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Novel Understanding of the Histogensis of Central Giant Cell Tumour of Long Bones in Comparison to Giant Cell Lesions of the Jaw

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Abstract: Peripheral giant cell granuloma (PGCG) resembles central giant cell granuloma (CGCG) of the jaw, the former might be its soft tissue counterpart although, may have an osteolytic effect in addition central giant cell tumor of long bone (CGCT) is a benign tumor, locally aggressive and considered to be potentially malignant neoplasm at which giant cells may result from fusion of the mononuclear cells. The term "giant cell lesion" has given the impression of giant cells as being the major neoplastic component of these lesions. The aim of the study is to compare the expression of CD68, ki67 and osteopontin in PGCG, CGCG of the oral cavity and CGCT of long bone, for proper diagnoses and assessment of the behavior of these lesions, a trial to delineate histogenic origin of mononuclear stromal cells (SC) and multinucleated giant cells (GC) in such cases. Sections from three lesions were collected examined by Ki 67 as proliferating marker and for CD68 and osteopontin for staining the stromal and multinucleated giant cells. Positive immunostaining of stromal, giant cells for both CD68 and osteopontin may point to the delineation of giant cells in the three lesions is macrophages which might be reactive in nature rather than neoplastic. PGCG might be their soft tissue counterpart as bone destruction; the aggressiveness in the CGCG as well as CGCT could be due to inflammatory substances. Some CGCT of long bones may be misinterpreted as true giant cell rich tumors although they are inflammatory reaction mediated by osteopontin.

Key words: CD68 · Giant cell lesions · Ki67 · Osteopontin

INTRODUCTION

Multinucleated giant cells (GC) in fibroblastic vascularized stroma are the main histologic constituents of several lesions, including: central giant cell tumor (CGCT) [1], central giant cell granuloma (CGCG) and peripheral giant cell granuloma (PGCG) [2, 3, 4], the latter is a giant cell lesion occurring in the gingiva or alveolar mucosa of the oral cavity, it is considered as reactive lesion which may be caused by local trauma causing inflammatory reaction, nevertheless, developmental causes are mentioned by some authors [2, 3, 5]. PGCG microscopically resembles CGCG and some pathologists believe that it might be its soft tissue counterpart [6]. Furthermore, the central giant cell granuloma of the jaws occurs mostly in the mandible, it is considered a non-neoplastic benign condition, although, in some instances it may have an aggressive osteolytic proliferation, therefore, it is considered to have variable clinical behavior [3]. In some cases the "cupping" superficial resorption of the underlying alveolar bone that is sometimes seen in PGCG might cause confusion with the CGCG eroding through the cortical plate into the gingiva [6]. Central giant cell tumor of long bone (CGCT) is a benign bone tumor, occurring at the epiphyses and sometimes metaphysis of long bones, with local aggressiveness, high rate of recurrence and a possibility of developing lung metastases has been reported [7, 8], so that it is considered to be low grade or potentially malignant neoplasm [9]. On occasion giant cell tumors of

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bone undergo frank malignant transformation to undifferentiated sarcomas [10]. CGCT, till present, is considered to be a distinctive neoplasm of undifferentiated cells. The giant cells are mostly result from fusion of the proliferating mononuclear cells. Radiologically, it is usually lytic and expansile without prominent peripheral sclerosis and periosteal reaction. The term "giant cell lesion" has given the impression of giant cells as being the major neoplastic component of these lesions [1]. Osteopontin (OPN) which is a secreted phosphorylated glycoprotein, may play a role in osteoclast differentiation and osteoblast recruitment and function. OPN roles in osteoclast migration to sites of resorption and is crucial bone turnover. [11,12], in addition it is up regulated in some diseases such as inflammation as it mediates various cellular functions such as adhesion, migration and survival of some types of cells as macrophages, T-cells and dendritic cells. Intracellular OPN is also expressed in some cells as macrophages. OPN modifies chronic inflammatory response, as absence in OPN in OPN-null mice coincide with deficiency in macrophage accumulation [11, 13]. Moreover, it has significant role in stimulation, migration and retention of macrophages at sites of inflammation, at which it might have role in monocyte-macrophage differentiation, [11, 14]. Monocytes do not express OPN, even upon activation, until they assume characteristics of macrophages [15]. When cells express CD68, it confirms their histiocytic origin. CD68 is a member of the lysosome associated membrane protein family; it is only expressed in the cells of the monocyte/macrophage lineage [16]. Adding that, CD68-positive cells are also used clinically as marker of inflammation and tumor progression [17].

