Original Paper, Oncology

Frequency of Bone Metastases with Metabolic Super Scan in Cancer Breast Patients

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Background: Metabolic skeletal changes particularly metabolic super scan (MSS) may provide unsuitable soil for survival and growth of tumor cells in cancer breast patients. We aimed to verify the presence of MSS in cancer breast patients in correlation with the available biomarker for bone metabolism and to explore its influence on the frequency of bone metastases compared to patients without MSS features. **Methods:** From July 2008 to December 2010, 450 histo-pathologically proved breast cancer patients referred to nuclear medicine department in national cancer institute (NCI), Cairo University, Egypt underwent whole body bone Scan. Bone metabolism was evaluated by laboratory biomarkers of serum alkaline phosphatase (ALP), serum calcium and parathormone (PTH) levels in addition to serum creatinine to assess renal function. Statistical correlations were done between bone scan, clinico-pathological data, radiological findings and serum biomarkers. Follow up within 16-24 months was done. **Results:** Based on the presence or absence of MSS features, breast cancer patients were classified into two groups: I) MSS group: - included 99 patients, with a mean age of 60.5 years ± 19.5. (II) Non-MSS group: included 351 patients with a mean age of 57 years ± 22.0. Patients based data analysis showed that MSS was seen in 22% of the studied breast cancer patients with more prevalence in the post menopausal women and those treated with hormonal and bisphosphonate therapy. Moreover, a significantly lower frequency of bone metastases was noticed in MSS group (8%) compared to Non-MSS (18.2%) ($P<0.05$). On the other hand sensitivity, accuracy and PPV for detection of bone metastases were significantly lower in MSS group (75%,75%,64%) compared to Non-MSS (90%, 88.6% and 75%) group respectively ($P<0.05$). A comparable low yield was obtained in PPV and specificity in both groups with no significant difference ($P>0.05$). Apart from significant higher level of alkaline phosphatase in MSS compared to Non-MSS group, no significant difference was noticed in the rest of the estimated metabolic biomarkers between both groups. **Conclusion:** MSS seems to be associated with low frequency of bone metastases in MSS compared to Non-MSS in cancer breast patients. MSS appears to be linked to menopausal status and treatment with hormonal and bisphosphonate therapy. Unfortunately MSS may have a negative impact on the accuracy of bone scan in detection of bone metastases.

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INTRODUCTION:

The skeleton is among the most common metastatic sites in patients with breast cancer. In addition to portending a dire prognosis, bone metastases cause significant cumulative osseous morbidity [1,2]. Development of skeletal metastases in breast cancer patients may entail a multi-step process that involves sequences of molecular events directed by genetic alteration. However, the deposited tumour cells within the bone marrow will establish metastases and grow only if the marrow micro-environment is appropriate. The detailed step by step interaction between tumour cells and bone marrow micro-environment remained to be elucidated. However osteoclast seemed to play a central role in these events. Recently there is increased focus on the contribution of bone micro-environment to the development and progression of bone metastases [2, 3,4,5,6].

Biochemical markers are usually used to provide information regarding the changes occurring in bone metabolism especially osteoclastic activity[7]. Multiple limitations have been addressed by many authors that interfere with wide acceptance for their use in clinical practice. These include availability, cost, technical demands, multiplicity as well as lack of specificity and standardization. In addition, these laboratory assays suffer from cross reactivity, difference in immunoassays and are influenced by gender, age, diurnal variation as well as liver diseases [7,8,9,10,11].

Bone scan is a widely accepted tool with high sensitivity and efficacy in early detection and follow up of bone metastases in patients with breast cancer[1,2]. Bone scan may portray changes in skeletal metabolism, bone micro-environment and osteoclastic activity in a simple and easy way. MSS pattern entails peculiar scan features of diverse aetiology; however osteoclastic activity seems to play a major role through variable patho-physiological pathways. MSS changes may provide diffuse change in bone metabolism that disrupts the complex cross talk of cancer cells and cellular bone marrow micro-environment. To what extent these MSS changes may influence the development and growth of bone metastases remained unanswered crucial question in that field [10, 11,13,14,15].

