Molecular Perfusion Imaging with 99mTc-MIBI Lower Limb Muscle SPECT: In Diagnosis and Follow up of Peripheral Arterial Diseases (PAD)

Rashid Rasheed*
GINUM Cancer Hospital, Nizampur, Pakistan

Abstract
The study was conducted to evaluate the clinical utility of 99mTc-MIBI lower limb muscle perfusion single photon emission tomography (SPECT) for diagnosis and follow up of PAD.

Methods: The study was performed to develop normal values of lower limb muscle perfusion for normal local population (N=36) by using SPECT and planar scintigraphic techniques. Patients (N=44) with established PAD were compared with age matched data of normal population. Normal range developed for planar study (0.90±0.10 ± 2S.D) was used as reference for planar study in patient population.

Results: In SPECT study visual analysis correlated extremely well with clinical features. Comparison of patient SPECT data with normal population showed significant hypoperfusion of lower limbs in terms of counts (P-value <0.001). Follow up of patients (N=12) showed significant improvement of muscle perfusion in nine patients while three patients showed no improvement. Comparison of follow up planar study values with baseline were not conclusive for any improvement in limb perfusion (p-value ~ 0.98). Comparison of SPECT study with other modalities showed higher sensitivity than Doppler ultrasound (P-value <0.05), ABI (p-value <0.001) and planar scan (p-value <0.001) The sensitivity, specificity, positive predictive values and negative predictive values are 93%, 100%, 100%, 92% for 99mTc-MIBI; 64%, 100%, 100%, 69% for ABI; 77%, 100%, 100%, 78.3% for Doppler ultrasound and 75%, 100%, 100%, 76.6% for Planar scan respectively.

Conclusion: 99mTc-MIBI lower limb muscle perfusion scan is an excellent tool for diagnosis and follow up in PAD and may be a baseline tool for assessment of patient response to treatment.

Keywords: 99mTc-MIBI; Lower limb muscle perfusion; Peripheral Arterial Diseases (PAD); SPECT

Introduction
Peripheral arterial disease (PAD) comprises those entities which result in obstruction to blood flow in the arteries, exclusive of the coronary and intracranial vessels. Peripheral vascular disease is a slow and progressive circulation disorder. It may involve any of the vessels outside the heart and is a disease of the arteries, veins and lymphatic vessels. However, the legs and feet are commonly affected thus the name peripheral vascular disease (PVD) [1]. The present study was undertaken to evaluate the potential of 99mTc-MIBI in Lower Limb Muscle Perfusion SPECT, its feasibility as a clinical tool for diagnosis and follow-up of peripheral arterial diseases (PAD) and to establish normal values of Lower Limb Muscle Perfusion SPECT for local population. 99mTc-MIBI SPECT gives excellent 3D analysis of perfusion in all muscular compartments. All SPECT slices give a refined perfusion status in objective manure starting from knee joint to ankle joint. 99mTc-MIBI is taken up in the mitochondria of muscles and stays inside the cells, therefore can be used for imaging perfusion when tagged with 99mTcO4.

Materials and Methods
This pilot study was approved by the local ethical and radiation committee of Multan Institute of Nuclear Medicine (MINAR), and Gujranwala Institute of Nuclear Medicine (GINUM), Pakistan. The entire human study was performed according to provisions of the Declaration of Helsinki of 1975, as revised in 2008 (5), regarding medical research involving human subjects. The study was carried out over a period of six months and conducted according to the national rules and following the international standards. Patients included in this study were referral cases from Outpatient Department (OPD), Nishtar Hospital, Multan. Subjects selected in control group were non-diabetic, normotensive, nonsmokers, who were further screened by routine clinical investigations. All patients included in the study were true positive cases of Peripheral Arterial Diseases (PAD), because we were interested in such cases for evaluation and comparison of the diagnostic sensitivity of various diagnostic modalities related to peripheral arterial diseases. The clinical features due to apparent disease were used as gold standard. Doppler study and Ankle Brachial Index (ABI) were performed in all patients and all controls were screened for any underlying pathology. ABI was calculated as below:

\[ \text{ABI} = \frac{P_a}{P_b} \]

\( P_a \) is highest pressure obtained from the ankle vessels for that leg.
\( P_b \) is highest brachial pressure of two arms.

