Original paper

Prognostic value of p53 protein expression in giant cell tumor of bone

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Giant cell tumor of bone (GCTB) is a benign tumor with a tendency for local recurrence. GCTB may cause lung metastases, and secondary malignant GCTB is rare. Its histological appearance does not predict local aggressiveness and/or the metastatic potential of the tumor. We aimed to investigate the prognostic value of the Ki-67 proliferative index and p53 protein expression in GCTB in predicting local recurrence, lung metastasis, and malignant transformation. We retrospectively reviewed 42 cases of GCTB. The p53 expression was positive in 20 cases. We used 10% as a cut-off value for p53 expression. In 10 cases, there were local recurrences. Lung metastases were found in three cases and malignant transformation was found in one case with classical GCTB located in the sacrum three years following diagnosis. The Ki-67 index was higher in cases with recurrence, but this difference was not statistically significant. Of the recurrent cases, two had no p53 staining while eight had moderate-to-strong staining. The staining was usually weakly positive in the non-recurrent cases. In conclusion, we believe that p53 may be used as a marker for the biological behavior of GCTB.

Key words: giant cell tumor of bone, recurrence, Ki-67, p53.

Introduction

Giant cell tumor of bone (GCTB) is a benign bone tumor with a tendency for local aggressiveness. It was initially described by Jaffe *et al.* in 1940 [1, 2, 3]. It comprises 5% of all bone tumors. Predilection sites are around the knee joint and epiphyses of long bones. There is a slight predominance of male patients in the Asian countries and female patients in Western countries, and some reports have suggested that there is no difference at all [4, 5, 6, 7, 8]. There is a peak of incidence in the 3rd and 4th decades [1, 2].

Curettage is preferred in therapy over total excision, because the latter may result in a loss of function depending on the tumor site. Recurrence, however, is frequent following curettage and incomplete resection and is rare following wide resection. The recurrence rate within the first couple of years is approximately 25% [2, 4, 9].

Although histologically benign, GCTB may cause lung metastases in 2–3% of cases in the 3-4 years following diagnosis [2, 4, 10, 11]. Malignant transformation of GCTB is rare, i.e. less than 2%, and is usually found in cases with multiple local recurrences. Most of malignant GCTB develop either *de novo* or years after surgical therapy accompanied by radiotherapy [12, 13].

Histologically, the tumor consists of multiple multinucleated giant cells with a background of oval to round, spindle-like mononuclear cells [1, 5, 14]. This histological appearance does not predict local aggressiveness and/or metastatic potential of the tumor. Recently, the proliferation marker Ki-67 and a well-known tumor suppressing gene, p53, are frequently used as prognostic factors in most of the tumors. We

aimed to investigate the prognostic value of the Ki-67 proliferative index and p53 protein expression in GCTB in predicting local recurrence, lung metastasis, and malignant transformation.

Material and methods

This was a retrospective case-control study. Of the cases referred to the Surgical Pathology Department of Uludag University, School of Medicine between

Table I. Clinicopathological findings of cases (n=42)

Variable		No. of cases (%)
Gender	Female	22 (52)
	Male	20 (48)
Age (years)	≤ 20	4
	20-40	27
	≥ 40	11
Tumor site	Distal femur	8 (19)
	Proximal tibia	8 (19)
	Vertebra	5 (12)
	Proximal femur	4 (10)
	Sacrum	3 (7)
	Proximal fibula	3 (7)
	Distal ulna	2 (5)
	Distal radius	2 (5)
	Distal tibia	2 (5)
	Phalanx	2 (5)
	Others	3 (7)
Secondary aneurysmal bone cyst	Yes	12 (29)
	No	30 (71)
Vascular invasion	Yes	4 (9)
	No	38 (91)
Pathological fracture	Yes	2 (5)
	No	40 (95)
Onset of lung metastases	Yes	3 (7)
	No	39 (93)
Surgery	Curettage	27 (64)
	Resection	15 (36)
Recurrence	Yes	10 (24)
	No	32 (76)
Current status	Dead	2 (5)
	Alive	32 (76)
	NA	8 (19)
Median follow-up, months		34 (range 1-162)

NA – not available

2002 and 2013, 42 cases of GCTB were included in the study. Hematoxylin-eosin tissue sections of the resected tumor tissues were re-evaluated. The paraffin blocks best representing the tumor were selected for immunohistochemical staining. Follow-up information was obtained from hospital files and/or by contacting with the patients. Data on age, gender, tumor site, surgical procedure, pathological fractures, local recurrence, lung metastasis, malignant transformation, lymphovascular invasion, and secondary aneurysmal bone cyst presence were noted. The potential relationship between the clinical and pathological findings and Ki-67 proliferation index and p53 protein expression were evaluated.

