Original Article

Prediction of Clinical Course and Biologic Behavior of the Bone Giant Cell Tumor Using Bax and bcl-2 Markers

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ABSTRACT

Background and Objectives: **Giant cell Tumor of bone (GCT) is often regarded as a benign tumor, but its clinical course is unpredictable, has a high rate of recurrence, and even can metastases and transform to a malignant tumor. Histological features of the tumor often could not predict its future biologic behavior, so it is just called "Giant cell tumor" without indicating malignancy or benignicity. Several methods are suggested to predict the biologic behavior of this tumor. This study evaluated the relation between Bax & bcl-2 as proteins involved in cell proliferation and death (apoptosis) with histopathologic features and clinical course of GCT.**

Materials & Methods: **Paraffin- embedded tissue specimens of 40 GCTs of conventional, aggressive, recurrent, malignant, & metastatic types were evaluated by immunohistochemistry for Bax & bcl-2 markers. Clinicopathologic features and immunohistochemical results were statistically analyzed and presented in tables & diagrams.**

Results: **Age, sex, and pattern of skeletal involvement were the same as other worldwide reports. Expression of Bax & bcl-2 markers were significantly higher in malignant GCTs but no statistically significant difference was found in other subtypes for bcl-2 while there was statistically significant difference between subgroups for Bax.**

Conclusion: **Considerable expression of Bax & bcl-2 markers in a GCT could signal its malignant course, but low expression is not valuable in predicting the clinical course. In addition, it seems that secondary tumor nodules in lung are just simple implantation not true malignant metastases**

Keywords: Giant Cell Tumor, BAX Protein, c-bcl-2 Proteins, Prognosis

Introduction

Giant cell tumor of bone is usually a benign tumor, but the rate of recurrence is high. The behavior of the GCT is such that as many as 50% of all cases recur following curettage. Recurrent lesions may seed the joint and local soft and subcutaneous tissues, causing such local destruction, that limb function is severely compromised, and amputation may be inevitable. Rare conventional cases result in metastasis to the lungs, so most of these features fit with the definition of a low-grade malignancy (1).

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Despite advances in molecular biology, there are no established or molecular alterations in tumoral cells are capable to predict clinical outcome in patients with these tumors. Apoptosis is a genetically regulated cell death involved in the reduction of cells in normal as well as malignant tissues (2). Deregulation of the molecules controlling apoptosis may contribute to the process of tumorigenesis by reducing the rate of cell death, leading to the accumulation of genetic defects. The most important known molecules regulating apoptotic cell death are Bax, bcl-2, and p53 (3).

Recently, the bcl-2 family of proteins, including bcl-2, bcl-xL, bcl-xS, Mcl-1, Bax, and Bad, have been shown to play an important role in the regulation of apoptosis. Whereas the bcl-2, bcl-xL and Mcl-1 proteins appear to inhibit apoptosis, Bax, Bad, and bcl-xS proteins apparently promote apoptosis (4-7).

It has been shown that the proteins of the bcl-2 gene family heterodimerize and homodimerize with each other, and the relative proportions of these dimmers may determine whether a cell becomes apoptotic (7-9).

Overexpression of bcl-2 has been reported to protect tumor cells from apoptosis, (9, 10) whereas increased Bax expression promotes apoptosis induced by cytotoxic drugs and radiation (11-13). Conflicting results have been presented with regard to the correlation between bcl-2 and Bax expression and prognosis (14, 15). An elevated bcl-2/Bax mRNA expression ratio has been shown to correlate with poor clinical outcome in low-grade urinary bladder carcinoma (16).

The aim of this study was to assess the correlation of bcl-2 and Bax expression with the histological characteristics and clinical course and outcome of any type of Giant cell tumor of bone which is classified as aggressive (tumors which have destroyed cortex of the bone and have made aggression into adjacent soft tissue), recurrent (tumors which at least have recurred one time), malignant (tumors which fulfilled the histologic criteria of malignancy such cytonuclear features, frequent mitoses, necrosis etc), metastatic (tumors which have metastasis to the other organs) and conventional (tumors confined into the bone without any mentioned descriptive features for the other groups).

Materials and Methods

Paraffin embedded tissue specimens of forty Giant cell tumors including: 13 aggressive, 9 recurrent, 11 conventional, 6 malignant and 1 metastatic GCTs diagnosed were included in the study.

In addition, clinical data were elicited from the recorded files of the hospital archive and private office.

