Journal of Clinical Orthopaedics and Trauma 10 (2019) 1021–1026

Contents lists available at ScienceDirect

Journal of Clinical Orthopaedics and Trauma

journal homepage: www.elsevier.com/locate/jcot

Role of intravenous zoledronic acid in management of giant cell tumor of bone- A prospective, randomized, clinical, radiological and electron microscopic analysis

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ARTICLE INFO

Article history:
Received 12 May 2019
Received in revised form
10 September 2019
Accepted 12 September 2019
Available online 28 September 2019

Keywords:
Zoledronic acid
Bisphosphonates
Giant cell tumor
Apoptosis
Apoptotic index
Electron microscope

ABSTRACT

Background: The primary treatment of Giant cell tumor of bone is surgical management. Bisphosphonates are antiresorptive drugs which inhibit osteoclast mediated bone resorption and shown to have inhibitory effect on various tumors. The present study aims to establish clinical, ultrastructural and radiological response of intravenous zoledronic acid on giant cell tumor of bone.

Methodology: Design - Prospective randomized controlled study. A group of 30 patients of GCT bone were randomized into two equal groups. Patients in control group did not receive any adjuvant therapy before surgery. Patients in bisphosphonate group received three doses of intravenous zoledronic acid at four weeks interval prior to definitive surgery. The evaluation was done based on size of swelling, VAS score, plain radiograph, MRI and histopathological and Transmission electron microscopic examination findings.

Results: Significant reduction in VAS score (from mean 5.33 to 1.8), increased mineralization particularly at periphery of lesion in plain radiograph, statistically significant increase in mean apoptotic index, P value < 0.0001 (mean 41.46 in bisphosphonate group and 6.06 in control group) was noted in bisphosphonate group. No significant change in tumor volume is noted in MRI. No significant side effects were noted.

Discussion: One distinctive feature of pathogenesis of GCT bone is osteoclastogenesis which causes extensive bone destruction. Use of intravenous Zoledronic acid counteracts this bone destruction. Further, possible antiangiogenic effect of intravenous bisphosphonates inhibits tumor growth and provides symptomatic improvement.

Conclusion: IV Zoledronic acid alleviates pain, produce sclerosis and induce apoptosis hence decrease the rate of tumor progression and decrease the rate of local bone destruction, hence they are useful adjuvant to surgery in GCT.

1. Introduction

Giant cell tumor (GCT) of bone is a primary bone tumor with a prevalence of about 6% of all primary bone tumors.[1] A distinctive feature in the pathogenesis of this tumor is proliferation of osteoclasts. Stromal cells over express Ligand for receptor activator of nuclear factor kappa beta (RANKL). RANK is expressed by mononuclear cells, when activated by RANKL results in their proliferation and differentiation into osteoclast like giant cells which are responsible for osteolysis.[2–5] Treatment for GCT bone is primarily surgical, either curettage with or without use of adjuvants or wide excision with reconstruction. Wide excision usually requires sacrificing the adjacent joint as these lesions are commonly juxtaarticular. Functional outcome following subsequent reconstruction is suboptimal.[6–8] Although curettage retains joint mobility and function, when performed alone it results in very high recurrence rates varying from 10% to 50% in different series.[9,10] Adjuvants such as chemical (phenol, alcohol, H_2O_2), thermal (bone cement, liquid

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https://doi.org/10.1016/j.jcot.2019.09.011
0976-5662/

nitrogen) and mechanical (high speed burr) are useful to extend surgical margin and when combined with curettage, results in lower recurrence rates.[11,12] Of late, certain drugs used in the treatment of osteoporosis, such as bisphosphonates, are being investigated for the treatment of Giant cell tumor of bones.[13,14]

Bisphosphonates are analogues of inorganic pyrophosphate in which the oxygen atom of the P–O–P bond is replaced with a non-hydrolysable P–C–P bond.[15] Bisphosphonates inhibit osteoclast activity by several mechanisms which depend largely on their chemical structure. Bisphosphonates have been shown to induce apoptosis of tumor cells and inhibit tumor cell growth of a variety of tumor cell types which include breast, prostate, osteosarcoma and myeloma.[16–20] These drugs may potentially have anti angiogenic effects as well.[21] Current study aims to analyze the clinical and radiological effects of bisphosphonates in patients with Giant cell tumors of bone and to correlate them with ultrastructural changes and apoptosis of tumor cells using Transmission Electron Microscopy (TEM).

