Research Article

Histomorphological Changes in Campanacci Grade III Giant Cell Tumors After Use of Denosumab in a Neoadjuvant Setting

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Abstract

Objectives: To determine the histomorphological changes induced in Campanacci grade III giant cell tumors (GCTs) of bones by a short course of denosumab in a neoadjuvant setting.

Methods: This is a retrospective study of all adult patients with biopsy-proven GCTs of bones, Campanacci grade III, treated by denosumab at a single tertiary care hospital in Pakistan from January 2014 till December 2020. Data items were extracted from the case records of patients and histopathological reports. Optimal histological response was defined as ≥50% decrease in giant cells with fibrosis and ossification. Data analysis was done by SPSS version 22.0.

Results: The mean age of 28 patients was 31.8±12.4 years with 15 (53.6%) males and 13 (46.4%) females. Tibia, femur, radius, pelvic girdle, humerus and ulna were involved in 9 (32.1%), 8 (28.6%], 6 (21.4%), 2 (7.1%), 2 (7.1%) and 1 (3.5%) patients, respectively. The optimal histological response was obtained in 25 (89.3%) cases. Denosumab-induced changes were noted in all except one (3.6%) case. In 11 (39.3%) patients, complete necrosis was found. In rest, variable amount of viable tumor (range: 2 to 100%) and denosumab-induced changes were observed.

Conclusion: Denosumab in neoadjuvant setting induces significant histomorphological changes in high-grade GCTs.

Keywords: Curettage, denosumab, histopathological changes, giant cell tumors, surgical resection

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Giant cell tumors (GCTs) of bone are locally aggressive, low-grade tumors characterized by the presence of osteoclast-like giant cells (OLGCs) in an appropriate background, hence the name. GCTs are relatively rare tumors commonly occurring around the age of 35 years, with an incidence of 1.7 per million cases per year and a male to female ratio of 1:1.38. Distal femur is the most common site accounting for 35% of all cases of GCTs.[1] Overt malignant transformation is a rare finding occurring only in 2% of cases, in which the tumors most commonly metastasize to the lungs.[2] The lesions of GCT classically present with pathological fractures and bone pain occurring at a closed growth plate and appear as well-defined lesions with non-sclerotic margins on radiographical imaging.[3] Surgical excision of the lesions remains the treatment of choice for GCTs with intralesional curettage used for Campanacci grade I and grade II tumors. Resection and reconstruction approach is used for more aggressive grade III tumors involving cortical destruction.[4]

The RANK pathway that causes differentiation and activation of osteoclasts is the key signaling pathway of bone remodeling and is involved in the pathogenesis of GCTs. Dysregulation of RANK ligand (RANKL)-RANK-osteoprotegerin (OPG) signaling cascade leads to increased osteoclast-mediated bone destruction and progression of existing tumor.

[5] The use of a RANKL inhibitor, denosumab, is an approved therapy for blocking osteoclast maturation and reducing bone resorption.[6]

Recently, denosumab has been reported to completely block RANKL activity and therefore it has been postulated that administration of pre-operative denosumab may induce significant histopathological changes and make the surgical excision of GCTs quicker, easier and safer.[7, 8] However, these studies included GCTs of mixed Campanacci grades on which denosumab was used.[9-12] We have earlier reported on clinical outcomes of 70 patients with GCTs, out of which 29 received denosumab therapy.[8] Furthermore, there is a paucity of such studies, which specifically highlight the role of pre-operative denosumab in producing histopathological changes in Grade III GCTs before resection. Therefore, this study aimed to assess the spectrum and extent of histomorphological changes induced by a short course of denosumab in a neo-adjuvant setting in Campanacci grade III GCT patients planned duly for resection.

Methods

Study Design

The study was designed in a retrospective fashion by obtaining data regarding GCTs of bones treated between January 2014 to December 2020. The data was collected from patient record files of Orthopedic Surgery Department, Dr Ruth KM Pfau Civil Hospital, Karachi, Pakistan. Patients were contacted by the authors individually for written informed consent before registration. The study was conducted in accordance with the principles of the World Medical Association updated Declaration of Helsinki. The patients' data retrieved included their age, gender, bone involved, histopathology findings before and after denosumab cycles, and follow-up data.

