Peripheral giant cell granuloma: immunohistochemical analysis of different markers. Study of three cases

Falaschini S*, Ciavarella D*, Mazzanti R**, Di Cosola M***, Turco M***, Escudero N, Bascones A****, Lo Muzio L*

SUMMARY

Peripheral giant cell granuloma (PGCG) is a non-neoplastic lesion representing a local hyperplastic reaction to injury or inflammation. It is known to be a reactive soft tissue lesion that develops only within the oral cavity, with a slightly predilection for female sex. The usual localization for PGCG is the premolar region and the crest of the edentulous ridge.

This study presents three cases of PGCG, including 2 male and 1 female, with an age comprised between 25 and 35 years. All patients were treated with resection biopsy and no one relapsed.

With the aim of determine the probable origin of stromal mononuclear cells and multinuclear giant cells, each case was then studied by immunohistochemistry to evaluate the expression of endothelial and monocyte/macrophage lineage.

Immunohistochemical results showed a strong diffuse positivity for CD-68 in round mononuclear stromal cells and in multinucleate giant cells. These latter were immunonegative for CD-34 and only focally positive for á-1 antitrypsin.

These results suggest that multinucleated giant cell shows an osteoclast phenotype and that probably derive from monocyte/macrophage lineage and that do not derive from the endothelial cells of the capillary.

In second instance, we underlined the importance of an exhaustive dia.

Key words: Peripheral Giant Cell Granuloma (PGCG), immunohistochemistry.

Received: April 2007.
Acepted for publication: May 2007.

* Department of Surgical Sciences, "Universitá di Foggia", Foggia, Italia.
** Department of Dental Sciences, University of Palermo, Palermo, Italy.
*** Institute of Pathology, University of Ancona, Ancona, Italy.
**** U.O. Odontostomatologia e Chirurgia Orale Z.T 6 e 7 ASUR MARCHE, Italy.

Falaschini S, Ciavarella D, Mazzanti R, Di Cosola M, Turco M, Escudero N, Bascones A, Lo Muzio L. Peripheral giant cell granuloma: immunohistochemical analysis of different markers. Study of three cases. *Av. Odontoestomatol* 2007; 23 (4): 189-196.

INTRODUCTION

Peripheral giant cell granuloma is an infrequent reactive, exophytic lesion of the oral cavity, also known as giant-cell epulis, osteoclastoma, giant cell reparative granuloma, or giant cell hyperplasia. It is the most frequent giant cell lesion of the jaws, and originates from the connective tissue of the periosteum or from the periodontal membrane, in response to local irritation or chronic trauma (1). It is

more frequent in women than in men, with a slightly higher prevalence in the 30- to 70-year-old-age group, and affects largely the lower jaw (55%) than in the upper jaw (the reported proportion being 2,4:1) (2).

Cases of PGCG have been documented in children, where the lesion appears to be more aggressive, with absorption of the interproximal crest area, displacement of the adjacent teeth and multiple recurrences (3).

Clinically, it manifests as a soft to firm, bright nodule or as a sessile or pediculate mass, which is predominantly bluish red with a smooth shiny or mamillated surface, localized in the gingival tissue or alveolar processes of the incisor and canine region (1), through according to Pindborg the preferential location is the premolar and molar zone (4). The lesion ranges in size from small papules to enlarged masses, though reportedly rarely exceeding 2 cm in diameter, and are generally located in the interdental papilla, edentulous alveolar margin, or at marginal gum level (5). It is basically asymptomatic, in fact pain is not a common characteristic, and lesion growth in most cases is induced by repeated trauma such as with occlusion, in which case it may ulcerate and becomes infected (6, 7).

Although the pathogenesis of oral cavity PGCGs is still uncertain, local irritants such as calculus, bacterial plaque, periodontitis, periodontal surgery, ill-fitting dentures, overhanging restorations and tooth extractions are suggested as the etiological causes (8-10). These are soft tissue lesions that rarely affect the underlying bone, though the latter may suffer erosion (5, 11). Treatment comprises surgical resection, with extensive clearing of the base of the lesion to avoid relapses (12).

The present study describes three clinical cases of peripheral giant cell granuloma located in different areas. We analyzed the presence and tissue localization of several markers such as CD68, CD34 and α1- antitrypsin by immunohistochemistry, for the purpose of evaluating the origin of stromal mononuclear cells and multinucleate giant cells. Finally we underlined the importance of a early clinic, radiographic and histologic diagnosis to prevent possible damages to the teeth and adjacent bone

CLINICAL CASES

Case 1

A 27-years-old Bolivian woman without disease antecedents of interest or known drug allergies referred for resection-biopsy of a gingival epulis. The lesion was located between the second and third molar, measured 1.8×1.5 cm in size, was purple in colour and bleeding if touched (fig. 1a). The periapical X-rays showed a bone loss between 1.7 and 1.8, demonstrating the possible involvement of the periodontal ligament (fig. 1b).