2. Material and methods

The study was conducted after obtaining approval from Ethics Committee of the institute between April 2014 and December 2014. Sample size of 30 patients was set after consulting with statistician. Inclusion criteria was patients with histopathologically proven GCT of bones of extremities. Exclusion criteria were patients with metastatic GCT, GCT of axial skeleton, patients with altered renal functions, patients with known allergy to bisphosphonates and patients with age less than 12 years. Patients presenting to the orthopaedics outpatient department with complaints of pain and/or swelling and radiological features suggestive of GCT were evaluated. After obtaining Magnetic Resonance Imaging (MRI), biopsy was done to get a histopathological diagnosis. If the patient had biopsy done outside our institute, slides were reviewed by the pathologist to confirm the diagnosis. Patients in whom the diagnosis was GCT were considered for enrolment in the study. Patients explained about the nature of the study. Only those patients willing to participate in the study were included after obtaining written consent. Serum investigations including renal function tests, serum calcium, phosphate and alkaline phosphatase estimation were performed. Bone scan and Non Contrast Computed Tomograph (NCCT) chest was done to rule out metastatic disease. Patients who fulfilled the inclusion criteria were randomized in to two groups with 15 patients in each group using computer generated random number table. Group A was bisphosphonate group while Group B was control group.

Patients were evaluated on the following parameters

1. Demographic data
2. Clinical evaluation
3. Radiological evaluation

In clinical evaluation, history was obtained and clinical examination was performed. Patients were assessed for intensity of pain and size of the swelling if the swelling was apparent. Intensity of pain was measured using Visual Analogue Scale (VAS). Size of the swelling was recorded (in cm) by measuring its maximum length and breadth. Radiological evaluation was done on plain radiograph of the site with antero-posterior and lateral views and MRI of the site. On the basis of plain radiograph, patients were categorized according to their campanacci grade.

Patients in group A i.e. bisphosphonate group were given 5 mg Intra Venous (IV) zoledronic acid. 3 doses were given with a gap of 4 weeks between each dose. Renal functions and serum calcium were obtained before each dose. Each injection was given under observation, as slow IV infusion over 15 min, after pre hydrating with 500 ml IV normal saline. In each follow up, inquiry was done regarding any symptoms pertaining to the side effects which were explained at the time of informed consent such as fatigue, nausea, vomiting, jaw pain, fever and flu like symptoms or any other side effect if experienced by the patient.

After completion of 3 doses, final clinical data was collected to see for any change in VAS score and size of the swelling. Plain radiograph was repeated to see for any increase in mineralization, evident as increased sclerosis at the site of lesion. MRI of the site was repeated to assess any change in volume of the tumor or signal intensity. Radiological changes were assessed by radiologist. Tumor volume was calculated using the formula $\pi/6$ x maximum length x maximum breadth x maximum height of the tumor. Thirteen patients in group A underwent surgery. Twelve patients underwent extended curettage with bone grafting while in 1 patient wide excision followed by reconstruction using endoprosthesis was done. Two patients who were completely asymptomatic after the completion of therapy and plain radiograph demonstrated increase in mineralization, were given an option to undergo surgery or to postpone surgery and be under observation. Patients opted for postponement of the surgery. In these 2 patients, biopsy from the site was done under image intensifier to get tissue sample from the lesion for histopathology and electron microscopic evaluation. Patients in group B were not given any form of neo adjuvant therapy. All 15 patients underwent Surgery. Thirteen patients underwent curettage with bone grafting and in 2 patients wide excision followed by reconstruction using endoprosthesis was done. During surgery, 10% phenol, H_2O_2 and high-speed burr was used as adjuvant after curettage in all cases in both the groups. Surgical samples from both the groups were collected for histopathological and electron microscopic analysis. Histopathologic analysis was done by pathologist while ultrastructural changes were assessed by a designated faculty from electron microscope facility of the institute. All the samples from both the groups were assessed by same person to avoid interobserver bias. Radiologist was non blinded while pathologist and electron microscopy interpreters were blinded.

3. Result

A total of 32 Patients were enrolled in the study out of which 2 patients were excluded because of deranged renal functions. Out of 30 patients included in the trial 15 patients were randomized in to the bisphosphonate group while 15 patients were randomized in to the control group. All the 30 patients were available for the analysis of the outcomes. The age of the patients in the study ranged from 16 years to 58 years (Table 1). Distal femur and proximal tibia were the most common sites with 10 (33.33%) patients each, followed by distal radius with 5 patients (16.66%). There was no significant difference between the two groups in terms of demographic variables.