There are limited data in the literature that addresses the frequency and influence of MSS changes in development and progress of bone metastases in cancer breast patients. This encouraged us to perform the current prospective study that aims to verify the presence of MSS in breast cancer patients and its correlation with the available bone biomarkers and to explore its effect on the development of bone metastases.

MATERIAL AND METHODS:

This study was approved by the ethics committee at (NCI), Cairo University, Egypt. Informed consent was obtained from all patients or their relatives with a full description of the procedures. 450 Female patients with histo-pathologically proven breast carcinoma were enrolled in the study. The patients were seen in NCI from July 2008 to December 2010. All patients underwent routine laboratory investigations including complete blood count, liver and kidney functions. The staging procedure was based on clinical examination; routine digital mammography complemented with an ultrasound examination for breasts and axillae, chest X-ray and abdominal

ultrasound and computed tomography for chest (If clinically indicated). Histo-pathological diagnosis examination was performed through a free hand core biopsy for superficial tumour and ultrasound guided biopsy for deeply seated tumour. According to clinical staging, morbidity and hormone receptor status, patients were stratified to different NCI treatment protocols. All patients underwent standard Tc-99m MDP whole body bone scan. Tc-99m MDP whole body bone scan was obtained 3 hours following IV administration of 555-925 MBq Tc-99m MDP. Whole body scans were produced using a large field of view with variable angle dual head Siemens gamma camera. The acquisition was carried out in anterior and posterior views simultaneously with 180 degree positioning for the dual head gamma camera. The gamma camera was equipped with low energy general purpose (parallel hole) collimator. A Tc-99m photo peak of 140 KeV with a 20% window was used. The matrix used was 265x1028. Imaging was performed while the patient was lying supine at 5 cm distance from the collimator with 10 cm/min scan speed. Occasionally additional localized detailed images in anterior and posterior views were obtained for better exploration of indeterminate lesions using a 256 x 256 matrix. The laboratory assessment based on chemo-luminescence assay of serum alkaline phosphatase (ALP), calcium (Ca ++) and occasionally parathormone (PTH) levels were done as markers for bone metabolism. Serum creatinine was done as an indicator for kidney function. Male patients with breast cancer were excluded from the current study.The epidemiological factors studied were patients' demographic characteristics, stage at diagnosis, investigations, details of treatment, pathology including tumor type, nodal status, hormone receptor status and HER2/neu status (if available). All patients were pathologically staged using the sixth edition of the AJCC Cancer Staging Manual of Breast cancer criteria 16. Patients were followed up till the end of December 2012. Determination of true or false positive and/or negative bone scan lesions were based on qualitative assessment, Follow up bone scan, complementary radiological imaging as follows:

True-positive: focal intense uptake concordant with radiological findings for metastases and/or progressed during follow up.

True-negative: low grade focal lesion that is concordant with negative radiological findings for metastases and spontaneously regressed or remain stationary on follow up.

False-positive: Focal intense uptake discordant with negative radiological findings for metastases and/or shows no progression during follow up.

False-negative: lack of bone scan abnormality in the presence of specific radiological and/or occasionally PET/CT changes consistent with metastases.

STATISTICAL ANALYSIS:

Values of laboratory data were expressed as mean ± SD. Sensitivity, specificity, accuracy, positive and negative predictive values for bone scan results were calculated using the Willson score which was generated by the Open Epi program. The significance of the correlations was assessed with the Fisher z test. In addition, the multiple comparisons were adjusted by using the Bonferroni-Holm method. P-values less than 0.05 were considered to indicate significant differences.

RESULTS:

Interpretation of bone scans was carried out with complete unawareness of the rest of the patients' data. Six criteria were listed for the diagnosis of MSS features, namely: diffusely Enhanced skeletal uptake, high bone to background ratio, prominent long bones uptake, Non visualization of both kidneys, beading of the costo-chondral junctions and prominent sternal uptake. At least four criteria were required for diagnosis of MSS. Based on the presence or absence of MSS features breast cancer patients were divided into two groups; MSS group that included 99 patients (Figure 1), with mean age of 60.5 years ± 19.5 and Non-MSS group that included 351 patients with mean age of 57 years ± 22.0. The main clinico-pathological patient's criteria in both groups are illustrated in table 1. The included breast cancer patients were treated according to the standard NCI treatment protocols. Table 2 shows differences in treatment between MSS and Non-MSS groups. As shown in table 2 MSS seemed to be linked to hormonal and bisphosphonates treatment rather than other systemic therapy.

