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99m-Tc-MDP-Scintimammography in the Evaluation of Breast Masses or Tumor Angiogenesis

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Abstract. *Background: Vascular endothelial growth factor (VEGF) is a prognostic factor in breast cancer. One of the mechanisms of extra-skeletal uptake of Tc-99m-methylenediphosphonate (MDP) is suggested to be tumor vascularity. We studied the correlation between MDP uptake and VEGF and compared the diagnostic accuracy of mammography versus MDP-scintimammography(MDP-S). Materials and Methods: Fifty-four patients with suspicion of breast cancer were evaluated. Breast images were collected 5-10 minutes after injection of Tc-99m-MDP prior to biopsy. Tissue slides were stained using a rabbit-polyclonal anti-VEGF. Results: MDP-S showed a diagnostic accuracy of 83.3% in BIRADS category 4 lesions. Four out of 23 benign lesions were false-positive. The tumor to background (TM/BG) ratio of early images of MDP-S was correlated with VEGF staining (p: 0.014) and with tumor size (p: 0.006). Conclusion: Early images of Tc-99m-MDP-S may satisfactorily identify cancers with increased neovascularization. MDP-S seems to be an accurate imaging modality, especially in BIRADS category 4 lesions.*

Breast cancer is the leading cause of cancer among women worldwide (1). Declines in breast cancer mortality rates in the United States, Canada and Europe have been associated with improvements in effective adjuvant therapy and breast cancer screening (2, 3).

Mammography is particularly useful in detecting breast abnormalities, but has a relatively low specificity in diagnosing breast cancer (4,5). Thus, a large number of patients undergo a confirmatory surgical biopsy meaning that many unnecessary biopsies are taken from patients with benign lesions (6). The BIRADS is a quality-assurance tool devised for the standardization of mammographic reporting to reduce confusion in breast imaging interpretations and facilitate outcome monitoring (7) (Table I). For lesions in BIRADS category 4 or 5, management options include needle localization with excision or image-guided breast biopsy. Because most mammographic abnormalities are benign, surgical biopsies subject patients to the small but real risk of discomfort and cosmetic deformity, potentially with no real benefit (8). Thus, a wide spectrum of noninvasive imaging modalities has been proposed to overcome the limitations inherent in mammography. These include morphological (ultrasound, magnetic resonance) or functional imaging modalities, including radionuclide-based techniques.

Breast scintigraphy using different radiopharmaceuticals has been found to be a promising technique in the diagnosis of cancer (9-12). Tc-99m-methylenediphosphonate scintimammography (MDP-S) with early imaging after intravenous injection has demonstrated significantly improved results in differentiating malignant tumors from benign lesions (10,11). Several mechanisms have been described to explain the extra skeletal uptake of Tc-99m-methylenediphosphonate (MDP) including tumor vascularity, inflammation, local pH factor, altered calcium metabolism, hormonal influence and cell wall damage (13-15).

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a potent and widely distributed angiogenic peptide (16). This growth factor is a dimeric 34-42 kd glycosylated basic protein, with moderate affinity for heparin, encoded in four molecular isoforms (17). Expression of VEGF may be induced by several factors: hypoxia, altered tumor suppressor genes and also certain cytokines such as interleukin1β, IL-6, oncogenes, growth factors and hormones (18). VEGF expression is

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Table I. *BIRADS classification of mammographic abnormalities (ref 8).*

Category	Assessment	Recommendation
1	Negative	Routine screening
2	Benign finding	Routine screening
3	Probably benign finding	Short interval follow-up to establish stability
4	Suspicious abnormality	Not characteristic, but definite probability of malignancy; consider biopsy
5	Highly suggestive of malignancy	High probability of cancer; appropriate action should be taken

present in approximately half of ductal carcinoma *in situ* and in the majority of invasive breast cancers. This production is significantly associated with high intratumoral microvessel density and cancer prognosis (19, 20).

