

# Denosumab Therapy Related Changes in Giant cell Tumor (Osteoclastoma) of Bone : A New Osteosarcoma Mimicker

Pradnya Manglekar<sup>1</sup>, Sujit Joshi<sup>1</sup>, Yogesh Panchwagh<sup>1</sup>

## Abstract

**Objectives:** Giant cell tumor (Osteoclastoma) of bone is locally aggressive osteolytic tumor. Denosumab—A monoclonal antibody against RANKL is recently being used to treat this tumor. We discuss histopathological changes occurring in giant cell tumor of bone after Denosumab treatment.

**Method:** A retrospective study of 12 cases from January 2014 to March 2018. All patients included were diagnosed as giant cell tumor (osteoclastoma) of bone on needle or open biopsy. Subsequently these patients received Denosumab therapy followed by surgical resection (extended curettage or wide excision). Histomorphological features after Denosumab therapy were evaluated in these specimens and compared with morphological features of prior biopsy samples.

**Results:** Needle or open biopsy samples studied prior to receiving Denosumab therapy showed typical morphological features of osteoclastoma i.e. presence of uniformly distributed osteoclastic cells and sheets of mononuclear stromal cells. No atypical mitoses or matrix production noted. Post Denosumab therapy resection specimens showed marked reduction in number of osteoclastic giant cells. There was predominance of mononuclear stromal cells along with abundant, irregular new bone (osteoid) formation with osteoid matrix deposition. Occasional mitotic activity was seen. Few foci of necrosis were noted.

**Conclusion:** Denosumab treatment causes significant giant cell depletion accompanied by abundant new bone formation separated by cellular stromal proliferation. These features bear very little resemblance to their pre-treatment counterparts and can mimic morphological features of osteosarcoma and other bone forming tumors. Hence, one should be aware of these changes so that a misdiagnosis of osteosarcoma can be avoided.

**Keywords:** Giant cell tumor (osteoclastoma), Denosumab, RANKL.

## Introduction

Giant cell tumor of bone (osteoclastoma) is a benign locally aggressive osteolytic tumor that is composed of sheets of mononuclear neoplastic stromal cells and uniformly distributed multinucleate osteoclast-like giant cells[1]. Osteoclast precursors form a minor component of mononuclear cells seen within the tumor. It commonly affects individuals in the age group of 20 to 45 years with a slight female predominance. Common sites affected by the tumor include epiphysis and metaphysis of long bones, eg. distal femur, proximal tibia, distal radius and proximal humerus. Other uncommon sites include sacrum, pelvic bone, vertebrae, small tubular bones of

hands and feet[2]. Aggressive lytic behaviour of the tumor is attributed to the presence of multinucleate osteoclast-like giant cells that bring about bone lysis which is mediated by RANK – RANKL (Receptor activator of nuclear factor Kappa B - Receptor activator of nuclear factor Kappa B ligand) coupling. Denosumab is a human monoclonal antibody against RANKL that inhibits formation, activation and survival of these osteoclastic giant cells, thus preventing tumor associated bone lysis. It also induces new bone formation[3].

## Objective

To study histopathological characteristics of giant cell tumor of bone after Denosumab

treatment.

## Materials & Methods

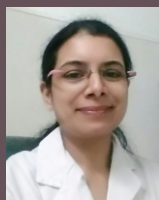
This is a retrospective study of 12 cases from January 2014 to March 2018 conducted in a tertiary care centre. All patients diagnosed as giant cell tumor (osteoclastoma) of bone on needle biopsy or open biopsy were included in the study. These patients were subjected to Denosumab therapy followed by extended curettage or wide excision. Effects of Denosumab treatment on tumor histomorphology were studied and compared with prior biopsy samples. The patient population included in the study comprised of 7 males and 5 females in the age group of 20 to 47 years (Fig.1). Amongst the 12 cases, 4 cases were from distal tibia, 2 cases from proximal humerus and one case each from distal femur, phalanx of hand, medial malleolus, patella, and iliac bone. One of the cases presented with lung

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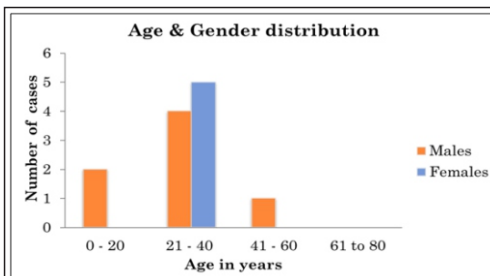
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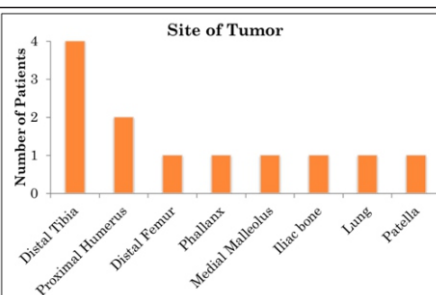
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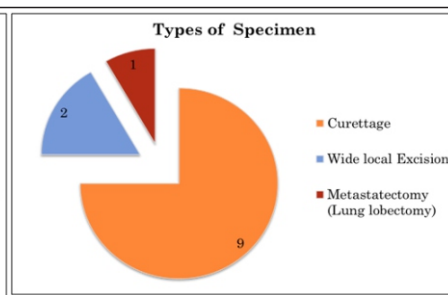
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**Figure 1:** Patient population included in the study comprised of 7 males and 5 females; youngest being 20 years of age and oldest being 47 years of age.