Patient selection
Thirty-six controls from normal population and forty-four patients (19 female and 25 male, mean ages, 33.9 y; range, 10–68 y) with suspected peripheral artery disease were selected for this study before starting any

*Corresponding author: Rashid Rasheed, GINUM Cancer Hospital, Nizampur, Sialkot Road, Gujranwala, Pakistan, Tel: +92 55 3493370; Fax: +92 55 3493375; E-mail: dr.nmd.paec@gmail.com

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imaging procedure. No patient had a history of allergy. Each subject or a patient gave written consent after receiving a full explanation of the clinical procedure. As described earlier, patients with suspected peripheral artery disease of the bone or limbs were included in the study. However, patients with limb lesions were preferred because they could be compared with the healthy contralateral side. Pregnant or lactating women were excluded from this study. Candidates with known hepatic or renal insufficiency or a history of allergy were also excluded.

99mTc-MIBI scintigraphy

Preparation of 99mTc-MIBI: Hexakis (2-methoxy-2-methylpropylisonitrile) - MIBI was obtained in a freeze-dried kit form from Pakistan Institute of Nuclear Science and Technology (PINSTECH). The kit was formulated as a five-patient vial which comprised 1.0 mg MIBI compound and 0.075 mg of SnCl2·2H2O. The kit was radiolabeled by adding 100-120 mCi of sodium pertechnetate from a freshly eluted technetium generator (PakGen; PINSTECH), shaking for about 20 sec and heating in boiling water for 10 min. Afterwards, the volume was raised to about 5.5 mL by addition of 0.5 mL of saline. The pH of the solution was in the desired range of 6–7. The entire vial content was mixed well and 1 mL out of it was injected intravenously to each patient.

Quality control: Radiochemical purity of the complex was determined by chromatographic techniques. Radiochemical purity of the 99mTc-MIBI was studied by using two simple chromatographic techniques, e.g., Instant Thin Layer Chromatography (ITLC-SG strips; Gelman Sciences) and Paper Chromatography (PC) by using Whatman No.3 chromatographic strips. The technique was employed to determine the percentage of hydrolyzed, (radionuclide bound to ligand) and of free pertechnetate. Acetone was used as a mobile phase for paper chromatography and saline was used for ITLC. Small aliquots from the reconstituted kit were spotted on the respective strips. The strips, after elution, were cut in fractions of 1cm and counted for radioactivity in a well type scintillation gamma counter (Scaler Timer–ST7, Thorn EMI – Nuclear Enterprises, United Kingdom).

99mTc-MIBI imaging: The E-Cam, equipped with a low-energy all-purpose collimator, was used for acquisition, and data were processed using the ICON 8.5 Macintosh system (Apple Computer Inc.). Target-to-nontarget ratio (T/NT) was calculated using the region ratio software of the E-Cam.

Patients preparation: Baseline investigations of all the subjects, including a complete blood examination (red blood cell count, total lymphocyte count, leukocyte count, erythrocyte sedimentation rate) and urea, creatinine, and liver function tests, were conducted. Urine samples were also collected beforehand for routine chemical and microscopic examination. A dose of 740–800 MBq (20 mCi) of 99mTc-MIBI was given intravenously to acquire images of the suspected area and the corresponding nontarget area. During the study, vital signs were monitored for any significant change from baseline. Blood and urine samples of the patients were checked after 5 d to document any change from prescan values that could be attributed to use of the radiopharmaceutical.

Biodistribution: Whole-body images of 3 candidates were used to calculate the biodistribution of 99mTc-MIBI. Urine voiding was avoided during the study up to 240 min. A region of interest was drawn around the whole body on anterior and posterior images, and counts with geometric mean method were considered 100% of the injected dose at that particular time. Similarly, regions of interest were drawn around the liver, kidneys, and urinary bladder, and the percentage injected dose at 30, 120, and 240 min was calculated using the following formula: percentage injected dose in an organ=100 × organ count at particular time/total-body count at that time. Other subjects were not included in the biodistribution study, as the objective was to have an overview of the behavior of the kit in humans.