The prognostic yield of MDP uptake is not well known. We intended to compare the diagnostic accuracy of mammography and MDP-S, and then to study the correlation between MDP uptake and VEGF staining in a group of patients scheduled for breast biopsies at Marmara University Hospital in Istanbul, Turkey.

Materials and Methods

Fifty-four eligible female patients were evaluated prospectively. Patient selection criteria were: age more than 18 years, clinical enlargement of a palpable mass lesion with BIRADS category 1-3 and BIRADS category 4 or 5 mammographic lesions of any size. Lactation, pregnancy, hypoplasic breast and prior history of breast cancer during the previous 2 years or prior biopsy on ipsilateral breast were accepted as exclusion criteria. The protocol was approved by the Ethical Committee of Marmara University Medical Faculty, and informed consent was signed by each patient. The mean age of the patients was 53.37 ± 13.1 years.

Only one of the patients had a prior history of breast cancer. Her breast cancer presented 3 years previously with a mass lesion in the contra-lateral breast. Physical examination of the breasts was done by an experienced breast surgeon. The decision to perform a breast biopsy was made by either the breast surgeon or, in the case of a non-palpable lesion, by an experienced radiologist. A histopathological diagnosis was obtained from excisional or core biopsy materials within 2 weeks of mammographic and scintimammographic evaluation.

Bilateral mammograms were obtained for all patients in craniocaudal and mediolateral projections using a dedicated screen film mammography unit (GE-Senographe Medical Systems, Milwaukee, USA). Additional projections, such as coned-down compression and magnification views or breast ultrasonography, were obtained in selected patients. Mammographic classifications of the lesions were done according to the BIRADS classification system by two experienced radiologists.

MDP-S was performed within one week of mammography using a single head gamma camera (GE Medical Systems) fitted to a low energy high-resolution collimator. The matrix size was 256x256 pixel; the photopeak was centered at 140 keV with a symmetrical 20% window. The patient was positioned on the gamma camera table in such a manner that the breast to be imaged was freely suspended. A kit preparation (Medrocis, CIS Bio International, France) of Tc-99m-MDP was used and 20 mCi of the radiopharmaceutical was injected intravenously into the arm contralateral to the breast lesion. Using a special scintimammography table with semicircular apertures, multiple planar images in the anterior views in the supine position with arms raised and both lateral views in prone position were acquired at 5-10 minutes following the intravenous injection of Tc-99m-MDP. An additional anterior image with the marker placed on the breast mass was also obtained after the first anterior scan was completed. Findings were interpreted by two nuclear medicine physicians as positive or negative according to the presence or absence of foci of increased MDP uptake over background in either breast, without reference to patient history or clinical findings. Tumor to background (TM/BG) ratios were calculated only for patients with focal uptake.

Formalin-fixed, paraffin-embedded breast cancer tissue sections were cut, mounted on poly-L-lysine-coated slides, deparaffinized in xylene and rehydrated in graded alcohol. Slides were stained using rabbit polyclonal VEGF antibody (VEGF 121, 165, 189, 206, BioGenex, San Ramon, CA, USA) for vessel count. Sections were blocked for endogenous peroxidase activity with hydrogen peroxide and antibody immunodetection was investigated with an immunoperoxidase technique using rabbit polyclonal anti-VEGF. Slides were counterstained with Mayer's haematoxylin. To check the quality of the immunohistochemical staining method, ten cases of benign breast lesion and intact breast specimens in all malignant cases were used as controls. Since 4 of these 10 benign lesions had false-positive MDP uptake, they were chosen as controls specifically.

Areas of invasive tumor containing the greatest numbers of capillaries and small venules (areas of most intense neovascularization), which most frequently occurred at the margins of the carcinoma, were examined by light microscopy and were found by scanning the tumor sections at low power (1x40 and 1x100). Only tumors that had good quality staining of microvessels with the immunoperoxidase technique were evaluated in this study. Samples of 3 malignant tumors and 1 MDP uptake-positive benign lesion were excluded from the study because of inadequate staining.