The aim of the study is to compare the expression of CD68, ki67 and osteopontin in cells of peripheral giant cell granuloma, central giant cell granuloma of the oral cavity and the central giant cell tumor of long bone, in order to be able to properly diagnose and assess the behavior of these giant cell lesions as well as a trial to delineate the histogenic origin of mononuclear stromal cells (SC) and multinucleated giant cells (GC) in such cases.

MATERIALS AND METHODS

A total of 41 cases were included in the present study. Tissue sections were taken from formalin-fixed paraffin-embedded blocks. Sections were stained for routine H&E examination and diagnosis; cases were obtained from the long bones CGCT (n=5), collected from the archives of the Pathology department, faculty of medicine, Cairo university. While, thirty-six cases from the mucosal as well as bony lesions of the jaws; PGCG (n=15), CGCG (n=21) were collected from the archives of the oral pathology department, faculty of dentistry, Cairo university. Patient's clinical data as well as radiologic findings were obtained from the files of all cases. The latter was performed to detect the site, extension of bone destruction and possibility of metastasis. Informed consent was taken from each patient.

Immunohistochemistry: Each of the selected paraffin sections were incubated with CD68, OPN for delineation of nature of the mononuclear and multinucleated giant cells and ki 67 antibodies as a proliferating marker (diluted 1:50, Clone 7A6, SantaCruz Biotechnology Inc., Santa Cruz, CA, USA). The immunohistochemical staining was performed using a highly sensitive polymer-based system (EnVision, Dako Corporation, Carpinteria, CA, USA) with the diaminobenzidine substrate solution as chromogen (Sigma, St. Louis, MO, USA). Sections were counterstained with Myer hematoxylin. Two experienced pathologists made an independent analysis of each case. Regardless the staining intensity; ten high-power fields (magnification, x 400) were examined and the percentage of mononuclear and multinucleated positively stained cells was obtained for each case. Only nuclear immunoexpression was evaluated for Ki 67 using image analysis system (Leica Qwin 500 image analysis system) by counting the positive nuclei in 500 cells in most positive fields, while cytoplasmic immunexpression was considered for CD68 and OPN.

Statistical Analysis: Pre-coded data was statistically analyzed by the Statistical Package of Social Science Software program (SPSS), version 21. Data was summarized using frequency and percentage. Comparison between groups was performed using Chi-square test or Fisher's exact test. Kappa measurement of agreement was calculated to assess the agreement between giant cells and stromal cells; marker sub-units. P values less than 0.05 were considered statistically significant and if less than 0.01 were considered highly significant.

RESULTS

A total of 41 cases were included in the present study, for the long bone cases CGCT (n=5), the age of the patients (one man and 4 women) ranged from 20 to 25 years, with a mean of 21.8 years. 3 were located in the distal femur, one in the proximal tibia and one in distal tibia. Follow up of the cases showed recurrence in one case. Thirty-six cases from the oral mucosa as well as

Table 1: Comparison between immunoexpression of different markers between CGCT of long bone and oral giant cell lesions

	CGCT (n=5)		Oral-GCL (n=36)			
Item	N	%	N	%	X^2	P value
Ki 67						
+VE	2	40.0	11	30.6	0.18	0.65 NS
-VE	3	60.0	25	69.4		
CD 68 (GC)						
+VE	2	40.0	33	91.7	3.98	0.02 S
-VE	2	60.0	3	8.3		
OPN (G C)						
+VE	3	60.0	15	41.7	0.60	0.64 NS
-VE	2	40.0	21	58.3		
OPN (G C)						
+VE	5	100.0	26	72.2	1.84	0.31 NS
-VE	0	0.0	10	27.8		
OPN (S C)						
+VE	5	100.0	19	52.8	4.0	0.07 NS
-VE	0	0.0	17	47.2		

X^2= Chi square value, S C = stromal cells, G C=giant cells, S= significant, NS= non-significant.

