Immunohistochemical Study Of Angiogenesis In Giant Cell Tumor Of Orthopedic Patients

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Abstract:

Objectives: To study the angiogenic activity in giant cell tumor of bone, by means of VEGF expression, in the endothelial cells using immunohistochemical staining.

Material and methods: A cross sectional study was conducted from March 2020 to April 2022. 50 cases of GCT of bone were included in this study. Tissue sections that were retrieved from archived tissue blocks were stained with immunohistochemical stain for VEGF protein.

Results: Our study revealed that thirty nine of the cases showed a positive staining for VEGF in the tumor cells (72.2 %). There was a significant association between the expression of VEGF in the tumor cells with tumor stage (p=0.003). There was also a significant association between the expressions of VEGF in the tumor cells with the presence of pulmonary metastases (p=0.015).

Conclusion: GCT of bone is frequently hypervascular; VEGF secreted by tumor cells elicits angiogenesis, which significantly contribute to the development of pulmonary metastasis. This provided confirmation for the prognostic significance of VEGF in predicting the behavior of GCT of bone and also the basis for therapeutics strategy targeting angiogenesis.

Key words: angiogenesis, patients and VEGF.

Introduction:

Giant cell tumor (GCT) of bone, also known as osteoclastoma, is a rare primary skeletal neoplasm with a tendency toward locally aggressive behavior. It was first described in 1940 by Jaffe, Lichtenstein and Portis as a distinct clinicopathological entity. It usually behaves in a

benign manner but has a prominent tendency to local recurrence and rarely to pulmonary metastases.

GCT is an infrequent and unpredictable bony lesion. Many previous studies that have been made to predict the behavior of GCT where failed to find a specific biological or histological parameters to establish the prognosis or aggressiveness of this lesion.

Histological grading has proved to be of little value, and since there is a great need for indicators of how aggressive the treatment should be in each particular tumor, different promising alternatives had been explored. Some investigators have focused their studies on angiogenesis in GCT of bone using both molecular techniques and immunohistochemistry.

Various factors are known to induce angiogenesis, but among those, proangiogenic capacity of VEGF, basic fibroblast growth factor and angiopoietins are well investigated and established to date (O'Reilly *et al.*, 1997). A few clinical reports have been found in the literature that addressed the expression of VEGF in GCT of bone. In previous studies the VEGF expression was 100% (Kumta *et al.*, 2003;Matsumoto *et al.*;Zheng *et al.*, 2000). Regarding the association between the VEGF expression in tumor cells and tumor stage in GCT of bone only a few studies have been reported using immunohistochemical and molecular techniques.

Zheng *et al.* 2000 and Matsumoto *et al* stated that there were higher levels of VEGF gene expression in stage III than in stage I and II.

The tumor is large at initial presentation. Grossly, greater than 6 cm in length and typically eccentric in the long axis of bone, but may be centrally located, involving the epiphysiometaphyseal region with extension to the adjacent intact cartilage (Michel Forest *et al.*,

1998.). The tumor is solid but often soft and friable; when it replaces the bone marrow, it appears brown or reddish in colour.

The tumor may show focal cystic areas that may appear ABC-like lesion grossly and histologically. Focal yellowish areas that represent lipid–laden macrophages, foci of hemorrhage and necrosis may be seen. However, bone production by the tumor is rare

(Vigorita and J., 2008).

Methods:

A cross sectional (retrospective) study is conducted. It is a study on angiogenic activity of giant cell tumor of bone.

This study was based on retrieved paraffin archival tissue blocks from the biopsy and en-block resected specimens within the histopathology department of Hospital, between the years of 2020 until 2022. These specimens had been reported by various independent pathologists in the department within that period. The demographic data and histopathological reports were obtained from registry book.

For statistical coherence, sample size for this study was calculated for each objective as follow

To determine the expression of VEGF in the tumor cells of GCT of bone

Sample size was calculated based on single mean formula:

$n= (1.96\sigma/\Delta)^2$

σ= standard deviation (SD)

If decided to obtain a more precise estimate, e.g. Δ=2.5

SD= expression of VEGF in the tumor cell from previous study (Kumta *et al.*, 2003)=9.70

$n= (1.96\sigma/\Delta)^2$

$n= (1.96x9.70/2.5)^2$

n= 58 +10% non response

n=66

Reagents

1. **VEGF Primary Antibodies**

A primary antibody was obtained from Santa Cruz biotechnology, (SC 7629). It is a rabbit polyclonal anti-human vascular endothelial growth factor (A-20) capable of recognizing the 165, 189 and 121 amino acid splice variants of human VEGF.

A concentration of 2µg/ml of antibody was used. Cells labeled by the antibody display cytoplasmic staining and/or membrane staining. The antibody labels the VEGF-121, VEGF-165 and VEGF-189 isoforms of the VEGF.