After the areas of highest neovascularization had been identified, all microvessels were counted on a 1x400 field and, according to their staining density, cases were scored as VEGF (3+) when cytoplasmic staining in all tumor tissue was strong and diffuse, (2+) when staining was moderate, (1+) when cytoplasmic staining was weak or focal, and negative when no staining was present. The VEGF immunoreactivity of the malignant lesions is illustrated in Figure 1. The correlation between TM/BG ratios and grade of VEGF staining were determined using Spearman's correlation test. Probability values less than 0.05 were considered significant.

Results

At the beginning of the study, 37 out of 54 (68.5%) patients were postmenopausal, while 17 (31.5%) were premenopausal.

Thirty-one out of 54 (57.4%) breast lesions were histologically diagnosed as malignant (29 invasive ductal, 1 mucinous and 1 mixed invasive carcinoma). The diameters

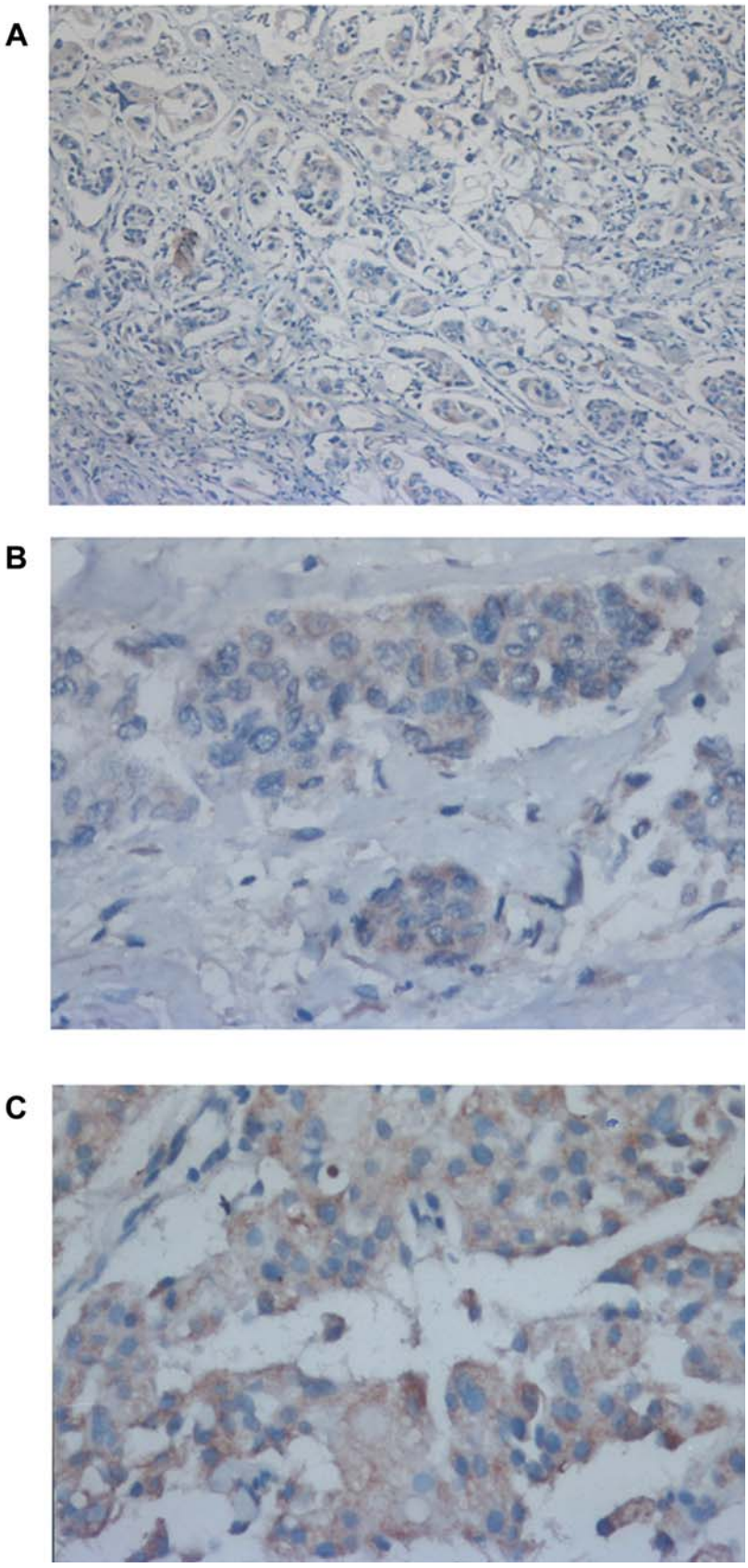

Figure 1. *A. Focal (1+) cytoplasmic VEGF staining (1x100); B. Moderate (2+) cytoplasmic VEGF staining (1x200); C. Strong and diffuse (3+) cytoplasmic VEGF staining (1x400).*

Table II. *Characteristics of malignant patients and tumors.*

Age (years)	Menopausal status	Tumor size (cm)	BIRADS category	TM/BG ratio	VEGF grade
68	(+)	6	5	2.90	IS
65	(+)	3.5	5	2.75	IS
49	(+)	2.5	5	1.92	IS
27	(-)	3.5	5	1.65	(-)
52	(+)	Inf	4	1.78	(-)
36	(-)	3.5	5	2.25	(-)
48	(+)	3.6	5	1.80	(+)
47	(+)	1	5	1.52	(+)
84	(+)	2.2	5	1.21	(+)
49	(+)	2.1	5	1.71	(+)
57	(+)	2	5	1.91	(+)
40	(-)	1.7	5	1.0	(+)
60	(+)	2.2	5	1.49	(+)
73	(+)	Inf	4	1.65	(+)
60	(+)	2.7	4	1.74	(+)
47	(+)	1.4	5	1.52	(+)
75	(+)	3.5	5	1.78	(+)