Immunohistochemical staining

Immunohistochemical staining was performed with the Leica Bond-Max automatic immunohistochemical stain device using Ki-67 (clone MIB-1, dilution 1 : 200, Dako, Tokyo, Japan), and p53 (clone DO-7, dilution 1 : 500, Dako, Tokyo, Japan) antibodies with the polymer DAB kit. As positive controls, colon adenocarcinoma was used for p53, and tonsil was used for Ki-67. No negative control was used in the study.

Microscopic evaluation

The Ki-67 proliferative index was scored semiquantitatively as low (+), intermediate (++), or high (+++), depending on the number of positive mononuclear cells in the most prominently stained area, using a ×40 lens, setting up threshold values as < 5%, 5-20% and > 20%, respectively.

Immunostaining for p53 protein was evaluated using × 40 magnification light microscopy. These results were categorized as negative or positive, according to a cut-off value of 10% to discriminate between negative and positive cases.

Statistical analysis

Statistical analyses were performed using the SPSS v20 software program (SPSS, Chicago, IL, USA). Data were expressed as the median value (minimum-maximum) and percentages for frequencies. For comparison between groups, the Mann-Whitney U and Fisher's exact tests were used where appropriate. A p value less than 0.05 was considered significant.

Results

There were 22 female (52.4%) and 20 male (47.6%) patients with a median age of 31.5 years (15-75 years). All clinical data are listed in Table I. Most of the tumors were located in the distal femur and proximal tibia of the knee region (38.8%, Fig. 1). Surgical therapy consisted of curettage in 27 cases and resection in 15 cases.
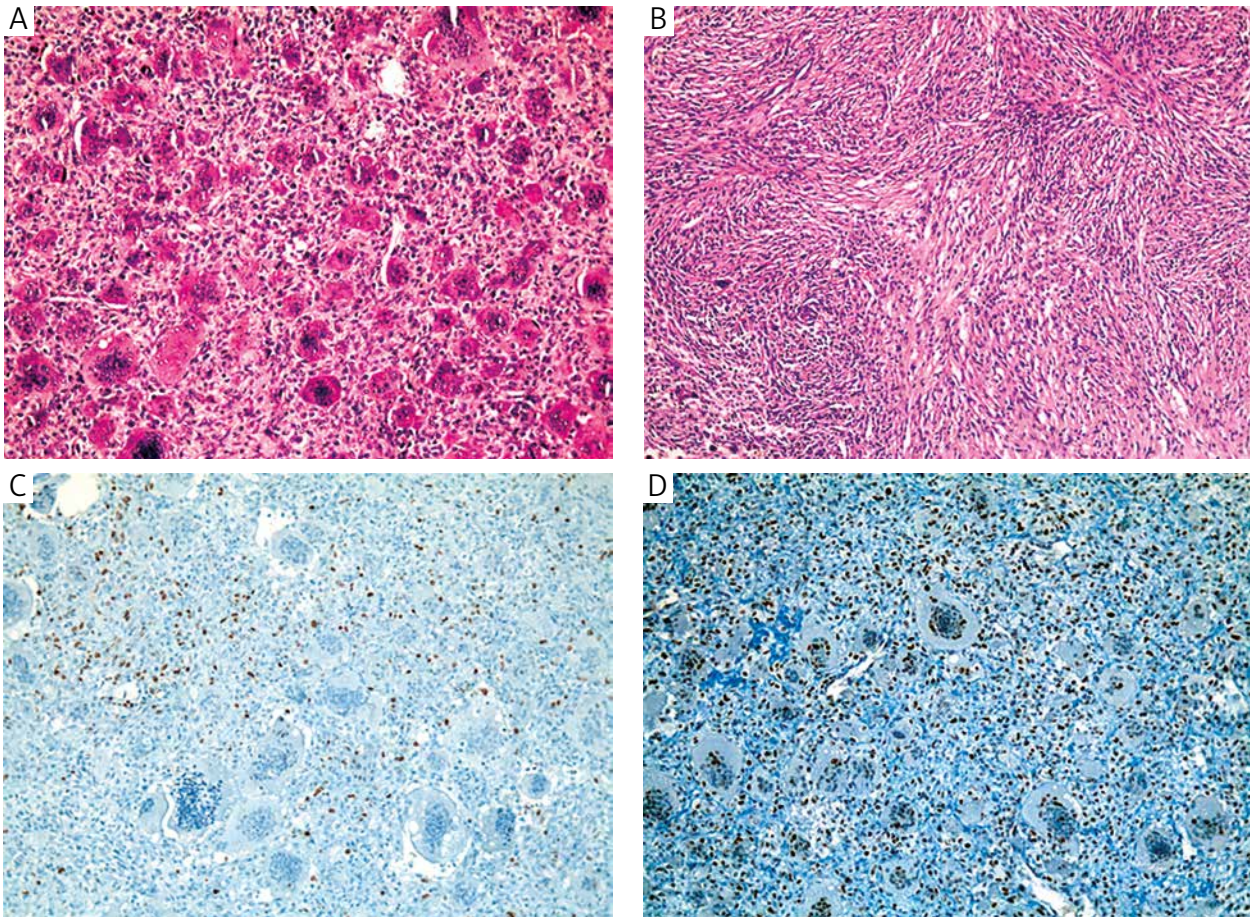
Fig. 1. A) Giant cell tumor in the distal femur in a 31-year-old woman. Multinucleated giant cells uniformly distributed among mononuclear stromal cells. B) Spindle cell proliferation with a storiform pattern associated with foamy cells (hematoxylin-eosin, ×200). C) Ki-67 index was moderate (= 10%, Ki-67, 200×). D) Nuclear positivity for p53 in both stromal and giant cells (p53, 200×)