Study protocol: In our study, the stress to the lower limb muscles was created by performing exercise of feet. Exercise was performed with 100 times flexions and extensions of feet simultaneously on both sides. At peak stress (after 100 flexions and extensions), 20 mCi (740 MBq) of 99mTc-MIBI was injected intravenously as a bolus dose and flushed with 5 mL of 0.9% normal saline. Flexion and extension was continued 50 times after the injection. The dose in all patients and controls was strictly standardized to reduce the error in the counts. In all patients, imaging time was also standardized to avoid any statistical error in the count data due to decay of the radionuclide. All the patients were imaged after 20 ± 5 min and studied by following the same-day stress 99mTc-sestamibi SPECT imaging protocol.

Results

Quality control tests

During labeling of MIBI with 99mTc - besides the bound 99mTc to MIBI complex, free pertechnetate (99mTcO4-) and reduced or hydrolyzed 99mTcO2 were also formed as separated by PC and ITLC. In PC, 99mTcO4 had an Rf of 0.00-0.01, while the 99mTc-MIBI and the reduced or hydrolyzed 99mTcO2 appeared at Rf 0.9-1.0. The reduced or hydrolyzed fraction was separated from the other two fractions by using saline, in which case the 99mTc-MIBI complex and the free 99mTcO2 appeared at Rf=0.00-0.01, and the 99mTcO2 was detected at Rf=0.9-1.0. The overall labeling yield of the 99mTc-MIBI complex as calculated by these methods was more than 98.6 ± 1.2%.

Quantitative SPECT perfusion curves

The leg can be divided in to 64 short axis slices. That is why SPECT offers a detailed compartmental and segmental analysis of the perfusion status of the leg, which is not possible in planar scan and in the Doppler study. As compared to the Doppler ultrasound, the uptake of the tracer is directly proportional to the blood flow that is why SPECT scan reflects the exact status of the perfusion of the limbs. All controls and patients were analyzed in terms of standardized counts of both lower limbs. Initially, counts of each slice out of 12 slices of each normal subject (N=36) were calculated for both limbs. Perfusion curves were generated for each limb by using mean counts from each slice as shown in Figure 2a and 2b. The upper and lower limits were defined as ±1.5SD of mean %age uptake counts of one particular slice for all 36 subjects. Afterwards, the counts of each slice from control group were compared with the counts of same slice from patient group to evaluate the significance of the difference. The P value was taken as <0.05 (Figure 1).

Planar study

The posterior static c alf images show the major part of the leg muscles. The advantage of the posterior images when compared with images in anterior views is that there is no tibial bone attenuation. The rectangular Region of Interest (ROI) was drawn on one side and mirror method was used for the contralateral side as shown in Figure 2c.

Case study

Patient presented with history of pain and blackish discoloration
of the right foot for last one month was treated locally with analgesics but no relief. On examination, all distal pulses were palpable except posterior tibial artery (PTA) on the right side. Ankle brachial index was calculated with values of 0.75 for right and 0.92 for left side.

**SPECT data visual analysis**

The reconstructed SPECT short axis slices of the perfusion scan showed perfusion defect in the posterior compartment of the right leg and there was normal perfusion in the anterior and lateral compartments of the right side. The visual inspection of the short axis SPECT slices helps to clearly differentiate between the different compartments, e.g., anterior lateral and posterior views. In this case, the short axis slices clearly show reduced perfusion in the posterior compartment of right leg, as shown in the Figure 3a. There is also decreased perfusion pattern on the left side. Moreover the SPECT study shows that the level of hypo-perfusion in the right limb starts from knee (Slice 1) to the ankle joint (Slice 12). This suggests that the level of obstruction is lying around the knee joint or above, thus we can predict the level of obstruction of artery easily. Follow up scan of same patient shows moderate improvement in the perfusion status of right limb as shown in Figure 3b.

