Original Article

Bone Tumors & Expression of SATB2 Immunohistochemical Stain in the Diagnosis of Osteogenic Sarcoma

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ABSTRACT

Objective: To determine the frequency & histopathological features of various benign and malignant bone tumors at Mayo Hospital/KEMU, Lahore. To evaluate the expression of Special AT-rich sequence-binding protein 2 (SATB2), a new novel immunohistochemical stain in the diagnosis of osteogenic sarcoma.

Methodology: It was a retrospective cross-sectional study of 83 cases of bone tumors received and reported from 1st July 2017 to 30th June 2019 at Mayo Hospital/ KEMU, Lahore. Relevant records & data, radiological findings, paraffin wax blocks & slides were retrieved and reviewed. Fresh slides were prepared wherever required. The immunohistochemical (IHC) stain of SATB2 was applied to the relevant malignant cases. Data was compiled & analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: Out of a total of 83 bone tumors, 56(67.46%) cases were classified as benign & 27(32.53%) cases as malignant. Four cases in the malignant category were metastatic in origin. The commonest benign tumor was osteochondroma comprising of 24(42.85%) cases followed by giant cell tumor consisting of 16(28.57%) cases. The most frequent malignant bone tumor was osteosarcoma composed of 14(51.85%) cases & chondrosarcoma was the 2nd commonest malignant tumor comprising of 5(18.85%) cases. In the benign cases, the age of the patients ranged from 12 to 47 years & in the malignant cases, the age ranged from 5 to 67 years. Nine (64.29%) cases of osteosarcoma were reported in children in the age range of 5 to 16 years. Overall humerus was the bone, most frequently affected by both benign & malignant bone tumors comprising 24(28.90%) cases followed by the femur which was affected in 21(25.3%) cases. Nuclear positivity for SATB2 was noted in 12(87.5%) cases of osteosarcoma.

Conclusion: Osteochondroma is the commonest benign bone tumor and osteosarcoma is the most frequent malignant bone tumor. The immunohistochemical stain SATB2 proves to be a very effective tool in the diagnosis of osteosarcoma.

Keywords: *Bone tumors. Immunohistochemical stain SATB2. Osteosarcoma. Osteochondroma.*

INTRODUCTION

Bone is a rigid firm mesenchymal body tissue comprising of cells embedded in a mineralized organic matrix having the tendency to develop a neoplasm either benign or malignant.[1] Bone tumors comprise only 0.5% of the total world cancer incidence.[2] When excluding myeloma and lymphoma bone tumors with a malignant biological tendency, constitute only 0.2% of all malignancies in adults and 5% of childhood malignancies.[3,4] They may be classified as 'primary tumors', which arise in the bone itself, and 'secondary tumors' which originate elsewhere from some other site and then produce their metastatic deposits to the skeleton.3 Thus bone tumors frequently cause a diagnostic challenge for general surgical pathologists. Clinical findings and radiological data are of utmost importance in accurately diagnosing malignant bone tumors for determining stage, prognosis & planning limb salvage surgery.[5] These tumors tend to occur at certain favored skeletal sites and in particular age groups or even specific locations in particular bones.[6] Secondary or metastatic bone tumors however involve the axial skeleton more frequently than the appendicular skeleton. Relevant data on age, gender, precise anatomical location of the lesion, and radiological correlation are of utmost importance in arriving at a definitive histological diagnosis.[7]

Advancements in the field of immunohistochemical stains and molecular studies have certainly made an accurate histological diagnosis of bone tumors much easier. Special AT-rich sequence binding protein (SATB2) is a potent nuclear matrix protein associated transcription factor and epigenetic regulator enhancing osteoblastic differentiation and bone regeneration.[8] It is also expressed in the glandular epithelial cells of the lower gastrointestinal tract thus facilitating the diagnosis of colorectal cancer too.[9] Hence SATB2 has now emerged as a new novel immunohistochemical stain for the confirmation of osteogenic sarcoma and to differentiate it from its potential mimickers.[8]

Diagnosis of osteosarcoma is a challenging task due to limited osteoid deposition in some cases and the presence of hyalinized stroma which mimics osteoid. In these cases, SATB2 plays a significant role in its diagnosis. The present study was conducted to determine the frequency of bone tumors in our setup & to evaluate the diagnostic utility of SATB2 in osteogenic sarcoma.