Table (1): The main clinico-pathological criteria in MSS and Non-MSS groups in 450 breast cancer patients:

	MSS Group n= 99 (22%)	**Non-MSS Group n= 351 (78%)**
Age: (years)		
• **Mean ± SD**	60.5± 19.5.	57±22
Menopausal Status:-		
Premenopausal	23 (5%)	100 (22%)
Postmenopausal	76 (17%)	251 (56%)
Histopathology:		
IDC	89 (20%)	287 (63.8%)
ILC	7 (1.5%)	32 (7.1%)
Other types	3 (0.5%)	32 (7.1%)
Staging :		
I	4 (0.8%)	8 (1.8%)
II	40 (9%)	155 (34.5%)
III	53 (11.8%)	176 (39%)
IV	2 (0.4%)	12 (2.7%)
Estrogen receptor		
Positive	70 (16%)	162 (36%)
Negative	20 (4%)	135 (34.4%)
Unknown	9 (2%)	36 (7.6%)
Progesterone receptor		
Positive	54 (12%)	118 (26.2%)
Negative	36 (8%)	199 (44.2%)
Unknown	9 (2%)	34 (7.6%)
HER2/neu receptor		
Positive	32 (3.1%)	90 (20%)
Negative	14 (7.1%)	24 (5.3%)
Unknown	53 (11.8%)	237 (52.7%)

*MSS== = Metabolic Superscan **SD = standard deviation ***n = number ****IDC = Invasive duct carcinoma ***** ILC = Invasive lobular carcinoma ******Staging using the sixth edition of the AJCC Cancer Staging Manual of Breast cancer criteria

Table 2:-Treatment applied to MSS and Non-MSS groups in 450 breast cancer patients:

Parameter	MSS Group		Non-MSS Group		*P*-value
	N=99	(22%)	N = 351	(78%)	
Surgery					
- **MRM**	80	81%	289	82.3%	
- **CBS**	18	18%	50	14.2%	>0.
- **Just Biopsy**	2	2%	12	3.5%	05
RTH					
• **Adjuvant**	97	98%	339	97%	>0.
• **Palliative**	2	2%	12	3%	05
CTH					
• **Adjuvant**	70	71%	279	79.5	
• **Palliative**	2	2%	12	3.5	>0.
• **Not given**	27	27%	60	17	05
Hormonal TTT					
• **Adjuvant**	85	86	274	78	<0.
• **Not given**	14	14	77	22	05
Bisphosphonate TTT					
• **Given**	65	65.5%	85	24.2	<0.
• **Not Given**	34	34.5%	266	75.8	05

*N ; number ** MRM : Modified radical mastectomy ***CBS ; Conservative breast surgery **** CTH = chemotherapy. ***** TTT: treatment.

In the current study, bone metastases were confirmed in 72 out of 450 studied patients. This confirmation was based on follow up bone scan, complementary X-ray, CT, MRI and/or F-18 FDG PET-CT. A significantly lower frequency of bone metastases was noticed in MSS group (8%) compared to Non-MSS group (18.2%) (Figure 2) (P<0.05). A similar mean age was demonstrated in patients with bone metastases in MSS (58 year ± 16.5) and Non-MSS (53 years ± 17) (P>0.05). The remaining clinico-pathologoical criteria for patients with bone metastases and their distribution among MSS and Non-MSS groups are illustrated in table 3. A relative higher prevalence of bone metastases was noticed in post-menopausal and positive hormonal receptors status compared to pre-menopausal and negative hormonal receptors patients in both groups (P<0.05). IDC was the most common pathological subtype in patients with metastases in MSS (8/8) and Non-MSS (59/64) group. Out of 72 metastatic patients 21, 31 and 12 in Non-MSS and 1, 5, 2 in MSS were stage II, III and IV at initial presentation respectively. None of the patients with bone metastases were in stage I at initial presentation in both groups.

