

Clinical Evaluation of ^{99m}Tc -MDP Three Phase Bone Scan and ^{99m}Tc -UBI₂₉₋₄₁ Scintigraphy Imaging in Preferentially Detection Infection from Sterile Inflammation among Diabetic Patients with Suspected Foot Infection

Alireza Doroudi^{1*}, Hamidreza Samaee¹, Seyyed Mostafa Saadati², Faramarz Ahmadi², Ali Kiasat², Mostafa Erfani³

¹School of Pharmacy of Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran. ²Nuclear Medicine Department of Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran.

³Radiation Application Research School, Nuclear Science and Technology Research Institute [NSTRI], Tehran, Iran.

ABSTRACT : Ubiquicidin 29-41 (UBI₂₉₋₄₁) is an antimicrobial peptide with six positively charged residues which preferentially binds to the negatively charged groups present on the microbial membrane by electrostatic interaction at the foci of infection. For this reason UBI₂₉₋₄₁ has been suggested to label by ^{99m}Tc (^{99m}Tc -UBI₂₉₋₄₁) directly or by using coligand reagents like 6-hydrazinopyridine 3-carboxylic acid (HYNIC) and tricine [^{99m}Tc /Tricine/HYNIC]UBI₂₉₋₄₁ indirectly. The purpose of this study was to investigate the clinical efficacy of [^{99m}Tc /Tricine/HYNIC]UBI₂₉₋₄₁ scintigraphy imaging in combination with ^{99m}Tc -methylene diphosphonate (^{99m}Tc -MDP) three phase bone scanning among diabetic patients with suspected foot infection.

Ten diabetic patients 6 man and 4 women (mean age 62.45, range 57 -67 years) with suspected foot infection before antibiotic therapy for the present complication have been participated in this study. Every patient underwent ^{99m}Tc -MDP three phase bone scan and ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging.

All subjects had positive cultures. None of the patients reported any adverse reaction after the (740-920 MBq) ^{99m}Tc -MDP and the (555-740 MBq) ^{99m}Tc -UBI₂₉₋₄₁ injections. The visualized site of infection with ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy images were consistent with ^{99m}Tc -MDP three phase bone scanning. ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy image demonstrated rapid distribution at the suspected region to infection. This matter can provide scan interpretation of suspected infection as early as 5 min after injection. Not only there was no major improvement in scan contrast was observed up to 120 min after injection, but also there was no any appreciable distribution difference between soft tissue and bone at the site of infection. For preferentially discrimination diagnosis of infection from sterile inflammation lesions among diabetic patients with diabetic foot problem, ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging may be considered after the ^{99m}Tc -MDP three phase bone scan of the patient is positive.

It is suggested to preferentially diagnose infection from sterile inflammation lesion among patients with suspicious foot infection two scintigraphy imaging ^{99m}Tc -MDP and ^{99m}Tc -UBI₂₉₋₄₁ can be considered.

KEYWORDS: Diabetic foot infection; ^{99m}Tc -MDP; ^{99m}Tc -UBI₂₉₋₄₁; Ubiquicidin.

I- INTRODUCTION

Early diagnosis of osteomyelitis, the most complication of diabetic foot is still challenging, because several other factors like neuropathy, diabetic osteoarthopathy, inflammation and trauma with the similar presentation is commonly in these patients. Different techniques and facilities have been suggested to solve this dilemma. The available techniques like Radiography, Ultrasoundography, Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) have high sensitive, although these modalities lack specificity for infection, especially in the early phase, when anatomic structures have not been changed (1, 2, 3).

Radionuclide scintigraphy imaging has been extensively used to visualize infection from sterile inflammation.⁶⁷ Ga-citrate is the most primitive radioisotope for this purpose, but unfortunately it has several disadvantages like long physical half-life, high and multiple energy gamma radiation causing high radiation absorbed doses and high sensitive for both infection and non-infectious inflammation (4, 5). ^{99m}Tc -MDP radiopharmaceutical is commonly used for diagnosing of osteomyelitis among diabetic patients. Three phase bone scanning with ^{99m}Tc -MDP is more sensitive but low specificity and selectivity for the detection of infection. The neuroarthopathy,

osteoarthropathy, inflammation and trauma increase non-selectively the uptake of this radiotracer (6). Leukocytes labeled with ^{99m}Tc or ^{111}In is considered as a gold standard for the scintigraphy imaging of infection from sterile inflammation in nuclear medicine (7). In order to prepare the label leukocyte, it is necessary to take blood from the patient and the leukocyte must be separated, labeled and then reinjected to the patient. The procedure is time-consuming and potentially risk of contamination or transmission of blood-borne pathogens to patient or technician (8, 9). Ubiquitin (UBI) $_{29-41}$ is a small synthetic peptide with molecular weight 1.69 KD_a with the amino acid sequence Thr-Gly-Arg-Ala-Lys-Arg-Arg-Met-Gln-Tyr-Arg-Arg (Fig 1) has six positively charged residues (10, 11).