35	(-)	4	5	1.58	(+)
56	(+)	2	5	1.63	(+)
56	(+)	0.6	4	1.0	(+)
71	(+)	2.2	5	2.39	(++)
50	(+)	2.4	5	1.83	(++)
48	(-)	3.0	5	1.47	(++)
63	(+)	2.5	5	2.01	(++)
45	(-)	7	4	2.81	(++)
40	(-)	6	5	2.05	(++)
53	(+)	4.	4	1.64	(++)
54	(+)	4	5	2.75	(++)
56	(+)	3.6	5	4.76	(+++)
76	(+)	3	5	2.03	(+++)
70	(+)	2.5	5	1.87	(+++)

BIRADS, Breast Imaging Reporting and Data System ;TM/BG, tumor to background uptake of Tc-99m-MDP; VEGF,vascular endothelial growth factor ; IS, inadequate staining; Inf, inflammatory

Table III. *Demographic characteristics of patients.*

	Malignant	Benign
Age (range)	55 (27-84)	50 (23-75)
Postmenopausal (patients/total)	24/31	12/23
BIRADS classification		
Category 1-3	0	5
Category 4	6	18
Category 5	25	0
Positive MDP uptake	29	4

of the malignant lesions range from 0.6 cm to 7 cm. Twenty-three (42.5%) lesions were histopathologically diagnosed as benign (9 fibrocystic changes, 5 fibroadenoma, 4 fat necrosis, 1 ductal hyperplasia, 1 tubular adenoma, 1 mastitis, 1 nodular adenosis and 1 intraductal papilloma). Tables II and III show the demographic features of our patient population and tumor characteristics.

On mammographic evaluation, 25 breast lesions were evaluated as BIRADS category 5, 24 as category 4 and 5 as category 1-3. Overall, mammography showed true-positive in all 31 cancers and true-negative in only 5 out of 23 benign lesions. Thus, in our patient population, mammography had 100% sensitivity, 21.7% specificity, 66.6% diagnostic accuracy, 100% negative (NPV) and 63.2% positive predictive value (PPV), respectively. Of 24 breast lesions interpreted as BIRADS category 4 on mammography, 6 (25%) were malignant and 18 (75%) were benign.

