Effect of Thermoablation with pH Change on Giant Cell Tumor of Bone: An In Vitro Study

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ABSTRACT

Objective: To evaluate the effect of pH on the apoptosis and necrosis rate of giant cell tumor of bone (GCTB) cells during thermoablation.

Materials and Methods: GCTB tissues were collected from 15 patients. Cells were incubated at 25 °C , 37 °C, 45 °C, and 50 °C, with the variation of the pH at 4.7, 5.6, 6.5, 7.4, 8.3, and 9.2 for 20 minutes (in triplicate for each condition). The effect of thermoablation and pH variation on GCTB cells death was evaluated by staining with Annexin V-FITC and propidium iodide solution after 3 days of incubation. The fluorescence intensity was evaluated by flow cytometry to evaluate the percentage of tumor cells death.

Results: Thermoablation alone increased the percentage of tumor cells death. However, when combined with an increase in pH, the percentage of GCTB cells death increased more. Conversely, lowering the pH did not increase the tumor cells death compared with thermoablation treatment alone, while changing the pH alone had only a low effect on increasing the percentage of GCTB cells death.

Conclusion: Thermoablation the temperature between 37 °C and 45 °C plus a pH level slightly higher than physiologic pH (between 7.4 and 8.3) for 20 minutes increased GCTB cell death. However, determining the optimum condition to kill tumor cells while causing minimal harm to normal cells requires more study.

Keywords: Thermoablation; pH; giant cell tumor of bone; in vitro (Siriraj Med J 2024; 76: 339-345)

INTRODUCTION

Giant cell tumor of bone (GCTB) is a locally aggressive primary intermediate bone tumor, accounting for 6% of all primary bone tumors.[1,2] Extended intralesional curettage, together with bone grafting/cementing, is a mainstay surgical treatment for this condition.[3] However, intralesional curettage alone results in an approximately 15%–25% recurrence rate,[4,5] and thus adjuvant treatment is often recommended.[6] Adjuvant treatment, such as with ethanol,[7] phenol,[8] botulinum toxin,[9] raloxifene,[10] argon beam,[11] warm Ringer's lactate solution,[12] or liquid nitrogen,[13] can reduce tumor recurrence. However, in some situations, such adjuvant treatment is not suitable since it could damage nearby important tissues, such as nerves or arteries.[14] The comparison of adjuvant treatments for GCTB has been reviewed in other publications.[15,16]

A new generation of adjuvants are now becoming available from the successful use of certain drugs in osteoporosis treatment, since the target of antiresorptive drugs (i.e., bisphosphonates[17] and denosumab[18]) in such treatments. Bisphosphonates can reduce the local recurrence of GCTB, especially for patients who have undergone intralesional curettage, but is not recommended for those who have undergone wide resection.[19] Using denosumab for the treatment of GCTB was found to be related with osteonecrosis of the jaw or atypical long

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bone stress reactions during treatment and a rebound in hypercalcemia[20,21] after discontinuing denosumab for the treatment of GCTB. Malignant transformation is also possible during denosumab treatment, probably due to immunosuppression from the inhibition of RANKL.[22] The cost of these new drugs is also problematic for many developing countries. Hence, adjunctive therapy may serve as a viable consideration for patients encountering financial constraints.

Our group reported an alternative adjuvant technique using thermoablation[23,24] for the treatment of GCTB. This adjuvant has the benefits of being cheap and it will not damage nearby structures. In thermoablation, the process is performed after the extended intralesional curettage step has been done, whereby a 45 °C lactate-ringer solution (LRS) is irrigated into the GCTB lesion using a sterile IV set to a free flow rate for 20 minutes, followed by bone grafting or cementing and then augmentation of the bone, and finally wound closure. Unfortunately, not all tumor cells may be eradicated by the thermoablation, and some tumor cells can survive, causing a recurrence in the patient. Therefore, to improve thermoablation adjuvant therapy, we realized that something else would need to be included in the solution to ensure the death of all the tumor cells.