Table 2: Comparison between the diagnostic groups as regard different markers expression.

	G1		G2		G3		
Item	N	%	N	%	N	%	P value
Ki 67	-	-	-	-	-	-	G1*G2= 1.0 (NS)
+VE	2	40.0	8	38.1	3	20.0	G2*G3= 0.3 (NS)
-VE	3	60.0	13	61.9	12	80.0	G1*G3= 0.6 (NS)
CD 68 (GC)	-	-	-	-	-	-	G1*G2= 0.004 (S)
+VE	2	40.0	21	100.0	12	80.0	G2*G3= 0.06 (NS)
-VE	3	60.0	0	0.0	3	20.0	G1*G3= 0.1 (NS)
CD 68 (SC)	-	-	-	-	-	-	G1*G2= 1.0 (NS)
+VE	3	60.0	11	52.4	4	26.7	G2*G3= 0.2 (NS)
-VE	2	40.0	10	47.6	11	73.3	G1*G3= 0.3 (NS
OPN (GC)	-	-	-	-	-	-	G1*G2= 1.0 (NS)
+VE	5	100.0	21	100.0	5	33.3	G2*G3= <0.001 (S)
-VE	0	0.0	0	0.0	10	66.7	G1*G3= 0.03 (S)
OPN (SC)	-	-	-	-	-	-	G1*G2= 0.6 (NS)
+VE	5	100.0	17	81.0	2	13.3	G2*G3= <0.001(S)
-VE	0	0.0	4	19.0	13	86.7	G1*G3= 0.001 (S)

G1 = CGCT (n=5), G2 = CGCG (n=21), G3 = PGCG (n=15). S= significant, NS= non-significant.

Table 3: Agreement between CD 68 expressions in the giant cells (GC) and stromal cells (SC) within each diagnostic group separately.

			CD 68 (GC)						
			+VE		-VE				
Item			N	%	N	%	K	SE of K	Agreement strength
CD 68 (SC)	GCT	+VE	2	40.0	1	20.0	0.615	0.318	Good
		-VE	0	0.0	2	40.0			
	CGCG	+VE	11	52.4	0	0.0	0.0	0.0	Poor
		-VE	10	47.6	0	0.0			
	PGCG	+VE	4	26.7	0	0.0	0.167	0.111	Poor
		-VE	8	53.3	3	20.0			
	Total	+VE	17	41.5	1	2.4	0.147	0.095	Poor
		-VE	18	43.9	5	12.2			

K= Kappa measurement of agreement, SE of K = Standard error of Kappa.

Table 4: Agreement between Osteopontin (OPN) in the giant cells (GC) and stromal cells (SC) within each diagnostic group separately.

			OPN (GC)						
			+VE		-VE				
Item			N	%	N	%	K	SE of K	Agreement strength
OPN (SC)	GCT	+VE	5	100.0	0	0.0	--	--	Identical findings
		-VE	0	0.0	0	0.0			
	CGCG	+VE	17	81.0	0	0.0	0.0	0.0	Poor
		-VE	4	19.0	0	0.0			
	PGCG	+VE	2	13.3	0	0.0	0.471	0.232	Moderate
		-VE	3	20.0	10	66.7			
	Total	+VE	24	58.5	0	0.0	0.626	0.119	Good
		-VE	7	17.1	10	24.4			

K= Kappa measurement of agreement, SE of K = Standard error of Kappa

NB: Kappa could not be calculated when both variables GC & SC are fully agreed at one choice as it means they are identical i.e. both methods gives the same result without any difference to be statistically tested.

bony lesions of the jaws with male to female ratio 2:1 the age of the patients ranged from 21- 41y with mean age 26y. 10 cases of the mandible, while 26 cases of the maxilla; cases were diagnosed by H&E as PGCG (n=15); only four cases showed cupping of the underlying bone, CGCL (n=21); showed well-circumscribed multilocular defects with no obvious cortical expansion, five of them showed definitive communication with the oral cavity. The molars in some cases were notable for resorption of the roots, three of maxillary cases showed destruction of the sinuses, these cases were cellular lesions with vascular stroma and prominent mitosis by H&E, yet abnormal mitosis or necrosis was not prominent in the cases.