Recurrence

In 10 cases, there were local recurrences (23.8%). The clinical findings in the recurrent cases are listed in Table II. The initial surgical procedure in recurrent cases was curettage in seven cases and wide resection in three cases.

The recurrence rate was 26.92% (7/26) in the curettage cases and 18.75% (3/16) in the resection cases. Statistical analysis revealed no significant association between recurrence and the type of surgery (p = = 0.490). Recurrences in the resection cases were most likely due to unsuccessful surgery because of the complicated tumor site, i.e. vertebrae. In recurrent cases, three had multiple recurrences. One case had two and the other two cases had four recurrences. The patient with the tumor located in the clivus had undergone operations twice and received radiotherapy as the resection was incomplete. The residual tumor was under control and the patient did not have any complaints.

The patient with a tumor located in the distal tibia had four recurrences in the past decade (Fig. 2). She was treated with curettage every time. She received radiotherapy and hyperbaric oxygen therapy following the last operation. She has been in follow-up for four years without recurrence. The patient with a tumor located in the proximal femur underwent prosthesis implantation following the 4th recurrence. One year after the implantation, the patient is well and has no complaints.

Lung metastases

Lung metastases were found in three cases (7.14%). Two of the patients were female and one patient was male. In cases with tumors in the vertebra and proximal tibia, the metastases were found at the time of diagnosis. In the case with the tumor in the thoracic vertebra, the metastasis was found at the time of recurrence. One of the patients had dropped out of the follow-up evaluation, while one patient received denosumab, a novel RANKL inhibitor (receptor activator of nuclear factor κ-B (NF-κB) ligand), and the other patient received bisphosphonate. Both are still under treatment.