**Comparison of SPECT perfusion curves**

The data plotted against the normal profile curves clearly demonstrate the difference from normal curves due to decreased perfusion in the posterior compartment of the right leg. It is clearly shown in the curves that the level of the perfusion defect is located at the knee joint (Figures 4a and 4b). As the anterior muscle compartment shows normal perfusion on the right side, this means that the obstruction lies at the origin of the posterior tibial artery (PTA) or proximal. Later on, Doppler study of both limbs was performed which also shows the thrombus at the origin of the PTA with dampened downstream flow, as shown in Figure 5a. Follow up study showed persistent thrombus in Figure 5b at origin of right PTA despite patient’s clinical symptoms had improved. The improvement of the perfusion of the limb was excellently manifested on $^{99}$Tc-MIBI SPECT perfusion scan and Doppler scan was unable to demonstrate the improvement.

**Inter-comparison of diagnostic modalities**

Doppler ultrasound is an investigation of choice in all the patients with peripheral arterial diseases. We performed color Doppler study in all 44 patients and results were compared with ABI, Planar scan, SPECT scan and clinical features. As all selected cases in this study were true positive, e.g., all patients had clinically established disease in one or both limbs so they served the basis for the evaluation and comparison of the diagnostic capability of all the techniques.

**Sensitivity and specificity**

Comparison of SPECT study with other modalities showed higher sensitivity than Doppler ultrasound (P-value<0.05), ABI (P-value<0.001) and planar scan (P-value<0.001). The sensitivity, specificity, positive predictive values and negative predictive values for $^{99}$Tc-MIBI are 93%, 100%, 100%, 92%, respectively, for ABI, these values are 64%, 100%, 100%, 69%; for Doppler ultrasound, these values are 77%, 100%, 100%, 78.3% and for planar scan, these are 75%, 100%, 100%, 76.6%, respectively.

**Discussion**

Peripheral arterial disease (PAD) is an important healthcare problem and is an indicator of widespread atherosclerosis in other vascular territories, such as the cerebral and coronary circulations [2]. Radiopharmaceuticals including $^{99}$Tc-transferrin, $^{99}$TcO$_4^-$, $^{99}$Tc-DTPA, $^{99}$Tc-pyrophosphate and $^{11}$C, $^{18}$Na, $^{18}$F, $^{82}$K, $^{99}$Tc-MIBI, $^{133}$Xe, $^{131}$I-iodoantipyrene were used as well [5,6]. $^{99}$Tc-MIBI (methoxy-isobutyl-isonitrile), which was developed primarily as a myocardial perfusion agent, has also proved valuable agent for skeletal muscle perfusion studies [7,8]. Technetium has superior imaging and dosimetry characteristics in comparison to other radionuclides [8]. For many years $^{99}$Tc-MIBI has been used in investigating peripheral arterial diseases using planar, whole body static and SPECT techniques. More emphasis was given to MIBI because of its uptake in the soft tissue. As MIBI is taken up in multiple areas of the body, there is potential diversity of usage of this tracer.

In the recent years, positron emission tomography (PET) imaging has also shown excellent results in the investigation of peripheral arterial diseases. But high cost, small half life of the isotopes and...
limited availability are the major limitations of this technique [9]. Though SPECT studies have been done in the past in investigation of peripheral arterial diseases but it has not been developed as a clinical tool for diagnosis and follow up. Owens et al. reported two cases in which he showed the usefulness of 99mTc-MIBI in diagnosing chronic compartmental syndrome [10]. Oshima et al. quantified leg perfusion by using thallium-201 and showed that this technique is useful in investigating peripheral arterial diseases [11].