Immunostaining for Bax and bcl-2

Immunohistochemical studies were performed on formalin fixed and paraffin embedded tissue from surgical biopsy specimens using the streptavidin-biotin-peroxides method (Novostain Super ABC, Novocastra Laboratories Ltd, Newcastle, UK) with monoclonal antibodies specific for Bax (dilution 1:50, DAKO, Glostrup, Denmark) and bcl-2 (dilution 1:50, DAKO). Appropriate positive and negative controls were used for our staining technique (17). Cells were examined at 10 optical fields and at x400 magnification. Perinuclear cytoplasmic staining was interpreted as being positive for bcl-2; and cytoplasmic staining was interpreted as being positive for Bax.

We separated the percentage of Bax and bcl-2 by quick score that is based on both intensity and proportion of cytoplasmic staining. The proportion of malignant cell staining- positivity throughout the section is termed category A and is assigned score from 1-6 (1= 0-4%, 2= 5-19%, 3=20-39%, 4= 40-59%, 5= 60-79%, 6=80-100%) and intensity of stain (category B) is graded as 0= Neg, 1= week, 2= intermediate, 3= strong. Category A is added to B to form an additive quick score from 1-9 and AxB gives a multiplicative quick score between 0-18 (18).

Data was analyzed by SPSS software (Ver. 11.5) and Fisher's exact test and Kruskal Wallis test were used to study the significance of differences. It was chosen as statistically significant if the *P*-value was less than 0.05.

Results

Demographic and clinical characteristics of patients with different subtypes of GCT were assessed and the results are shown in Table 1.

Table 1: Demographic characteristics of patients with different subtypes of Giant cell tumor of bone

Variable	Conventional GCT	Aggressive GCT	Recurrent GCT	Malignant GCT	Metastatic GCT	Total	*P* value
N	11	13	9	6	1	40	
Age Mean±Sd	26.36±7.90	29.15±13.59	29.55±6.82	33.83±8.18	44.00	29.55±10.12	0.390
Sex F:M	6:5	11:2	7:2	3:3	1:0	28:12	0.354

Immunohistochemical staining for Bax and bcl-2 markers were done and mean percent of staining and intensity were assessed in all subgroups and then were encoded according to quick score of which the results are shown in Table 2.

Comparing positivity of staining of Bax and bcl-2 showed statistically significant differences between the subgroups for both Bax and bcl-2 ($P<0.001$ for both) and higher positivity of staining for Bax and bcl-2 were significantly more frequent in malignant GCT.

Table 2: Staining positivity for Bax and bcl-2 among patients with different subtypes of Giant cell tumor of bone

Staining positivity		Conventional GCT N (%)	Aggressive GCT N (%)	Recurrent GCT N (%)	Malignant GCT N (%)	Metastatic GCT N (%)
1+	**Bax**	-	-	-	-	-
	bcl-2	9(81.8)	1(7.7)	1(11.1)	-	-
2+	**Bax**	1(9.1)	-	-	-	1(100)
	bcl-2	2(18.2)	8(61.5)	1(11.1)	-	1(100)
3+	**Bax**	2(18.2)	-	-	-	-
	bcl-2	-	3(23.1)	5(55.6)	-	-
4+	**Bax**	6(54.4)	3(23.1)	-	-	-
	bcl-2	-		1(11.1)	1(16.7)	-
5+	**Bax**	2(18.2)	9(69.2)	8(88.9)	-	-
	bcl-2	-	1(7.7)	1(11.1)	5(83.3)	-
6+	**Bax**	-	1(7.7)	1(11.1)	6(100)	-
	bcl-2	-	-	-	-	-
Total		11	13	9	6	1