MDP-S showed foci of pathologically increased MDP uptake in 29 (93.5%) out of 31 cancers, with 2 false-negative results (Figure 2). Of the 2 MDP-S false-negative breast lesions, one was smaller than 1 cm (0.6 cm) and the other, with a 2.6 cm diameter, was deeply located in the axillary fold of the right breast. In 19 (82.6%) out of 23 benign breast lesions, MDP-S showed negative MDP uptake. There were 4 false-positive cases; 2 patients with fibrocystic changes, one with fibroadenoma and another one with mastitis. With these results, MDP-S showed 93.5% sensitivity, 82.6% specificity, 88.8% diagnostic accuracy, 87.8% PPV and 90.4% NPV, respectively.

Five out of 6 mammographically category 4 malignant lesions showed focal radiotracer uptake. Of 18 category 4 benign lesions, 15 (83.3%) were reported as negative uptake (true-negative) and 3 (16.6%) as false-positive. This results in 83.3% diagnostic accuracy of MDP-S in BIRADS category 4 lesions.

Three out of 31 malignant and 1 out of 10 benign lesions were excluded from the study because of inadequate VEGF staining. Overall, VEGF staining was (-) in 3, (1+) in 14, (2+) in 8 and (3+) in 3 patients. Out of four benign lesions with false-positive MDP uptake, VEGF staining was (1+) in one, negative in two and inadequate in another lesion. Because of the small sample size of benign lesions, further statistical analysis of this group was considered unsuitable, as it would not reflect the general population.

A significant correlation was found between TM/BG ratios for early images and grade of VEGF staining (tumor angiogenesis) in 28 malignant lesions – three cancer patients were excluded due to inadequate VEGF staining – (rs: 0.460, p: 0.014) (Figure 3). There was also a significant correlation between TM/BG ratios and tumor size in this group of patients (rs: 0.479, p: 0.006).