In our study, all patients were clinically diagnosed cases of peripheral arterial disease (true positive cases) because our aim was to evaluate the diagnostic sensitivity of different techniques e.g., Doppler vs. 99mTc-MIBI scan, so we avoided the costly procedure of angiography. As there were no previous normal values of lower limb muscle perfusion developed for our local population, in the first part we developed our own normal values for local population. Arteriography shows only the morphologic occlusive features and does not reveal the compensatory capacity of the collaterals. In this study, we therefore, used 99mTc-MIBI leg SPECT with single-headed gamma camera to quantify leg muscle perfusion. It takes 20 min to complete the study and reveals a three-
dimensional image of leg muscle. Although $^{99m}$Tc-MIBI leg SPECT images would be inferior for spatial resolution in terms of anatomic information to X-ray computed tomography, but it reflects local perfusion and also enables a transverse image of leg perfusion. In our study, 10/44 (22.7%) patients had normal Doppler flow but they were hypoperfused. This is also confirmed in the study of Dabrowski et al. in which he showed the significance of $^{99m}$Tc-MIBI scan in picking up the hypoperfusion of the muscles in patients with diffuse atherosclerosis with normal blood flow indices [12]. Thus, analysis of $^{99m}$Tc-MIBI SPECT can provide quantitative information and permit an objective, reproducible test, independent of observed bias in detecting ischemia of lower limb muscles. Local perfusion was also quantified by this method before and after treatment, e.g., in follow-up to assess for disease response to treatment.

Posterior static images of all normal subjects and patients were also taken to evaluate and compare the diagnostic capability of $^{99m}$Tc-MIBI planar and $^{99m}$Tc-MIBI SPECT scan. The results of planar scan were disappointing as it could only pick the disease in the unilateral and advanced cases. Secondly, all counts were diluted to whole length of the leg, not to the same area of defect, thus losing the diagnostic count difference. In SPECT study, the counts were normalized within the slice to maintain the diagnostic difference among the normal and diseased slices. Thirdly, there is no information about compartmental perfusion status in planar scans so we cannot evaluate which artery is blocked, e.g., anterior tibial artery or posterior tibial artery. Planar scan sensitivity reflective of improvement in lower limb muscle perfusion at follow up was also not significant (p-value=0.98). The qualitative-quantitative method of perfusion assessment, as applied in this study, seems sufficient for evaluation of the undertaken therapy and prognosis of peripheral arterial diseases. It seems important that both qualitative (visual) as well as quantitative analysis, when performed separately, demonstrated a high degree of concordance with the clinical evaluation. When juxtaposed with the Doppler ultrasound method, the scintigraphic perfusion method possessed higher diagnostic efficacy. It was able to demonstrate improvement of perfusion even in cases, in which no increase of the flow-rate in large arteries could be detected by means of Doppler ultrasound. Scintigraphy, which reflects limbs perfusion at the level of myocytes, has proved, therefore, to be objective method for evaluation of lower limb muscle perfusion.

This method of quantification of lower limb perfusion shows that there are many advantages of $^{99m}$Tc-MIBI lower limb muscle perfusion SPECT over other diagnostic modalities. First of all, it shows the perfusion status of the limb at microvascular level or at the cellular level. Images of the $^{99m}$Tc-MIBI lower limb muscle perfusion SPECT produced an excellent visual interpretation of the lower limb perfusion. We can easily identify the slice with abnormal perfusion. The three dimensional compartmental visual analysis is another additional advantage of this technique over other diagnostic modalities. All compartments, e.g., anterior, lateral and posterior fascial compartments can be seen in every slice at a glance and can be visually analyzed in terms of perfusion defect. These three dimensional images give further additional information about the vessel blockage, e.g., if the defect is in the posterior compartment then it means that posterior tibial artery may be blocked and if the defect is in the anterior or lateral compartments, then anterior tibial or peroneal artery, respectively, may be blocked. Another advantage of the $^{99m}$Tc-MIBI lower limb muscle perfusion SPECT is that when the data are plotted in terms of quantification curves then one can easily analyze the point of drop of the perfusion curve at a particular level which indicates the level of blockage of the vessel in the leg. This finding could be of great help in deciding the level of amputation in the surgical aspect. We followed the patients through surgical wards and found high correlation of our results with the actual level of amputations performed by the surgeon.