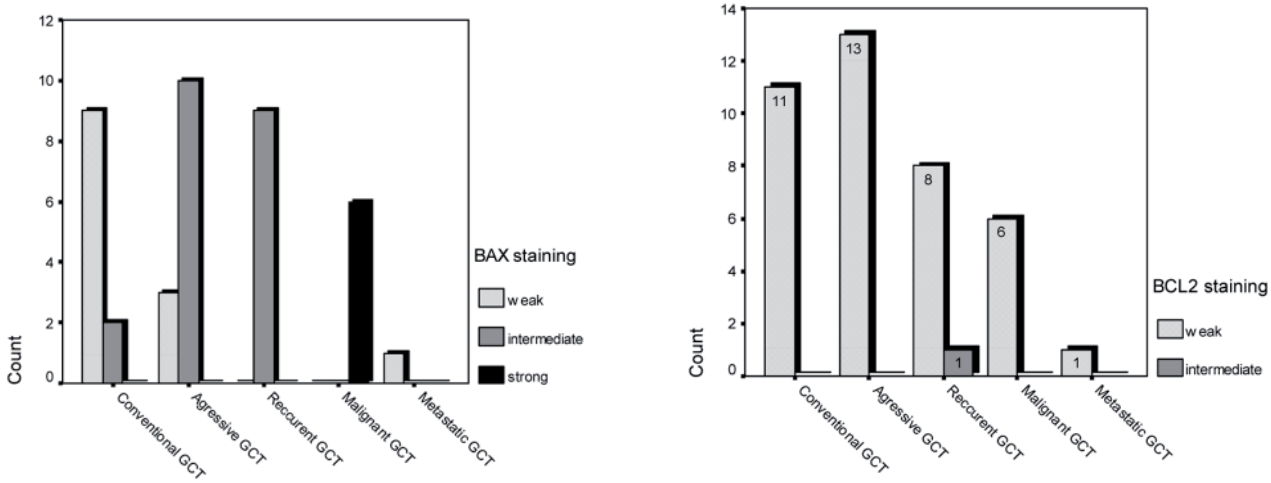

Fig. 1: Staining severity for Bax and bcl-2 among patients with different subtypes of Giant cell tumor of bone

Staining severity was also assessed for bcl-2 and Bax but the differences between the subgroups were not significant for bcl-2 (P= 0.473) while there was statistically significant difference between subgroups for Bax (P<0.001) [Fig. 1].

Finally, we calculated additive and multiplative quick score of which the results are shown in Table 3.

Table 3: Quick scoring for Bax and bcl-2 among patients with different subtypes of Giant cell tumor of bone

Quick score		Conventional GCT	Aggressive GCT	Recurrent GCT	Malignant GCT	Metastatic GCT	*P* value
Additive score	**Bax**	5.0±1.09	6.6±0.50	7.1±0.33	9.0±0.0	3.0	<0.001
	bcl-2	2.1±0.40	3.4±0.96	4.1±1.36	5.8±0.40	3.0	<0.001
Multiplative score	**Bax**	4.6±2.33	8.5±1.98	10.2±0.66	18.0±0.0	2.0	<0.001
	bcl-2	1.18±.40	2.4±0.96	3.5±2.55	4.8±0.40	2.0	<0.001

Discussion

Giant cell tumor of bone is a very peculiar and interesting tumor due to its biological behavior and the phenomenon of metastases of a histologically benign tumor (19). About 50% of GCTs recur following curettage. Though GCT is generally considered as a benign tumor, it can pursue an aggressive course and surprisingly few of these tumors could transform to a malignant neoplasm or metastases, so these features fulfill the criteria for regarding this tumor as a low grade malignancy(1).

Factors regulating the local recurrence and metastatic potential of this benign tumor depend on its aggressiveness, which can be better assessed by clinical and radiological parameters rather than the histopathological appearance (20). It is believed that the intracellular balance of two apoptosis-related proteins, bcl-2 and Bax, regulates the cell proliferation and cell death. Evidence from animal models likewise demonstrates that the balance of bcl-2 and Bax is also critical in determining cell death in the CNS (21-23). Bcl-2 is an anti-apoptosis protein whose overexpression contributes to the uncontrollable proliferation of gliomas and other neoplasm's (22, 24). In contrast, Bax, an antagonist of bcl-2, is an apoptosis promoter and its overexpression often results in cellular apoptosis (22, 24, 25).

In the present study 40 giant cell tumors of bone including 13 aggressive, 9 recurrent, 11 conventional, 6 malignant and 1 metastatic GCTs were assessed for these two markers.

The mean age of our studied patients was about 29.5 years (range 16-56) without any significant difference in five subgroups.

In a similar study by Tunn *et al.* about Giant cell tumor of bone among 87 patients, which was performed in 2003, the average age of the patients was 28.2 (range 8-72) years (19).

In Bertoni study about malignancy in giant cell tumor of bone in 2003, patient age ranged from 20 to 68 years (median, 62 years) for primary malignant GCT and from 30 to 77 years (median, 40 years) for secondary malignant GCT(26).

In our study sex distribution between different subtypes of giant cell tumor of bone, did not show any statistically significant difference, but overall females were more frequent than males (female to male ratio: 2.3). This pattern of sex involvement is higher than other worldwide reports with a female to male ratio of 1.3 (27).

The most common site of involvement was about the knee joint in our study and there was not any significant side preference of tumoral involvement in our patients.

Zhang *et al.* studied case analysis on treatment and recurrence of giant cell tumor of bone in 2006 in China .They found that Giant cell tumor of the bone usually recurs around the knee joint, especially at the proximal tibia, usually graded as Grade II or III by the Campanicci's radiological grading system (28).