METHODOLOGY

The present study was a two year retrospective analysis on all bone tumors received at the Department of Pathology, King Edward Medical University, Lahore, Pakistan from 1st July 2017 till 30th June 2019. The study

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was approved by the ethical committee of the institution. All the histopathological reports of these cases, maintained in the histopathological section, were reviewed and Haematoxylin and Eosin (H & E) stained slides of every case were re-examined. Further sections were cut from paraffin blocks wherever required. The slides were re-examined to analyse data regarding age, gender, anatomical location, radiological findings, and histopathological diagnosis. All cases irrespective of their age and gender were considered. Hematological malignancies like myelomas and lymphomas were excluded from the study as their diagnosis requires bone marrow biopsy. Inflammatory bone lesions, tumor-like conditions, and primary tumors of odontogenic origin were also excluded from the study.

In suspected and diagnostically uncertain cases of osteogenic sarcoma & its variants showing scanty osteoid, the immunohistochemical stain SATB2 was performed according to the instructions and guidelines of the kit (Dako Company). The stained slides were examined under the microscope to confirm the diagnosis and to exclude other bone sarcomas that cause diagnostic confusion like lymphomas, Ewing sarcoma family of tumors (ESFT), chondrosarcoma, and aneurysmal bone cyst.

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0. Frequency and percentages were calculated.

RESULTS

In the present study, a total of 104 bone lesions were reported over a period of two years. Out of 104 bone lesions, 83(79.8%) were bone tumors. Inflammatory bone lesions & tumor-like conditions constituted 21(20.2%) cases and were excluded from the study. Benign tumors accounted for 56 (67.46%) cases and malignant tumors for 27(32.53%) cases giving a benign to a malignant ratio of almost 2:1. Out of the 27 malignant bone tumors, 23(85.18%) cases were of primary bone origin and 4(14.81 %) cases were metastatic in origin (Table 1).

The mean age of the patients presented with bone tumors was 23±3.1 years and age ranged from 5 to 67 years. Sixty one (58.7%) patients were males and 43(41.3%) were females. In the benign category, patients ranged in age from 12 to 47 years whereas in the malignant group patients age ranged from 5 to 67 years.

Considering the benign category of bone tumors, the commonest tumor reported was osteochondroma which consisted of 24(42.85%) cases out of 56 benign bone tumors and its most common site was the upper end of the humerus (13 cases) followed by the lower end of the femur and tibia (5 cases each). Only one case of osteochondroma was reported in the scapula (Table 1 & 2). Cases of giant cell tumor constituted 16(28.57%) cases of which 7 were reported in the femur and 3 each in the humerus and tibia, 2 in the radius, and 1 in the ulna. The frequency and site of distribution of other benign tumors are shown in Table 1 and 2. Microscopic pictures of benign tumors are shown in Figure 1, 2 & 3. Regarding the malignant bone tumors which comprised of 27 cases, the commonest primary malignant tumor was osteogenic sarcoma composed of 14(51.85%) cases. In 5 cases, the distal femur was involved, 3 cases were reported each in the tibia and humerus, 2 cases in the ulna, and 1 case was reported in the radius. Chondrosarcoma was the second commonest malignant bone tumor constituting 5(18.51%) cases. Two cases were reported in the distal femur and 1 each in the jaw, ulna, and rib (Table 1, 2). Two cases of Ewing sarcoma family of tumors were located in the shaft of the radius and 1 in the humerus. The single (3.70%) case of chordoma was located in the sacrum. The anatomical location of these cases is depicted in Table 2. Figure 4, 5, 6 & 7 showed microscopic picture of