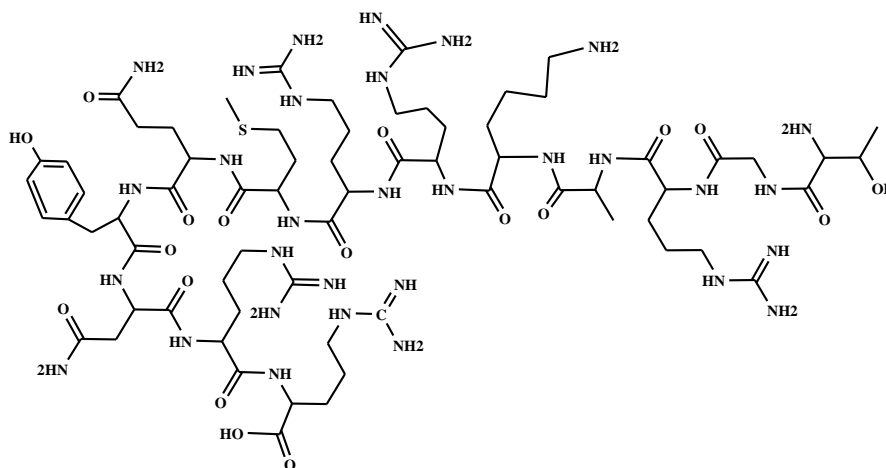


Figure 1. The Structure of UBI $_{29-41}$

UBI $_{29-41}$ can bind to the negatively charged groups present on the microbial membrane by electrostatic interaction (12). So that UBI $_{29-41}$ is suitable candidate to label by ^{99m}Tc directly ^{99m}Tc -UBI $_{29-41}$ or by using coligand reagents like 6-hydrazinopyridine 3-carboxylic acid (HYNIC) and tricine [^{99m}Tc /Tricine/HYNIC]UBI $_{29-41}$ indirectly [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ for scintigraphy imaging of infection (13,14,15,16). ^{99m}Tc -UBI $_{29-41}$ and [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ have been shown rapid accumulation to the infection area and fast clearance with minimum liver uptake and hepatobiliary excretion.

This study was conducted to evaluate the value of [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ and ^{99m}Tc -MDP scintigraphy imaging in detection of infection and differentiation of infection from sterile inflammation in diabetic patients with suspected diabetic foot infection.

II. MATERIALS AND METHODS

All chemical materials have been purchased from Merck or Fluka. The chemicals and solvents were of the highest purity and analytical grade and used without further purification. The freeze-dried kit of MDP, [Tricine/HYNIC] UBI $_{29-41}$ and $^{99}\text{Mo}/^{99m}\text{Tc}$ generator have been provided by Radioisotope Division of Atomic Energy Organization of Iran.

2.1. Labeling of MDP by ^{99m}Tc

(740-920 MBq) freshly eluted $^{99m}\text{TcO}_4$ was added to MDP vial and shaken for 30 sec and mixture was placed for 10 min at the room temperature.

2.2. Labeling of [Tricine/HYNIC] UBI $_{29-41}$ by ^{99m}Tc

Labeling of the kit [Tricine/HYNIC] UBI $_{29-41}$ was performed by adding 0.5 ml of saline in an evacuated vial and shaken, the mixture was allowed to preincubate for 5 min at room temperature then (555-740 MBq) of freshly eluted $^{99m}\text{TcO}_4$ in 0.5 ml of saline was added to the vial and incubated for 10 min in water bath at 100 $^\circ\text{C}$.