Patients were grouped according to their Campanacci grading on plain radiograph. Most of the patients were having grade 2 lesions (Table 1). Mean VAS score before initiation of bisphosphonate therapy was 5.33 ± 1.77. Mean VAS score after receiving 3 zoledronic acid injections was 1.8 ± 0.6. The difference between the two sets of VAS score was statistically significant (Table 3). Mean tumor volume before and after therapy in the bisphosphonate group was 89.42+-34.84 and 89.70+-38.11 respectively. Difference was statistically insignificant, indicating that bisphosphonates are successful in controlling the growth of the lesion (Table 3). Ultrastructural changes which were studied using transmission electron microscopy included both nuclear and cytoplasmic. Nuclear changes included pyknosis, clumping of chromosomes, nuclear fragmentation and formation of blebs in nuclear membrane

Table 1
Table showing demographic characteristics of both the groups and distribution according to camapnacci grading on plain radiograph.

	Bisphosphonate group (n = 15)	Control group (n = 15)	p- value
Mean Age	32.86	31.46	0.486 (using Mann-Whitney *U* test)
Gender			0.5 (using Mann-Whitney *U* test).
M	9 (60%)	8 (53.33%)	
F	6 (40%)	7 (46.66%)	
Campanacci grading			
Gr 1	3 (20%)	3 (20%)	
Gr 2	8 (53.33%)	7 (46.66%)	
Gr 3	4 (26.67%)	5 (33.33%)	

Table 2
Table showing distribution of ultrastructural changes and mean apoptotic index in both bisphosphonate and control group.

Ultrastructural Changes	Bisphosphonate group (n = 15)	Control group (n = 15)	p-value
Nuclear changes			
Pyknosis	10	6	
Clumping of chromatin	15	15	
Nuclear fragmentation	15	15	
Blebs in nuclear membrane	12	10	
Cytoplasmic changes			
Dense mitochondria	15	15	
Disorganized Endoplasmic reticulum	15	15	
Cell junction break	10	6	
Mean apoptotic index	41.46	6.06	0.001 (using Mann-Whitney *U* Test)

Table 3
Table showing changes in VAS score, size of swelling and mean tumor volume as measured on MRI before and after completion of bisphosphonate therapy.

(n = 15)	Pre bisphosphonate therapy	Post bisphosphonate therapy	p- value
Mean VAS score	5.33+-1.77	1.8+-0.6	0.001 (using Wilcoxon Signed Ranks test)
Mean size of swelling (cm)	10.56+-12.49	10.73+-12.8	0.609 (using Wilcoxon Signed Ranks test)
Mean tumor volume (cm^3)	89.42+-34.84	89.70+-38.11	0.706 (using Wilcoxon Signed Ranks test)

