A single ^{99m}Tc-MIBI study to predict response to neoadjuvant treatment in sarcoma patiens

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Abstract

Technetium-99m methoxy isobutyl isonitrile (^{99m}Tc-MIBI) was used as a tumour imaging agent to predict the response of neoadjuvant treatment in patients with bone and soft tissue sarcoma. *Our study included* 31 patients (M:F = 23:8), 17 having osteosarcomas and 14 with soft-tissues sarcomas. Scintigraphy with ^{99m}Tc-MIBI was performed before the initiation of the neoadjuvant treatment. Static images were acquired at 10 and 60min post-injection and lesion to normal (L/N) ratios and washout rates (WR%) were calculated. Tumour response was assessed by detecting percent necrosis in a surgically resected specimen. Responses were correlated and compared with WR%. *Percentage of tumour necrosis* was 71.35±20.20% (mean±SD) with eight good and 23 poor responses. On visual analysis, 16 showed homogeneous, 11 heterogeneous and 4 doughnut shaped pattern of uptake. Seventy five percent of good responders had homogeneous uptake. Early and delayed L/N ratios were significantly different in both good and poor responders (P=0.006 and P<0.001, respectively) but correlated poorly with the tumour necrosis values in the specimen (R=0.23 and 0.06 respectively). Mean washout rate was 26.13±11.25% (median = 29%) and there was weak correlation between tumour necrosis and WR% (r=-0.32, P=0.029). The mean WR% of good responders was 15.0±10.0% and that of poor responders was significantly higher (30.1±8.8%, P=0.003). Good responders by 88% were below the median cut-off value. *In conclusion* WR% of ^{99m}Tc-MIBI may be used before surgery to identify poor responders to neoadjuvant treatment in patients with bone and soft tissue sarcomas.

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Introduction

Neoadjuvant treatment is the chemotherapy employed before primary treatment for a malignancy is initiated which in case of sarcomas is surgery [1]. It reduces the size of the surgical resection, provides space for post-operative treatment inhibition due to delayed wound healing, and reduces the progression of micro-metastases. However ineffective treatment due to multi-drug resistance (MDR) may cause unnecessary delay in surgery and is related to higher incidence of metastases [2, 3]. Therefore it is important to detect early the poor responders who might benefit from alternate treatment regimens. Response to treatment in solid tumours is effectively assessed after the completion of the treatment using percent necrosis in the resected specimen [4]. Nearly all medical imaging modalities attain specific features to assess response to treatment, including the recently proposed criteria of RECIST and PERCIST using CT and PET respectively [5-7]. All these criteria require at least two studies usually before and after the commencement of treatment.

Technetium-99m-methoxyiso-butylisonitrile (MIBI) was first reported as a tumour imaging agent [8]. Its handling in the tumour cells is similar to most of the anti-cancer drugs. Its efflux, being under the influence of P-glycoprotein (Pgp) and multidrug resistance associated protein-1 (MRP-1), may confer the ability to predict the effectiveness of chemotherapy in a single study prior to its commencement in various types of tumours [9-13]. Bone and soft-tissue tumours are frequently studied together for the role of ^{99m}Tc-MIBI in assessment of tumour response because efflux rate of ^{99m}Tc-MIBI correlates well with Pgp expression in patients with musculoskeletal sarcomas [14-18]. Some researchers doubt the usefulness of ^{99m}Tc-MIBI scan in these tumours [19] while others have shown that it does have a predictive value in these cases [14, 20]. In a recent study the comparison of uptake ratios of ^{99m}Tc-MIBI before and after the neoadjuvant treatment has shown its effectiveness but the procedure require two MIBI studies [21].

In our present work we used a single ^{99m}Tc-MIBI study to predict treatment response before starting neoadjuvant treatment in newly diagnosed cases of bone and soft tissue sarcomas and compare this to percent necrosis in a surgically resected specimen.