Table II. Clinical data of recurrent giant cell tumor cases

Case	Age	Sex	Site	First surgical procedure	Number of recurrences	Time to first recurrence	Final surgical procedure	Postrecurrent follow-up (months)
1	56	F	vertebra (C3)	resection	1	12 months	wide resection	96
2	37	F	proximal femur	curettage	4	5 months	prothesis replacement	12
3	23	F	proximal tibia	curettage	1	28 months	curettage and bone grafting	35
4	34	F	distal tibia	curettage	4	30 months	curettage and bone grafting -radiotherapy	40
5	34	M	clivus	curettage	2	5 months	wide resection -radiotherapy	29
6	23	F	vertebra (Th11)	resection	1	15 months	wide resection	12
7	27	M	vertebra (Th7)	resection	1	5 months	wide resection	13
8	38	M	phalanx	curettage	1	15 months	amputation	2
9	25	F	proximal tibia	curettage	1	9 months	curettage and bone cementing	4
10	17	F	phalanx	curettage	1	3 months	curettage and bone grafting	5

M – Male; F – Female
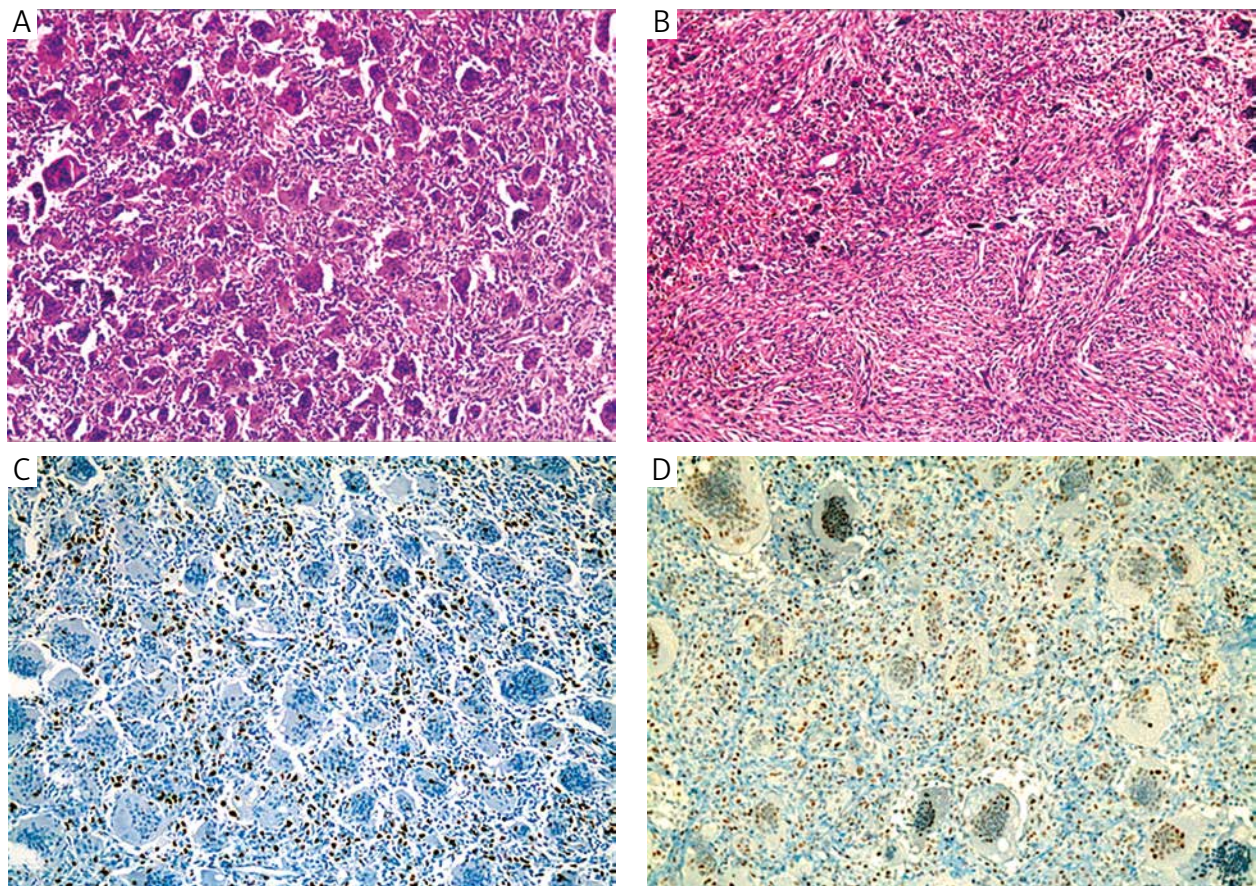
Fig. 2. A) Recurrent giant cell tumor in the distal tibia in 34-year-old woman. B) Histological features typical of a giant cell tumor merge into an area predominantly consisting of spindle cells (HE, ×200). C) Ki-67 index was high (= 35%) in the stromal cells (Ki-67, 200×). D) Overexpression of nuclear p53 is evident in the recurrent tumor (p53, 200×)