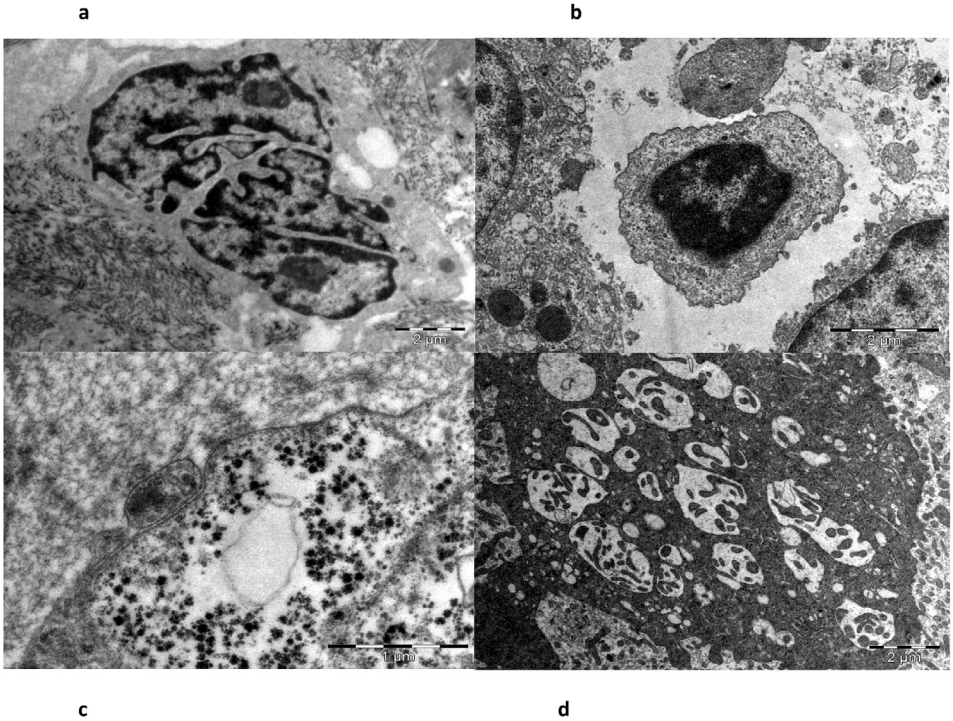

Fig. 1. a - Electron microscopy picture showing nuclear fragmentation. **b** - Electron microscopic image showing nuclear condensation. **c** - Electron microscopic image showing electron dense cytoplasmic deposits, probably due to cytoskeletal disruption. **d** - Electron microscopic image showing cytoplasmic vacuoles with partially digested material, probably phagolysosomes.

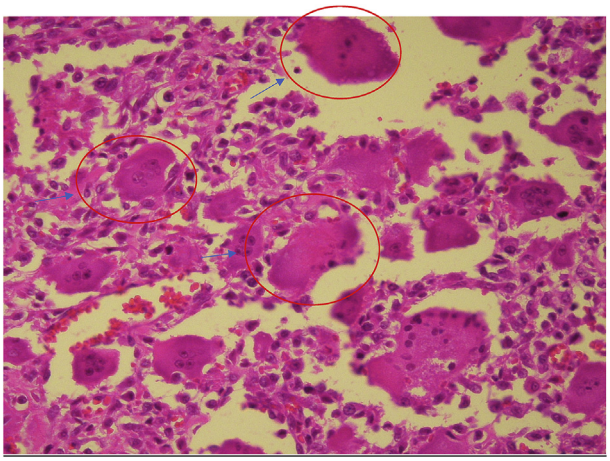

Fig. 2. Light microscopy image showing ground glass appearance of giant cells following bisphosphonate therapy.

(Fig. 1a and b). Cytoplasmic features include mitochondrial edema, disorganized endoplasmic retinaculum, and cell junction break (Fig. 1c and d). The changes were evident in both the group so Apoptotic index was calculated to compare the apoptotic changes. It was calculated using the formula,

Apoptotic index = (Number of cells demonstrating features of apoptosis/ Total number of cells) × 100

Mean apoptotic index as calculated, in bisphosphonate group and control group was 41.46 ± 12.74 and 6.06 ± 2.60. The apoptotic index in the bisphosphonate group was significantly higher than the apoptotic index in the control group (Table 2). Light microscopic examination was done after H and E staining, to see for any post bisphosphonate therapy degenerative changes like nuclear shrinkage, nuclear hyperchromacity, chromatin homogenization, cytoplasmic eosinophilia and reduction in number of giant cells (Fig. 2). We could appreciate nuclear hyperchromacity in 6 out of 15 patients, cytoplasmic eosinophilia in 8 out of 15 patients and reduction in number of giant cells in 3 out of 15 patients.

4. Discussion

Giant cell tumors have remained an enigma to musculoskeletal oncologists with their epiphyseal location, benign locally aggressive nature with ability to metastasize and the challenges for surgical reconstruction. Plenty of efforts have been made to reduce recurrence following curettage by mechanical, chemical and thermal means. The latest addition to the armamentarium are pharmacological agents such as Bisphosphonates and Denosumab. Our present study compares the apoptotic index in the Giant Cell Tumor following Bisphosphonate therapy against a control group of patients. To our knowledge, this is the first study in the literature to assess the in-vivo effects of Zoledronic acid on GCTs by electron microscopy. Our results demonstrate that Zoledronic acid administration leads to significant apoptosis of tumor cells in GCTs. The increased apoptotic index in the Bisphosphonate group was independent of grade of the tumor.