Treatment consists of lesion resection under infiltrating anaesthesia, followed by the total avulsion of 17 and 18. The histological study confirmed the diagnosis of peripheral giant cell granuloma, described as ulcerated fibrous epulis with moderate chronic lymphoplasmacellular inflammatory infiltrate. Control examination after six days showed the complete restitution ad integrum of the tissue; six months after resection there were not signs of relapse.

Case 2

A 25-years-old Italian male referred for removal of a tooth, 15, because it is very damaged and difficult to

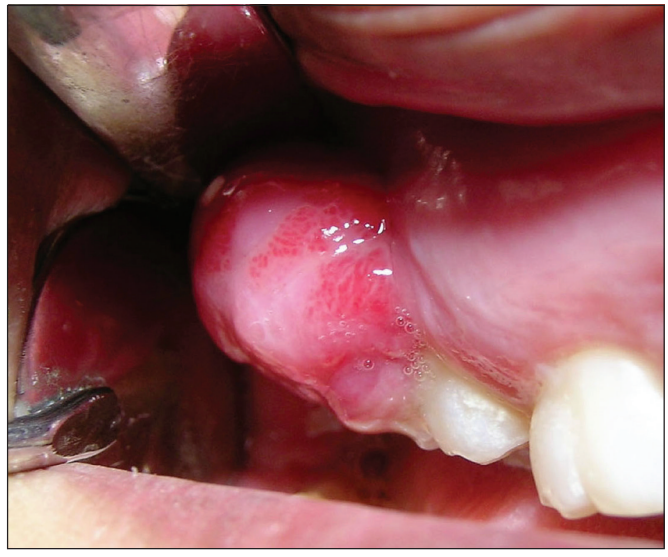

Fig. 1a. Case 1: The peripheral giant cell granuloma localized between second premolar and third top molar.

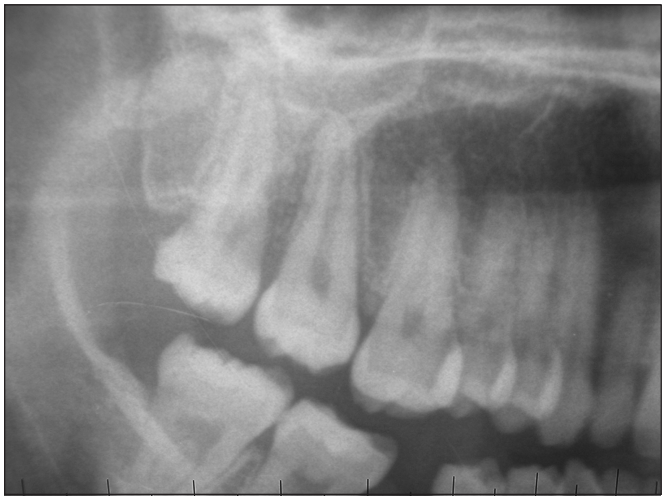

Fig. 1b. Case 1. Periapical radiography.

restore. Intraoral examination revealed also the presence of bad oral hygiene with several amounts of plaque on the surface of all teeth, and the absence of 27, 34 and 46. The 15 avulsion was performed under infiltrating local anaesthesia. There were no complications in the immediate postoperative period, but after a week, when the patient returned to the dentist to removal the suture, he showed an exophytic lesion in the treated region. It had a pedunculate base, soft consistency and purple in colour, measuring 1.5×2 cm in size (fig. 1c). Resection biopsy was performed, and the histological study showed the presence of peripheral giant cell granuloma.

Case 3

A 35-years-old Italian male with a history of cardiac infarction referred with an exophytic lesion located between 42 and 43. Clinical exploration revealed a soft large-base purple coloured lesion, measuring 1.5×2 cm in size (fig. 1d). The patient referred only slight discomfort during mastication and bleeding with the brushing. Tooth 43 was vital, there was no painful to percussion and no mobility to palpation and the oral hygiene was very deficient. The X-rays study did not show bone disruption and radicular reabsorption of 43. The lesion was treated by surgery using infiltrating local anaesthesia, with a curettage of the radicular surface after the resection. The histological diagnosis was peripheral giant cell granuloma.

Each case was then studied by immunohistochemistry to evaluate some inflammatory, endothelial and stromal markers.