Some previous studies have reported apoptosis induction by changing the cellular pH by several mechanisms.[25] Matsubara et al., for instance, found that additional stress, such as extracellular pH, influenced the invasiveness and survival of osteosarcoma.[26] Therefore, we hypothesized that alteration of the pH of the solution that carries the heat for thermoablation after extended intralesional curettage could boost the tumor cells death rate without affecting normal cells. Consequently, the objective of this study was to examine whether thermoablation with a changed pH of the solution used for thermoablation could increase the percentage of tumor cells death without harming the normal osteocytes and chondrocytes *in vitro*.

MATERIAL AND METHODS

Patients

The protocol was approved by the Institutional Review Board (COA no. Si 711/2014). Included patients were those with giant cell tumor of bone (GCTB) confirmed by a pathological report from a tissue biopsy, who provided written informed consent the day before surgery, and agreed their left-over tumor tissue could be collected for the study. On the operation day, after the surgeon had performed the curettage and collected enough tissue for the pathological study, the remaining tissue samples were collected using a sterile technique and sent directly to the laboratory for analysis for this study. Cell preparation was conducted individually for each patient, ensuring there was no cross-contamination between patients. The procedure for each specimen was carried out on separate days.

Primary culture

The giant cell tumor tissue samples were washed with sterile PBS and minced by a sterile blade. The tissue was digested in 0.5 mg/mL collagenase in serum-free DMEM supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin at 37 °C for 30 minutes with gentle agitation. The mixture was filtered through a 70 mm cell strainer (Falcon®, Corning, NY, USA) to obtain a single cell suspension that was then washed 3 times with 10% DMEM by centrifugation at 900 g for 5 minutes. The obtained pellets were resuspended in 10% DMEM and the total cells were counted using a hemocytometer.

Differential pH and temperature treatment

Cell suspensions of 1×10^6 cells/mL were aliquoted into 300 µL medium at different pH levels (pH 4.7, 5.6, 6.5, 7.4 (physiologic pH of blood), 8.3, and 9.2) in 0.5 mL Eppendorf tubes. The cell suspensions were incubated at 25°C (considered as room temperature), 37 °C (considered as body temperature), 45°C, and 50°C for 20 minutes. The cells in each tube were seeded in each well at 300,000 cells/well and incubated in a CO2 incubator at 37 °C for 3 days. The experiment is conducted in triplicate for every condition, except for the chondrocyte and osteocyte conditions, owing to the constrained availability of primary cells.

The temperature in this study was elevated to 45°C and 50°C, drawing upon the principles of hyperthermia-based therapies.[27] Also, temperatures above and below physiological conditions to investigate the potential impact of temperature variations on treatment efficacy. We selected a range of pH values from 4.7 (acidic) to 9.2 (alkaline) to explore the impact of acidity/alkalinity on treatment effectiveness. This range covers the physiological pH of blood (7.4). We include both acidic and alkaline environments to understand the potential impact of pH manipulation on the treatment's ability to kill tumor cells. However, the current understanding of bone cells behavior at extreme pH is limited.

Apoptosis analysis

The Annexin V-FITC Apoptosis Detection Kit (Sigma, St Louis, MO) was used to measure the cytotoxic activity of the giant cells. The cells were resuspended in

500 µL binding buffer. The cell suspension was stained with 5 µL Annexin V-FITC and 10 µL propidium iodide solution. The tube was incubated at room temperature for 10 minutes and protected from light. The fluorescence intensity of the cell suspension was determined using a flow cytometer (FACSCalibur, BD Biosciences).

Thermoablation responder was defined as when the change in temperature resulted in a difference in the percentage of total cells death of more than 50%, while partial responder was a difference of 20%–50%, and non-responder meant less than 20% cell death difference between the different temperatures at pH 7.4. pH responder was defined as when the change in temperature resulted in a difference in the percentage of total cells death of more than 50%, while partial responder was a difference of 20%–50%, and non-responder meant less than 20% cell death difference between the different pH levels at the temperature of 37°C.

