and possibly differentiate bacterial infection from sterile inflammation in suspected diabetic foot.

METHODS

Patient Selection

Fifteen diabetic foot patients (10 males and 5 females, mean age: 58 y, range: 48-79) with suspected bacterial infection of bone or soft tissue were entered in this study. First, all the patients underwent a routine three phase bone scan and later a ^{99m}Tc-UBI scan was performed in all the patients. This study was approved by the ethics committee of Tehran University of Medical Sciences. After full explanation of the procedure, informed consent was obtained from all patients. In addition all pregnant or lactating women, patients with history of antibiotic therapy for their present condition, known hepatic disorder, renal disease or history of hypersensitivity state, were excluded from this study.

Three Phase Bone Scan

After preparing the radiopharmaceutical, 900-950 MBq of Tc-99m methylene diphosphonate (MDP) was administered intravenously. Dynamic 3 second images were obtained for 60 seconds after injection (angiographic phase) and blood pool phase images were obtained from the feet during following four minutes. Delayed skeletal phase images were obtained at 2-4 hours after injection.

^{99m}Tc-UBI 29-41 Scintigraphy

After labeling of Ubiquicidin (UBI) 29-41 with ^{99m}Tco₄⁻, 555-740 MBq of the radiotracer was injected intravenously while the patient was in supine position. The study was performed in dynamic phase consisting of 10 frames of 60s each, and static phase, performed in two stages at 60 and 120 minute. Delayed phase was performed on anterior and posterior whole body projections. Also spot views of the suspected site (ulcerated foot), liver and kidneys were obtained using low energy general purpose (LEGP) collimator. First, the spot view of the diabetic foot with collection of 500 kilo count was acquired, and then the same time was used for acquisition of other spot views. Visual score (0 -3) was used to categorize studies as positive or negative, with scores of 0 (minimal or no uptake; equivalent to soft tissue) and 1 (mild; less uptake than liver) being considered as negative, and scores of 2 (moderate; uptake equal to or greater than liver) and 3 (intense; uptake equal to or greater than kidneys) being considered as positive studies. All the scans were interpreted by three nuclear medicine physicians, and their agreed opinion was considered as the final interpretation.

Bacterial culture

After imaging, samples for culture were taken from the infected site by endocrinologist. Samples were taken from the infected wounds, using a sterile swab when possible or by fine needle aspiration in case of closed infections. The inoculation was on blood agar and MacConkey agar culture media and was followed by incubation at 37°C for 48 h. The microbiologist interpreting bacterial cultures was unaware of the patient history and scintigraphic findings. Bacterial culture was used as the gold standard for definitive diagnosis of infection.

On the basis of bacterial culture as the gold standard test, the scans were interpreted as

true negative, false negative, true positive and false positive.

RESULTS

All subjects tolerated intravenous injection of ^{99m}Tc-UBI 29-41 well. No adverse reaction or significant change in vital signs was observed in any patient during or after acquisition. Of 15 studies performed with ^{99m}Tc-UBI and bone scan, including 5 female and 10 male (mean age, 58 y; range, 48-79 y) all had positive culture. The Group D Strep-Enterococcus was the most cultured microorganism (single organism or as a part of multiorganisms) (60%). In 9 patients several microorganism were present (20%) (Table 1).

The bone scans were positive for osteomyelitis in 12 cases (80%), showing increased activity in all three phases of the study, while in three patients (20%), the

scan was positive for the first two phases and negative for the third phase, indicating soft tissue infection.

From a total of 15 patients, six patients had positive and nine patients had negative ^{99m}Tc-UBI scintigraphy (sensitivity: 40%). As there were only 6 cases of positive ^{99m}Tc-UBI in 12 patients with positive bone scans for osteomyelitis, the sensitivity of ^{99m}Tc-UBI for detection of osteomyelitis was 50%. No patient with soft tissue infection (cellulitis) observed on bone scan, had positive ^{99m}Tc-UBI study. However, the sensitivity of three phase bone scans for detection of infection (including both cellulitis and osteomyelitis) considering culture results was 100%.

Comparing visual assessment and scoring of 60 minute ^{99m}Tc-UBI images with 120 minute images, no significant difference was seen.

Table 1. ^{99m}Tc-UBI, bone scintigraphy and the bacterial culture results.

