^{99m}Tc-MIBI whole body scintigraphy and P-glycoprotein for the prediction of multiple drug resistance in multiple myeloma patients

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Abstract

Multi-drug resistance (MDR) is a major challenge in the treatment of multiple myeloma (MM). There is low sensitivity of technetium-99m methoxy isobutyl isonitrile (^{99m}Tc-MIBI) whole body scan (WBS) in the detection of active MM lesions, because ^{99m}Tc-MIBI is washed out from malignant cells in the presence of P-glycoprotein (PGP). The objective of the present cohort study was to evaluate of ^{99m}Tc-MIBI WBS in the prediction of MDR in MM patients during a course of one year follow up. *Thirty four patients* with MM (25 male, 9 female of mean age 54.12±11.46 years) entered the study. Thirteen patients had no previous history of treatment and 21 had a history of previous chemo-radiotherapy. The diagnosis and staging of the disease were based upon routine laboratory and clinical criteria like bone marrow plasma cell count, serum M component, calcium, albumin, β_2-microglobulin. Measurements of PGP and WBS using ^{99m}Tc-MIBI were performed before initialization of treatment and the response to treatment was assessed one year later. The baseline ^{99m}Tc-MIBI WBS were considered positive for the detection of active lesions when at least one area of non-physiologic increased activity was noted. The follow up ^{99m}Tc-MIBI WBS was positive for MDR when the patient had active disease but normal WBS. *Our results* showed that for WBS, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy in active state for the diagnosis of MDR were 38.9%, 62.5%, 70%, 31.2% and 46.1%, respectively. Also the above values for the detection of MDR, using PGP values were 50%, 50%, 69.2%, 30.8% and 50%, respectively. The relative risk of resistant to multiple regimens of chemotherapy after one year follow up in patients with negative to patients with positive ^{99m}Tc-MIBI WBS was 1.02 (0.60-1.72). *In conclusion*, we found a low sensitivity of WBS and of PGP in the detection of MDR in patients with active MM. However, both WBS and PGP have 70% and 69% positive predictive value for MDR.

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Introduction

Multiple myeloma (MM) is one of the most frequent B cell malignancies representing 14% of all haematological malignancies in adult population [1]. Different radiotracers have been introduced for the evaluation of MM lesions. Technetium-99m methoxy isobutyl isonitrile (^{99m}Tc-MIBI) is a lipophilic cationic agent, believed to be retained in cells by electronegative cellular and mitochondrial membrane potentials [2] and has been shown to have a potential value for the assessment of disease activity in MM patients [3]. The value of fluorine-18 fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) in the evaluation of MM lesions has recently been reported [4, 5]. ^{18}F-FDG can detect more lesions than the ^{99m}Tc-MIBI in patients with MM [4] and may perform better than ^{99m}Tc-MIBI in the detection of focal lesions, whereas ^{99m}Tc-MIBI may be superior in the visualization of diffuse disease [5].

Previous studies confirmed that over-expression of P-glycoprotein (PGP), the physiologic function of which is facilitating cholesterol trafficking from plasma membrane to endoplasmic reticulum, is a major factor for the reduction of drug sensitivity of the tumoral cells [6, 7]. P-glycoproteins are encoded by a small gene family, consisting of two members in humans (MDR1 and MDR2) [6]. Class I PGP (MDR1) coded in chromosome 7 at q21–31, decrease intracellular concentrations of a wide variety of structurally diverse chemotherapeutic drugs resulting in multidrug resistance (MDR), whereas the closely related class II PGP (MDR2) is not associated with such effects [6]. Based on previous reports, MDR1 expression in MM is an independent prognostic factor associated with poor long-term outcome [7]. Also a significant negative relation between PGP expression and response to chemotherapeutic regimens has been found, using in vitro immunohistochemical methods [7].