Subjects and methods

The present study was conducted at our Nuclear Medical Centre, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan after approval from the local ethical committee. A total of 31 patients were diagnosed and were retrospectively registered, since ^{99m}Tc-MIBI is performed before histopathology, on the basis of histopathology. Patients were 23 males and 8 females aged from 9 to 78 years (mean age±SD 35.7±16.8 years) and were finally included after taking their informed consent. Seventeen patients were diagnosed as osteosarcomas and the remaining were soft-tissues sarcomas (Table 1, Fig. 1).

Table 1. *Pre and post treatment findings*

No	Diagnosis	Visual Pattern	Post treatment necrosis	Treatment response	Wash-out (%)
1.	Fibrosarcoma	Homogeneous	80%	Poor	28
2.	Synovial sarcoma	,,	90%	Good	13
3.	Osteosarcoma	Doughnut	99%	,,	18
4.	,,	,,	80%	Poor	26
5.	,,	Heterogeneous	70%	,,	22
6.	Neurofibrosarcoma	Homogeneous	50%	,,	34
7.	Dermatofibrosarcoma	Heterogeneous	65%	,,	34
8.	Osteosarcoma	,,	80%	,,	32
9.	,,	Homogeneous	60%	,,	22
10.	Synovial sarcoma	,,	99%	Good	30
11.	Rhabdomyo-sarcoma	,,	40%	Poor	31
12.	Osteosarcoma	Heterogeneous	90%	Good	20
13.	,,	Doughnut	75%	Poor	30
14.	,,	Homogeneous	99%	Good	2
15.	Synovial sarcoma	,,	80%	Poor	26
16.	Fibrosarcoma	,,	90%	Good	4
17.	Synovial sarcoma	,,	90%	,,	8
18.	Osteosarcoma	,,	80%	Poor	27
19.	,,	,,	70%	,,	33
20.	,,	Doughnut	10%	,,	33
21.	,,	Heterogeneous	40%	,,	31
22.	,,	,,	50%	,,	13
23.	,,	,,	75%	,,	29
24.	,,	,,	80%	,,	42
25.	,,	,,	60%	,,	9
26.	Liposarcoma	Homogeneous	90%	Good	25
27.	Synovial sarcoma	Heterogeneous	60%	Poor	51
28.	Osteosarcoma	Homogeneous	80%	,,	30
29.	Liposarcoma	,,	50%	,,	37
30.	Lympangio-sarcoma	Heterogeneous	60%	,,	32
31.	Synovial sarcoma	Homogeneous	70%	,,	40

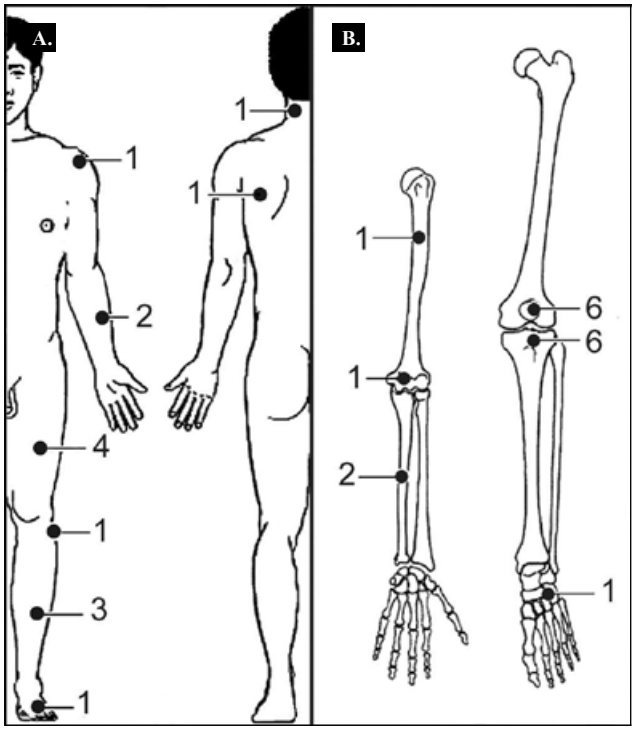

Figure 1. Anatomical distribution of soft-tissue sarcomas (**A**) and osteosarcomas (**B**) in patients (n=31).