Table 3: Distribution of bone metastases in MSS and Non-MSS group in 450 breast cancer *patients (n= 72) :*

Parameter	MSS Group		Non-MSS Group		P-value
	N=8	(8%)	N = 64	(18.2%)	
Menopausal Status					
- **Pre-menopausal**	3	3%	22	6.2%	>0.05
- **Post-menopausal**	5	5%	42	12%	
Pathological Type					
• **IDC**	8	8%	59	16.8%	>0.05
• **ILC**			2	0.6%	
• **Other type**			3	0.8%	
Initial Staging					
• **Stage I**	0	0	0	0	
• **Stage II**	1	1%	21	6%	
• **Stage III**	5	5%	31	8.8%	>0.05
• **Stage IV**	2	2%	12	3.4	
Estrogen Receptors					
• **Positive**	6	6%	34	9.6	<0.05
• **Negative**	2	2%	16	4.6	
• **Unknown**	0	0%	14	4	
Progesterone receptors					
• **Positive**	6	6%	31	8.8	<0.05
• **Negative**	2	2%	19	5.4	
• **Unknown**	0	0%	14	4	
HER2/neu receptors					
• **Positive**	2	2	21	6%	
• **Negative**	1	1	3	0.8%	
• **Unknown**	5	5	40	11.4%	

*N ; number ** IDC : Invasive Duct carcinoma *** Staging using the sixth edition of the AJCC Cancer Staging Manual of Breast cancer criteria.

Out of the 72 patients with confirmed bone metastases, bone scan was able to detect bone metastases in 64 patients. In the remaining 8 patients bone metastases were confirmed by MRI in three (bone marrow based metastases), by CT in two (dominantly lytic metastases) and by PET-CT in two patients (one with bone marrow based and other mixed lytic and sclerotic metastases). Table 4 shows a comparison between bone scan results in breast cancer patients with bone metastases in MSS and Non-MSS groups. Based on the net results of true positive, false positive, true negative and false positive in each group, there was a relative high significant

difference between sensitivity, negative predictive value and overall accuracy in Non-MSS (90%,86.5% and 75%) group compared to MSS (75%,75% 64%) respectively (P<0.05).

On the other hand, a comparable low yield in respect to specificity and positive predictive value was obtained in both groups. The relative high false positive number in Non-MSS group may contribute in such finding. Bone infarction, trauma and degenerative process were the main causes of false positive in Non-MSS group, While avascular necrosis of femoral head and osteomyelitis were the main causes for false positive in MSS group. Two out of the eight false negative bone scan results were missed in MSS group (one patient was attributed to dominantly lytic lesion and the other due to bone marrow based metastases).The remaining six false negative bone scan results were missed in Non-MSS group (bone marrow based lesions in three patients, dominantly lytic metastases in two patients, and mixed sclerotic and lytic lesion in one patient).

Table 4: Comparison between MSS and Non-MSS in respect to bone metastases in 450 cancer breast patients (n=72):

	MSS Group (6/8)	Non-MSS Group (58/64)	P-value
True Positive	6	58	
False Positive	2	9	
True Negative	1	4	
False Negative	2	6	
Sensitivity	75%	90%	>0.05
Specificity	33%	40%	<0.05
PPV*	33%	40%	<0.05
NPV**	75%	86.6%	>0.05
Overall Accuracy	64%	75%	>0.05

**PPV= positive predictive value **NPV = Negative predictive value.

Laboratory Results:

In the current study the values of serum level of creatinine, calcium and alkaline phosphatase were estimated in both groups. Table 5 shows a comparison between different values of laboratory results in both groups. Apart from a significant higher levels of alkaline phosphatase in the MSS group compared to Non-MSS group (P<0.05), No significant difference could be detected in the values of serum creatinine and calcium level in both groups. Parathyroid hormone (PTH) was assessed in a limited number of patients (50 MSS and 30 in Non-MSS group). There was a non significant limited higher level of PTH in MSS 80 (± 20 pg/ml) compared to 60 ± (15 pg/ml) in patients with non MSS.