It has been postulated that after entering the cytoplasm, ^{99m}Tc-MIBI is recognized by MDR-1 PGP and transported by this PGP out of the malignant cell [8]. In fact, it has been shown that ^{99m}Tc-MIBI is a transport substrate for PGP. Over-expression of PGP in in-vitro samples of resistant tumoral cells was reported to decrease ^{99m}Tc-MIBI uptake [8, 9]. In breast and lung carcinoma samples, also high PGP expression was significantly associated with fast washout of ^{99m}Tc-MIBI [10, 11]. Others found that increased PGP expression was correlated with a low accumulation of ^{99m}Tc-MIBI in the bone marrow, in leukaemia patients [12]. As reported for other types of malignancies in the above-mentioned studies, the net in vivo uptake of ^{99m}Tc-MIBI may be used for non-invasive assessment of MDR and PGP over-expression in MM. Furthermore, a recent study showed that breast cancer with absolute lack of tracer uptake on early images have a defective apoptotic program and high levels of B-cell CLL/lymphoma 2 (Bcl-2) [13, 14]. This observation may also have an important clinical implication for patients with MM. No study has been performed to examine the ability of ^{99m}Tc-MIBI scintigraphy to predict response to chemotherapy in MM patients. According to the evidences supporting the lack of abnormal uptake in the active lesions of patients with MM and MDR, we sought to determine weather this event may help diagnose and predict MDR in clinical assessments. Our study was conducted prospectively to compare the level of PGP expression and ^{99m}Tc-MIBI scintigraphic results in order to predict the outcome of treatment with multiple drugs and chemotherapy in MM patients.

Patients and methods

From November 2006 to September 2008, thirty four consecutive MM patients, 25 male 9 female, with mean age 54.12±11.46 years entered a cohort study. The diagnosis of MM was based on the Durie and Salmon (1975) criteria [15]. Patients were excluded if they were pregnant, unwilling or unable to cooperate, or were considered bone marrow transplant candidates.

Thirteen patients had no previous history of treatment (were recently diagnosed) and 21 had a history of previous chemo-radiotherapy (8 in remission and 13 relapsed). The criteria proposed by Bataille et al. (1992) [16] were used to determine disease activity. Finally, twenty six patients who fulfilled the criteria of active MM disease at onset of the study (including 13 recently diagnosed and 13 previously diagnosed patients with relapsed disease) were prospectively evaluated. For each patient, thorough hematological and biochemical investigations including hemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver and kidney function tests, serum β_2 microglobulin, serum M component, 24h urinary excretion of Bence Jones protein, bone marrow and plasma cell count, serum levels of albumin and calcium were performed. Thereafter, all patients underwent ^{99m}Tc-MIBI WBS and PGP measurements using bone marrow (n=17) or peripheral blood samples (n=17).

Informed consent was assigned by all patients and documented in their medical record. The project was approved by the Ethics Committee and the Research Council of Tehran University of Medical Sciences.

P-glycoprotein measurement

P-glycoprotein over-expression was defined as the detection of positive immune-reactive cells in flow cytometric analysis of bone marrow or whole blood samples. Cells (5 x 10^5) were incubated for 30min with a matched-isotype control antibody IgG2a (Dako Corporation, Glostrup, Denmark) and with the monoclonal antibody 4E3 (7.5 mg/l, Dako Corporation, Carpinteria, USA), which recognizes an external epitope of P-gp. Cells were washed twice in PBS/BSA/NaN3 and incubated for 20min with FITC-labeled goat F(ab')$_2$ anti-mouse IgG (Caltag Laboratories, Burlingame, UK) [17].

All samples were assessed by a pathologist (ME) who was unaware of the patients clinical and laboratory data.

^{99m}Tc-MIBI imaging and interpretation

A commercial MIBI kit (AEOI, Tehran, Iran) was used and the labeling and quality control procedures were performed according to the manufacturer's instructions. Each patient received 740MBq ^{99m}Tc-MIBI via an antecubital vein. Whole-body anterior and posterior scans were obtained after 20 and 60min using a large field of view dual head gamma camera (Solus, ADAC, Milpitas, CA) equipped with a low-energy high resolution parallel hole collimator. A 20% window around the 140keV energy peak of ^{99m}Tc-MIBI was used. Patients were in the supine position during the image acquisition.

All scintigrams were interpreted by two experienced nuclear medicine specialists (BF, MS), who were unaware of patients clinical and laboratory data and positive lesions were identified by consensus. Different patterns of scan results are demonstrated in Figures 1 and 2. The baseline ^{99m}Tc-MIBI WBS was considered positive for MIBI avid lesion, if at least one region of non-physiologic increased radiotracer activity was found. On the other hand, baseline ^{99m}Tc-MIBI WBS was considered to be positive for the presence MDR if the patient had clinically active disease, but with no ^{99m}Tc-MIBI avid lesion (Fig.1B). A WBS with diffuse (Fig. 1A) or focal (Fig. 2A) abnormal uptake of ^{99m}Tc-MIBI was considered negative for MDR.