The lyophilised MIBI kit (Cardiolite®) manufactured by Bristol-Myers Squibb Medical Imaging, Inc, USA was labelled with freshly eluted pertechnetate ($^{99m}TcO_4^-$). Nearly 500MBq of ^{99m}Tc-MIBI were injected into the anticubital veins of the patients keeping all aseptic and radiation safety measures. Scintronix gamma camera (Scintronix USA Inc., Woburn Mass., USA) was used to acquire images. A 20% energy window was set at 140keV with a low energy high resolution collimator. Early and delayed 5min images of each patient on a 256X256 matrix were acquired after 10 and 60min. Patients were positioned supine under the head of the gamma camera with the field of view including the lesion and also the contralateral normal area of the body for comparison.

Neoadjuvant treatment was started by the oncologist according to standard protocols. The tumour was surgically resected by the surgeon after treatment and the specimens were sent for histopathology. Seven blocks in average were cut according to the size of the specimens and at least two slides from each block were analysed. Treatment response was categorized according to previously published criteria of percent necrosis i.e. ≥90% as good response, <90% as poor response [4].

Images acquired on gamma camera were analysed visually and semi-quantitatively. Visually, lesions were classified into homogenous uptake, heterogenous uptake or doughnut shaped pattern. For semi-quantitative analysis regions of interest (ROI) were drawn over the lesion and a normal adjacent or contralateral area on both early and delayed images. Counts per pixel were recorded as 'L' for lesion and 'N' for normal site. Lesion to normal (L/N) was calculated and applied in the following formula in order to estimate the percent washout rate [14, 15].

$$WR\% = \frac{ER - DR}{ER} \qquad Equation1$$

Where, ER (early ratio) is L/N ratio at the 10min post injection image and DR (delayed ratio) is L/N ratio at the 60min post injection image.

Chi-square test was used to see the association between neoadjuvant chemotherapy and visual patterns of ^{99m}Tc-MIBI uptake while Student's t-test was applied to compare the means of ER, DR, and WR%. P-value below 0.05 was considered as statistically significant. Relationship between these values as well as WR% and percent necrosis were assessed using simple regression analysis and Pearson correlation coefficient.

Table 2. *Observed frequencies of responses in various ^{99m}Tc-MIBI uptake patterns*

MIBI Patterns	Good responders	Poor responders	Total	Percent of good responders
Homogeneous	6	10	16	38%
Heterogeneous	1	10	11	0.1%
Doughnut	1	3	4	25%
Total	8	23	n=31	

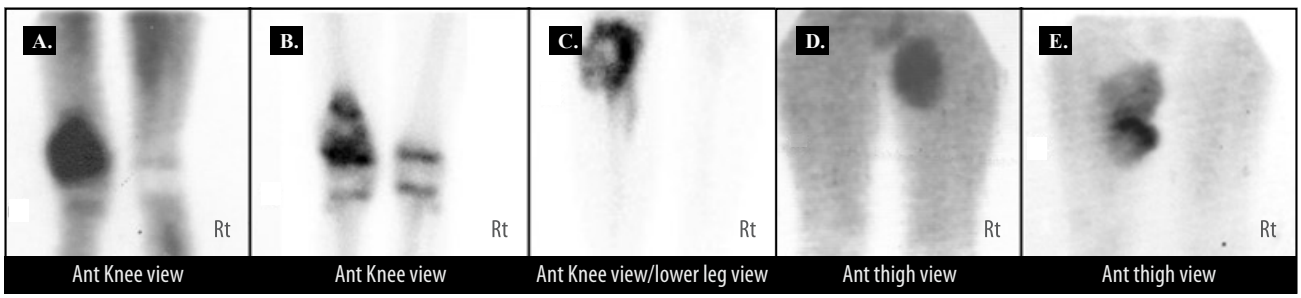

Figure 2. Visual patterns of ^{99m}Tc-MIBI uptake. Osteosarcomas showing homogeneous (**A**), heterogeneous (**B**) and doughnut pattern (**C**); soft-tissue sarcomas showing homogenous (**D**) and heterogeneous (**E**).