Inclusion and Exclusion Criteria

We included patients who had a diagnosis of Campanacci grade III GCTs proven by histopathology and graded by Campanacci grading system.[13] These patients were given 3-4 doses of once weekly subcutaneous injection of denosumab, 120 mg, before definitive surgical procedure of resection. After resection, tumor specimens were obtained and sent for histopathology. Patients without age or other relevant data were excluded. We also excluded those patients who had grade I or II GCTs, primary and secondary malignant GCTs before the start of denosumab, history of prior use of denosumab or bisphosphonates, non-compliant with denosumab therapy, lost to follow-up, non-availability of biopsy before start of denosumab, previous surgery for GCTs, or lack of consent by the patients.

Study Population

We found 70 patients with GCTs of bones from hospital records. Out of these, 28 candidates fulfilled the inclusion and exclusion criteria. The demographic, clinical, radiological and follow-up details were documented from the clinical charts of each patient individually. The patients were also followed-up for reappearance of signs and symptoms of GCT clinically and radiographically. The histopathology report was reviewed on first follow-up. Viable tumor and denosumab-induced changes were reported as percentages by taking proportion of values before (Figs. 1 and 2) and after denosumab therapy from respective histopathology reports.
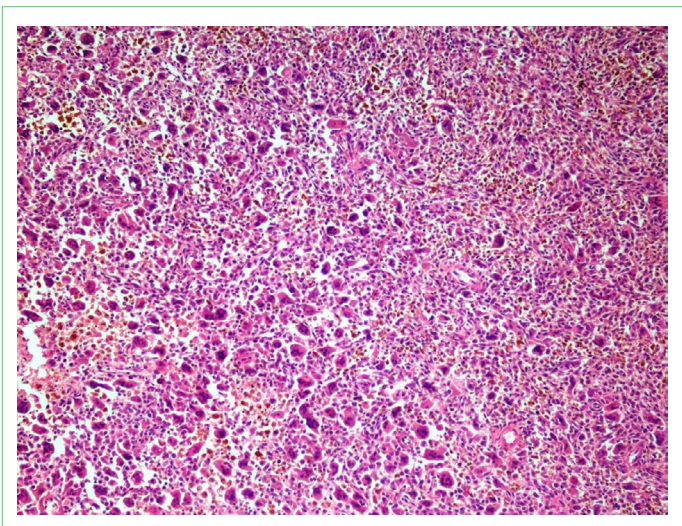

Figure 1. Histology of giant cell tumor of bone before denosumab treatment. Low-power view shows even sprinkling of osteoclast-like giant cells in the background of mononuclear stromal cells. (H&E, ×100).
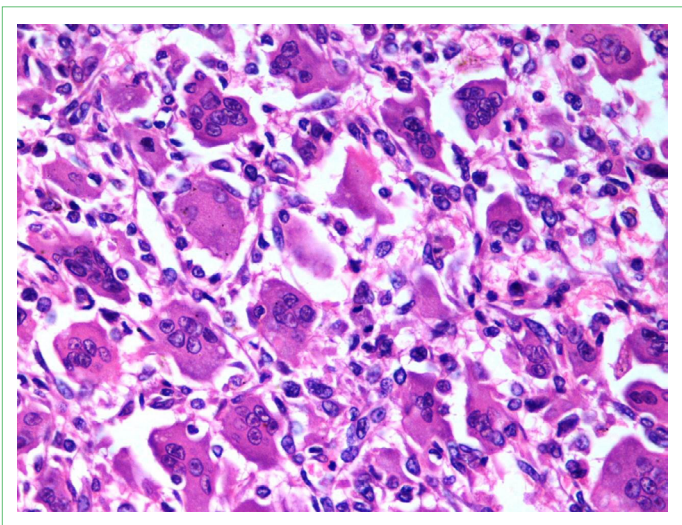

Figure 2. Histology of giant cell tumor of bone before denosumab treatment. High-power view shows even sprinkling of osteoclast-like giant cells in the background of mononuclear stromal cells. Note the similarity of nuclear features of stromal cells and giant cells (H&E, ×400).

Outcome Analysis

This study focused on the histopathological changes produced after administration of denosumab in GCTs of bones. The primary end-point was the frequency of optimal histological response, defined as ≥50% reduction in giant cells associated with fibrosis and ossification. In addition, proportions of all major histological changes such as viable tumor, necrosis, foamy macrophages and malignant change after denosumab were noted and described. Percentages were computed as proportion of value of outcomes in the total pathological field.

Statistical Analysis

Data were analyzed using SPSS software (version 22.0; IBM, Armonk, NY, USA). The descriptive statistics are presented as means±standard deviation (SD) with minimum and maximum for continuous variables. Categorical variables are presented as frequencies and percentages. No statistical tests were applied.