Vascular invasion

Vascular invasion was found in four cases (9.52%). The only recurrence in this group of cases was found in the case with the tumor in the distal tibia. The vascular invasion had no relationship with lung metastases.

Aneurysmal bone cysts and pathological fractures

In 12 cases (28.57%), we found secondary aneurysmal bone cysts. Pathological fractures were present in two cases (4.76%) with tumors in the distal femur and carpal bones.

A malignant transformation was found in one case (2.38%) with classical GCTB located in the sacrum three years following diagnosis (Fig. 3). The patient did not receive radiotherapy following initial treatment, and the transformation was into osteosarcoma. The patient underwent wide resection followed with chemotherapy. Unfortunately, the patient died seven months later with local recurrences and lung metastases.

Outcome

The median follow-up period was 34 months (1-162). We could not obtain follow-up information in eight cases. Of the 34 cases with follow-up information, two patients are dead: one aforementioned case with local recurrence and lung metastases, and one due to reasons irrelevant to the diagnosis.

Immunohistochemistry

Immunohistochemical evaluation revealed Ki-67 proliferative indices of 1-60% (Fig. 1C, 2C). The Ki-67 index was higher in cases with recurrence, but this difference was not statistically significant (p = = 0.731). Ki-67 proliferative indices were 22% (1-40) in recurrent cases, 13% (1-40) in non-recurrent cases, and 60% in the malignant case.