Immunohistochemical Results: Ki67 Results: All cases used in this study were immunopositive for ki67 (100%). No significant difference were detected when comparing the long bones lesions with the oral lesions (both peripheral and central) (P=0.65) as seen in Table 1. Cells had low proliferative index, immunopositivity is below 6% of tumor cells (Figs a, b, c).

No significant difference was also found between all lesions when comparing long bone lesions, central oral lesions and peripheral oral lesions (Table 2), [the cut-off point was counted and correlated to the higher count regarding the non neoplastic PGCG (6%)]. The more aggressive cases of CGCG of the oral cavity showed mitotic index between 2-5% while those of CGCT of long bones showed mitotic index 1-3 %.

CD68 Results: CD68 immunostaining was expressed in 40% and 91.7% of cases, within the giant cells, of CGCT of long bones and those of the oral cavity respectively. Statistically significant difference was detected (P=0.02) as shown in Table 1. While, no statistical significance was found between the expression of the CD68 in the stroma between the long bones and the oral lesions. Regarding the SC, CD68 was expressed in the SC of CGCT of long bones more than in oral lesions as shown in Table 1, but this difference was statistically insignificant (P=0.64).

Table 2 showed only significant difference between expression of CD68 in giant cells between the long bone lesions and the central oral lesions, while no significance was found between expression of CD68 in GC between any central lesion and peripheral oral granuloma and between expressions of CD68 in SC between all diagnostic groups. These results are assured in Table 3 where positive expression for CD 68 in both giant cells and stroma together were seen in 40%, 52.4 %, 26.7% of CGCT of long bones, CGCG and PGCG respectively (Figs d, e, f).

OPN Results: Table one showed that OPN is expressed in 100% of lesions of the long bones and less in the oral lesions (both peripheral and central) but this difference in expression is statistically not significant. Table 2 demonstrates that in oral lesions OPN was expressed in the GC of 33.3% and 86.7% in SC of PGCG. In CGCG it was highly expressed in both GC (100% of cases) and SC (81%) noting that the aggressive lesions showed expression in both GC and SC. For giant cell tumors of the long bone OPN was expressed in 100% of the cases in both GC and SC, statistically significant tables (Table 2). Table 4 demonstrate OPN immunostaining expression in both stromal and giant cells, immunoexpression was notable in 100%, 81%, 13.3% in CGCT of long bones, CGCG, PGCG, respectively (Figs g, h, I).

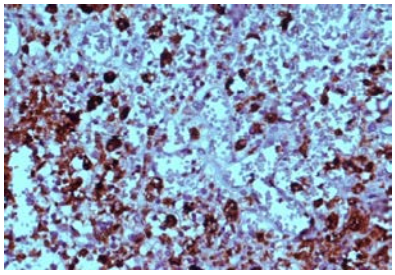

Fig. a: Case of PGCG of jaw with relative high proliferative index detected by Ki 67 immunostaining (high magnification)

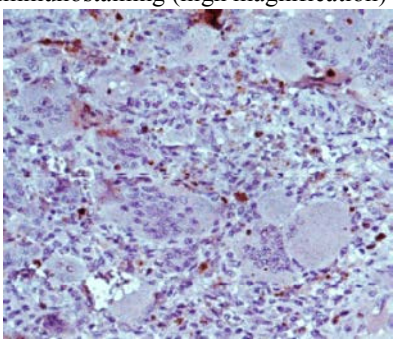

Fig. b: Ki 67 Immunostaining expression showing low proliferative activity of case of CGCG of jaw (High magnification)

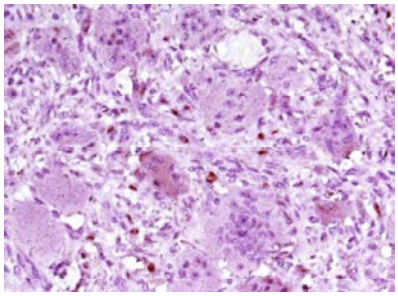

Fig. c: Few cells were immunostained by Ki 67 indicating low proliferative activity of case of CGCT of long bone (High magnification)