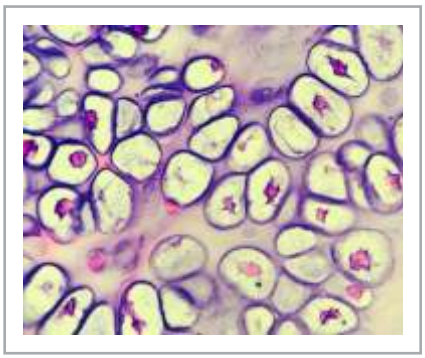

Figure 1: Chondroma Showing Cartilaginous Matrix with Scattered Uniform Appearing Chondrocytes in Lacunae (H & E stain, 400x magnification)

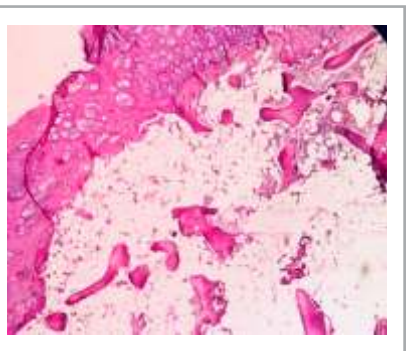

Figure 2: Exostosis (Osteochondroma) Showing a Cartilaginous Cap with Maturation into Trabecular Bone and Underlying Hematopoietic Marrow (H & E stain, 100x magnification)

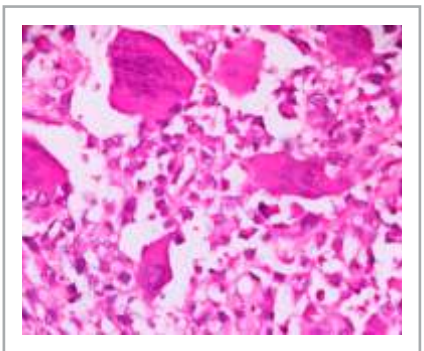

Figure 3: Giant Cell Tumor (Osteoclastoma). Numerous Multinucleated Osteoclast Giant Cells Scattered in a Background of Uniform Mononuclear cells (H & E stain, 200x magnification)

malignant tumors.

Metastatic (secondary) bone tumors comprised of 4(14.81%) cases, of which 2 were metastatic deposits of renal cell carcinoma (Figure 8), one presented as a pathological fracture in the femoral neck & one as a jaw tumor. One case of a rib tumor was a metastasis from breast cancer and one case of jaw tumor was metastases from cancer of the prostate (Table 2).

Regarding age distribution, patients with osteogenic sarcoma ranged from 5 to 54 years of age. Nine out of 14(64.29%) cases of osteogenic sarcoma were seen in children in the age range of 5 to 16 years. Chondrosarcoma occurred between 41 to 67 years of age and the 3 cases of Ewing sarcoma family of tumors were seen in the age range 7 to 15 years. A single case of chordoma was reported in the sacrum in a 32-year-old male patient. All 4 cases of metastatic deposits were above 60 years of age.

Out of a total of 4 cases of osteogenic sarcoma, 6 were of the telangiectatic type, 3 were chondroblastic, 1 was a small cell variant and the rest were the conventional subtypes.

Expression of the novel immunohistochemical nuclear stain SATB2 was studied in 14 suspected cases of osteogenic sarcoma. Twelve (87.71%) cases showed strong brownish-black nuclear positivity whereas 2 showed weak nuclear positivity (Figure 9).