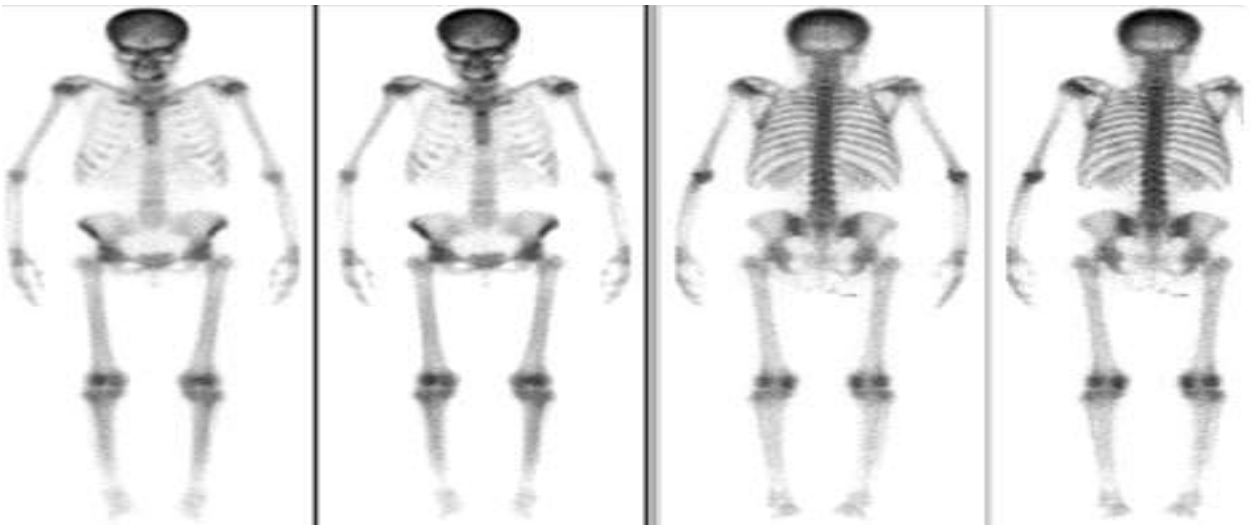

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Fig1: Postmenopausal female patient with stage III Estrogen receptor positive IDC in left breast underwent Lt MRM & received PO combination CTH & RTH followed by hormonal and Bisphosphonate therapies. Patient had elevated levels of alkaline phosphatase (260 U/L) & parathyroid hormone (90 pg/ml) and normal levels of serum creatinine (1.4ng/ml) and calcium (9.2pg/ml). FU bone scan showed MSS features including high bone to back ground uptake, prominent calvarial & long bone uptake as well as faint kidneys with no evident osseous deposits.