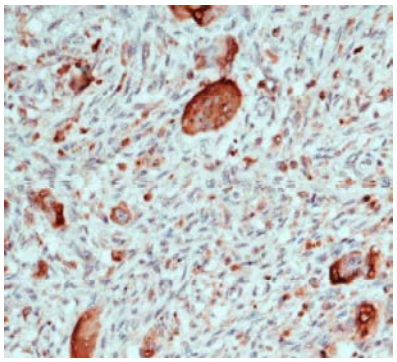

Fig. d: SC and GC showing positive CD 68 immunostaining expression in case PGCG (low magnification)

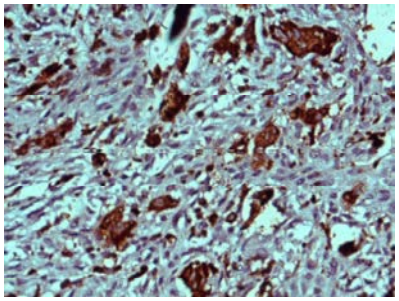

Fig. e: SC and GC showing positive CD 68 immunostaining expression in case CGCG of jaw (high magnification)

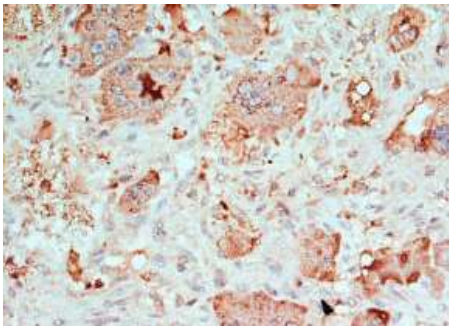

Fig. f: SC and GC showing positive CD 68 immunostaining expression in case CGCT of long bone (high magnification)

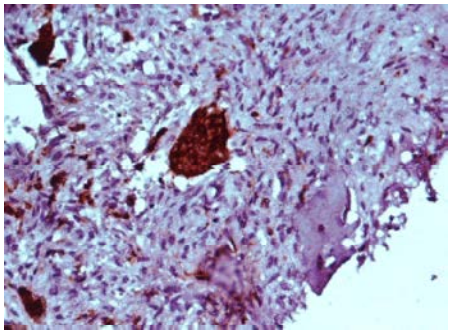

Fig. g: A case of PGCG destroying the underlying bone showing positive OPN immunostaining (high magnification)

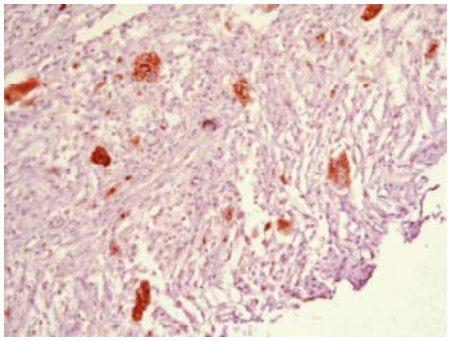

Fig. h: CGCG of jaw showing positive immunostaining for OPN (low magnification)

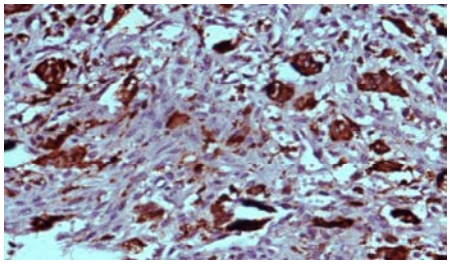

Fig. i: A case of CGCT of long bone; SC and GC showed positive immunostaining by OPN. (high magnification)