Statistical analysis

The percentages of cell death were assessed in relation to either a temperature of 37°C (under consistent pH) or pH 7.4 (under consistent temperature), compared with various alternative conditions. For instance, when examining a temperature of 45°C, the percentages of cell death for pH levels of 4.7, 5.6, 6.5, 8.3, and 9.2 were juxtaposed with those for pH 7.4 using a paired T-test. Similarly, when focusing on a pH of 8.3, the percentages of cell death at temperatures of 25°C, 45°C, and 50°C were compared with those at 37°C. A p-value below 0.05 indicated a statistically significant distinction. All statistical analyses and graph generation were conducted using PASW Statistics, version 18.0 (SPSS Inc., Chicago, IL, USA). Error bars on the graphs denote the standard error of the mean.

RESULTS

All the GCTB patient's demographic data enrolled in this study are shown in Table 1. Of the 18 included patients, 3 patients did not have a high enough cell count to undergo the full protocol. Only 15 patients had enough cells to go through all the experiments and be included

TABLE 1. Patient demographic data and the response of each patient to thermoablation and pH changes *in vitro*.

Patient number	Sex	Age at surgery	Final diagnosis	Thermoablation response	pH adjustment response	Recurrent after surgery within 1 year
1	F	32	Recurrent GCTB of right proximal humerus with lung metastasis	Y	Y	N
2	M	35	GCTB of right distal femur	N	N	N
3	F	25	Recurrent GCTB of right distal femur	N	N	N
4	F	56	Recurrent GCTB of sacrum	Y	N	7 months
5	M	39	GCTB of right distal radius	Y	N	N
6	M	24	GCTB of right proximal fibular	Y	N	N
7	F	42	GCTB of left acetabulum	Y	N	1 year
8	F	29	Recurrent GCTB of right distal femur	P	N	N
9	M	19	GCTB of right distal femur	Y	N	1 year
10	F	30	GCTB of left proximal tibia	Y	N	N
11	M	54	Recurrent GCTB with secondary ABC of right proximal tibia	N	N	N
12	M	29	Recurrent GCTB of right distal femur	Y	N	N
13	M	31	Recurrent GCTB of right distal femur	Y	N	N
14	F	70	GCTB left proximal tibia	Y	N	N
15	M	37	Recurrent GCTB of right distal radius	Y	N	N

Abbreviations: GCTB giant cell tumor of bone, ABC aneurysmal bone cyst, M male, F female, Y yes (more than 50% of total cells death), N no (less than 20% of total cells death), P partially (20-50% of total cells death).

in the data analysis. We could obtain only one osteocyte and chondrocyte control from subject number 13, who had undergone a wide resection operation.

The profiles of the osteocyte and chondrocyte total cell death after treatment with the different temperature and pH solutions for 20 minutes are shown in Fig 1a and b. At temperatures 25 ºC, 37 ºC, and 50 ºC, across all pH levels, there is no discernible disparity in the percentage of cell death observed among osteocytes, chondrocytes, or tumor cells (Fig 3). When examining solely the temperature of 45 ºC, osteocytes exhibit a survival rate of approximately 40% within the pH range of 5.6-8.3, whereas chondrocytes demonstrate survival rates exceeding 50% when the pH falls within the range of 6.5-7.4. However, chondrocyte survival notably diminishes as the pH increases to 8.3, resembling the pattern observed in tumor cells (Fig 3).

There were four response patterns of giant cell tumor cells to various temperature and pH, as shown in Fig 2, and as described in the following. Tumor cells from 1 patient (patient number 1) responded to either thermoablation or the increase in pH (pattern a). Two of the patients (patient numbers 14 and 15) responded only to thermoablation but not to the pH change (pattern b). The majority (patient numbers 4, 5, 6, 7, 8, 9, 10, 12, and 13) of patients showed a response (pattern c) whereby the percentage cell death increased after both the temperature and pH increased. Three patients (patient numbers 2, 3, and 11) did not show a response to both temperature or pH change; however, the total cell death in patients number 3 and 11 was more than 80% for all the temperature and pH levels tested, with the majority of cell death in both patients from the early apoptosis of the cells.