2. **CD34 Primary Antibodies**

A monoclonal mouse anti-human antibody Clone: QBEnd-10 Isotype: IgG1, kappa. The antibody is manufactured and marketed by Dako Denmark A/S. CD34 protein with product code No. M7165. In formalin fixed, paraffin embedded tissue section, the antibody labels endothelial cells.

Statistical analysis

Data was entered and analysed using PASW Statistics 19.0. All continuous variables were described using mean (SD) whereas for categorical data as frequency (%). Possible association

between tumor characteristics with VEGF expression were analysed using Pearson Chi-square test for categorical data and independent *t*-test for numerical data.

Results:

A total of 50 cases were included in this study. There was only slight preponderance of male compare to female. The minimum age was 9 years and the oldest patient was 63 years with the mean age 31.02 years.

Tumor Characteristic

The most common site of the giant cell tumor of bone was the lower extremities (36; 66.7%) followed by the upper extremities (18; 33.3%). 39 cases were stage III (72.2%), 14 cases were stage II (25.9%) and only one case was stage I (1.9%). From 54 patients only 12 (22.2%) patients had pulmonary metastasis (Table 1).

Table1: Tumor characteristics among study subjects (N=54)

Characteristics	Frequency (n)	Percentage (%)
Site		
Upper Extremities	18	33.3
Lower Extremities	36	66.7
Stage		
Stage I	1	1.9
Stage II	14	25.9
Stage III	39	72.2

Pulmonary Metastasis		
Present	12	22.2
Not Present	42	77.8

Because the sample size for this study was small and to achieve a significant statistical results, we combined the tumors occurred in the radius, ulna, humerus and other upper limb bone as one category labeled as upper limb. For the same reason we combined the tumors occurred in the tibia, femur, sacrum and other lower limb bone as one category labeled as lower limb.

General comment on immunohistochemical staining

Our immunohistochemical staining showed good and reproducible results as successful optimization was achieved, whereby the positive control as well as the actual tests showed positivity. The tests showed different intensity of the staining and semiquantitative score was given to the expression of VEGF protein in the tumor cells. The positivity of CD34 in the blood vessels was used to count the blood vessels for microvessel density.

The positivity for VEGF protein was limited to the cytoplasm without membrane staining. In most of the cases, staining for VEGF was diffuse throughout the tumors. However, the intensity varied considerably among tumors cells and a semiquantitative score was given consequently. Figures 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 show negative control, positive control and actual tissue positivity for expressions of VEGF and CD34 immunostaining.

Both positive and negative controls were run with the test. The positive control was used to ensure that the test is working. It was best to use the tissue of known positivity as a control. We used tonsil as a positive control for CD34.

For positive control of VEGF; we used a normal kidney tissue. The negative control for CD34 and VEGF were performed by omitting the primary antibodies. Generally, immunohistochemical staining showed good and reproducible results after optimizing the test before the actual run of the study.

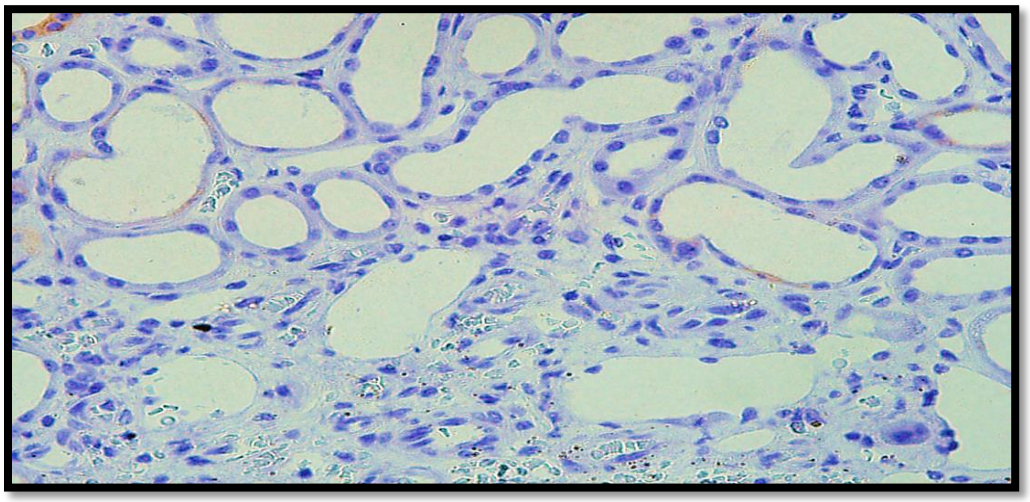

Figure1 : VEGF negative control (X400 magnification)