**Figure 2:** Distribution of cases according to site of tumor.



**Figure 3:** Different types of specimen received after Denosumab therapy.

metastasis in which lung lobectomy was done. (Fig. 2). Post Denosumab treatment, we received 9 curettage specimens, 2 wide local excisions and 1 lung lobectomy (Metastatectomy). (Fig. 3). Formalin fixed paraffin embedded tissue sections (5μ thickness) were prepared from pre-treatment and post-treatment specimens and stained with hematoxylin and eosin (H&E). Radiological assessment was done at the time of diagnosis (before Denosumab treatment) and prior to surgical procedures after denosumab therapy (Fig. 4 & 5).

### Observations & Results

On histomorphology, pre-treatment biopsy specimens showed tumor composed of uniformly distributed multinucleate osteoclastic giant cells against a background of mononuclear stromal cells. The mononuclear stromal cells showed round to oval nuclei, inconspicuous nucleoli and eosinophilic cytoplasm with ill-defined cytoplasmic borders. Sparse mitotic activity was observed. There were no atypical mitotic figures. Cellular atypia, osteoid matrix deposition and new bone formation were not observed in any of the cases (Fig. 6). Histological sections from post-

treatment specimens showed significant reduction in number of osteoclastic giant cells. Predominance of mononuclear cellular stroma was observed which showed mild cellular atypia in the form of hyperchromatic nuclei and high Nucleus : Cytoplasm ratio. Prominent nucleoli were seen in some of the cells. (Fig. 7). This was accompanied by abundant osteoid matrix deposition and irregular new bone formation (Fig. 8). Occasional mitosis and occasional focus of necrosis were seen. One of the cases showed focal clusters of foamy macrophages (Fig. 9). We also received lung lobectomy done in a case of metastatic giant cell tumor (osteoclastoma) of bone. It also showed similar changes in histomorphology after Denosumab treatment as were seen in other specimens. (Fig. 10). Pre-treatment radiological assessment showed expansile multiseptate lytic lesion whereas post-treatment radiological assessment showed a prominent sclerotic rim to the tumor which indicated new bone formation (Fig. 4 & 5).

### Discussion

Histologic analysis of tissue specimens from patients with giant cell tumor of bone after

denosumab therapy showed significant reduction in the number of osteoclastic giant cells accompanied by cellular stromal proliferation and exuberant, irregular new bone formation and osteoid matrix deposition. Cellular stroma showed mild atypia in the form of hyperchromatic nuclei, high nucleus : cytoplasm ratio. Prominent nucleoli were seen in some cells. Sparse mitotic activity was noted. Normally, osteoblasts synthesize bone matrix and bring about its mineralization while osteoclasts are responsible for bone resorption. An equilibrium is maintained between these two processes with the help of several signaling pathways. One such pathway involves three factors : 1) RANK (Receptor activator of nuclear factor Kappa B) – a transmembrane receptor expressed by Osteoclast precursors and osteoclasts, 2) RANKL (Receptor activator of nuclear factor Kappa B Ligand) which is expressed by osteoblasts and marrow stromal cells, 3) Osteoprotegerin – a decoy receptor for RANKL, secreted by osteoblasts and several other cell types. Coupling of RANK with RANKL activates transcription factor NF-κB (Nuclear factor Kappa B) which brings about differentiation of osteoclast

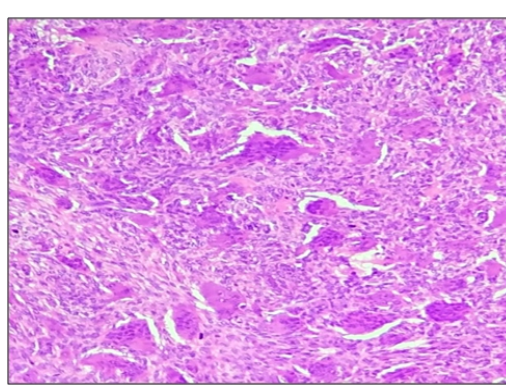
precursors into osteoclasts. Other pathway is WNT/β Catenin pathway in which WNT proteins produced by marrow stromal cells bind to LRP5 and LRP6 proteins on the surface of osteoblasts that activates β catenin and leads to production of osteoprotegerin. Osteoprotegerin can bind to RANKL and prevents its interaction with RANK [1]. RANKL plays a pivotal role in the pathophysiology of giant