Few authors had studied the microscopic effects of Bisphosphonates. In an in vitro analysis, Chang et al. showed that that there was a dose-dependent increase in apoptosis in GCT cell treated with zoledronate and pamidronate.[22] They also hypothesized that by inducing apoptosis; bisphosphonates can clear the remaining microscopic tumor tissue after surgery and thus can reduce recurrence following Curettage. Cheng et al. studied apoptosis in stromal cells of GCT bone treated with bisphosphonates both in vitro and in vivo.[23] They found that mean apoptotic index increased from 7.6% to approximately 54% in the stromal tumor cells and 29.7% to approximately 73.9% in the giant cells following treatment with pamidronate. It was also found that in cell cultures treated with bisphosphonates that zoledronic acid was most potent in inducing cell death. Cheng et al. found similar ultrastructural changes in multinucleated giant cells following treatment with pamidronate and also correlated it increased apoptotic index in GCT specimens following bisphosphonate treatment.[24]

The current study also aimed to assess clinical, radiological and histopathological effects of bisphosphonates in patients of giant cell tumor of bone of extremities. We found that there was significant reduction in pain as perceived by the patient, and documented as VAS score in all the 15 patients (100%) in the bisphosphonate group. Similar results are shown by other authors. Balke et al. retrospectively assessed the effects of bisphosphonates given to patients with either inoperable GCT of spine and sacrum or recurrent, metastatic GCT.[13] They noted pain relief in majority of the patients receiving bisphosphonate which was not restricted to the type of bisphosphonate or the method of administration. However, they did not measure the severity of pain by any method. A case control study by Tse et al. showed similar results.[25] Significant symptomatic improvement was also reported in the study by Cornelis et al. who studied long term efficacy of bisphosphonates in patients with benign bone diseases including GCT bone.[14] Mechanism of pain relief following bisphosphonate therapy can be due to several factors. It may be due to inhibition of ongoing osteolysis or due to inherent analgesic properties of bisphosphonates as shown in some studies. Strang P showed analgesic effect of bisphosphonates in patients with bone pain due to skeletal metastasis in breast cancer.[26] He proposed that analgesic effect consists of an acute effect caused by reduction of pain-producing substances such as prostaglandins, lactic acid and proteolytic enzymes and a more sustained and long-lasting effect due to inhibition of osteoclastic activity. In another recent study on mice, Kim et al. demonstrated analgesic effect of bisphosphonates.[27] They concluded that the analgesic effect of bisphosphonates is more for non-nitrogen containing bisphosphonates, was unrelated to antiresorptive effect and was possibly via some interaction with neurons.

Another clinical variable studied was size of the tumor. Size of the swelling was relatively stable throughout the duration of study irrespective of the radiological grade. This holds implication in cases of GCT which are inoperable due to their vicinity to vital organs, or in cases where surgery needs to be postponed due to some underlying medical co morbidity. Tse et al. found that 20 out of 24 (83.3%) patients experienced subjective decrease in swelling although they did not report any objective measurements.

On radiological evaluation, MRI showed no significant change in tumor volume or signal intensity on T2 images of GCTs treated with Zoledronic acid. This observation is significant because it objectively proves the effect of Zoledronic acid in halting tumor progression. The result of our study was similar to the observations of Balke et al. in which they showed that following bisphosphonate therapy tumors did not regress or progress but remained stable in size. Another striking observation in our study was increased mineralization in the site of lesion on plain radiograph (Fig. 3a and b). All the patients had evidence of increased sclerosis in the site of lesion. Increased mineralization was not restricted to the Campanacci grading or site of the lesion. Increased mineralization was most prominent in the periphery of the lesion. Results were in concordance with the previous studies. Tse et al. showed that after 2 doses of pamidronate injection 90 mg by IV infusion, the radiographs of the patients showed increased radio density within the