Follow-up

During the one year period of follow-up, all patients were treated by 6 courses of melphalan and prednisone (MP) or a combination of MP with vincristine and cyclophosphamide (VMCP) or 4 courses of vincristine, adriablastin and dexamethasone (VAD) and every 4 weeks were re-assessed clinically and the response to chemotherapy treatment mentioned before was evaluated. Patients were considered to have reached partial or complete remission and were drug sensitive when achieving a serum monoclonal component (MC) reduction equal to at least 50% and bone marrow plasma cells of <5%. All other patients were considered to have active disease with

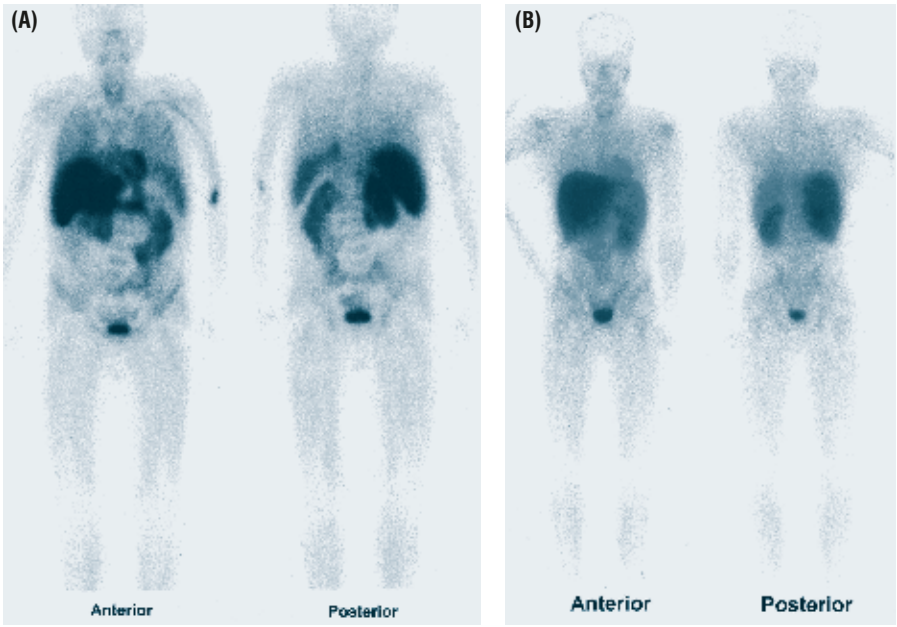

Figure 1. Whole body scan with ^{99m}Tc-MIBI in different patients with active multiple myeloma disease: The first case represents diffuse abnormal uptake throughout the axial skeleton (A), the second one shows a negative scan for active lesion (B).