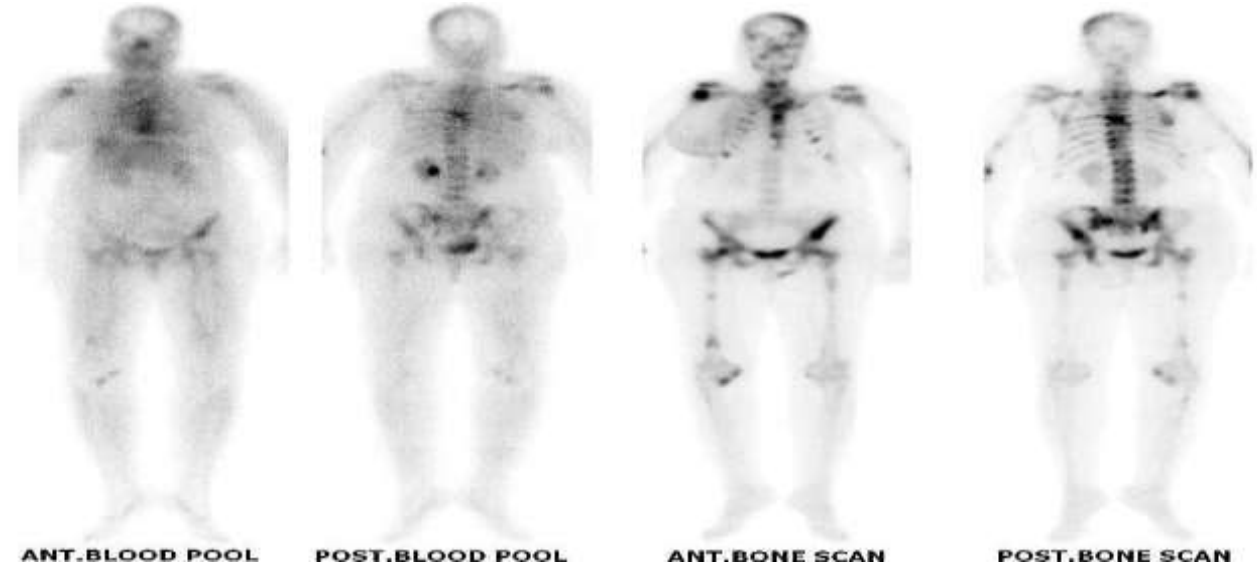

Fig2: Postmenopausal female Patient with stage III Estrogen Receptor positive IDC in left breast underwent Lt MRM & received PO combination CTH & RTH followed by hormonal therapy. Patient had elevated levels of alkaline phosphatase (130 U/L) & parathyroid hormone (40 pg/ml) and normal levels of serum creatinine (1.2ng/ml) and calcium (9.2pg/ml).FU bones scan showed multiple Osseous deposits with no evidence of MSS features

Table 5: laboratory results in MSS and Non-MSS groups in 450 breast cancer Patients (n=450):

Parameter	MSS Group		Non-MSS group		P-value
	Mean	SD	Mean	SD	
S. Cr (n. 0.5-1.5 ng/ml)	1.1	± 0.4	1	± 0.3	>0.05
Alk. Ph(n.53-128 U/L)	220	± 40	90	± 40	<0.05
Serum Ca (8.4-10.4pg/ml)	9.3	± 0.5	9	±0.6	>0.05

* SD: Standard diviation ** S.Cr : serum creatinine *** Alk Ph : Alkaline phosphatise **** Ca:Calcium.

DISCUSSION:

Breast cancer is the most common malignant disease in women. It forms a major health problem in western countries. Bones metastases are frequent in patients with breast cancer especially the advanced one. In addition to its relatively poor prognosis, bone metastases cause a significant cumulative morbidity in breast cancer patients[1,2]. In spite of its clinical importance, the underlying cellular and molecular mechanism driving bone metastases in breast cancer patients remains elusive. Recently, in addition to the progress in understanding the phenotype of cancer cells, there is increased focus on the tumour micro-environment and its influence on the development and growth of bone metastases in breast cancer. Better understanding of the influence of cellular changes in micro-environment of bone on the entire multi-step metastatic process is required in clinical practice[4,5,15,17,18]. It was possible in the present study to explore the probability of changes in bone micro-environment via using MSS and its influence on frequency of bone metastases in patients with breast cancer.

In the current study, MSS features were seen in 22 % of the studied breast cancer patients. These MSS features were more frequent in post-menopausal patients especially those with positive hormonal receptors status compared to pre-menopausal and negative hormonal receptor status. The major action of estrogen hormone is to inhibit osteoclastic activity which occurs through different several mechanisms. The fall in estrogen at time of menopause results in elimination of its inhibitory effect on the osteoclasts and consequent enhanced bone resorption. As osteoblastic response follows osteoclastic activity in co-ordinated pattern, the net result will be enhanced skeletal uptake to bone tracer. This preferential bone uptake produces MSS features[11,12, 17,18,19,20]. Moreover a relative significant increase in the prevalence of MSS among IDC pathological subtype as well as stage II and III was noticed in the current study. This can be explained by the high percentage of such pathological subtype and staging categories in this work as they constitute 84% and 94% of the studied group of patients respectively. Correlation between HER2/neu receptor status and MSS cannot be explored as it was tested in a limited number of the studied patients.