2.3. Quality Control

Radiochemical purity analyses were performed by Instant Thin-Layer Chromatography (ITLC) by using Whatman No.3 filter paper chromatography as the stationary or solid phase and different solvent systems as mobile system. Samples of the preparations containing labeled peptide or MDP (2 μl) were applied at the approximately 1 cm from the bottom of ITLC strips and allowed to dry at the room temperature and then placed in air-tight containers with different solvent systems. Then the strips were cut to $\frac{1}{3}$ lower and $\frac{2}{3}$ upper and

counted for 2min under a single head gamma camera equipped with a low energy all-purpose collimator. Using an energy peak centered a 140 Kev.

The quality control of [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ was performed: 2-butanone for free $^{99m}\text{TcO}_4$ ($R_f=1$), 0.1 M Sodium Citrate, $P_H=5$, to determine the non peptide-bound ^{99m}Tc coligand and $^{99m}\text{TcO}_4$ ($R_f=1$) and Methanol/ 1 M Ammonium Acetate 1:1 for ^{99m}Tc colloid ($R_f=0$), R_f values of [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ in each system equal 0.0 and 0.8-1 respectively.

The quality control of ^{99m}Tc -MDP was performed: Methanol/Acetone 50:50 $^{99m}\text{TcO}_4$ ($R_f=1$), ^{99m}Tc -MDP and reduced ^{99m}Tc ($R_f=0$). By using Phosphoric acid 15% as another mobile solvent, ^{99m}Tc -MDP and $^{99m}\text{TcO}_4$ moved to the solvent front, where as reduced ^{99m}Tc remained at the point of spotting.

2.4. Patients

This clinical study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences and then registered to the Iranian Registry of Clinical Trials [IRCT]. The registration number of this trial was IRCT 201106196653N1.

Patients suspicious for diabetic foot infection were introduced to the department of Nuclear Medicine and Molecular Imaging of Golestan hospital. The patients have entered to this study that the subjects have not recently received any antibiotic therapeutic agents for their present situation in order to prevent any bias in the interpreting of radioisotope scanning. Each patient gave written consent after receiving a full explanation of the procedure and the aim of this study. Ten patients were included 6 males and 4 women (mean age =62.45 year and age range =59 to 67 year) participated in this approach. Patients with history of therapeutic antibiotic therapy for their present condition or during this study, renal disease or hepatic disorder were excluded from this investigation.

2.5. Imaging protocol

For all studies: a single-headed camera (E-Cam, Siemens, USA) employing a low-energy, high-resolution collimator was used. 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. Every patient has been participated to this study to undergo ^{99m}Tc -MDP three phase bone scan and near about 48 hours later [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging.

2.6. Three phase bone scan

The (740-920 MBq) of ^{99m}Tc -MDP was injected intravenously. Images were included blood flow (phase 1), blood pool (phase 2) and following local increased uptake in the region of interest on delayed phase (phase 3) were obtained (Fig 2).