p53 was positive in 20 (47.62%) and negative in 22 (52.38%) of the cases (Fig. 1D, 2D). Of the recurrent cases, two had no staining while 8 had positive staining. A statistically significant relationship was found between p53 positivity and local recurrence (Table III, p = 0.030 using Fisher's exact test).
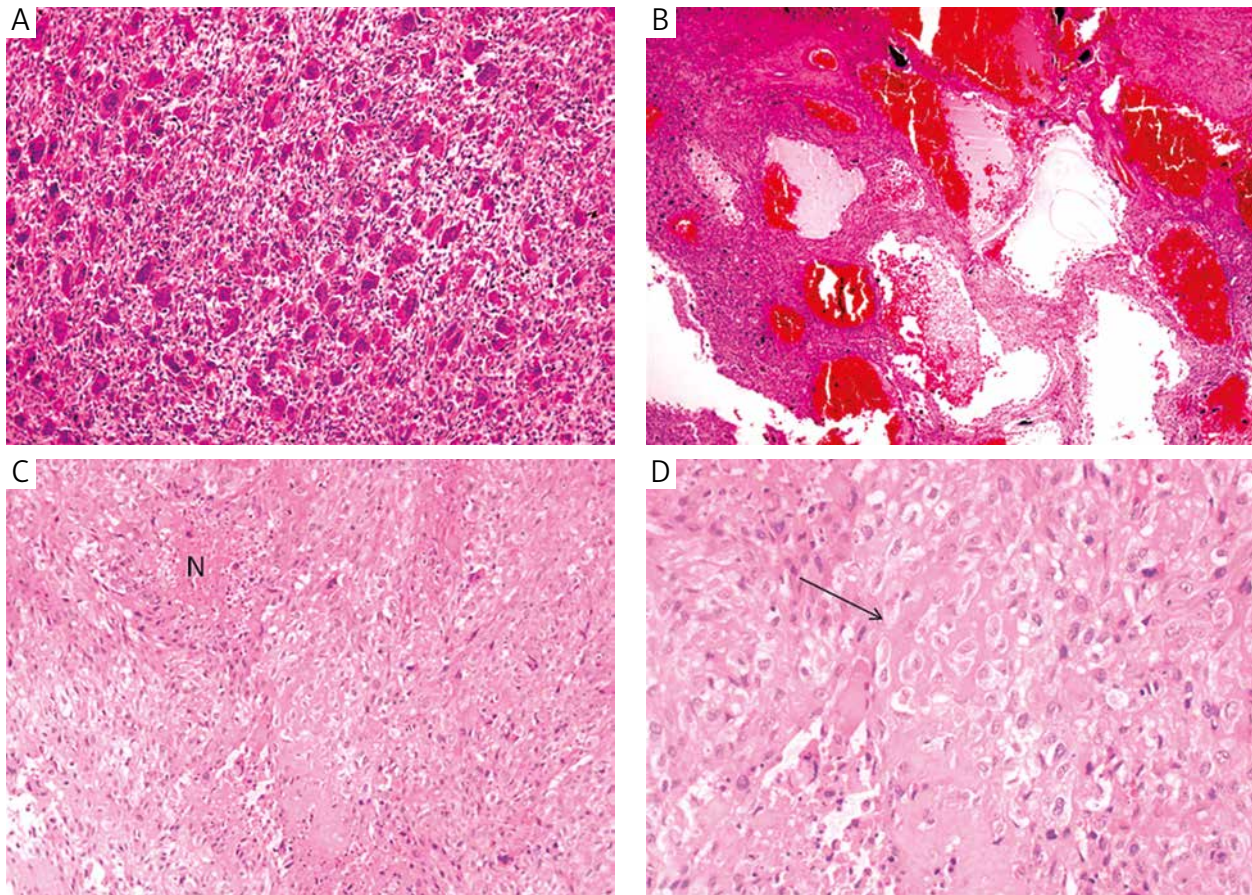
Fig. 3. A) Malignant transformation of the giant cell tumor in the sacrum in a 46-year-old woman. The primary tumor consists of fusiform stromal cells and scattered multinucleated osteoclast-like giant cells (HE, 200×). B) Aneurysmal bone cyst-like areas with vascular channels lined by osteoclastic giant cells (HE, 200×). C) Recurrent tumor in the same patient. Tumor consists of cellular proliferation of short spindle-shaped cells and an area of necrosis (N) within the sarcomatous tumor cells (HE, ×200). D) The tumor contains osteoid production associated with anaplastic tumor cells (↓, HE, ×200)

Table III. P values for the relation between recurrence and clinicopathological findings.

		RECURRENCE		P
		NEGATIVE (n = 32)	POSITIVE (n = 10)	
Gender	M/F	17 (53)/15 (47)	3 (30)/7 (70)	0.284
Tumor site	Axial skeleton	5 (15.62)	4 (40)	0.181
	Nonaxial skeleton	27 (84.38)	6 (60)	
Sc. ABC	Negative	22 (68.75)	8 (80)	0.696
	Positive	10 (31.25)	2 (20)	
Surgery	Curettage	18 (56.25)	7 (70)	0.490
	Resection	14 (43.75)	3 (30)	
p53	Negative	20 (62.50)	2 (20)	0.030*
	Positive	12 (37.50)	8 (80)	
Ki-67	Negative	15 (46.87)	3 (30)	0.473
	Positive	17 (53.13)	7 (70)	
Vascular invasion	Negative	29 (90.62)	9 (90)	1.000
	Positive	3 (9.38)	1 (10)	

M – Male; F – Female, Sc. ABC – Secondary aneurysmal bone cyst
* Statistically significant using Fisher's exact test ($p < 0.05$)

Discussion

Many studies have reported on GCTB concerning the benign and yet aggressive behavior of the tumor and frequent recurrences if not adequately treated. Despite the use of Enneking's staging system and treatment plans, the recurrence rates are as high as 11.2-42% [4, 5, 6, 7, 14, 15]. Some reports indicated that 25% of the recurrences occurred within the first six-month period, 97% in the first couple of years, and 100% in the first three-year period [16, 17, 18]. In our series, the recurrence rate was 23.80%, with eight cases (80%) within the first two-year period. All recurrences were found within the first three-year period.