DISCISSION

Whether the CGCGs of the jaws and the CGCTs of long bones are really single pathologic process is an unanswered question [2, 18]. Conflicting behavior of the CGCGs, including, higher proliferative activity [2, 19] and aggressive osteolytic behavior challenged researches to study the pathogenesis, nature and origin of giant cells leading to understanding the diversity of behavior of such lesions [2]. In this study a total of 41 cases were included, CGCT (n=5), with recurrence in one case. 36 cases from the oral mucosa as well as bony lesions of the PGCG (n=15), while CGCL (n=21). Of the all cases there was no great difference between Ki 67 immunostaining of stromal cells in CCGT of long bones and PGCG as well as oral CGCG. Cells showed positive immunostaining had low proliferative index below 6% of tumour cells, the more aggressive cases of CGCG of the oral cavity showed mitotic index between 2-5% while those of CGCT of long bones showed mitotic index 1-3 %. Pointing to that the possibility of recurrence in case of CGCT of long bone or the ability of the lesion to cause destruction of the surrounding tissue might not be due to its proliferative power but rather caused by any other cause, inflammatory reaction is not excluded. Although Yuhree Kim *et al.* [1] stated that the stromal cells of the GCT are generally believed to be the major neoplastic and proliferative component of these lesions. Our study was in agreement with Osaka *et al.* [20], who suggested that the tumors with some of the low-ki67 staining of the cells may be of the growth cessation type or the continuously slow-growing type and these tumors might be primary, recurrent. This is also in accordance with Regezi [21] and Lau *et al.* [22], who explained that the growth process of both central and peripheral giant cell lesions is similar based on the immunohistochemical expression of TNF-α, IL–6 and IL–1β. This means that the aggressive behavior, seen in some cases, is not related to the cellular proliferation. However, Regezi [21] stated that the mononuclear spindle shaped cells of the PGCG and CGCG have been suggested that they represent the proliferating part of the lesion responsible for the biologic activity of these tumors, these mononuclear cells resembles immature osteoblasts by releasing some factors including NF-êB legend, RANKL, colony stimulating factor and other factors.

In present study CD68 immunostaining was expressed in 40% and 91.7% of cases within the giant cells of CGCT of long bones and those of the oral cavity respectively. Regarding the immunostaining results of OPN, it was expressed in the GC of 33.3%, while 86.7% in SC of PGCG, in oral CGCG it was highly expressed in both GC (100% of cases) and SC (81%) noting that the aggressive lesions showed expression in both GC and SC, as infiltration of CD68-positive cells is used clinically as a marker of inflammation and tumor progression. Increased trabecular bone without a corresponding decrease in osteoclasts suggests that loss of CD68 negatively impacts osteoclast function without reducing osteoclast numbers *in vivo*. Papanicaulao *et al.* [2] noticed that spindle shaped cells in both PGCG and CGCG releases TNF-α which is responsible for stimulating osteoclastic bone resorption, but it is significantly more in CGCG [2]. This may explain the difference in behavior and rate of bone resorption between the two lesions. Gamberi *et al*. [23] found that IL-6 is expressed by giant cells in GCTs and may be responsible for its biologic aggressiveness. Friedrich et al. stated that, due to the presence of bone resorption adjacent to PGCGs, this lesion have similar cellular composition as giant cell lesions of different sites and the giant cells in these lesions express the same osteolytic proteases and osteoclast activating cytokines involved in bone metabolism [24]. The locally aggressive osteolytic activity that giant-cells exhibit is further explained by the expression of other matrix metalloproteinases such as type IV collagenase (MMP-2) [1].

For giant cell tumors of the long bone in our study, OPN was expressed in 100% of the cases in both GC and SC. The positive expression of CD68 in both giant cells and stroma together were seen in 40%, 52.4 %, 26.7% of CGCT of long bones, CGCG and PGCG respectively. While for OPN immunostaining, expression in both stromal and giant cells was notable in 100%, 81%, 13.3% in CGCT of long bones, CGCG, PGCG respectively, indicating that the giant cells in all studied lesions, for most of the cases, might be of the same origin and are of macrophage lineage. Lund *et al.* [11] considered that OPN is important in promoting migration and retention of macrophages in sites of inflammation, it is also important in regulation of foreign body giant cells formation [11]. The poor matrix