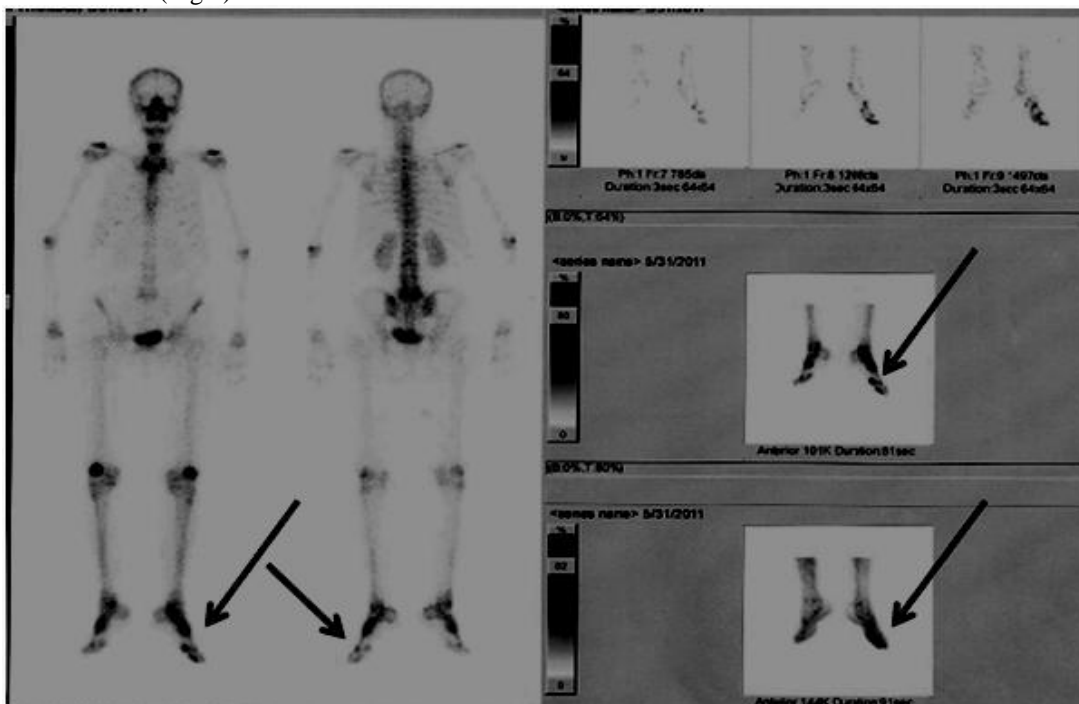


Figure2. Three phase bone scan in the patient: showed increased radiotracer uptake in left big toe bone structure in all phases of study.

2.7. [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ scintigraphy

The (555-740 MBq) [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ was injected intravenously, while the patient in supine position the dynamic study was performed immediately after injection of the label peptide with 60s for each frame up to 45 min. Whole-body anterior and posterior images followed by spot views of the region of interest were also obtained at 60 and 120 min. (Fig 3)

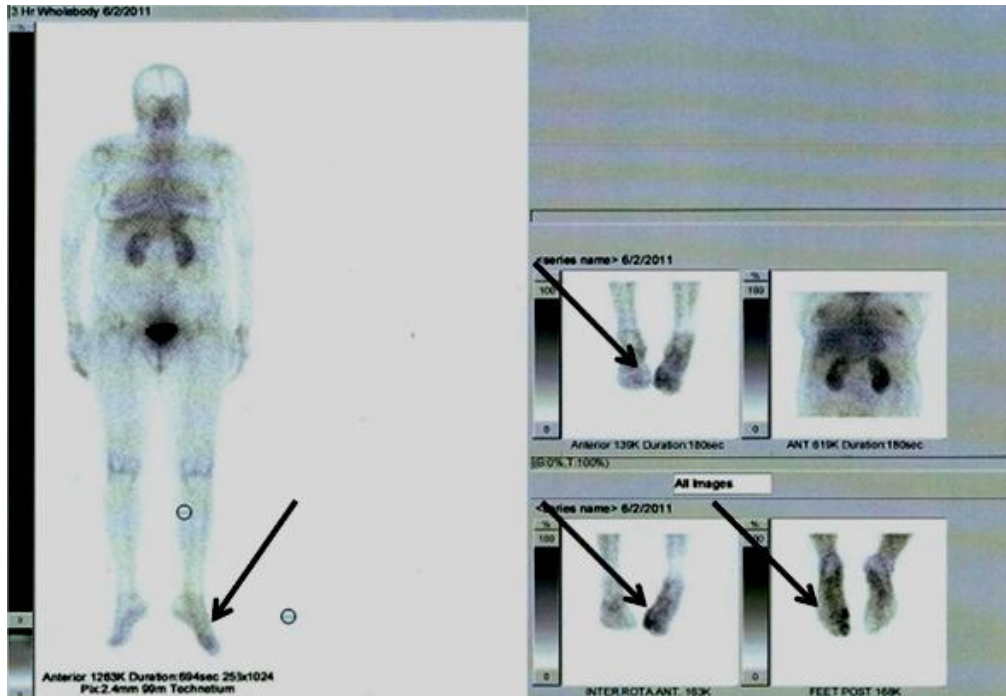


Figure3. The [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ scintigraphy imaging in the same patient showed lesser intensity of uptake in organ of interest compare to early images. Note the distribution of radiotracer in whole body and comparison with renal and liver uptake. There is moderate intensity accumulation of radiolabel [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ in left big toe (bones and soft tissue accumulation)

2.8. Bacteria Culture

When the scintigraphy imaging studies have performed .The samples for culture were taken by specialist. The samples were taken from the infected sites, by 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 hours. The Bacteria culture was used as the gold standard test for diagnosis of infection.

III. RESULTS

3.1. Quality control

The average radiopharmaceutical purity of [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ and ^{99m}Tc -MDP prepared from freeze-dried kits were above 80 % and 95 % respectively.