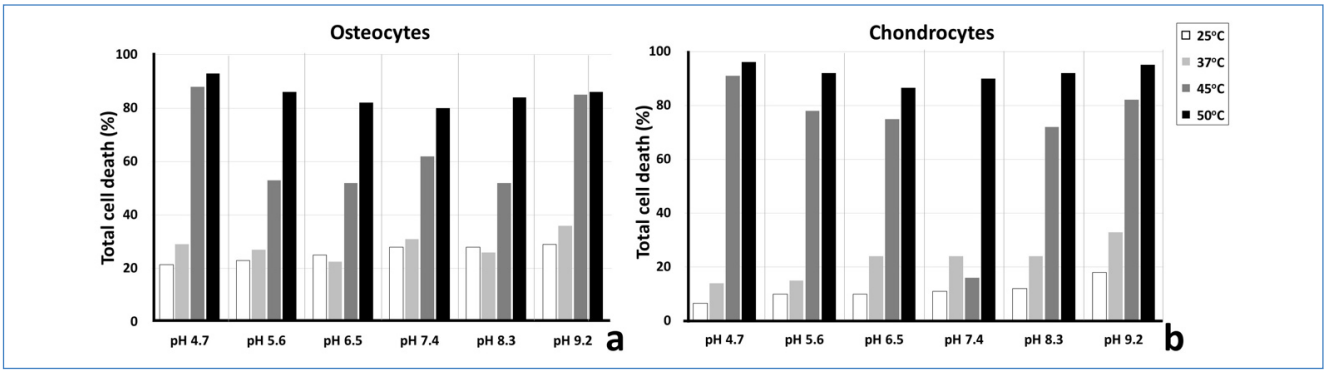

Fig 1. Percentage of osteocytes (a) and chondrocytes (b) cell death with various temperature and pH invitro.