support to the vessels may lead to frequent and profuse intraosseous hemorrhage attracting blood-derived monocytes with active conversion into osteoclasts, resulting in GCTB formation [9], therefore all giant cell lesions might not be a true neoplasm but reactions to several insults such as hemorrhage. Moreover, the diversity of behavior may be due to the action of macrophages and the idea that osteopontin might release chemical mediators that causes collagen lysis, so contributes in extracellular matrix destruction. This matches the study by O'regan and Berman [15], who proposed in their review that OPN appears to function at the inflammatory phase of a pathological response and despite of being a multifunctional protein, it regulates aspect of inflammation and tissue repair. It is also suggested that it may regulate bone resorption and repair. In addition, Ul Hapue *et al.* [9] and Osaka *et al*. [20] stated that some rare benign GCT, proven immunohisto chemically, were found with lung metastases, suggests that it might be associated or misdiagnosed as other tumors such as giant cell–rich osteosarcoma and malignant fibrous histiocytoma of bone.

CONCLUSION

There was no great difference in proliferative activity in CGCT of long bones, CGCG and PGCG of the jaw. The positive immunostaining of stromal and giant cells for both CD68 and osteopontin may point out that the delineation of giant cells in the three lesions is macrophages, as these lesions might be reactive in nature rather than neoplastic. PGCG might be their soft tissue counterpart as the bone destruction; aggressiveness in the CGCG as well as CGCT could be due to inflammatory substances affecting the collagen matrix. Some CGCT of long bones may be misinterpreted as true tumors rich in giant cells although they are inflammatory reaction mediated by osteopontin. So we recommend further immunostaining study of stromal cells as well as giant cells with both CD68 and osteopontin for those metastasizing and non-metastasizing tumors to compare the exact cellular histogenesis.

REFERENCES

1. Yuhree Kim, M.D., B.S. Saqib Nizami, M.S. Hana Goto and F. Y. Lee, 2012. Modern Interpretation of Giant Cell Tumor of Bone: Predominantly Osteoclastogenic Stromal Tumor. Clinics in Orthopedic Surgery, 4: 107-116.
2. Panagiota Papanicolaou, Evanthia Chrysomali, Evangelia Stylogianni, Catherine Donta and Dimitris Vla-chodimitropoulos, 2012. Increased TNF-α, IL-6 and decreased IL-1β immunohistochemical expression by the stromal spindle-shaped cells in the central giant cell granuloma of the jaws. Med. Oral Patol. Oral Cir. Bucal., 17(1): 56-62.
3. Motamedi, M.H., N. Eshghyar, S.M. Jafari, E. Lassemi, F. Navi, F.M. Abbas, *et al*., 2007. Peripheral and central giant cell granulomas of the jaws: a demographic study. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 103: e39-43.
4. Liu, B., S.F. Yu and T.J. Li, 2003. Multinucleated giant cells in various forms of giant cell containing lesions of the jaws express features of osteo-clasts. J. Oral Pathol. Med., 32: 367-75.
5. Katsikeris, N., E. Kakarantza-Angelopoulou and A.P. Angelopoulos, 1988. Pe- ripheral giant cell granuloma. Clinicopathologic study of 224 new cases a review of 956 reported cases. Int. J. Oral Maxillofac. Surg., 17: 94-99.
6. Padam Narayan Tandon, S.K. Gupta, Durga Shanker Gupta, Sunit Kumar Jurel and Abhishek Saraswat, 2012. Peripheral giant cell granuloma: Contemp. Clin. Dent., 3(Suppl1): S118-S121.
7. Amalia Conti, Gemma Caballero Rodriguez, Antonella Chiechi, Rosa Maria Dégano Blazquez, Victoria Barbado, Tibor Krènacs, Chiara Novello, Laura Pazzaglia, Irene Quattrini, Licciana Zanella, Piero Picci, Enrique De Alava and Maria Serena Benassi, 2011. Identification of Potential Biomarkers for Giant Cell Tumor of Bone Using Comparative Proteomics Analysis. American Journal of Pathology, 178(1): 88-97.
8. Mathias Werner, 2006. Giant cell tumour of bone: morphological, biological and histogenetical aspects. International Orthopaedics (SICOT), 30:484–489 DOI 10.1007/s00264-006-0215-7.
9. Anwar Ul Haque and Ambreen Moatasim, 2008. Review Article: Giant Cell Tumor of Bone: A Neoplasm or a Reactive Condition? Int. J. Clin. Exp. Pathol., 1: 489-50.
10. Ira J. Miller, Alan Blank, Suellen M. Yin, Allison Mcnickle, Robert Gray and Steven Gitelis, 2010. Case Report: A case of recurrent giant cell tumor of bone with malignant transformation and benign pulmonary metastases. Diagnostic Pathology, 5:62 doi: 10.1186/1746-1596-5-62.