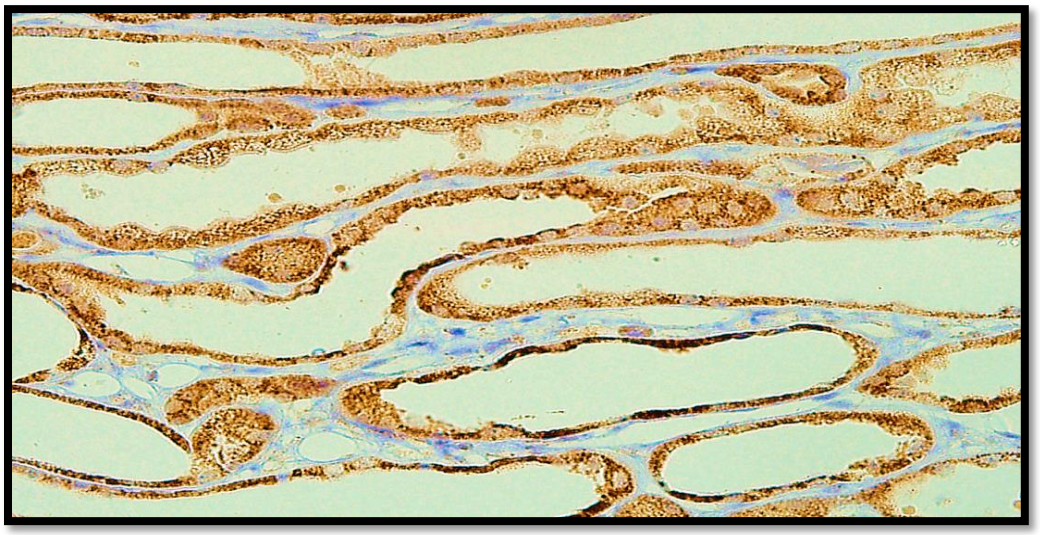

Figure2 : VEGF positive control (X 400 magnifications)

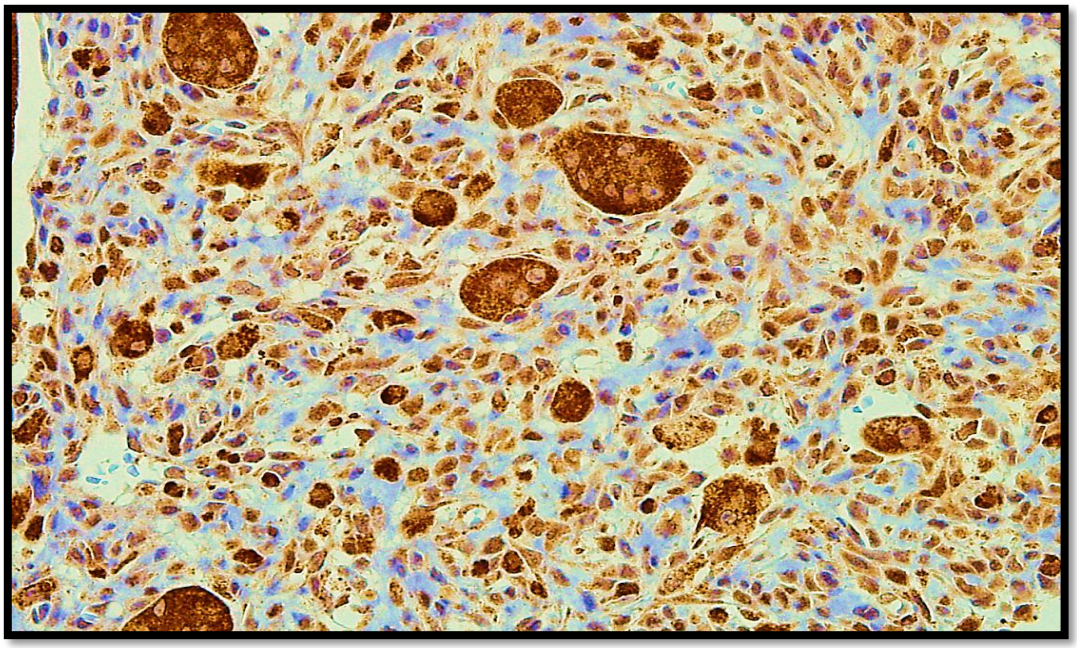

Figure3: VEGF cytoplasmic positivity in tumor cells (X 400 magnification)