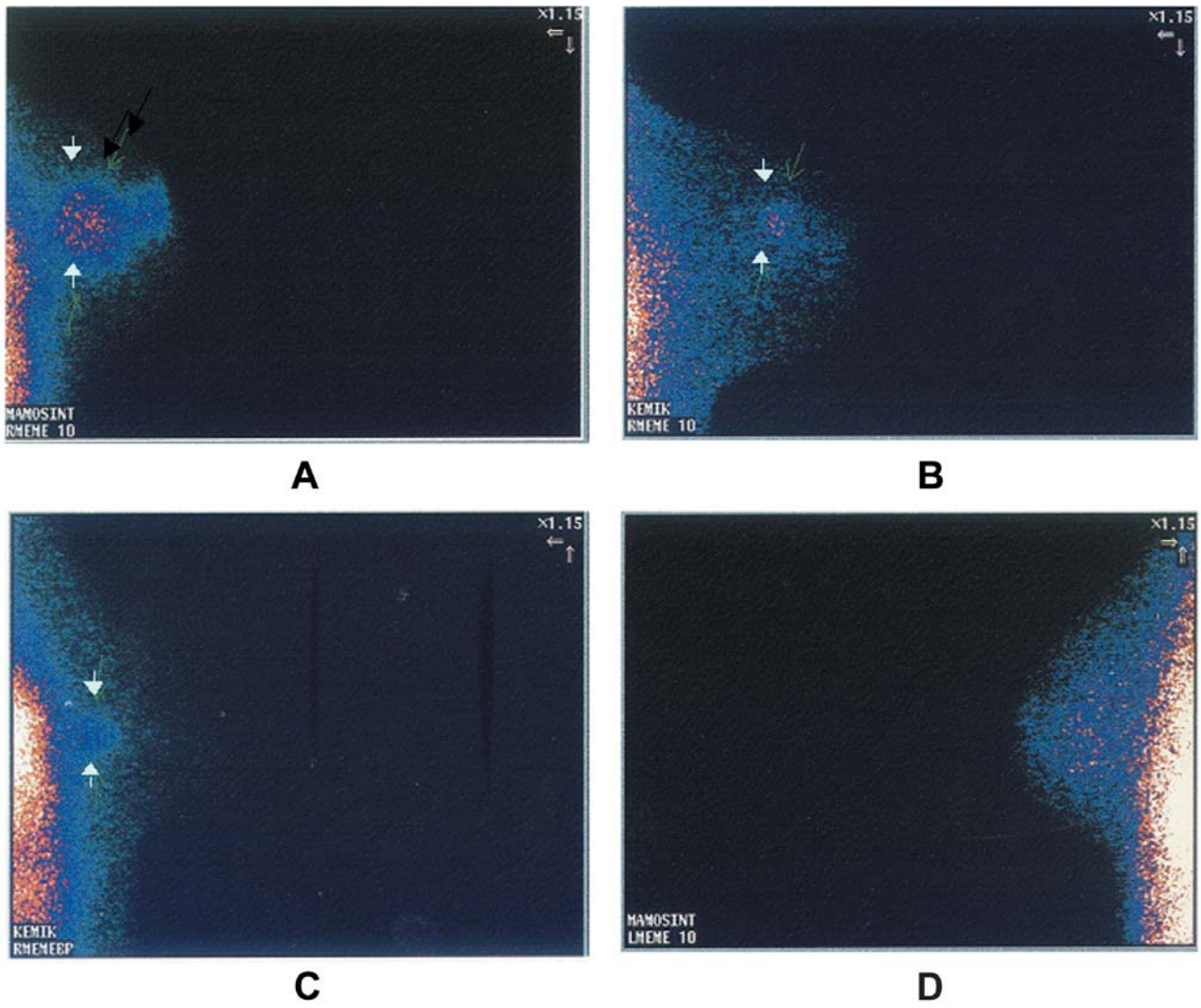

Figure 2. *Results of MDP-S in different malignant patients (abnormal MDP uptake shown with arrows in A, B, C) and benign lesion (no uptake seen in D).*

Discussion

Mammography is the most common modality used for the detection of breast cancer. Despite the major advantages, it has some limitations in clinical practice (21). Although mammography has a relatively high sensitivity in the range of 85% to 90%, especially in examination of fatty breasts of older women, it is less reliable for detecting lesions in patients with dense breasts, severe dysplastic disease, breast implants, or in patients evaluated following breast surgery or radiotherapy, with false-negative rates between 25% to 30% (22). An important disadvantage of mammography is its low specificity and low predictive value of only 10% to 35% for nonpalpable cancers. Consequently, many mammography-directed surgical breast biopsies are benign (6). Thus the addition of different noninvasive diagnostic techniques will be useful in mammographically suspicious breast lumps. In this study, the sensitivity, specificity, diagnostic accuracy, PPV and NPV of mammography were 100%, 21.7%, 66.6%, 63.2% and 100%, respectively. These values are compatible with the high sensitivity and low specificity of mammography in the literature. The reason for 100% sensitivity and NPV of mammography in our study is because over 90% of patients with abnormal mammographic features were selected for breast biopsies. In a recent study, the PPV of mammography was found to be 0%, 29% and 84% for BIRADS categories 3, 4 and 5 lesions, respectively (23). The equivalent values in our study were 0%, 16.6% and 100%, respectively.

Scintigraphic imaging of the breast using different radionuclides has demonstrated encouraging results (10-12).

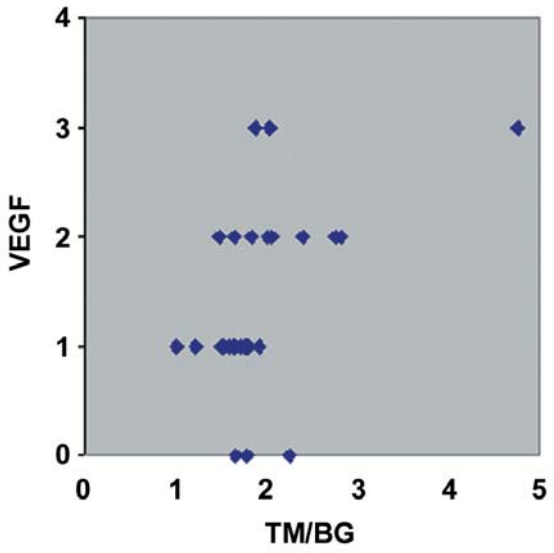

Figure 3. *The correlation between VEGF and tumor to background (TM/BG) ratio. Higher uptake of MDP is related to higher angiogenesis (rs:0.460, p:0.014).*