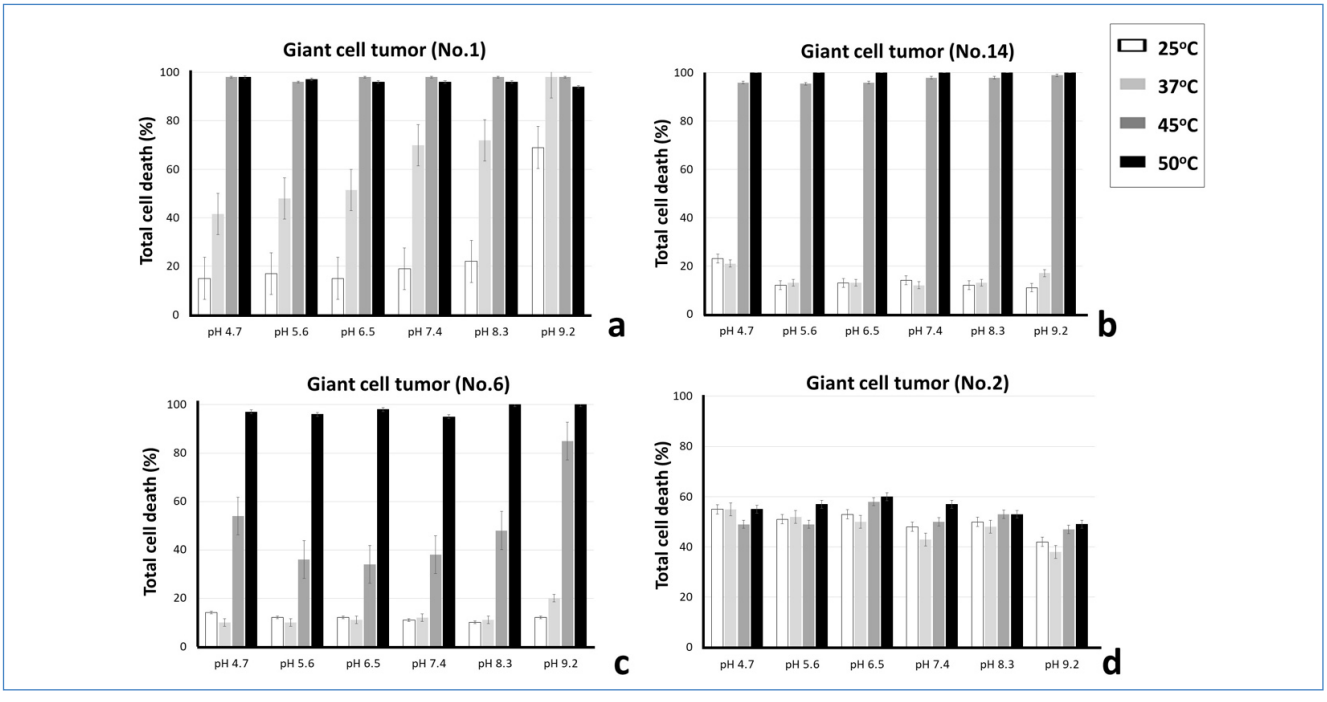

Fig 2. Shows 4 different patterns of giant cell tumor response to various temperatures and pH. The percentage of tumor cell death changes in response to both temperature and pH changes (a), only temperature but not pH changes (b), change of pH after temperature changed (c) and no different percentage of cell death after changes in both temperature and pH (d). The error bar represents the standard error of the mean.

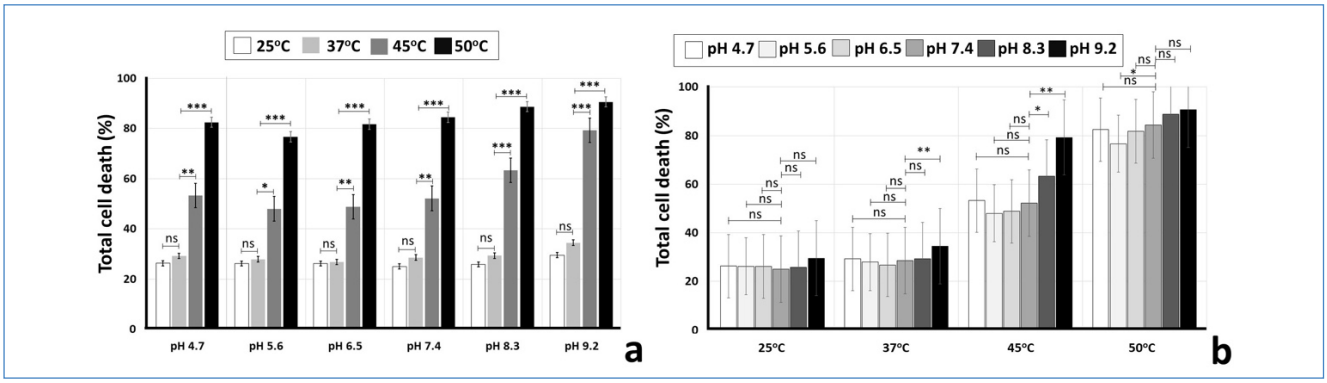

Fig 3. Shows the overall percentage of total GCTB cell death after being treated with various temperatures and pH for 20 minutes in vitro. Under consistent pH, the percentages of cell death at temperatures of 25 °C, 45 °C, and 50 °C were compared with those at 37 °C (a). Under consistent temperature, the percentages of cell death for pH 4.7, 5.6, 6.5, 8.3, and 9.2 were compared with those for pH 7.4 (b).
* = p-value < 0.05, ** = p -value < 0.01, *** = p-value < 0.001

The data from the 15 patients were pooled, and the average percentage of total cell death at various temperature and pH levels are shown in Fig 3. Thermoablation seemed to have some potential to increase the percentage of cell death. Changing the pH alone had only a low effect on inducing tumor cell death. However, the combination of increased temperature and pH around the tumor cells environment showed a trend in increasing the percentage overall giant cell tumor cell death.