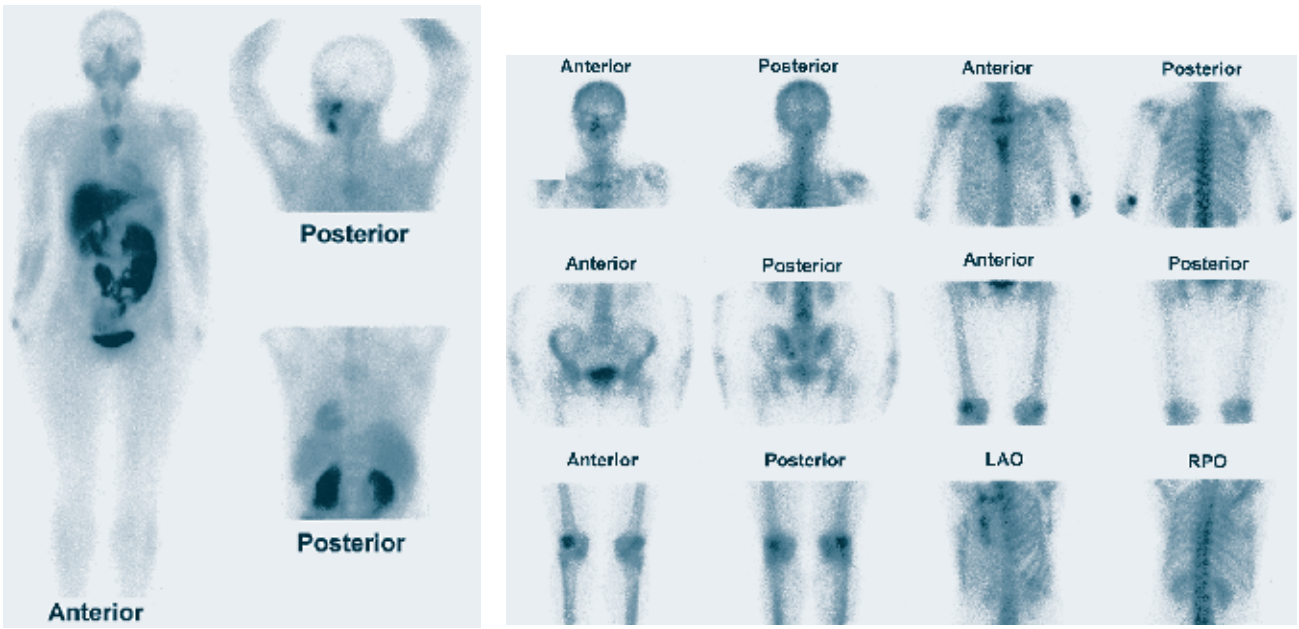

Figure 2. ^{99m}Tc-MIBI whole body scan reveals a focal active lesion in the sternal manubrium in a patient with multiple myeloma (A). A photon-deficient area is noted in the multiple spot views of whole body bone scan with ^{99m}Tc-MDP, corresponding to the active lesion delineated on ^{99m}Tc-MIBI images (B). LAO: left anterior oblique; RPO: right posterior oblique.

no response to treatment and were drug resistant [9]. The treatment protocol was modified to other regimens (mentioned above) when the patient did not achieve remission. Patients in complete or partial remission after the above-mentioned protocol did not receive any maintenance treatment.

Statistical analysis

Baseline ^{99m}Tc-MIBI imaging and PGP measurements were used to differentiate between MDR and drug-sensitive patients in order to predict the clinical outcome and treatment response. Using patients' outcome as the gold standard, the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of ^{99m}Tc-MIBI WBS and PGP measurements for prediction of MDR were calculated. The relative risk (RR) of MDR at endpoint of the study based on the baseline ^{99m}Tc-MIBI WBS was defined as the ratio of the probability of MDR in negative to the probability of MDR in positive ^{99m}Tc-MIBI WBS at the onset of the study. This ratio was expressed as a 95% confidence interval. Kappa value as a measure of agreement between ^{99m}Tc-MIBI WBS and PGP results was also calculated and the cut-off value of more than 0.5 was noted as significant agreement. Also a P value of less than 0.05 was considered statistically significant.

Results

At onset of the study, 26 out of 34 (76.5%) studied patients were in active state and 8 cases (23.5%) were in remission based on the previously mentioned criteria. Regarding the above mentioned criteria, the sensitivity, specificity, PPV, NPV and accuracy of WBS for determining the active disease were 61.5%, 75%, 88.9%, 37.5% and 64.7%, respectively. In active cases, the respective values for the diagnosis of MDR using WBS findings were 38.9%, 62.5%, 70%, 31.2% and 46.1%, respectively. The above values for the detection of MDR using P-PGP values were 50%, 50%, 69.2%, 30.8% and 50%, respectively. Table 1 shows the results of ^{99m}Tc-MIBI WBS in comparison with outcome after one year follow up in 26 patients with active MM at onset of the study. The relative risk (RR) of resistance to multiple regimens of chemotherapy after one year follow up in patients with negative to the patients with positive ^{99m}Tc-MIBI WBS was 1.02 (0.60-1.72). It means that the risk of MDR during one year follow up for a patient with positive ^{99m}Tc-MIBI WBS at onset of evaluation is not significantly different from that risk for a patient with negative ^{99m}Tc-MIBI WBS (P=0.946). The agreement between baseline PGP measurement and baseline ^{99m}Tc-MIBI WBS was not significant (kappa=0.23).