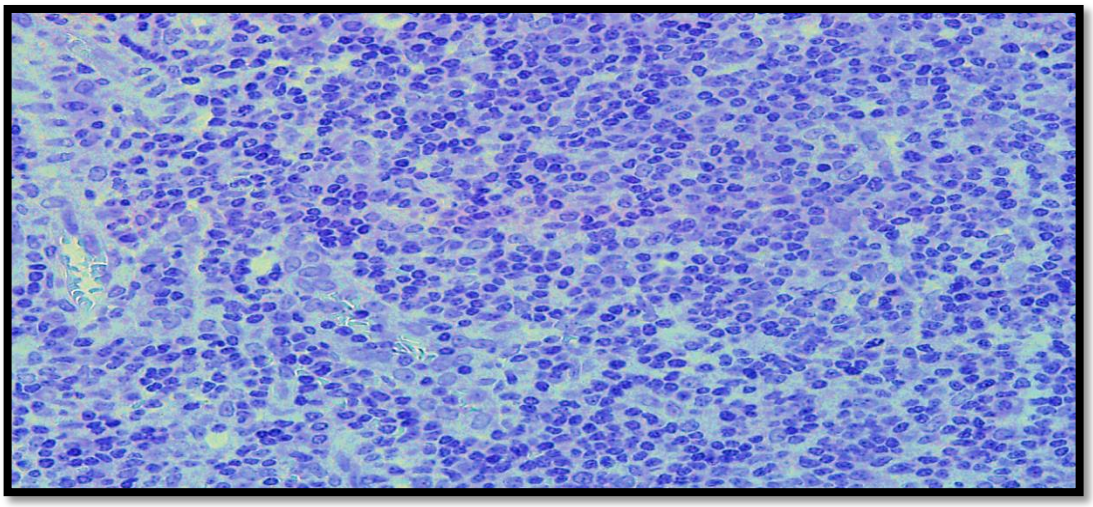

Figure4 : CD34 negative control (X 400 magnification)

Expression of VEGF in the tumor cells

A total of 54 cases were included in this study. Expression of VEGF in tumor cells was grouped into two categories (positive, negative) based on semiquantitative score of both intensity and the percentage of the staining cells. The findings are summarized in (Table 5.2) below. Based on the table, positive VEGF staining were seen in 39 (72.2%) of 54 cases. Negative VEGF staining was seen in 15 (27.8%).

Table2: Expression of VEGF in the tumor cells (TC) among study subjects (N=54)

	VEGF-TC	
	Negative	**Positive**
Tumor cells	15 (27.8%)	39 (72.2 %)

Association between expression of VEGF in tumor cells with tumor characteristics:

The association of expression of VEGF in tumor cells with tumor characteristics shown in (Table 5.3). Based on the table VEGF expression in tumor cells is significantly associated with two parameters; aggressive tumor stage and presence of pulmonary metastases. Pearson Chi square test showed ρ-value of (0.003) and (0.015) respectively, which indicates a significant association. No significant association was found between the VEGF expression and the tumor site.

Discussion:

Giant cell tumor of bone is an infrequent and unpredictable lesion. Despite many years of considerable speculation, the behavior of GCT is still uncertain. This study is based on the assumption that there could be a direct correlation between the angiogenesis and the aggressive

behaviour of GCT. The chief endpoint of the study is the aggressive behaviour of the tumor, taking in a consideration the tendency for recurrence and pulmonary metastasis.

The evaluation of angiogenesis was done by study the immunohistochemical expression of two markers; VEGF and CD34. The immunohistochemistry was performed on the tissue obtained almost from a resected specimen and few a biopsy samples. It was discovered in this study two patients presented with local recurrence prior to wide resection and one patient had multiple recurrences after that. This information was obtained from reviewing the patient's records. All patients were treated at the same institution, by the same orthopedics (Orthopedic Oncology), oncology and pathology staffs, and data about the clinicopathological variables evaluated were available for almost all patients; all of which were represent the major power of this study. However, restriction of the samples to those that fulfilled the inclusion criteria, thus small sample size creates one of the limitations for this study.

GCT of bone is a benign but locally aggressive tumor that most commonly occurs in young adults between 20 and 40 years of age (UNNI, 1998;Bridge *et al.*, 1992). This is concordance with the current study that the majority of the patients were between 18-45 years with the mean age 30.98 years. Though some studies have stated that GCT shows a slight female preponderance (55%) (UNNI, 1998). However, in this study there was a slight male predominance (32%) which is consistent with most studies that maintain that there is no sex predilection in GCT (Wulling *et al.*, 2001).

The epiphyseal region of long tubular bones especially the distal femur and the proximal tibia are the main sites for GCT, 49% were located in the lower extremities, 13% were reported in the upper extremities (Wulling *et al.*, 2001). Similar findings were seen in this study, were 66.7% of

GCT were reported to arise from the lower extremities, 33.3% were located at upper extremities. Study done by Zheng *et al.* (2000) stated that the majority of their tumors were stage I and II (62.5%), this is in contrast to our study which revealed that the main bulk of tumors were stage III (72.2%) and only 27.8% were stage I and II. Our study revealed that 22.2% of patients had pulmonary metastases. These finding are consistent with observations reported by (Faisham *et al.*, 2006) which stated that the pulmonary metastases was 30% in their series.

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