DISCUSSION

The standard treatment of choice of GCTB is still extended intralesional curettage with bone grafting/cementing.[3] The use of adjuvant treatment can increase the success rate of the surgery by decreasing the recurrence rate. Thermoablation has also been reported to be an adjuvant for extended intralesional curettage.[23,24] However, to the best of our knowledge, using a pH adjusted medium together with thermoablation has not been tested and reported yet. Consequently, we performed an *in vitro* study of GCTB treatment with thermoablation with a variation of the pH of the solution too. Thermoablation alone still showed promising results as an adjuvant for extended intralesional curettage in GCTB treatment. Increasing the temperature to around 45°C could increase the percentage of tumor cells death, while normal bone and cartilage cells still displayed a high percentage of survival. Increasing the pH of the solution to a basic range would probably be better than an acid range, in that it should provoke a greater percentage of overall tumor cells death. However, changing the pH alone seemed to have no additional effects over thermoablation alone. Both increasing and decreasing the pH of the thermoablation solution showed only slight or no effects on tumor cell death but increased the rate of normal cell death. Therefore, the adjustment of the pH of the thermoablation medium is probably not necessary, since the benefit gained from changing the pH was not much in the current condition involving 20 minutes incubation time.

Our study reveals four distinct patterns of GCTB tumor cell response to variations in pH and temperature (Fig 2). Unfortunately, the relatively small sample size in this study precludes the feasibility of conducting robust statistical analyses to isolate and identify the specific factors contributing to these divergent responses. Nonetheless, we acknowledge the potential importance of further exploring this phenomenon. Therefore, we propose that future genetic investigations may hold promise in elucidating the underlying determinants governing these response patterns, thereby addressing this intriguing aspect of our findings.

Upon scrutiny of the effects of extreme pH levels, particularly at 45°C, it becomes evident that both chondrocytes and osteocytes face challenges in survival under conditions of extreme acidity (pH 4.7) and extreme alkalinity (pH 9.2). However, intriguingly, tumor cells exhibit a potentially higher survival rate in acidic environments, contrary to previous findings.[25] This discrepancy prompts consideration of the intracellular pH dynamics within tumor cells, which notably resemble osteoclast-like multinucleated cells. A comparative analysis reveals that while chondrocytes maintain an estimated intracellular pH range of 6.9-7.2[28] and osteocytes around 5.8-6.2[29], osteoclasts exhibit a lower pH range of 5.5-6.0[30], attributed to their primary function in bone resorption. This distinction in intracellular pH levels leads us to postulate that giant cell tumors of bone (GCTB) possess an enhanced ability to withstand acidic environments relative to other bone cell types due to their inherently lower intracellular pH. Nonetheless, further investigation is warranted to elucidate the underlying mechanisms and

implications of this observed phenomenon, which could yield valuable insights into the behavior and resilience of tumor cells under acidic microenvironments, contributing to our understanding of tumor biology and potentially informing therapeutic strategies targeting pH-associated vulnerabilities in cancer.

Thermoablation also has some limitations in clinical practice. Although *in vitro* studies have shown promising results, in clinical practice, it is not easy to control the temperature of the solution at 45°C for all 20 minutes of irrigation. Also, this procedure prolongs the operation time and can lead to an increase in intra- and post-operative complications,[31] such as inappropriate blood loss or surgical site infection. Future research avenues in the utilization of thermoablation as an adjunctive therapy should prioritize efforts aimed at reducing thermoablation duration. This can be achieved through the implementation of commercially available intravenous (IV) fluid warmers such as the ThermoTouch IV Fluid Warmer (Smiths Medical), Level 1 Fluid Warmer (Meditech), or Warm-It Express IV Fluid Warmer (Parker Laboratories). These devices offer precise control over the temperature of the output fluid, maintaining it within the range of 34 to 42°C. Coupled with the identification of the optimal thermoablation period (up to 20 minutes) to minimize intraoperative time, this approach holds promise for mitigating post-operative complications.