Another advantage of $^{99m}$Tc-MIBI lower limb muscle perfusion SPECT over Doppler was that it could be easily performed in those few cases as well where leg was partially covered by dressing applied over wounds which easily picked up the hypoperfusion from the tissues under the dressings in contrast to Doppler, and it was due to unique quality of gamma ray imaging. As compared to the other diagnostic modalities like computed tomographic angiography (CTA), catheter angiography and magnetic resonance angiography, theradiosotope technique by using MIBI is cost effective and widely available almost at every nuclear medicine center. Regarding radiation exposure, there was six times less dose than CTA or catheter angiography. Moreover, due to easy availability of $^{99m}$Tc-MIBI, it can be used as a follow up tool to evaluate the response to treatment.

The findings of statistical analysis were very striking in our study. The chi-square test was applied to evaluate the significance of difference in multiple ways. In comparison of $^{99m}$Tc-MIBI SPECT perfusion scan with ankle brachial index, there was a significant difference with p-value<0.001, thus showing a clear advantage of $^{99m}$Tc-MIBI SPECT perfusion scan over ankle brachial index. The comparative difference of $^{99m}$Tc-MIBI SPECT perfusion scan from planar scintigraphy was also significant with p-value of <0.001. The most striking finding of the study was the comparison of Doppler ultrasound with $^{99m}$Tc-MIBI SPECT perfusion scan. The chi square test gave p-value of <0.05 which means that there is a significant difference between the results of the two investigations. This proves the diagnostic advantage of $^{99m}$Tc-MIBI SPECT perfusion scan over Doppler ultrasound. The reason of this finding was inability of the Doppler ultrasound to pick up the disease in those patients in which there was gangrene of the toes with normal indices of Doppler flow study. This was due to the reason that we did not take into account the diffuse atherosclerotic changes with normal blood flow as disease positive criteria for gangrenous patients because...
diffuse atherosclerosis changes were also seen in the patients without critical limb ischemia and without gangrene. These findings are supported by the study of Dabrowski et al., in which the 99mTc-MIBI perfusion scan study picked up improvement of perfusion of lower limb muscles and Doppler ultrasound was non-conclusive after lumbar sympathectomy in chronic arteriosclerotic ischemia of lower limbs [13]. This basic limitation of Doppler ultrasound was observed in ten patients when compared to 99mTc-MIBI SPECT perfusion scan. High values of specificity and positive predictive values are due to the design of study, e.g., due to selection of true positive cases for comparison of diagnostic modalities and secondly due to the fact that all subjects selected as control were normal adults with no previous history of disease. Keeping in view these parameters, there were technically no false positive cases in our study, which led to such high specificity and positive predictive values.

In our study, we tried to study maximum number of patients as follow up cases but it was possible in fourteen out of forty four patients, who showed willingness for follow up scan. Two patients with extensive gangrene of lower limb expired and remaining twelve patients were called for follow up scan after at least 2-6 months. Out of twelve patients called for follow up scans, nine patients showed improvements in the perfusion levels and three patients did not show any improvement even after three month of follow up (Table 1).

These results of follow up data were judged clinically on the basis of the improvement of symptoms, healing of wounds, improvement in the Doppler flow indices and scintigraphic SPECT curves. Overall slicewise comparison of baseline and follow up SPECT study showed that there was improvement of perfusion of both limbs on follow up (p-value<0.001). Planar study was non-conclusive for any improvement in lower limb muscle perfusion at follow up (P-value ~ 0.98). The present data confirmed observations made by Duet et al. [14] and Tellier et al. [13] concerning a potential usefulness of scintigraphy in the diagnosis of chronic hypoperfusion of lower limbs.

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References


Table 1: Chi square values.

<table>
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<th>Study</th>
<th>Chi-SQ</th>
<th>DF</th>
<th>P-Value</th>
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<tr>
<td>MIBI with Doppler ultrasound</td>
<td>4.423</td>
<td>1</td>
<td>&lt;0.035</td>
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<tr>
<td>MIBI with ABI</td>
<td>11.344</td>
<td>1</td>
<td>&lt;0.01</td>
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<tr>
<td>MIBI with Planar Scan</td>
<td>5.436</td>
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<td>&lt;0.02</td>
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<tr>
<td>MIBI with True Positive</td>
<td>3.106</td>
<td>1</td>
<td>0.078</td>
</tr>
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