3.2. Human study

All patients have participated in this investigation had positive culture. Staphylococcus bacteria were the most cultured pathogen as a single or as a part of multiorganisms (10 patients).In four patients Staphylococcus were present with several microorganisms like Streptococcus, Klebsiella, and Pseudomonas.

For interpretation of [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ scintigraphy images two criteria were used. First the distribution of [^{99m}Tc /Tricine/HYNIC] UBI $_{29-41}$ in the suspected infection region and the contra lateral healthy side as a non-target region was considered. Second visual score(0-3) was used The scores of 0 equivalent to soft tissue (no uptake),1 less uptake than liver was considered as negative,2 uptake equal to or greater than liver and 3 uptake equal to or greater than kidneys were considered as positive. For interpretation of ^{99m}Tc -MDP three phase bone scan only the distribution of ^{99m}Tc -MDP in suspected infection area with contra lateral healthy side was used to interpret the three phase bone scan. All the scintigraphy images were interpreted by three independent nuclear medicine physicians and their agreed opinion was considered as the final decision.

During this study none of the patients reported any adverse reaction like nausea, vomiting, diarrhea, bronchospasm, hypotension, hypertension, tachycardia, bradycardia, fever, chills, muscle cramps and allergic

reaction after ^{99m}Tc -MDP and [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ injections. [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging showed rapid distribution at the suspected region to infection. This can provide scan interpretation of suspected infection as early as 5 min after injection. Not only there was no major improvement in scan contrast was observed up to 120 min after injection, but also there was no any appreciable distribution difference between soft tissue and bone at the site of infection. The results of these scans reiterated this matter [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ for the characteristics of ligand molecule to reach at the binding sites as early as possible.

The visualized site of infection with [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy images were consistent with ^{99m}Tc -MDP scans. In our study, sensitivity, specificity and positive predictive value (PPV: proportion of patients with infection who were diagnosed correctly) of radionuclide scintigraphy imaging were 100%.

IV. DISCUSSION

Foot infection is the most serious and common complication among diabetic patients. Diabetic patients with foot infection are very sensitive to osteomyelitis which may lead to amputation. For this reason early diagnosis and effective treatment of osteomyelitis is very important in the medicine. The diagnosis of infection from sterile inflammation because of co-existence of neuropathy, vasculopathy, macroangiopathy, osteoarthropathy, inflammation and trauma in diabetic patients is challenging and critical. Several techniques and facilities have been suggested to solve this problem. Plain radiography is usually considered as the first step to assess foot infection but the sensitivity for radiography has been reported to range 43 to 75 % and selectivity from 75 to 83 % (17).

UBI₂₉₋₄₁ is a synthetic antimicrobial peptide and this molecule has 6 positively charged and binds directly to the negatively charged groups present on the microbial membrane by electrostatic interaction, at the site of infection without any accumulation in sterile inflammatory processes.

UBI₂₉₋₄₁ peptide molecule is suitable candidate for labeling with ^{99m}Tc for scintigraphy imaging to detect infection from sterile inflammation lesion. UBI₂₉₋₄₁ in dose 400 μg can be labeled with ^{99m}Tc directly or by using coligand reagents in dose 40 μg labeled with ^{99m}Tc indirectly. ^{99m}Tc -UBI₂₉₋₄₁ has been shown appreciable results for differentiation between infection and inflammation in human studies. Akhtar et al, investigated eighteen patients with suspected bone, soft tissue or prosthesis infection by ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy scanning. They reported the sensitivity, specificity, positive predictive value (ppv), negative predictive value (npv) and overall diagnostic accuracy ^{99m}Tc -UBI₂₉₋₄₁ radioisotope imaging for infection localization were 100 %, 80%, 92.9%, 100% and 94.4% respectively (18). Assadi et al, studied the accuracy of ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging in detection of osteomyelitis in comparison to ^{99m}Tc -MDP and magnetic resonance imaging. The result of above mentioned study indicated the accuracy of ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging to visualize osteomyelitis was 100 % (19). They found that the detection of osteomyelitis with ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging is a promising modality with high accuracy. Sepulveda-Mendes et al, studied 196 patients with FUO (Fever Unknown Origin) by ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging. They reported sensitivity, specificity, accuracy, ppv and npv of ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging to visualize the infection foci were 97.52%, 95.35%, 96.61%, 96.72% and 96.47% respectively (20). They concluded that ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging could be the gold standard in molecular imaging of infection sites. Melendez-Alafort et al, assessed 6 children with suspected bone infection by ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging. They reported ^{99m}Tc -UBI₂₉₋₄₁ radioisotope imaging has adequate biokinetic properties and ability to detect infection (21).