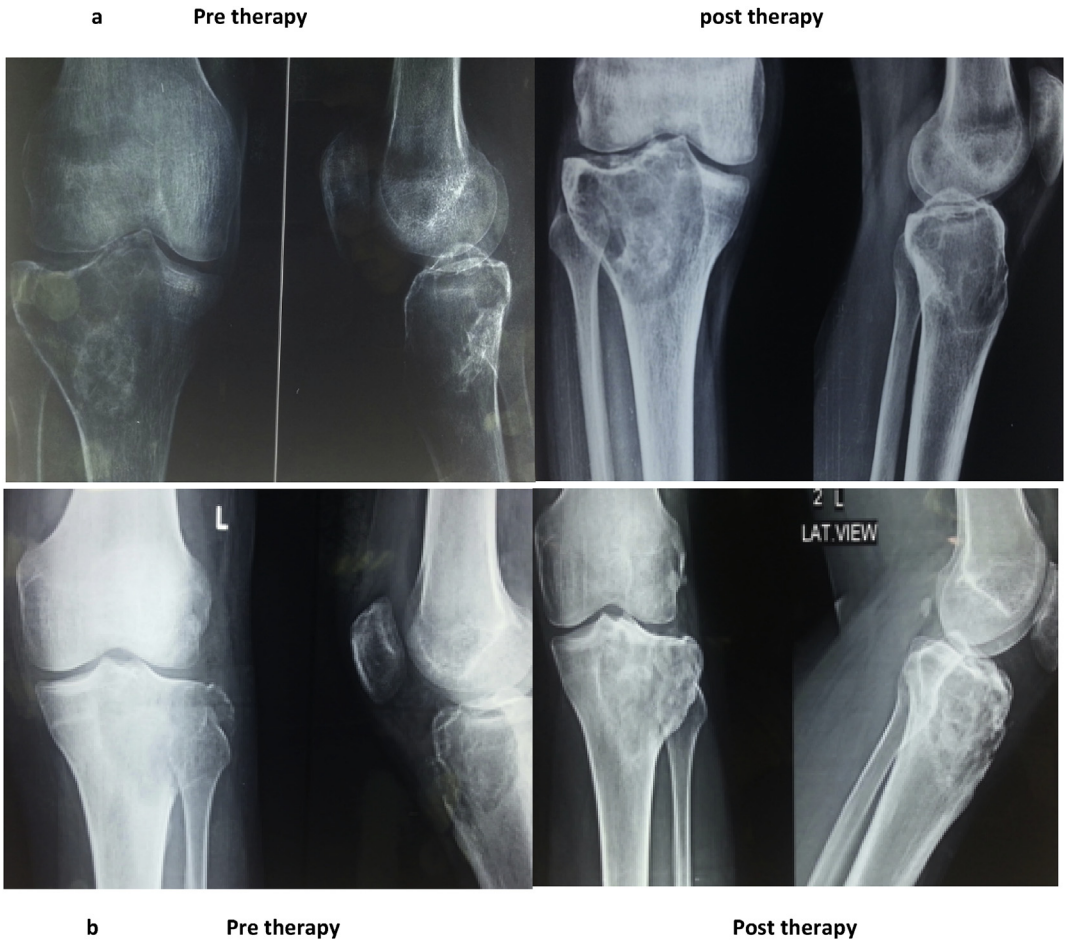

Fig. 3. **a** and **b** – Plain radiograph showing increased sclerosis in the lytic lesion post bisphosphonate therapy.

lesion particularly in its perimeter. Gille et al. in his case report on GCT cervical spine reported marked calcification on the site of lytic lesion following administration of zoledronic acid.[28] Fujimoto et al. described a patient with GCT sacrum which was considered inoperable and hence was started on alendronate infusion.[29] Follow up CT scans revealed calcification at the lytic site and significant reduction in tumor volume. Increased mineralization on CT imaging of the lesion was also shown by Cornelis et al. as described earlier. Increased mineralization, particularly in the periphery can make a tumor well contained and more amenable to complete removal by curettage, especially those with cortical breach and soft tissue extension. Complete removal will indirectly reduce the recurrence rate thus improving the functional outcome.

Our study has certain limitations. It can be argued that Electron microscopy is a cumbersome and more subjective means to measure apoptosis with inter observer variability as compared to modern flow cytometry-based methods. But it provides an excellent opportunity to study ultrastructural and morphological changes of apoptosis. The results of the present study correlates well with the earlier studies used to assess bisphosphonates induced apoptosis in GCT cells. As this is a pilot study which had prospectively recruited patients and randomly distributed them into two groups, our numbers are limited. However, our results demonstrate that Zoledronic acid had significant clinical, radiological and electron microscopic effects in this small group of patients. Larger studies with follow-up may help us to understand whether these effects lead to reduction in recurrence rates of GCTs following extended curettage.

To summarize, preoperative Zoledronic acid to diagnosed GCT patients will help in relieving pain, halting tumor progression, and inducing significant apoptosis of tumor cells. Routine use of Zoledronic acid may become the standard of care following further research.

Declaration of competing interest

There is no conflict of interest. Authors have nothing to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcot.2019.09.011.

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