Table 1: Histological Categorization and Frequency of Benign and Malignant Bone Tumors

Histological Subtype	Frequency	Percentage (%)
Benign Bone Tumors (n=56) 67.46%		
Exostosis (Osteochondroma)	24	42.85%
Giant Cell Tumor	16	28.57%
Chondroma	7	12.5%
Aneurysmal Bone Cyst	4	7.14%
Fibrous Histiocytoma	3	5.35%
Chondroblastoma	1	1.78%
Chondromyxoid Fibroma	1	1.78%
Total Benign Tumors	**56**	**100%**
Malignant Bone Tumors (n=27) 32.53%		
Osteogenic Sarcoma (Primary)	14	51.85%
Chondrosarcoma (Primary)	5	18.51%
Ewing Sarcoma Family of Tumors/ESFT (Primary)	3	11.11%
Chordoma (Primary)	1	3.70%
Metastatic	4	14.81%
Total Malignant Tumors	**27**	**100%**

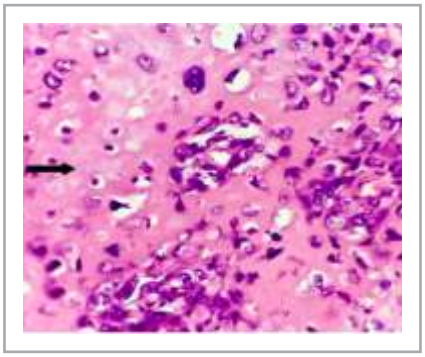

Figure 4: Osteogenic Sarcoma with a Lacelike Unmineralized Osteoid Matrix (Arrows) Deposited between Malignant Tumor Cells (H & E stain, 200x magnification)

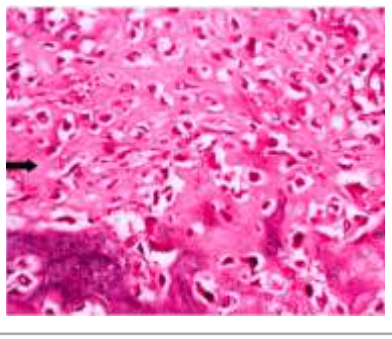

Figure 5: Chondroblastic Variant of Osteogenic Sarcoma Showing Abundant Osteoid (Arrows) and Chondroid Differentiation (H & E stain, 100x magnification)

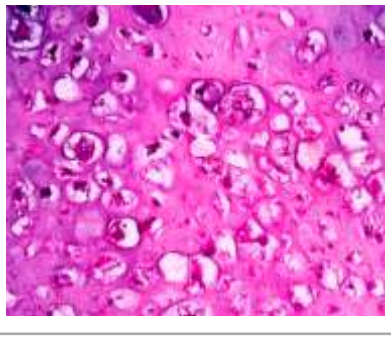

Figure 6: Chondrosarcoma (Grade 3) Showing Hyaline Cartilaginous Matrix with Admixed many Bizarre Looking Anaplastic Chondrocytes (H & E stain, 200x magnification)

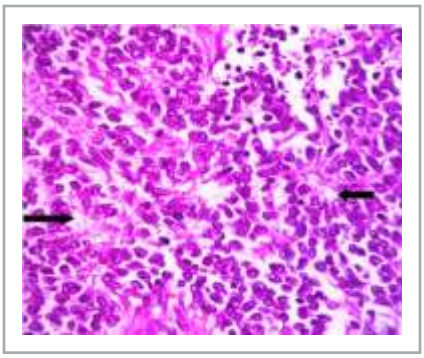

Figure 7: Ewing Sarcoma Family of Tumors (ESFT) Comprising of Small Round Blue Cells Arranged in True Rosettes and Perivascular Peudorosettes (Arrow) (H & E stain, 400x magnification)

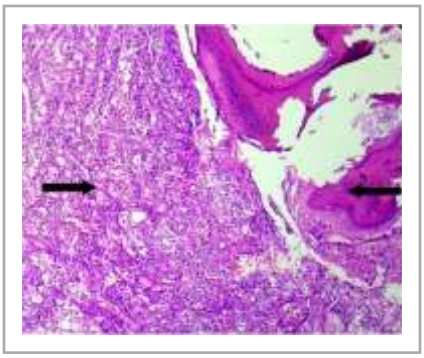

Figure 8: Metastatic Renal Cell Carcinoma (Arrow on the left side) in Bone (Arrow on the right side) (H & E stain, 100x magnification)