Gandomkar et al, evaluated [^{99m}Tc /Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging in 7 patients with suspected bone or soft-tissue infection and reported sensitivity and specificity 100% (22).

Vallejo et, al evaluated 13 patients with suspected mediastinitis after cardiac surgery by ^{99m}Tc -UBI₂₉₋₄₁ scanning. They reported the sensitivity, specificity, ppv, npv and overall diagnostic accuracy for detecting patients with mediastinitis by ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging were 83%, 100 %,

100 %, 87% and 92 % respectively (23). Relative high accuracy, sensitivity and specificity of ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging in the detection of infection foci indicate high potential of this radiopharmaceutical directly tags the microorganisms at the site of infection (infection-specific radiotracer). This radiotracer binds directly to the bacterial cell membrane at the site of infection. It attaches to the negatively charged groups present on the bacterial cell wall by electrostatic interaction. Therefore it can be considered as a suitable agent in comparison to radiopharmaceutical agents that have an indirect approach. Such promising results may be partly due to the pathogen inducing infection. Staphylococcus aureus is one of such infection pathogens. Akhtar et al, studied the bacterial infection seeking potential of ^{99m}Tc -UBI₂₉₋₄₁ in Staphylococcus aureus and Escherichia coli induced infections. They showed that ^{99m}Tc -UBI₂₉₋₄₁ accumulated less in Escherichia coli than in Staphylococcus aureus (24). In another attempt Fard-Esfahani et al, investigated ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging particularly in 15 diabetic patients with suspected bone or soft-tissue infection and reported the sensitivity of 50% for ^{99m}Tc -UBI₂₉₋₄₁ scanning in those patients (25). The result of that study is contrary with other approaches. The explanation for low sensitivity of ^{99m}Tc -UBI₂₉₋₄₁ scintigraphy imaging in the above

mentioned assessment could be related to the presence of multiorganisms especially E-coli at the site of infection. In our investigation Staphylococcus bacteria were the most microorganisms observed in the cultures of the patients. For this reason all the scintigraphy images were positive. This matter emphasizes high sensitivity of radiotracer scintigraphy imaging to detect infection sites. There was no culture negative case to assess the potential of radiotracer scintigraphy imaging for preferential diagnosis of infection from sterile inflammation process. All patients underwent ^{99m}Tc-MDP three phase bone scan. The results of three phase bone scan of the above mentioned subjects were positive. [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging study has been performed approximately about 48 hours after the three phase of bone scan has been done. None of the patients reported any adverse reaction after the radiopharmaceuticals were administered. [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ demonstrated rapid distribution at the suspected area to infection. There was not distribution difference between soft-tissue and bone at the site of infection. Therefore, the [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging could not differentiate soft-tissue from bone infection. The rapid distribution of this radiopharmaceutical to the interested area indicates the specificity of [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ to detect infection. It can provide interpretation of [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ scan as early as 5 min after injection. For discrimination diagnosis of infection from sterile inflammation lesions among diabetic patients with diabetic foot problem, [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging may be considered after ^{99m}Tc-MDP three phase bone scan of the patient is positive. Our study suffers from one main limitation regarding number of patients attended in this experiment. Beside above mentioned drawback, the most patients had Staphylococcus aureus positive cultures, which are explained by the fact that Staphylococcus aureus is the most common cause of diabetic foot infection in this setting. Larger study populations will increase higher probability of covering other pathogenic microorganisms provide a more detailed information about the diagnostic value of ^{99m}Tc-MDP and [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ scintigraphy imaging to detect infection from sterile inflammation lesions among diabetic patients with diabetic foot problem.

V. CONCLUSION

If the microorganisms involved in the pathogenesis of foot infection among diabetic patients are sensitive to UBI, two scintigraphy imaging ^{99m}Tc-MDP and [^{99m}Tc/Tricine/HYNIC] UBI₂₉₋₄₁ can be considered as a gold standard.