In our study immunohistochemical evaluation, using antibodies to Bax and bcl-2 was performed in primary conventional, malignant, recurrent, aggressive, and metastatic Giant cell tumors.

Comparing positivity of staining of Bax and bcl-2 showed statistically significant differences between

these subgroups for both bcl-2 and Bax ($P<0.001$ for both) and higher positivity of staining for Bax and bcl-2 were significantly more frequent in malignant GCT but the differences between the subgroups were not significant for bcl-2 ($P= 0.473$), while there was statistically significant difference in staining severity between these subgroups for Bax ($P<0.001$).

Evaluation of additive and multiplative quick score among GCT subgroups showed statistically significant difference for both Bax and bcl-2 and scores were significantly higher in malignant subtype of GCT.

This implies that malignant transformation makes the cells to express these markers more frequently and so it could be regarded as an indicator for malignant transformation and unfavorable clinical course. Conversely and surprisingly, negativity of cells for these two markers cannot predict the tumor biologic behavior, so the dilemma of the prediction of the Giant cell tumor course and behavior remains unresolved. Following an extensive research on published and net literature, we only found few articles based on studies similar to the present study.

Pammer *et al.* studied expression of regulatory apoptotic proteins in peripheral giant cell granulomas and lesions containing osteoclast-like giant cells in 1998 in Austria, they found that strong expression of Bak and Bax in the majority of giant cells. In contrast, giant cells show only weak positivity for bcl-2 and moderate positivity for bcl-x. Mononuclear cells were negative to weakly positive for bcl-x. Only scattered mononuclear cells were positive for Bak, Bax and bcl-2. The frequency of apoptotic nuclei detected by TUNEL-staining compared to regular nuclei was 18 times higher in giant cells than in mononuclear cells. In summary, their findings support the presumption that giant cells of bone and soft tissue tumors are reactive cell forms and not of neoplastic origin (29).

Osaka *et al.* studied clinical and immunohistochemical characteristics of benign giant cell tumor of bone with pulmonary metastases in Japan and reported that examination of Ki-67 should be carried out for aggressive type of giant cell tumor (30).

Kaseta *et al.* studied prognostic value of Bax, bcl-2, and P53 staining in primary osteosarcoma in 2008 in Greece. The increased apoptotic rate as determined by an elevated Bax/bcl-2 protein expression ratio or by the Bax (+)/bcl-2(-)/P53 (+) protein expression pattern, appears to identify groups of osteosarcoma patients with unfavorable prognosis (18).

Sulh *et al.* studied proliferation index (PI) and vascular density of giant cell tumors of bone in 1996 in USA. They found that the degree of tumor cell proliferation and vascularity are not useful parameters to predict the recurrence of GCT of bone and that are no significant differences between the PI of mononuclear round-ovoid cells and mononuclear spindle cells (31).

Oda *et al.* suggest that multiple oncogene or tumors suppressor gene mutations as same as P53, H-ras, C-myc, MMP-9 and MIB-1 may play an important role during malignant transformation in conventional giant-cell tumors (32).

Lee *et al.* studied gene expression profiling which identified p63 as a diagnostic marker for giant cell tumor of the bone in 2008 in USA .They found that strong p63 nuclear staining was present in giant cell tumor of the bone and p63 can be used as a diagnostic marker to aid the clinical diagnosis of giant cell tumor of the bone (33).

In our study, the reactivity of stromal cells for these two markers in a metastatic nodule of GCT in lung was very low so this may indicate that secondary lung nodules of this tumor are not true secondary malignancy but just a simple implantation or seeding without further malignant growth potential.

This is in accordance with Mira who in his interesting book about Bone tumors claims that secondary nodules of a GCT of bone are not true metastases but simple implantation (27).

Conclusion

It seems that further studies must be performed to detect parameters, which would enable us to have a better understanding of the histogenesis of the giant cell tumor of bone and to make us capable for predicting the clinical course of the tumor. The clinical applicability and prognostication value of the parameters studied in this study are yet to be determined.

Despite studies conducted to clarify the true nature of the Giant cell tumor of bone and to make the pathologist able to predict the biologic behavior of this tumor, and the clinician to plan a corresponding

modality of treatment, yet this goal has not been achieved and at the present time no unique parameter has been introduced to be confidently applied for this purpose.

Furthermore, malignant transformation and metastases are very rare in giant cell tumor of bone so sample volume in all reported studies has not been adequate for such a confident conclusion.

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