The definitive treatment for GCTB is surgery, including curettage, curettage and bone grafting, curettage and bone cementing, local use of phenol, formaldehyde or similar additional reagents, marginal excision and wide resection [2, 4, 14, 19]. Campanacci et al. suggested that the surgical margin is the most important prognostic factor, i.e. the presence of a tumor at the margin increases the recurrence rate [20]. This may explain the higher recurrence rates found in curettage and grafting patients. Some researchers reported that adjuvant agents may help to decrease the risk of recurrence [14, 19, 21]. Recurrent lesions are usually treated with a repeat curettage. The recommended wide resection in recurrences is sometimes not preferred, because it may lead to a loss of the joint adjacent to the tumor [18]. Local radiotherapy was recommended to control the local recurrences in the past, but was condemned due to the potential relationship between former radiotherapy and malignant transformation. However, radiotherapy is still used to treat tumors located in the vertebrae as a technically complete resection is not feasible [2]. Surgical treatment consists of curettage, curettage and grafting, curettage and bone cementing, and wide resection in our series. The highest recurrence rate was found in curettage and grafting, and curettage and bone cementing cases. The recurrence rates were lower in the wide resected cases. However, we did not find any statistical relationship between type of surgery and recurrence rate in our series. Local radiotherapy was used in three recurrent cases in the proximal femur, distal tibia, and clivus.

Although some studies support relationships between recurrence and cortical destruction, soft tissue invasion, the presence of pathological fractures, tumor size, early ages, gender, and tumor site, e.g. distal radius, proximal femur, axial skeleton, or small bone involvement, others have suggested that there are no relationships at all [4, 5, 7, 9, 14, 19, 21, 22]. Cases involving the axial skeleton have been reported to have a particularly high recurrence rate [5]. Recurrence sites were the vertebrae (n = 3), phalanx (n = 2), clivus (n = 1), and proximal femur (n = 1) in our series and all were sites leading to inadequate surgical intervention.

In cases of young patients, specifically under 30 years, the recurrence rate was high. This may be due to a high bone turnover rate in the young [19]. The youngest patient with a recurrent tumor in our series was a 17 year-old female. Eight patients were between ages 20 and 40, and the oldest was 56 years old.

There is debate regarding the relationship between pathological fractures, soft tissue invasion and recur-

rence [4, 19, 21]. In our series one of the two cases with pathological fractures had a recurrence in the phalanx.

Jiang *et al*. reported that the recurrence rate was higher in female patients in a study of 140 cases [6]. Similarly, in 7 of the 10 recurrent cases in our series the patients were female.

Lung metastases are very rare in GCTB, and occur in 1-6% of all cases. In such cases, the primary tumor site is usually the distal radius expressing locally aggressive behavior. Lung metastases are diagnosed within 3-4 years following initial diagnosis, and both genders are equally affected [4, 5, 7, 8, 19]. Moreover, some studies suggest that embolization of tumor cells during surgery for recurrent lesions underlies lung metastasis, whereas some studies suggest that there is no such relationship at all [4, 18, 19, 23]. Interestingly, there are tumor thrombi in the vasculature peripheral to the tumor in one-third of the cases. However, this vascular invasion is not related to lung metastasis [4]. Lung metastases are more frequent in cases of recurrence [10, 11]. Most studies assume that local recurrence of GCTB is a risk factor for lung metastasis [11]. Chung Ming Chan *et al*. reported that the risk of lung metastasis of GCTB appears to increase with younger age at presentation, axial location, local recurrence, and Enneking stage-3 primary tumor [10]. Limited reports suggest early surgical removal of lung metastases as the preferred mode of treatment. Neoadjuvant chemotherapy, bisphosphonates, interferon α, and RANKL inhibitors are recommended as other treatment choices in such cases [8, 11, 14, 23]. Denosumab is a novel drug that inhibits normal and tumor associated osteolysis by preventing the function of RANKL, and may be an effective alternative based on the fact that giant cell tumors overexpress RANKL and its receptor. In our series, two patients had lung metastasis at the time of initial diagnosis, and one patient had lung metastasis at the time of local recurrence. Four patients with vascular invasion did not have lung metastasis.