Tc-99m-MIBI scintimammography has been used in most of the studies (24-27). The sensitivity and specificity of scintimammography using Tc-99m-MIBI ranged from 70-97% and 70-100%, respectively. Extra-osseous accumulation of bone-seeking agents has been reported in certain malignant tumors such as neuroblastoma, soft-tissue sarcoma, osteosarcoma and lymphoma. Similar diphosphonate uptake was seen in large primary breast tumors, cystosarcoma phyllodes and benign breast lesions such as chronic mastitis, fibroadenoma, fat necrosis and hamartoma (14,15,28-30).

Tc-99m-MDP, a member of the diphosphonate family, is widely used for bone scan. It is a readily available and affordable agent used on a routine basis in nuclear medicine departments. The procedure used in this study can be easily performed using the same kit and gamma camera during a routine session of bone scintigraphy. It has the advantage of obtaining a baseline bone scan for staging with no extra radiation burden in malignant and suspicious breast lesions. Berg *et al.* reported the first use of Tc-99m-MDP for breast cancer imaging in 1973 (28). However, there are a few reports investigating the prognostic yield of this radiotracer uptake in breast cancer. The most important one was done by Dimonte *et al.* (31). In this retrospective study, the investigators compared the TM/BG ratio with the lesions' histiotype, diameter, grading and the tissue concentrations of hormonal receptors, cathepsin D, type 1 thymidine kinase, pS2 and p53 proteins in 44 primary breast cancers. They found no statistically significant correlation between the TM/BG ratio and these prognostic factors.

Piccolo *et al.* (10) studied 330 women with histologically proven primary breast carcinoma using Tc-99m-MDP. They found a sensitivity of 92%. Twenty out of 25 cancers missed were less than 10 mm and 5 were less than 15 mm in diameter. The specificity was 91.5% in a group of 70 histologically proven benign breast lesions. In another study, the sensitivity and specificity of Tc-99m-MDP were reported as 96% and 58%, respectively, in 96 patients with 109 breast lesions (11). We have found that Tc-99m-MDP has a sensitivity of 93.5%. MDP-S showed 2 false-negative results; one was 6 mm in diameter, the other was deeply located at the axillary fold of the right breast. The specificity of MDP-S was high compared to that of mammography (82.6% *vs.* 21.7%). In fact only 4 cases among 23 histopathologically proven benign breast diseases were classified as false-positive. Thus our results were consistent with the existing literature.

In the present study, 18 out of 24 category 4 lesions were false-positive on mammographic evaluation. This comprises 36.7% of all mammographically biopsy-requiring lesions and 75% of category 4 lesions. In contrast, the number of false-positives for MDP-S was much lower with 3 out of 24 (12.5%) in category 4. Here, MDP-S reduced the false-positive results from 75% to 12.5% in category 4 lesions, avoiding unnecessary biopsies in 15 out of 18 lesions (83.3%). Thus, Tc-99m-MDP seems to be a useful imaging modality in differentiating benign from malignant breast lesions, but especially useful in BIRADS category 4 mammograms to avoid unnecessary biopsies.