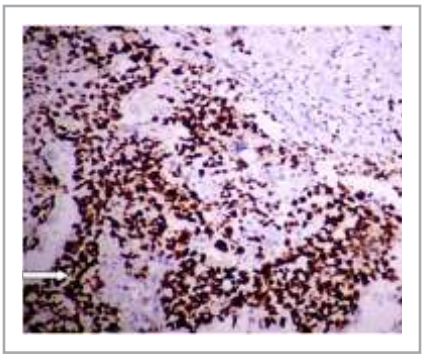

Figure 9: The Novel Immunohistochemical Stain SATB2 Showing Positive Nuclear Staining (Arrow) in Osteogenic Sarcoma (IHC stain, 100x magnification)

Table 2: Distribution of Bone Tumors According to Site

Benign	Phalanx	Sacrum	Humerus	Radius	Ulna	Femur	Tibia	Rib	Scapula	Jaw	Total
Osteochondroma			13			5	5		1		**24**
Giant cell Tumor			3	2	1	7	3				**16**
Enchondroma	2		1			1	3				**7**
Aneurysmal Bone Cyst			1	1	2						**4**
Benign Fibrous Histiocytoma			2	1							**3**
Chondromyxoid fibroma					1						**1**
Chondroblastoma							1				**1**
Malignant											
Osteogenic sarcoma			3	1	2	5	3				**14**
Chondrosarcoma					1	2		1		1	**5**
Ewing Sarcoma Family of Tumors (ESFT)			1	2							**3**
Chordoma		1									**1**
Metastatic						1		1		2	**4**
Total	2 (2.4%)	1 (1.2%)	24 (28.9%)	7 (8.4%)	7 (8.4%)	21 (25.3%)	15 (18%)	3 (3.6%)	1 (1.2%)	3 (3.6%)	83

DISCUSSION

Primary bone tumors comprise only 0.5% of the total world cancer incidence.[2] The diverse spectrum of bone lesions makes accurate diagnosis a challenging task. To establish an accurate diagnosis, a team approach is mandatory, taking into account all relevant clinical data, age, radiological studies, surgical notes & biopsy. Histopathological diagnosis helps in planning appropriate treatment protocols and to estimate the prognosis in individual cases.[7] In the present study, a total of 83 bone tumors were reported out of which there were 56 benign tumors (67.46%) and 27(32.53%) malignant tumors giving a ratio of almost 2:1 which concludes that the benign bone tumors far outnumber the malignant tumors. A study carried out in Iraq at the Kerbela University, Alwasiti Teaching Hospital from November 2015 to July 2017, concluded that out of a sample size of 119, benign cases reported were 100(84%) and malignant cases were 19(16%).[4]

In the present study, the most common benign bone tumor encountered was osteochondroma (42.85%), commonly known as exostosis followed by the giant cell tumor (28.57%), and chondroma (12.5%). The most common malignant tumor reported was osteogenic sarcoma (51.85%) and chondrosarcoma (18.51%). A study carried out in Mexico at a large

referral center in 2008, supports similar results with the most common benign tumors being osteochondroma (43.7%), followed by giant cell tumor (14.6%) and enchondroma (10.1%). Similarly, in the same study, the most common malignant tumor reported was osteosarcoma (46.6%) followed by chondrosarcoma (8.7%).[10] In a research conducted at a Medical College in Himmatnagar Gujrat, India in 2016, the most common benign bone tumors reported were osteochondroma & giant cell tumor and the most common malignant bone tumor was osteogenic sarcoma followed by chondrosarcoma.[11] However, in a study carried out in Patil Medical College in Pune, India in 2017, the most common benign tumor reported was giant cell tumor (30%).[7]

In the present study, the mean age of the patients presented with bone tumors was found to be 23±3.1 years with the youngest and the oldest patients being 5 and 67 years, respectively. Sixty one (58.7%) patients were males and 43(41.3%) were females. Similarly, in a study carried out in Mexico, the tumors affecting males were 53.7%, and 46.3% of females were diagnosed with bone tumors.[10]