Results

A total of 28 patients fulfilled the inclusion criteria and were included in the study. The mean age of all patients was 31.8±12.4 years (range: 7-60 years) with 15 (53.6%) patients being males and 13 (46.4%) females. According to the site of bone involvement, tibia, femur, radius, pelvic girdle, humerus and ulna were involved in 9 (32.1%), 8 (28.6%), 6 (21.4%), 2 (7.1%), 2 (7.1%) and 1 (3.5%) patients, respectively. The main demographic, clinical and histopathological characteristics of all participants are shown in Table 1. The optimal histological response to treatment with denosumab was obtained in 25 (89.3%) cases (Fig. 3). Only three cases (10.7%) showed <50% reduction in giant cells. Denosumab-induced changes were noted in all except one (3.6%) case. The extent of these changes varied from 5% to 100% of the entire tumor. In 11 patients (39.3%), complete tumor necrosis was found. In rest, variable amount of viable tumor (ranging from 2-100%) was observed. It was less than 25% in 14 (56%) cases. Specifically, two (8%) patients with GCTs of pelvis also reported significant tumor necrosis with viable tumor mass of 0% and 15%. Complete disappearance of giant cells was seen in 16 (57.1%) cases (Fig. 4), rest showed variable degree of reduction in giant cells with 8 (28.6%) patients showing <25% giant cells. No necrosis was found in 14 (50%) cases, whereas variable amount of necrosis was found in remaining 14 (50%) cases with 11 (39.28%) cases showing <25% necrosis. Variable amount of fibrosis ranging from 5 to 70% was noted in all but 3 (10.7%) cases (Fig. 5). No foamy macrophages were detected in 7 (25%) cases, rest of the cases showed variable numbers of macrophages in the remaining tumor. Similarly, no ossifica-

tion was found in 2 (7.1%) cases, rest of the cases showed variable degree of ossification ranging from 5 to 50% (Fig. 5). Only one (3.6%) patient developed malignant change in previously benign GCT four weeks after the completion of denosumab therapy. The patient did not show tumor reduction as well.

Discussion

The main treatment for GCT of bones is surgery. However, for relatively high grade tumors, the therapy is still controversial.[14-16] As described by Campanacci et al., grade III GCTs do not have well-defined tumor margins and may show soft tissue extensions.[13] Hence, grade III GCTs may extend over large areas whereby they require large resections and more difficult reconstruction with poorer functional outcomes. Moreover, increased friability of grade III GCTs due to absence of osteosclerotic rim makes the tumor resection and handling very difficult. In 2013, FDA licensed the use of denosumab therapy for surgically unresectable GCTs, while a number of studies have also been published on the utility of drug for downstaging in resectable GCTs. However, there is still paucity of studies on histopathological changes produced by denosumab specifically in grade III GCTs that may benefit the surgeons by providing them with well-defined osteosclerotic rim for easier resection and handling. This is one of the largest studies on the semi-quantitative assessment of morphological changes induced by denosumab used in a neoadjuvant setting.

From these results, it is obvious that significant amount of viable tumor remained after denosumab therapy. Previously some studies utilized denosumab as sole treatment approach rather than as adjuvant.[17-19] This study affirms the notion that remaining tumor after denosumab therapy is significant enough to cause future recurrence. Hence, many studies where denosumab has been coupled with less morbid procedures such as intralesional curettage after downstaging showed high recurrence rates.[20-25] We would hypothesize that patients with grade III GCTs should undergo resection surgery after denosumab therapy which would be less morbid due to lesser resections. However, large sample-sized comparative studies with only grade III GCTs are necessary to prove this hypothesis. Denosumab has been reported as a potential cause of malignant transformation in previously benign GCTs according to published reports.[26-29] The present study showed malignant progression in one candidate (3.6%) only after 4-weeks of therapy. In addition, there was a limitation of time factor where we performed resections after 4-weeks of therapy whereas there might be a possibility of more frequent sarcomatous changes in grade III GCTs on longer treatment regimens and prolonged follow-ups.