It is impossible to predict local aggressiveness and metastatic potential from the histological appearance of GCTB alone [1, 2]. Many studies have been performed to identify the prognostic markers in biopsies to predict clinical behavior. The Campanacci staging system, Ki-67 proliferative index, and p53 positivity are among the most popular prognostic markers [9, 16, 24, 25]. Ki-67 is a nuclear protein found in proliferating cells. It is expressed in the active phases of the cell cycle, i.e., G1, S, G2, and M, as it is not found in the G0 phase. Thus, it is a perfect marker of proliferating cells in both normal and tumor tissue. Usually the Ki-67-positive fraction of cells is correlated with the course of the disease, as it not only indicates the portion of mitotic cells but also indicates the portion of all proliferating cells. The percentage of cells expressing cellular staining with the immuno-

histochemical stain for Ki-67 gives the proliferative index. The index is usually high in aggressive tumors and is regarded as a poor prognostic factor [25, 26, 27]. In our series, giant cells were Ki-67-negative in all cases and mononuclear cells were Ki-67-positive in various proportions of tumor cells. This finding suggests that mononuclear cells are responsible for the proliferative activity in GCTB. Furthermore, several studies report that the Ki-67 index in recurrent cases is higher than in the primary tumor, and some studies report that there is no difference between the primary tumor and the recurrent tumor [16, 26, 27]. Ismail *et al*. emphasized that Ki-67 is not a useful immunopathological marker in predicting local recurrence and lung metastasis of GCTB [25]. We did not find a statistically significant relationship between the Ki-67 index and local recurrence and lung metastasis.

There is a small number of studies suggesting that p53 expression is an important prognostic marker in predicting local recurrence and lung metastasis [9, 16, 24]. Although originally responsible for tumor suppression, the mutant p53 gene can no longer suppress tumor growth, and furthermore, the mutant p53 protein promotes tumor growth and dissemination. The presence of mutant p53 protein is an indicator of tumor aggressiveness in various tumors. The p53 gene mutation has been rarely reported in GCTB [12, 13, 16, 28]. Papanastassiou *et al*. emphasized a correlation between the p53 mutation and local recurrence and tumor aggressiveness in GCTB [24]. In our series, a statistically significant relationship between p53 expression and local recurrence was revealed (p = 0.022). Of the cases with lung metastasis, weakly positive staining was found in cases with the primary tumor in the tibia and vertebra.

Malignant transformation in GCTB is rare (1.4-6.6% of all cases) and typically develops following multiple local recurrences. Most of the malignant GCTB evolve years after surgery and are associated with previous radiotherapy or occur *de novo*. On the other hand, cases of sarcomatous transformation in sites of GCTB following surgery alone have also been reported (so-called primary malignant GCTB) [1, 12, 13, 17, 29, 30]. Usually the malignant transformation of the GCTB has the morphology of osteosarcoma, fibrosarcoma, or so-called malignant fibrous histiocytoma (secondary malignancy in GCTB) [5, 29]. In most cases, the malignant transformation is diagnosed within five years following primary resection. Various oncogenes and tumor suppressor genes, including p53 and H-ras, are thought to affect this transformation [12, 13, 17, 30]. Okubo *et al*. reported that p53 and high GPX-1 expression is of great help in diagnosing malignant transformation in GCTB [12]. Prognosis is poor in cases with malignant transformation, and the five-year survival rate is 0-50%. A 46-year-old female patient with sacral GCTB in our series had os-

teosarcomatous transformation three years following initial therapy. She did not have a history of radiotherapy. Unfortunately, she died seven months after wide resection and chemotherapy due to recurrence and multiple lung metastases. Immunohistochemistry revealed a weak positivity for p53 in the primary GCTB and strong positivity for p53 in the recurrent malignant tumor. The Ki-67 index was also low in the primary tumor and high in the recurrent tumor.

Conclusions

In conclusion, the only prognostic factor with a statistically significant relationship we could find was p53 expression. However, we provide evidence suggesting that primary sites, including the proximal femur, axial skeleton, and small bones, and female gender may increase the risk of recurrence, although the results were not statistically significant. We conclude that the prognosis of malignant GCTB is poor, and p53 expression may be helpful in predicting clinical behavior, including local recurrence. Further studies with a larger number of cases may result in a better understanding and more information regarding this matter.

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