Table 1. *^{99m}Tc-MIBI WBS results in comparison with outcome after one year follow up in 26 patients with active multiple myeloma at onset of the study*

Result of ^{99m}Tc-MIBI WBS	Outcome after one year follow up		
	Response to the treatment	No response to the treatment	Total
Negative (No ^{99m}Tc-MIBI-avid lesion)	3	7	10
	(30%)*	(70%)	(100%)
	(37.5%)**	(38.9%)	(38.5%)
Positive (^{99m}Tc-MIBI-avid lesion)	5	11	16
	(31.2%)	(68.8%)	(100%)
	(62.5%)	(61.1%)	(61.5%)
Total	8	18	26
	(30.8%)	(69.2%)	(100%)
	(100%)	(100%)	(100%)

* Row percent ** Column percent

Discussion

According to the results of our previous study, in MM patients with history of previous treatment, the sensitivity of ^{99m}Tc-MIBI WBS for the detection of active disease was less than 61% [3], which is close to the result of the current study. In both our previous and current studies, the number of patients with MDR was high. In our previous study, 67% of patients with false negative ^{99m}Tc-MIBI scan, showed no response to multiple treatments [3], pointing to an opinion that low sensitivity of ^{99m}Tc-MIBI for the detection of MM lesions may be due to MDR, which is a problem interfering with ^{99m}Tc-MIBI uptake [8, 9, 12]. Based on our present study results, in MM patients neither the baseline ^{99m}Tc-MIBI WBS nor PGP findings were of importance in the detection of MDR.

Other authors had no false positive and 2/9 false negative MM cases on follow-up of ^{99m}Tc-MIBI scintigraphy and concluded that this scintigraphy had high sensitivity and specificity in tracing active nonsecretory myelomatous lesions [18], which is discordant with our results. This discrepancy can be explained by the small number of patients [18]. Another study also suggested a potential role of ^{99m}Tc-MIBI washout, in predicting the response to chemotherapy in patients with MM, as they found that disease free survival was significantly better in patients with lower washout of ^{99m}Tc-MIBI. In this study, patients treated with melphalan were excluded and 87.5% of patients in remission had low washout [9].

Other previous studies have confirmed that the intensity of ^{99m}Tc-MIBI uptake in the lesions detected by the baseline scans correlated with disease activity as determined by lactate dehydrogenase, C-reactive protein, beta2-microglobulin, serum ferritin [19] and also ESR, interleukin-6, soluble interleukin-6 receptor, serum calcium and bone alkaline phosphatase [20]. A negative correlation has been found between ^{99m}Tc-MIBI intensity and osteocalcin, type I procollagen carboxyterminal propeptide [20], serum thymidine kinase, and cross-linked carboxyterminal telopeptide of type I collagen [21]. It has been stated that ^{99m}Tc-MIBI scintigraphy can detect bone marrow lesions in MM patients that may not be detected by other imaging modalities and can be useful, especially in cases of solitary myelomas to exclude other involved sites [22]. In addition, ^{99m}Tc-MIBI scintigraphy can be a prognostic factor related to MM activity and MDR [19]. Others in 46 MM patients have shown that ^{99m}Tc-MIBI scintigraphy is a better tool in detecting biologically active myeloma lesions as compared to conventional skeletal radiographs [23]. In that study, ^{99m}Tc-MIBI scintigraphies remained positive in all patients during chemotherapy, and there was a direct correlation between scan result and clinical outcome of patients following high-dose treatment. Also some authors found that a diffuse ^{99m}Tc-MIBI pattern reflected a higher bone marrow plasma cells number [23, 24]. Moreover, in some, histologically or cytologically verified MM patients, soft tissue MM lesions can be correctly diagnosed by ^{99m}Tc-MIBI scans, while all plain radiographs are negative [23]. A more recent relatively large prospective study confirmed that ^{99m}Tc-MIBI scintigraphy, has showed high sensitivity (92%) and specificity (96%) in the diagnosis and follow-up of MM patients after chemotherapy, in parallel with the activity of MM bone disease [25].

Conversely, some other authors pointed out the limited value of ^{99m}Tc sestamibi in the follow-up evaluation due to the development of the multidrug resistant phenotype, which causes a decrease of net tracer accumulation [26]. This conclusion is concordant with our study. However, in our study no agreement was noted between negative ^{99m}Tc-MIBI WBS and PGP over-expression in the detection of MDR. Interestingly, some authors also reported that myeloma cells that express Bcl-2, a powerful inhibitor of tumor apoptosis that is associated with an entirely different form of drug MDR will

not accumulate the ^{99m}Tc-MIBI, even in the early time images [13]. Thus, a fraction of the cases with no ^{99m}Tc-MIBI uptake may be associated with this kind of MDR rather than PGP over-expression. This may be subject for further evaluation.

In conclusion, according to our results, a baseline ^{99m}Tc-MIBI scan is a useful indicator of active MM disease but this modality as well as PGP values do not predict drug resistance.

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