Table 1. The main demographic, clinical and histopathological characteristics of participants receiving denosumab therapy (n=28)

| S. No	Age (yr)	Gender	Location	Viable tumor	Denosumab-induced changes	Fibrosis	Macrophages	Necrosis	Ossification	Percent reduction in giant cells
1	36	F	Distal femur	70%	30%	5%	5%	5%	15%	0
2	40	M	Proximal tibia	60%	40%	20%	5%	10%	5%	0
3	7	F	Distal femur	5%	95%	50%	25%	10%	10%	<25%
4	32	M	Distal radius	20%	80%	20%	10%	10%	40%	<25%
5	21	M	Proximal femur	0%	100%	35%	20%	10%	35%	0
6	15	F	Distal femur	0%	100%	50%	0	0	50%	0
7	28	M	Distal radius	40%	60%	0	10%	0	50%	40%
8	23	F	Distal radius	0%	100%	25%	25%	0	50%	0
9	25	M	Distal ulna	90%	10%	5%	0	0	5%	>50%
10	30	F	Proximal tibia	0%	100%	70%	10%	10%	10%	0
11	38	M	Proximal tibia	0%	100%	70%	10%	10%	10%	0
12	18	F	Proximal tibia	100%	0%	0	0	0%	0%	<25%
13	60	M	Proximal humerus	0%	100%	45%	20%	0	45%	0
14	40	M	Distal femur	0%	100%	20%	20%	10%	50%	0
15	36	F	Distal femur	30%	70%	25%	10%	10%	25%	<25%
16	50	F	Distal radius	60%	40%	10%	10%	0	20%	0
17	21	F	Distal radius	10%	90%	25%	15%	0	50%	<25%
18	23	M	Proximal femur	80%	20%	10%	5%	0	5%	>50%
19	32	M	Proximal tibia	75%	25%	10%	10%	0	5%	<25%
20	24	M	Proximal tibia	0%	100%	50%	20%	20%	10%	0
21	53	F	Proximal tibia	80%	20%	10%	5%	0	5%	0
22	50	F	Distal tibia	5%	95%	20%	20%	5%	50%	0
23	25	F	Distal femur	95%	5%	0	0	5%	0	>50%
24	24	M	Proximal humerus	0%	100%	40%	20%	0%	40%	0
25	40	M	Proximal tibia	0%	100%	50%	0	0%	50%	0
26	45	F	Iliac bone	0%	100%	35%	35%	20%	10%	0
27	30	M	Pelvic bone	15%	85%	45%	0%	20%	20%	<25%
28	24	M	Distal radius	2%	98%	44	0	0	44	<25%
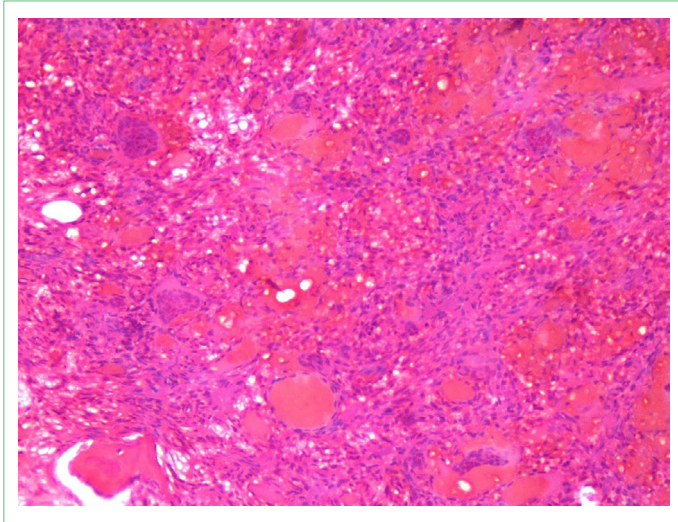
Figure 3. Low-power photomicrograph of a giant cell tumor histology after denosumab treatment. There is >50% reduction in the number of osteoclast-like giant cells in the background of hemorrhage. (H&E, ×100).
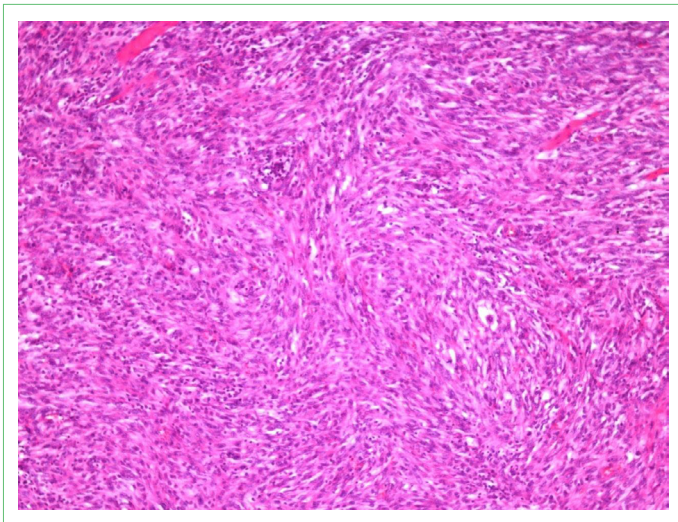