Patient ID	Age (year), Sex	Microorganism*	^{99m} Tc-MDP	Culture	^{99m} Tc-UBI 60 min	^{99m} Tc-UBI 90 min
1	62, Male	E coli, GDSE, SE	Osteomyelitis	+	+	+
2	54, Male	PA	Osteomyelitis	+	-	-
3	62, Male	GDSE, SE	Cellulitis	+	-	-
4	62, Male	GDSE, E coli	Osteomyelitis	+	-	-
5	59, Male	E coli, SE, SH, KP	Osteomyelitis	+	-	-
6	48, Female	GDSE	Osteomyelitis	+	+	+
7	60, Female	SH, PSPP	Osteomyelitis	+	-	-
8	62, Female	E coli, SSPP	Osteomyelitis	+	+	+
9	79, Female	GDSE	Osteomyelitis	+	-	-
10	49, Male	GDSE, SA, SE, KSPP	Osteomyelitis	+	-	-
11	(60, Male)	E coli, GDSE, SE	Osteomyelitis	+	+	+
12	53, Male	SA	Osteomyelitis	+	+	+
13	54, Female	GDSE	Cellulitis	+	-	-
14	50, Male	SA, PA	Cellulitis	+	-	-
15	56, Male	E coli, GDSE, SA	Osteomyelitis	+	+	+

*GDSE: Group D Streptococcus [Enterococcus], SE: Staphylococcus epidermidis, PA:Pseudomonas aeruginosa, SH: Staphylococcus haemolyticus, KP: Klebsiella pneumonia, PSPP: Pseudomonas SPP, KSPP: Klebsiella SPP, SSPP: Streptococcus Spp, SA: Staphylococcus aureus

Sex, whole body scan and the type of microorganism had no impact on ^{99m}Tc-UBI scan results.

DISCUSSION

The current study was conducted to evaluate ^{99m}Tc-UBI 29–41 potential as an infection-imaging agent and its ability to discriminate between bacterial infections and sterile inflammations in diabetic foot patients.

Most of diabetic patients experience infection during life (18, 19). Foot infections and their sequelae are among the most common and severe complications of diabetes mellitus. As diabetic patients with foot infections may develop osteomyelitis (50-60%) which might progress to amputation, early diagnosis of osteomyelitis is critical (5, 6, 20). So the major problems are differentiating infectious from noninfectious condition, and discriminating soft tissue infection from bone infection (21).

Diagnosing of infection in patients with diabetic foot and ulcers is challenging (18). The accompanying neuropathy, vasculopathy, immune deficiency, in these patients makes the diagnosis and treatment of osteomyelitis more difficult (6, 21). Clinical and laboratory features of skeletal infections may be confusing and are nonspecific and confirming bone infection often requires several diagnostic procedures (18, 22). Plain films should always be the first step in the imaging assessment of osteomyelitis, however, the sensitivity for X-ray radiography has been reported to range from 43% to 75%, and the specificity from 75% to 83% (22). Although other available anatomical methods such as ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) are sensitive, they are neither reliable in diagnosis patients with infection. These modalities lack specificity for infection, particularly in early phase, when anatomic structures haven't yet been distorted.

Over years, scintigraphic methods have become an important part of the diagnostic procedures for osteomyelitis. These methods have the advantage of discovering infection all over the body. The standard approach for bone scintigraphy with ^{99m}Tc-MDP to assess for osteomyelitis is to perform a three-phase procedure. The positive uptake on all three phases is highly sensitive for osteomyelitis but not specific. Consequently, the bone scan is often used as a screening test (22, 23). We used this method to evaluate the possibility of infectious process of the diabetic foot and to differentiate between osteomyelitis from cellulitis.

Several other radioactive agents including gallium-67 citrate, and indium-111 labeled white blood cells have also been used. These agents are highly sensitive but have the inconvenience of low specificity (22-24). Also [¹⁸F]fluorodeoxyglucose positron emission tomography (PET) has been found to accumulate non-specifically at the sites of infection and inflammation (22). Skeletal infection continues to be a common and difficult condition in clinical practice and early accurate diagnosis is very challenging. From the Ga-67 citrate imaging to ¹⁸F-FDG PET imaging, the leading question of discriminating infection from sterile inflammatory condition has been unanswered (25, 26).

To address this question radiolabeled antimicrobial peptides (AMPs), such as ^{99m}Tc-UBI 29-41 were introduced. These agents seemed to be highly infection-specific. ^{99m}Tc-UBI 29-41 (Ubiquicidin) allows rapid visualization of gram-positive and -negative bacterial infections with little or no accumulation in sterile inflammatory processes, indicating that this peptide directly tags the microorganisms at the site of infection (infection-specific tracer) (15-17, 27, 28). These agents bind directly to the bacterial cell membrane in the site of infection. It binds to the negatively charged groups present on the bacterial cell envelope by electrostatic interaction. Hence, it can be

considered as a better agent comparing to pharmaceuticals that have an indirect approach. Among these peptides, ^{99m}Tc-UBI 29–41 has shown hopeful results for differentiation between infection and inflammation in animal models (17, 28-30).

Although there have been a few studies on humans, suggesting efficacy of ^{99m}Tc-UBI in differentiation of infection from sterile lesions (15, 16, 25, 27), none of them have been performed exclusively on diabetic foot.