Results

Absolute values of percent necrosis of the tumour in all patients are shown in Table 1, while mean±SD of these values was 71.35%±20.20%. A total of eight patients showed good response to neoadjuvant treatment, 5 with osteosarcoma and 3 with soft-tissue sarcoma.

On visual analysis (Fig. 2) 16 (52%) patients showed homogenous uptake while 11 patients had heterogenous uptake. Doughnut shaped uptake patterns was seen in four patients. Table 2 shows frequencies of different visual patterns in good and poor responders. These frequencies were found to have association when compared by χ^2 test (P-value <0.05).

Mean±SD of early ratio (ER) was 2.39±0.90 while that of delayed ratio (DR) was 1.75±0.66 and comparison showed P value <0.001. Table 3 shows values of ER and DR calculated in various ^{99m}Tc-MIBI uptake patterns and treatment responses. Both ratios were significantly different among good responders and poor responders (P=0.006 and P<0.001, respectively). Comparison of the means of these ratios in various uptake patterns is given in Table 4. The means of L/N ratios of good responders shown in Table 3 were statistically not different from poor responders (P-value for early = 0.30 and for delayed = 0.86).

Washout rates % calculated using equation 1 are shown in Table 1. Mean±SD of these values was 26.13%±11.25%. The range of the values was from 2 to 51, minimum value in Pt# 14 showing good response to treatment (99% necrosis) and maximum in Pt# 30 showing poor response (60% necrosis). The median of the values was 29%.

No significant difference was seen between washout rates of various types of ^{99m}Tc-MIBI uptake patterns. Means±SD values of WR% regarding homogeneous, heterogeneous and doughnut patterns were 25.2±12.9, 27.5±10.4 and 27.5±6.6, respectively the highest and lowest values in these patterns were 40% and 2%; 51% and 9%; 33% and 18%, respectively.

When washout rates were compared to responses to neoadjuvant treatment, means±SD of WR% in good responders was 15.0%±10.0% while that of poor responders was 30.1%±8.8% (Pvalue=0.003). Correlation between WR% and absolute values of post-neoadjuvant treatment necrosis was weak and negative with r-value of -0.39 (P=0.029). We also compared percent washout rates with treatment response of the resection material by using the median of WR% i.e. 29%, as a cut-off value. These results are shown in Table 5.

Discussion

While analysing our data we arrayed responses to treatment in absolute percentages seen in the resected specimen and also categorized them to good and poor responses. It is widely accepted that necrosis of <90% is a bad prognostic sign due to the presence of resistant tumour cells and necrosis >90% is a good response to treatment. Nevertheless related literature documents a wide range of good responders (10% to 87%) seen by the various authors in their populations [3, 12, 14, 22-24]. This discrepancy is due to variation in assessment of response i.e., clinical, radiological or histological; size and type of population i.e., osteosarcoma, soft-tissue sarcoma or both; and treatment i.e., systemic or intra-arterial, with or without radiotherapy. Histological response is frequently assessed by analysing viable versus necrotic tissue in surgically removed specimen. We considered necrosis ≥90% as a good response while <90% as poor response [4]. In our study eight out of total 31 (26%) patients showed good response to neoadjuvant treatment.