ACKNOWLEDGMENTS

This study is part of pharm-D thesis of Hamidreza Samaee and special thanks to Ahvaz Jundishapur University of Medical Sciences for the financial support and also to Radiation Division of Atomic Energy Organization of Iran for providing the freeze-dried [Tricine/HYNIC] UBI₂₉₋₄₁kits and ⁹⁹Mo/^{99m}Tc generator. Abbreviations Bq: Becquerel; FUO: Fever unknown; HYNIC: 6-Hydrazinopyridine 3-carboxylic acid; In: Indium MDP: Methylene diphosphonate; MO: Molybdenum; NPV: Negative predictive value; PPV: positive predictive value TcO₄: Pertechnetate; Tc: Technetium; UBI: Ubiquicidin Competing interests.

The authors declare that they have no competing interests.

REFERENCES

- [1] A. Hartemann-Heurtier A. E. Senneville E. Rewiew Foot osteomyelitis. Diabetes Metab 34,2008, 87-95
- [2] W.T. Yuh, J.D. Corson, H.M. Baraniewski, K. Rezai Asad, A.R. Shamma, M.H. Kathol, Y. Sato, G.Y. El-houry, D.R. Hawes, C.E. Platz, R.R. Cooper, R.J. Corry. Osteomyelitis of the foot in Diabetic Patients :Evaluation with plain film, ^{99m}Tc-MDP bone scintigraphy, and MR Imaging. AJR Am J Roentgenol 152,1989, 795-800
- [3] P.R. Cavanagh, M.J. Young, J.E. Adams, K.L. Vickers, A.J. Boulton. Radiographic abnormalities in the feet of patients with diabetic neuropathy. Diabetes Care 17,1994, 201-209
- [4] C.J. Palestro. The current rule of gallium imaging in infection. Sem Nucl Med 14,1994, 128-141
- [5] P. Laverman, C.P. Bleeker-Rovers, F.H.M. Corstens, O.C. Boerman, W.J.G. Oyen. Development of infection and inflammation targeting compounds. Curr Radiopharm 1,2008, 42-48
- [6] H.A. Bohcheliani, A.D. Klisarova, L.A. Koeva Radioimmune imaging of diabetic foot infection Tc-^{99m}-labelled antigranulocyte antibody in combination with Tc-^{99m}-methylene diphosphonate bone scintigraphy. Turk J Med Sci 32,2002,255-259
- [7] S. Crerand, M. Dolan, P. Laing, M. Bird, L.M. Smith, L. Klenerman Diagnosis of osteomyelitis in neuropathic foot ulcers. J Bone and Joint Surg 78,1996, 51-55
- [8] R.E. Weiner, M.L. Thakur. Radiolabeled peptides in diagnosis and therapy. Semin Nucl Med 16,2001,296-311
- [9] W.J.G. Oyen, R.A.M.J. Claessens, J.R. VanHorn, J.W.M. Vander Meer, F.H.M. Corstens. Scintigraphic detection of bone and joint infection with indium-111 labeled nonspecific polyclonal human immunoglobulin G. J Nucl Med 31,1990,403-412
- [10] L. Melendez-Alafort, F.M. Ramirez, G. Ferro-Flores, C.A. Murphy, M. Pedraza-Lopez, D.J. Hnatowich. Lys and Arg in UBLA specific site for a stable Tc-^{99m} complex. Nucl Med Biol 30,2003, 605-615
- [11] G. Ferro-Flores, F.M. Ramirez, L. Melendez-Alafort, C.A. Murphy, M. Pedraza-Lopez. Molecular recognition and stability of ^{99m}Tc-UBI₂₉₋₄₁ based on experimental and semiempirical results. Appl Radiat Isot 61,2004,1261-1268
- [12] G. Ferro-Flores, C.A. Murphy, M. Pedraza-Lopez, L. Melendez-Alafort, Y.M. Zhang, M. Rusckowski, D.J. Hnatowich. In Vitro and in Vivo assessment of ^{99m}Tc-UBI specificity for bacteria. Nucl Med Biol 30,2003,597-603
- [13] M.M. Welling, S. Mongera, A. Lupetti, H.S. Balter, V. Bonetto, U. Mazzi, E.K.J. Pauwels, P.H. Nibbering. Radiochemical and biological characteristics of ^{99m}Tc UBI₂₉₋₄₁ for imaging of bacterial infections. Nucl Med Biol 29,2002,413-422