Sepúlveda-Méndez et al ^{99m}Tc-UBI study on patients with FUO concluded a high specificity of ^{99m}Tc-UBI for localizing infection foci (95.35%), the sensitivity was 97.52%, the positive predictive value, the negative predictive value and the accuracy was 96.72%, 96.47%, 96.62%, respectively. The observed agreement between the bacterial culture and the molecular image was 96.62% (P=0.0001) (15). Akhtar et al included eighteen patients in their study with suspected bone, soft-tissue, or prosthesis infections. The overall sensitivity, specificity, and accuracy of that study were 100%, 80%, and 94.4%, respectively (25). In the human study performed by De Murphy patients with fever of unknown origin, osteomyelitis, diabetic foot, prosthesis infection, septic arthritis, or bacteraemia were imaged with ^{99m}Tc-UBI 29-41 scintigraphy and high sensitivity (96.3%), specificity (94.1%), and accuracy (95.3%) with high positive predictive (95.1%) and negative predictive values (95.5%) were achieved (27). Meléndez-Alafort L et al performed ^{99m}Tc-UBI imaging in 6 children with suspected bone infection compared the results with Ga-67 citrate images. All infection positive ^{99m}Tc-UBI images were in agreement with those obtained with Ga-67 citrate (16).

In the current study, fifteen diabetic foot patients with suspected bacterial infection (either soft tissue or bone), without history of antimicrobial therapy, underwent ^{99m}Tc-UBI 29-41 study and three phase bone scan.

Bacterial culture from the lesion was used as the gold standard, which was positive in all 15 cases. From these 15 cases, only six patients had positive ^{99m}Tc-UBI scan, resulting in a sensitivity of only 40% which is not in accordance with other studies (15, 16, 25, 27). Bone scans were positive for osteomyelitis in 12 patients, while only six of these cases had positive ^{99m}Tc-UBI scan (50%). ^{99m}Tc-UBI study was negative in the three patients with soft tissue infection. It can thus be concluded that ^{99m}Tc-UBI study is of more value for detection of infection if osteomyelitis is present, though its overall sensitivity is not such to be recommended in diabetic foot patients.

However, as all patients in our study had positive bone scintigraphy (indicating either cellulitis or osteomyelitis) in addition to positive bacterial culture, the sensitivity of bone scan for detection of infection was 100%, emphasizing on high sensitivity of bone scan for infection/inflammation diagnosis as was already demonstrated (10, 22). In our study there was no culture negative case to assess the potential of bone scan for distinguishing sterile from infectious process.

Although, our study argues against the results obtained by others, it should be noted that this study included exclusively diabetic foot patients. Foot infections in diabetes mellitus are rarely due to a single microorganism (31, 32) as was the case in our study. The presence of multiorganisms in the site of infection may be a possible explanation for the low sensitivity acquired in diabetic feet as ^{99m}Tc-UBI [29-41] does not show the same affinity for all kinds of microorganisms. In a study by Akhtar et al relatively low ratios of accumulation of ^{99m}Tc-UBI [29-41] were observed in *E. coli* infections compared with those of the *S. aureus* group, which is suggested to be due to a lower virulence of the former. Other possible reasons may include low affinity of this peptide for *E. coli* microbial membranes (33). *E. coli* was one of the organisms

observed frequently in the culture of diabetic foot in our cases, which may have caused less accumulation of ^{99m}Tc-UBI [29-41].

The other possible explanation for low sensitivity in our results could be the chronic nature of infection in diabetic foot patients. It has been shown that ^{99m}Tc-UBI [29-41] has slight uptake in chronic infection while acute infection shows intense accumulation of the radiopharmaceutical (16). As Welling et al showed antimicrobial peptide ubiquicidin (UBI) binds to bacteria and to leucocytes at the site of infection (17), fewer amounts of leucocytes and active bacteria present in long-standing infectious lesions could account for less concentration of radioactivity and resultant negative scan. In addition to chronic character of diabetic foot infection, vascular insufficiency which is common in these patients might be another reason of less delivery of radiotracer to the site of infection (31).

Moreover comorbidities are common in long-standing diabetes mellitus (31). The presence of these conditions such as cardiac dysfunction and renal insufficiency may affect radiotracer distribution in the organs and cause lower target to back ground ratio leading to negative scan. However in our study renal disease was among the exclusion criteria practically excluding this possible explanation.

As this study was one of the first clinical studies performed in humans and included exclusively diabetic patients, limited number of patients were incorporated which can be considered as a drawback of our study. Performing larger studies with more patients may compensate for this shortcoming and reveal more conclusive results.

CONCLUSION

^{99m}Tc-UBI [29-41] scintigraphy cannot be used for differentiation of bacterial infection from sterile inflammation in diabetic foot.

Low sensitivity for detection of bacterial infection makes this agent less than favorable for diagnostic application in this group of patients.

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