Figure 5. Low-power photomicrograph of morphology of another giant cell tumor after denosumab treatment. There is complete disappearance of osteoclast-like giant cells and the tumor is replaced by fibrosis and ossification (H&E, ×100).

Figure 4. Low-power photomicrograph of morphology of another giant cell tumor after denosumab treatment. There is complete disappearance of osteoclast-like giant cells (H&E, ×100).

From this study, it emerged that vast majority of grade III GCTs showed a favorable histological response, particularly a reduction in the number of giant cells, to even a short course of denosumab therapy. As is well established, the number of giant cells is directly proportional to bone resorption.[30, 31] Hence, a decrease in the number of giant cells may render the tumor to lose less bone, forming a rim around the margins. RANKL inhibition plays a pivotal role in reducing these giant cells. A higher number of macrophages after denosumab therapy shows that denosumab fails to block stimulating factor for monocytes that has been established in literature.[6, 32, 33-37] The presence of stro-
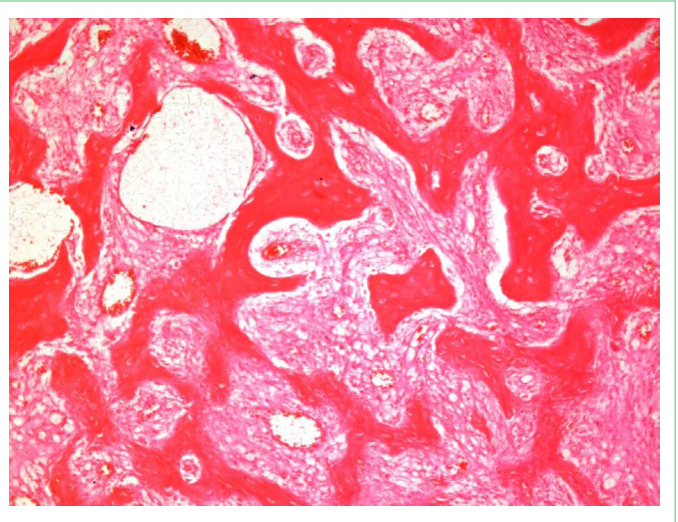
mal cells in the viable tumor indicates a possible mechanism for tumor recurrence after cessation of denosumab where the stromal cells may release RANKL which can lead to maturation of the macrophages into giant cells leading to reactivation of the disease. Hence, after downstaging with denosumab, grade III GCTs need to be resected.

During the outcome analysis, we focused primarily on four parameters; reduction in giant cells, necrosis, fibrosis, and ossification. Reduction in the number of giant cells is the cardinal morphological feature of denosumab treatment. Different studies have defined different cut-off points for defining optimal response to the drug. We used the definition proposed by Treffel et al.[37] Necrosis has been an important marker for predicting poor prognosis.[34] Tumors with greater proportion of necrosis are considered highly aggressive as this area reflects rapid mitotic activity which outgrows the vascular supply forming necrotic patches.[35, 36] Our results showed that complete necrosis was found in a small but significant number of cases. Denosumab is not directly cytotoxic and hence may not be directly responsible for this necrosis. However, the drug played a vital role in increasing fibrosis and osteoid matrix making the tumor area less friable and easier to resect with well-defined boundaries.[37] The reduction in giant cells leads to lesser bone resorption which enhances the matrix deposition in tumor areas.

Conclusion

In conclusion, denosumab produces significant changes after 4-weeks of once weekly cycle by increasing the solid components of tumor and reducing the tumor giant cells. However, maximum benefit from the drug might only be

obtained with resection in grade III GCTs as the drug fails to regress the tumor completely in majority of patients. The viability of tumor may subsequently lead to recurrence of the tumor in the long-term.

Disclosures

Ethics Committee Approval: Ethical clearance was granted by the ethical committee of Civil Hospital, Karachi vide approval number: IRB-87/DOS/CHK/2023, dated: 17 January 2023.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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