This study has some limitations to note. First, the majority of our subjects were not primary cases of GCTB, since this study required a large amount of tumor cells. However, the majority of first diagnosed lesions of GCTB did not have enough tumor cells for both the pathological study and the *in vitro* study. We therefore needed to include the recurrent GCTB patients who tend to have a larger lesion size, which could give enough cells for the varied conditions in the *in vitro* study. Secondly, our experimentation involving osteocytes and chondrocytes is constrained by the availability of a singular set of experiments due to the limited quantity of cells obtainable from a single subject. Future studies investigating the survival rates of those normal cells, under relevant experimental conditions simulating the proposed treatment modalities may guide surgeons in selecting the most suitable adjuvant therapy for GCTB cases where the tumor is located adjacent to a joint. Thirdly, one of our patient's age was much more than the normal range of GCTB; however, all the patients in our study had a definite pathological study confirming GCTB.

In conclusion, the suggested optimal condition for the use of thermoablation as an adjuvant is a temperature around 45°C and pH between 7.4–8.3 for 20 minutes. Future studies should aim to abbreviate the duration of thermoablation by fine-tuning the temperature to fall within the range of 37 to 45°C and adjusting the pH between 7.4 to 8.3, all while employing a reduced thermoablation timeframe.

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REFERENCES

1. Athanasou NA, Bansal M, Forsyth R, Reid RP, Sapi Z. Giant cell tumour of bone. In: Fletcher CD, Bridge JA, Hogendoorn PC, editors. WHO Classification of Tumours of Soft Tissue and Bone: Lyon, France: IARC Press; 2013. p. 321-4.
2. Cowan RW, Singh G. Giant cell tumor of bone: a basic science perspective. Bone. 2013;52(1):238-46.
3. Vaishya R, Pokhrel A, Agarwal AK, Vijay V. Current status of bone cementing and bone grafting for giant cell tumour of bone: a systemic review. Ann R Coll Surg Engl. 2019;101(2): 79-85.
4. Liu YP, Li KH, Sun BH. Which treatment is the best for giant cell tumors of the distal radius? A meta-analysis. Clin Orthop Relat Res. 2012;470(10):2886-94.
5. Chakarun CJ, Forrester DM, Gottsegen CJ, Patel DB, White