11. Susan Amanda Lund, Cecilia M. Giachelli and Marta Scatena, 2009. The role of osteopontin in inflammatory processes J. Cell Commun. Signal., 3: 311-32.
12. Chellaiah, M.A., N. Kizer, R. Biswas, U. Alvarez, J. Strauss-Schoenberger, L. Rifas, S.R. Rittling, D.T. Denhardt and K.A. Hruska, 2003. Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. Mol. Biol. Cell, 14(1): 173-189.
13. Tsai, A.T., J. Rice, M. Scatena, L. Liaw, B.D. Ratner and C.M. Giachelli, 2005. The role of osteopontin in foreign body giant cell formation. Biomaterials, 26(29): 5835-5843.
14. Weber, G.F., S. Zawaideh, S. Hikita, V.A. Kumar, H. Cantor and S. Ashkar, 2002. Phosphorylation-dependent interaction of osteopontin with its receptors regulates macrophage migration and activation. J. Leukoc. Biol., 72(4): 752-761.
15. O'regan, A. and Jeffrey S. Berman, 2000. Osteopontin: a key cytokine in cell-mediated and granulomatous inflammation. J. Exp. Path., 81: 373-390.
16. Maria do Socorro Aragão, Marta Rabello Piva, Cassiano Francisco Weege Nonaka, Roseana de Almeida Freitas, Lélia Batista de Souza, Leão Pereira Pinto, 2007. Central giant cell granuloma of the jaws and giant cell tumor of long bones-an immunohistochemical comparative study. J. Appl. Oral Sci., 15(4): 310-316.
17. Jason W. Ashley, Zhenqi Shi, Haibo Zhao, Xingsheng Li, Robert A. Kesterson and Xu Feng, 2011. Genetic Ablation of CD68 Results in Mice with Increased Bone and Dysfunctional Osteoclasts. PLoS One, 6(10): e25838.
18. Saw, S., N. Thomas, M.J. Gleeson, I. Bódi, S. Connor and T Hortobágyi, 2009. Giant cell tumour and central giant cell reparative granuloma of the skull: do these represent ends of a spectrum? A case report and literature review. Pathol. Oncol. Res., 15: 291-5.
19. De Souza, P.E., J.F. Paim, J.N. Carvalhais and R.S. Gomez, 1999. Immunohistochemical expression of p53, MDM2, Ki-─67 and PCNA in central giant cell granuloma and giant cell tumor. J. Oral Pathol. Med., 28: 54-58.
20. Osaka, S., H. Sugita, E. Osaka, Y. Yoshida, J. Ryu, A. Hemmi and K. Suzuki, 2004. Clinical and immunohistochemical characteristics of benign giant cell tumour of bone with pulmonary metastases: case series. Journal of Orthopaedic Surgery, 12(1): 55-62.
21. Regezi, J.A., 2004. Comments on the pathogenesis and medical treatment of central giant cell granulomas. J. Oral Maxillofac. Surg., 62: 116-118.
22. Lau, Y.S., A. Sabokbar, C.L. Gibbons, H. Giele and N. Athanasou, 2005. Phenotypic and molecular studies of giant cell tumors of bone and soft tissue. Hum. Pathol., 36: 945-54.
23. Gamberi, G., M.S. Benassi, P. Ragazzini, L. Pazzaglia, F. Ponticelli and C. Ferrari, *et al.*, 2004. Proteases and interleukin-6 gene analysis in 92 giant cell tumors of bone. An Oncol., 15: 498-503.
24. Friedrich, R.E., J. Eisenmann, K. Röser, H.A. Scheuer and T. Löning, 2010. Expression of proteases in giant cell lesions of the jaws, tendon sheath and salivary glands. Anticancer Res., 30: 1645-52.