Both hormonal and bisphosphonate therapies have been widely used in

treatment of breast cancer patients. Beside their anti-tumour effect, both can target osteoclastic activity through different pathways. Due to its estrogen antagonist properties hormonal therapy may simulate menopausal changes on bone with consecutive enhanced bone resorption in a manner similar to post-menopausal changes resulting in appearance of MSS features. On the other hand bisphosphonates are analogues of pyrophosphate that bind to remodeling bone and are internalized by osteoclast during bone resorption, resulting in inhibition of osteolysis activity. The net result will be reduced bone resorption and increased bone formation. Consequently preferential bone uptake to the tracer (MSS pattern) may occur. Both therapies produce MSS changes through disruption of coordination between osteoclast and osteoblast via different patho-physiological pathways. The duration of hormonal and bisphosphonate therapies may contribute to the severity and magnitude of MSS changes[15, 21,22,23,24]. In agreement with the fore mentioned data, this study shows more prevalence of metabolic superscan pattern in breast cancer patients treated with hormonal and/or Bisphosphonate therapy.

Bone metastases were confirmed in 72 out of 450 studied breast cancer patients. The frequency of bone metastases was significantly lower in MSS (8%) compared Non-MSS (18.2%) group (P<0.05). The changes that occur in the cells of bone micro-environment with MSS may provide unsuitable soil that disrupts the growth and maturation of tumour cells. Also, this may weaken the sanctuary nature of bone marrow to protect metastatic breast cancer cells. In addition, the anti-tumour effect of hormonal and bisphosphonate therapy may contribute in such finding [4, 17,19,20,21,22,23,24]. Further elaborate research data are needed focusing on the relation of characteristic changes in each micro-environment which may improve intervention for the sake of patient.

In agreement with published data, the current study shows a negative impact for MSS changes on the reliability of bone scan for detection of bone metastases in MSS compared to Non-MSS group of patients[13, 15, 18]. This was more obvious in sensitivity, NPV and overall accuracy rather than specificity and PPV. MSS may hide a true positive lesion leading to more false negative results. In addition the lesions complicating MSS may further lower the specificity of bone scan for detection of bone metastases.

Renal osteodystrophy is a well known cause for MSS[25]. In attempt to explore the contribution of renal factor in the existed MSS, serum creatinine level was tested in the 450 studied breast cancer patients. There were no significant differences in the values of serum creatinine level in MSS group compared to non MSS. This tends to reduce renal osteodystrophy as a contributing factor in MSS changes in the current study.

In respect to the studied bio-markers there was a significant increase in the values of alkaline phosphatase in both groups. On the other hand similar values for calcium and parathyroid hormone were recorded for both MSS and Non-MSS groups with no significant difference in-between. These results disagree with other authors[7, 26] who demonstrate elevated PTH and serum calcium levels in MSS compared to Non-MSS patients. This discrepancy may be attributed to different patho-physiological mechanism for MSS as well

as variation in duration and different population of the study.

The strength of the study lies in its novel prospective nature in addressing the effect of MSS on the frequency of bone metastases in breast cancer patients. The study enclosed female breast cancer patients of similar age group.

The limitation of the study is inability to correlate MSS with more specific bone biomarker. This study runs on a short term basis on heterogeneous group of patients with variation in duration of disease and different therapeutic modalities. The accuracy of the results based on a short term follow up period. The study did not correlate the magnitude and severity of MSS with the duration of the used hormonal or bisphosphonate therapy. Moreover it did not consider the aetiological based potential characteristic difference in MSS pattern among the studied breast cancer patient.

CONCLUSION:

MSS was seen in 22% of the studied breast cancer patients. MSS was more prevalent in post-menopausal patient, and/or those treated with hormonal and bisphosphonate therapy. MSS tends to reduce the frequency of bone metastases in breast cancer patients. However it has a negative impact on the accuracy of bone scan results in identification of bone metastases.

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