EA, Matcuk GR, Jr. Giant cell tumor of bone: review, mimics, and new developments in treatment. Radiographics. 2013;33(1):197-211.
6. Szendröi M. Giant-cell tumour of bone. J Bone Joint Surg Br. 2004;86(1):5-12.
7. Jones KB, DeYoung BR, Morcuende JA, Buckwalter JA. Ethanol as a local adjuvant for giant cell tumor of bone. Iowa Orthop J. 2006;26:69-76.
8. Quint U, Müller RT, Müller G. Characteristics of phenol. Instillation in intralesional tumor excision of chondroblastoma, osteoclastoma and enchondroma. Arch Orthop Trauma Surg. 1998;117(1-2):43-6.
9. Namazi H. Botulinum toxin: a novel adjuvant for giant cell tumor. Ann Surg Oncol. 2008;15(7):2066-7.
10. Namazi H. Raloxifene can reduce giant cell tumor recurrence: a new adjuvant therapy. Ann Surg Oncol. 2008;15(6):1783-4.
11. Lewis VO, Wei A, Mendoza T, Primus F, Peabody T, Simon MA. Argon beam coagulation as an adjuvant for local control of giant cell tumor. Clin Orthop Relat Res. 2007;454:192-7.
12. Waikakul S, Asavamongkolkul A, Phimolsarnti R. Use of warm Ringer's lactate solution in the management of locally advanced giant cell tumor of bone. Int J Clin Oncol. 2016;21(1):177-85.
13. van der Heijden L, van der Geest IC, Schreuder HW, van de Sande MA, Dijkstra PD. Liquid nitrogen or phenolization for giant cell tumor of bone?: a comparative cohort study of various standard treatments at two tertiary referral centers. J Bone Joint Surg Am. 2014;96(5):e35.
14. Ruggieri P, Mavrogenis AF, Ussia G, Angelini A, Papagelopoulos PJ, Mercuri M. Recurrence after and complications associated with adjuvant treatments for sacral giant cell tumor. Clin Orthop Relat Res. 2010;468(11):2954-61.
15. Tsukamoto S, Mavrogenis AF, Kido A, Errani C. Current Concepts in the Treatment of Giant Cell Tumors of Bone. Cancers (Basel). 2021;13(15).
16. Schoutens C, Verspoor FG. Heat treatment for giant cell tumors of bone: A systematic review. J Orthop Surg (Hong Kong). 2023;31(3):10225536231202157.
17. Balke M, Campanacci L, Gebert C, Picci P, Gibbons M, Taylor R, et al. Bisphosphonate treatment of aggressive primary, recurrent and metastatic Giant Cell Tumour of Bone. BMC Cancer. 2010;10:462.
18. van der Heijden L, Dijkstra PDS, Blay JY, Gelderblom H. Giant cell tumour of bone in the denosumab era. Eur J Cancer. 2017;77:75-83.
19. Shi M, Chen L, Wang Y, Wang W, Zhang Y, Yan S. Effect of bisphosphonates on local recurrence of giant cell tumor of bone: a meta-analysis. Cancer Manag Res. 2019;11:669-80.
20. Setsu N, Kobayashi E, Asano N, Yasui N, Kawamoto H, Kawai A, et al. Severe hypercalcemia following denosumab treatment in a juvenile patient. J Bone Miner Metab. 2016;34(1):118-22.
21. Uday S, Gaston CL, Rogers L, Parry M, Joffe J, Pearson J, et al. Osteonecrosis of the Jaw and Rebound Hypercalcemia in Young People Treated With Denosumab for Giant Cell Tumor of Bone. J Clin Endocrinol Metab. 2018;103(2):596-603.
22. Errani C, Tsukamoto S, Mavrogenis AF. How safe and effective is denosumab for bone giant cell tumour? Int Orthop. 2017;41(11):2397-400.
23. Phimolsarnti R, Charoenlap C, Ariyaboonsiri B, Wongkajornsilpa A, Waikakul S. In vitro effects of thermoablation on apoptosis of giant cell tumor of bone: a preliminary report. J Med Assoc Thai. 2012;95 Suppl 9:S138-45.
24. Pimolsanti R, Wongkajornsilpa A, Chotiyarnwong P, Asavamongkolku A, Waikakul S. Effects of thermoablation with or without caffeine on giant cell tumour of bone. J Orthop Surg (Hong Kong). 2015;23(1):95-9.
25. Matsuyama S, Reed JC. Mitochondria-dependent apoptosis and cellular pH regulation. Cell Death Differ. 2000;7(12):1155-65.
26. Matsubara T, Diresta GR, Kakunaga S, Li D, Healey JH. Additive Influence of Extracellular pH, Oxygen Tension, and Pressure on Invasiveness and Survival of Human Osteosarcoma Cells. Front Oncol. 2013;3:199.
27. Kok HP, Cressman ENK, Ceelen W, Brace CL, Ivkov R, Grüll H, et al. Heating technology for malignant tumors: a review. Int J Hyperthermia. 2020;37(1):711-41.
28. Browning JA, Wilkins RJ. Mechanisms contributing to intracellular pH homeostasis in an immortalised human chondrocyte cell line. Comp Biochem Physiol A Mol Integr Physiol. 2004;137(2):409-18.
29. Choi JUA, Kijas AW, Lauko J, Rowan AE. The Mechanosensory Role of Osteocytes and Implications for Bone Health and Disease States. Front Cell Dev Biol. 2021;9:770143.
30. Nordström T, Rotstein OD, Romanek R, Asotra S, Heersche JN, Manolson MF, et al. Regulation of cytoplasmic pH in osteoclasts. Contribution of proton pumps and a proton-selective conductance. J Biol Chem. 1995;270(5):2203-12.
31. Cheng H, Clymer JW, Po-Han Chen B, Sadeghirad B, Ferko NC, Cameron CG, et al. Prolonged operative duration is associated with complications: a systematic review and meta-analysis. J Surg Res. 2018;229:134-44.