- [14] L. Melendez-Alafort, A. Nadali, G. Pasut, E. Zangoni, R. De Caro, L. Cariolato, M.C. Giron, I. Castagliuolo, F.M. Veronese, U. Mazzi. Detection of sites of infection in mice using ^{99m}Tc labeled PN2S-PEG conjugated to UBI: a comparative biodistribution study. *Nucl Med Biol* 36,2009,57-64
- [15] M. Gandomkar, R. Najafi, M. Mazidi, M. Goudarzi, S.H. Mirfallah. New peptide based Freeze-Dried kit [^{99m}Tc-HYNIC]-UBI 29-41 as a human specific infection imaging agent. *Iran J Nucl Med* 16, 2008, 25-30
- [16] M. Gandomkar, R. Najafi, M. Mazidi, S.H. Mirfallah, M. Goudarzi. Three different procedures in labeling of Uniquicidin with technetium 99m: a comparative study. *Iran J Radiat Res* 7,2009, 97-104
- [17] T.A. El-Maghraby, H.M. Moustafa, E.K. Pauwels. Nuclear medicine methods for evaluation of skeletal infection among diagnostic modalities. *Q J Nucl Med Mol Imaging* 50, 2006,167-192
- [18] M.S. Akhtar, A. Qaisar, J. Irfanullah, J. Iqbal, B. Khan, M. Jehangir, M.A. Nadeem, M. Aleem Khan, M.S. Afzal, I. Ul-Haq, M.B. Imran. Antimicrobial peptide ^{99m}Tc-Ubiquicidin 29-41 as human infection –Imaging agent: clinical trial. *J Nucl Med* 46,2005,567-573
- [19] M. Assadi, K. Vahdat, I. Nabipour, M.R. Sehhat, F. Hadavand, H. Javadi, A. Tavakoli, J. Saberrifard, M.R. Kalantarhormoz, A. Zakani, M. Eftekhari. Diagnostic value of ^{99m}Tc-ubiquicidin scintigraphy for osteomyelitis and comparisons with ^{99m}Tc-methylene diphosphonate scintigraphy and magnetic resonance imaging. *Nucl Med Commun* 32,2011,716-723
- [20] J. Sepulveda-Mendez, M. Consuelo Arteaga, R.B. Juan Carlos, M. Pedraza-Lopez. Specificity of ^{99m}Tc-UBI for detecting infection foci in patients with fever in study. *Nucl Med Commun* 31,2010,889-895
- [21] L. Melendez-Alafort, J. Rodriguez-Cortes, G. Ferro-Flores, M. Consuelo Arteaga, Herrera-R. Rodriguez, E. Mitsoura, C. Martinez-Duncker. Biokinetics of ^{99m}Tc-UBI 29-41 in humans. *Nucl Med Biol* 31,2004,373-379
- [22] M. Gandomkar, R. Najafi, M. Shafiei, M. Mazidi, M. Goudarzi, S.H. Mirfallah, F. Ebrahimi, H.R. Heydarpor, N. Abdie. Clinical evaluation of antimicrobial peptide [^{99m}Tc/Tricine/HYNIC]ubiquicidin 29-41 as a human-specific infection imaging agent. *Nucl Med and Biol* 36, 2009,199-205
- [23] E. Vallejo, I. Martinez, A. Tejero, S. Hernandez, L. Jimenez, D. Bialostozky, G. Sanchez, H. Ilarraza, G. Ferro-Flores. Clinical utility of ^{99m}Tc-labeled Ubiquicidin 29-41 antimicrobial peptide for the Scintigraphic detection of mediastinitis after cardiac surgery. *Arch Med Res* 39,2008,768-774
- [24] M.S. Akhtar, J. Iqbal, M. Aleem Khan, J. Irfanullah, M. Jehangir, B. Khan, I. Ul-Haq, G. Muhammad, M.A. Nadeem, M.S. Afzal, M.B. Imran. ^{99m}Tc-labeled antimicrobial peptide Ubiquicidin (29-41) accumulated less in Escherichia Coli infection than in Staphylococcus aureus infection. *J Nucl Med* 45,2004, 849-856
- [25] A. Fard-Esfahani, D. Beiki, B. Fallahi, M.R. Moharjeri-Tehrani, M.R. Gharaie, N. Rouhipour, M. Dehghanian, M. Saghari, A. Emami-Ardekani, M. Eftekhari. Evaluation of ^{99m}Tc-Ubiquicidin 29-41 scintigraphy in differentiation of bacteria infection from sterile inflammation in diabetic foot. *Iran J Nucl Med* 18,2010,20-28