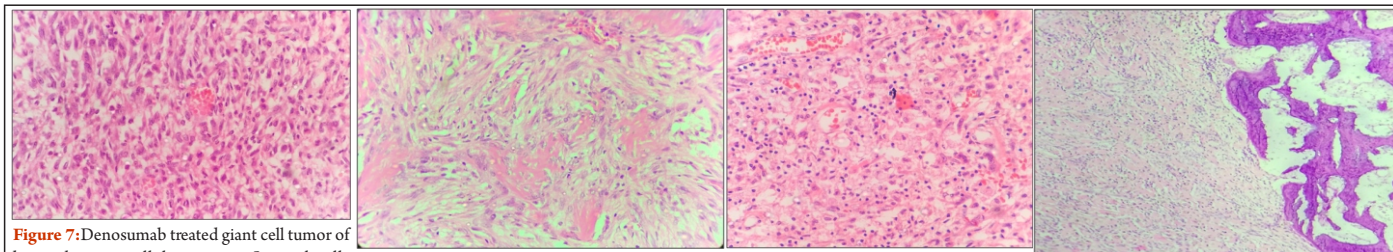
**Figure 4:** Pre-treatment Scanogram showing Expansile multiseptate lytic lesion in the distal end of femur extending to the sub-articular region.



**Figure 5:** Post-treatment radiograph showing a well demarcated lesion with thickened margins and suspicious new bone formation in the area of lysis.



**Figure 6:** Pre-treatment biopsy specimen showing uniformly distributed multinucleate giant cells against a background of mononuclear stromal cells. (H&E, 40x)



**Figure 7:** Denosumab treated giant cell tumor of bone showing cellular stroma. Stromal cells exhibit moderate hyperchromasia. Prominent nucleoli are seen in some cells. There is marked reduction in osteoclastic giant cells. (H&E, 40x).

**Figure 8:** Denosumab treated giant cell tumor showing osteoid matrix production and tumor of bone showing focal clusters of accompanying mononuclear stromal cells. (H&E, 40x).

**Figure 9:** Post denosumab treated giant cell tumor of bone showing focal clusters of accompanying mononuclear stromal cells. (H&E, 40x).

**Figure 10:** Metastatic deposits of Giant cell tumor in lung with Denosumab therapy related changes. (H&E, 20x).

cell tumor of bone. It is overexpressed by mononuclear neoplastic stromal cells which also produce several factors like SDF-1 (Stromal cell derived factor -1), MCP-1 (monocyte chemoattractant protein-1), M-CSF (Macrophage – colony stimulating factor). These factors help in recruitment of monocytes to the tumor site. Fusion of these monocytes leads to formation of multinucleate osteoclastic giant cells that express RANK. This is followed by RANK – RANKL coupling that activates osteoclastic giant cells. These osteoclastic giant cells further produce various factors like cathepsin K, TRAP (Tartarate resistant acid phosphatase) and V-ATPase[4]. These factors bring about lysis of bone matrix which leads to significant skeletal morbidity in patients with giant cell tumor of bone. Denosumab which is a newer drug being used in the treatment of giant cell tumor of bone is a human monoclonal antibody against RANKL. It binds to RANKL expressed on the surface of mononuclear neoplastic stromal cells. RANK – RANKL coupling is inhibited which in turn suppresses the osteoclast activity and survival. As a result, there is marked reduction in osteoclastic giant cells

following Denosumab therapy. Hence tumor associated bone lysis is prevented. Apart from RANKL, mononuclear stromal cells also express many characteristics of mesenchymal stem cells that have differentiated along the osteoblast lineage but not to a completely differentiated osteoblast phenotype. They minimally express osteocalcin and alkaline phosphatase which are supposed to be the markers of osteoblastic differentiation. It has been observed that these stromal cells are capable of differentiating into a mature osteoblast phenotype when separated from the osteoclastic component[6]. As a result, abundant new bone formation and osteoid matrix deposition is observed following denosumab therapy. These post-treatment changes in the form of new bone formation, osteoid matrix deposition accompanied by cellular stromal proliferation closely mimic bone forming tumor – osteosarcoma. If appropriate treatment history is not available, it can easily lead to a misdiagnosis of osteosarcoma. Wojcik J et al have also reported that denosumab treated giant cell tumor of bone exhibits morphologic overlap with malignant giant cell tumor of bone[7].

Rekhi B et al have reported a series of 27 cases of denosumab treated giant cell tumor of bone that appear as low grade osteosarcomas on histopathologic examination, but lack the clinical behaviour of an osteosarcoma[8]. A similar observation was made by Chang-Che Wu and Pin-Pen Hsieh who have reported a case of denosumab treated giant cell tumor of bone mimicking low grade central osteosarcoma[9].

## Conclusions

Denosumab treatment causes significant giant cell depletion accompanied by abundant new bone formation and foci of irregular osteoid matrix deposition closely mixed with cellular stromal proliferation. These features bear very little resemblance to their pre-treatment counterparts. In addition, these changes may mimic morphological features of osteosarcoma. Hence, it is important for us to be aware of these changes so that a misdiagnosis of osteosarcoma can be avoided.

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## How to Cite this Article

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