In the present study, the most common bone involved was the humerus (28.9%) followed by the femur (25.3%). In a study carried out in Beijing, China in 2015, the most common bone involved was femur (37.7%) followed by tibia (20.1%) and humerus (8.4%).[12] Similarly, in another research conducted at Regional Institute of Medical Sciences, Imphal, Manipur India in 2013, the most common bones of involvement were femur (30.6%) and tibia (29%).[1]

The most common variety of osteogenic sarcoma found in our study was telangiectatic osteogenic sarcoma. Telangiectatic osteogenic sarcoma is a rare kind of osteogenic sarcoma comprising 2-12% of all cases of osteogenic sarcoma. They tend to behave more aggressively than conventional osteogenic sarcoma. This variant causes diagnostic difficulty in differentiating it from the aneurysmal bone cyst and cavernous hemangioma thus the use of SATB2 immunostain helps in demonstrating malignant osteoid.[13] The small cell variant of osteogenic sarcoma also causes diagnostic confusion with lymphoma and Ewing sarcoma family of tumors.[8]

Immunohistochemical stain SATB2 helps in confirming the diagnosis in this scenario as it highlights the osteoid matrix. The osteoblastic and small cell subtypes express SATB2 more intensely than other histological types.[14]

We found 4 cases of metastatic bone tumors, 2 from renal cell carcinoma, 1 from carcinoma breast, and 1 from prostatic carcinoma. Secondary bone tumors usually come from the respiratory system, gastrointestinal tract, prostate, breast, kidney, and liver. The axial skeleton because of its rich content of red marrow, has a predilection for harbouring metastatic deposits than the appendicular skeleton.[7] Carcinomas have a much high tendency to metastasize to the bone than sarcomas.[2] In a study carried out in near east population in Beirut, Lebanon, the most commonly metastasizing tumors to the bone were from breast, lung, and thyroid origin.[15]

In the present study, SATB2 was performed in suspected cases of osteogenic sarcoma. Out of 14(51.85%) cases of osteogenic sarcoma, 12(85.71%) showed positive nuclear staining. The diagnosis of osteosarcoma can pose certain diagnostic challenges, particularly in those cases when no convincing bone or osteoid matrix is identified especially in limited small biopsy samples to differentiate between hyalinized collagen & osteoid. In these situations, SATB2 has shown to be a reliable diagnostic marker for osteoblastic differentiation.[9]

In a study by Machado et al., the value of SATB2 in osteogenic sarcoma was evaluated and it was found to be reactive in 90.4% cases of conventional osteogenic sarcoma, 87.5% of small cell variant of osteogenic sarcoma, 91.3% of osteoblastic osteogenic sarcoma, and in all cases of chondroblastic and parosteal osteogenic sarcoma. However, osteoblastic and small cell variants showed stronger staining than other histological types.[8] In another study carried out in 2016, 45 out of 48(94%) cases of osteogenic sarcoma were immunoreactive for SATB2.[16] The results of SATB2 positivity of these different studies are very similar to the present study with 85.71% positivity.

As bone tumors are rare, most of the pathologists have difficulty in diagnosing them. Hence it is of utmost importance to have the relevant clinical data, age, gender, anatomical sites, and radiographic findings. Immunohistochemistry has a limited role in the diagnosis of bone tumors however new research is constantly being done in the field of molecular pathology for the diagnosis of bone tumors.

CONCLUSION

Osteochondroma is the commonest benign bone tumor and osteosarcoma is the most frequent malignant bone tumor. The immunohistochemical stain SATB2 proves to be a very effective tool in the diagnosis of osteosarcoma. It is a novel marker for bone tumors with osteoblastic differentiation and may help to distinguish osteogenic sarcoma from its mimickers like Ewing sarcoma family of tumors, lymphomas, aneurysmal bone cyst, & chondrosarcoma but biopsy & histopathological evaluation still remains the gold standard for accurate diagnosis of bone tumors.

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