Table 3. *Mean±SD of lesions to normal ratios in ^{99m}Tc-MIBI uptake patterns and treatment response*

	Category	Early ratio	Delayed ratio
^{131}I-MIBI uptake pattern	Homogeneous	2.17±0.41	1.62±0.40
	Heterogeneous	2.19±0.58	1.57±0.37
	Doughnut	3.82±1.73	2.52±1.42
Treatment response	Good	2.10±0.32	1.79±0.32
	Poor	2.49±1.02	1.74±0.75

Table 4. *Comparison of means lesions to normal ratios between ^{99m}Tc-MIBI uptake patterns*

	Homogeneous and heterogeneous	Homogeneous and doughnut	Heterogeneous and doughnut
Early ratio	U=0.913	U=0.002	U=0.013
Delayed ratio	U=0.735	U=0.030	U=0.051

Table 5. *Treatment responses calculated with 29% as cut-off value of washout rate (P=0.007)*

	WR% <29%	WR% ≥29%
Good responders (n=8)	7	1
Poor responders (n=23)	8	15

The uptake of ^{99m}Tc-MIBI represents the presence of viable tumour cells. In the related literature various patterns of its uptake are categorized as homogeneous, heterogeneous and doughnut shaped [14]. We compared these patterns with types of neoadjuvant treatment response (i.e. good and poor) using χ^2 test and found a significant association between them (P<0.05). In a published study [14] the complete response (necrosis >90%) in homogeneous uptake was more than in our study (80% vs. 38%) probably due to lower number of overall good responses in our study. High percentage of poor responders was however seen cumulatively in heterogeneous and doughnut patterns in the aforementioned study as well as in our findings (85% and 87% respectively).

Lesions to normal (L/N) ratios were obtained from early and delayed digital images of the ^{99m}Tc-MIBI scan. There was significant difference between their means (2.39±0.90 and 1.75±0.66 respectively, P<0.05). In two other studies slightly higher values have been reported [15, 20]. We compared our data with the means given in these studies by one-sample t-test and found significant difference between the two (P<0.05) in both early and delayed L/N ratios. Early and delayed ratios in good responders were reported by others as 2.57±0.95 and 2.07±0.61 [14] and were significantly different from our values (P=0.004 and 0.041 respectively). These variations in results are expected because of different population studied i.e. different types of malignancies and inconsistent density of neoplastic cells.

When the means of L/N ratios were compared between good responders and poor responders in our study, there was no significant difference in early (P=0.30) and delayed images (P=0.86), suggesting that early or delayed images separately do not give sufficient information to evaluate drug resistance in bone and soft tissue sarcomas. This is in agreement with previously published data [15, 20]. Therefore washout rate has been introduced which is the measure of the efflux of ^{99m}Tc-MIBI from the lesion during 50 min studying early-to-delayed images.

Weak and negative correlation between percent values of post-neoadjuvant treatment necrotic tissue and ^{99m}Tc-MIBI WR% (r=-0.39) in our study was in agreement with others who showed r=-0.32 [14]. This suggests that WR% is unable to predict the absolute amount of necrosis that will be seen in the tumour after the neoadjuvant treatment. However, when the percent necrosis was categorized into good and poor responses, the WR% was found to be statistically different in the two categories (15.0±10.0% vs. 30.1±8.8% respectively, P=0.003) signifying that WR% of ^{99m}Tc-MIBI is a good parameter which may be used clinically to discriminate good from poor responders before the initiation of treatment.

The data of 24 patients in another study [20] showed similar results (18.5±11.1% vs 28.8±6.9% respectively, P=0.013) while in 25 patients by the same research group [15] slightly higher P value was observed (17.4±13.3% vs 26.7±9.0% respectively, P=0.09).

Further analysing our data we used median of the WR% (i.e. 29%) as cut-off value to separate good responders from the poor responders. It was found that 7 out of 8 (88%) good responders had WR% below this value and only one patient (Pt# 10) with synovial sarcoma having good histological response showed WR% marginally higher than 29% value (30%). On the other hand only 35% (8 out of 23) of the poor responders had WR% <29%. Elsewhere in the literature similar cut-off values of 25% [15] and 22% [20] have been reported which were derived as 1 SD below the mean WR%.

In conclusion, the washout rate of osteosarcomas and soft tissue sarcomas obtained by the ^{99m}Tc-MIBI study preoperatively may predict the response to treatment in appropriate clinical settings.

The authors declare that they have no conflicts of interest

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