Angiogenesis is the term used to identify the complex process leading to the formation of new blood vessels from the pre-existing vascular network (32). The growth of malignant tumors is dependent on the formation of new blood vessels (neovascularization) by the sprouting of endothelial cells from pre-existing vascular networks (particularly postcapillary venules). The secretion of growth factors such as VEGF by the tumor cells results in the activation of endothelial cells. This in turn leads to the proliferation and expression of cell surface receptors (*e.g.* VEGF receptor 2) and adhesion molecules (*e.g.* integrin $\alpha v\beta 3$). The endothelial cells secrete proteolytic enzymes such as matrix metalloproteinases that degrade the basement membrane and the extracellular matrix. First endothelial cells proliferate and migrate forming solid cords; then the inner layer of the endothelial cells undergoes apoptosis resulting in the formation of a vessel lumen. This immature vasculature undergoes remodeling and vascular myogenesis with stabilization of vessels by periendothelial cells and smooth muscle cells. The remodeling phase is often incomplete in tumor angiogenesis leading to increased vascular permeability for macromolecules (33).

VEGF is an endothelial cell mitogen that is angiogenic *in vivo*. Its permeability effect on capillaries is more potent than histamine, which results in its contribution to ascites in

ovarian cancer and to edema in brain tumors. VEGF is a homodimeric protein with a signal sequence secreted by various cells and by the majority of human tumor cells. More than 60% of breast cancers overexpress VEGF (34). In 1992, two independent studies performed by Horak *et al.* and Weidner *et al.* found that intratumoral microvessel density is a significant and an independent prognostic indicator in invasive breast cancer (35,36). In several different experimental studies, overexpression of VEGF was accompanied by marked tumor growth and angiogenesis and was a powerful prognostic factor in breast cancer (37).

Incomplete vessel maturation results in increased vascular permeability, which is often seen in, and is typical of, malignant neoplasias. This feature may be imaged and thus evaluated by diagnostic imaging modalities including contrast enhanced MRI and CT, Doppler ultrasonography with microbubble contrast agents and radionuclide imaging. Collingridge *et al.* have developed VG76e, a monoclonal antibody that binds to human VEGF labeled with iodine-124, for use with PET that allows noninvasive *in vivo* imaging of VEGF (38). Nonivasive measurement of VEGF *in vivo* can elucidate the role of VEGF in cancer biology and provide information about antiangiogenic agents. Kim *et al.* reported that the preoperative evaluation of primary breast cancer by power Doppler US with a microbubble contrast agent could predict tumor angiogenesis. However, the early uptake and washout index calculated from Tc-99m-MIBI scintimammography showed no correlation with intratumoral microvessel density or VEGF mRNA level (39).

In the literature, there is no study investigating the correlation between MDP uptake and angiogenesis. Considering angiogenesis as one of the extra-skeletal uptake mechanisms of Tc-99m-MDP, we aimed to search for the correlation between TM/BG ratio and immunohistochemically-assessed VEGF, which is a reliable indicator of angiogenesis and possibly an independent prognostic factor in breast cancer patients. Even though there were two false-negative cases with MDP uptake, a statistically significant correlation between MDP uptake and VEGF immunoreactivity was demonstrated (r_s: 0.460, p: 0.014) in our study. We also saw (1+) or negative VEGF staining of four cases with false-positive MDP uptake. This may suggest that not only neoangiogenesis but also some other factors can influence increased MDP uptake in benign lesions. The proposed mechanisms of delayed Tc-99m-MDP uptake in extra-skeletal neoplasms are multiple and include tumor vascularity, inflammation, local pH factors, altered calcium metabolism, hormonal changes and cell wall damage (13). Tc-99m-MDP accumulation is not specific to malignant lesions, since benign breast lesions such as chronic mastitis, fibro-adenoma, fat necrosis and atypical hyperplasia may also accumulate this agent (11). However, our study demonstrates that in malignant breast lesions, the uptake mechanism of Tc-99-MDP in early images might be especially associated with VEGF expression rather than the other uptake mechanisms reported for delayed images encountered on bone scans.

In conclusion, our study showed that MDP-S is an accurate test that differentiates malignant from benign breast lesions, particularly in patients with BIRADS category 4 mammograms. Also this is the first study to show that early tumoral uptake of Tc-99m-MDP might be used as a functional test for *in vivo* imaging of cancer angiogenesis. Confirmatory studies with larger patient groups and longer follow-up may confirm the effectiveness and prognostic value of MDP uptake.

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