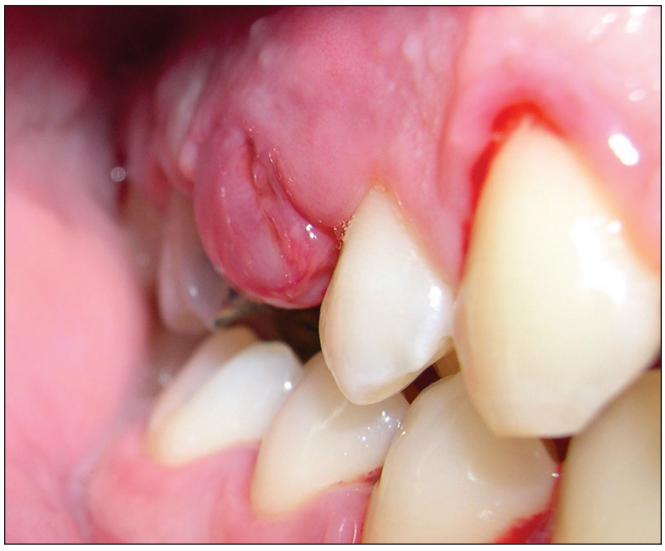

Fig. 1c. Case 2. The peripheral giant cell granuloma in the extraction place.

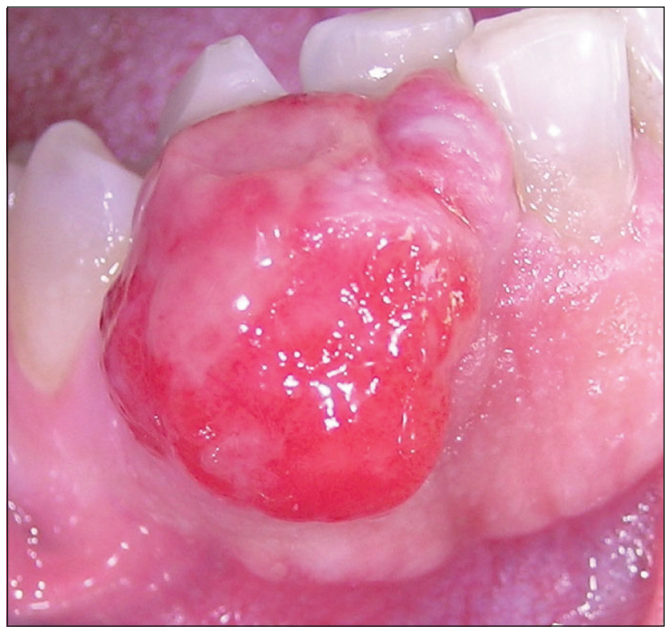

Fig. 1d. Case 3. The peripheral giant cell granuloma localized between 42 and 43.

Immunohistochemical analysis

A formalin fixed, paraffin embedded block from a representative area of the lesion was selected. Serial sections were cut at 4 micron and mounted. One slide of these was stained with hematoxylin and eosin, the others examined immunohistochemically by the avidin-biotin peroxidases complex method, using the following monoclonal primary antibodies: anti–CD68 (PG-M1, DAKO, Carpinteria, CA, USA) at diluition of 1:200, anti-CD34 (QBEND-10, DAKO, Carpinteria, CA, USA) at diluition of 1:500 and anti- α-1 antitrypsin (N1533, DAKO, Carpinteria, CA, USA) at diluition of 1:1000.

Immunohistochemically, macrophages and giant cells share similar antigenic inflammatory markers, such as those studied by us (13-15).

Immunohistochemistry is performed on the sections mounted on poly-l-lysine-coated glass slides. Deparaffinized and rehydrated sections are incubated for 30 minutes in 3% H_2O_2/methanol to quench endogenous peroxidase activity and then rinse for 20 minutes with phosphate-buffered saline (PBS) (Bio-Optica M107, Milan, Italy). Nonspecific protein binding is attenuated by incubation for 30 minutes with 5% horse serum in PBS. Specimens are incubated overnight with the monoclonal mouse antihuman CD34, CD68 and α-1 antitripsina protein. The antibody is applied directly to the section and the slides are incubated overnight (48C) in a humidified chamber. The sections are washed 3 times with PBS at room temperature. Immune complexes are subsequently treated with the secondary biotinylated antibody and then detected by streptavidin peroxidase, both incubated for 30 minutes at room temperature (Vectastain ABC kit, Vector Laboratories, Burlingame, Calif). After rinsing with 3 changes of PBS the immunoreactivity is visualized by development for 2 minutes with 0.1% 3,3V-diaminobenzidine and 0.02% hydrogen peroxide (DAB substrate kit, Vector Laboratories). Sections are counterstained with Mayer's haematoxylin, mounted with permanent mounting medium, and examined by light microscopy.

In each simple we analysed the differents histological and Immunohistochemical characteristic of 25 random lands using a conventional microscope with high potency of magnification (magnification 400×) (the pathologist performed a semiquantative analysis of the sample and he didn't use a software of the image).

The histological carachetristics of these lesions consisteded in an hyperplastic granulation tissue with many multinucleated giant cells (Figs. 2a, b). These giant cell were localized in the deep corion in a vascular stroma of ovoid and spindle-shaped fibroblastys. There were also several areas characterised by haemorrhage, underlined by the presence of Fe deposits.

Immunohistochemically, all lesions showed a consistent immunopositivity for CD68 in macro-

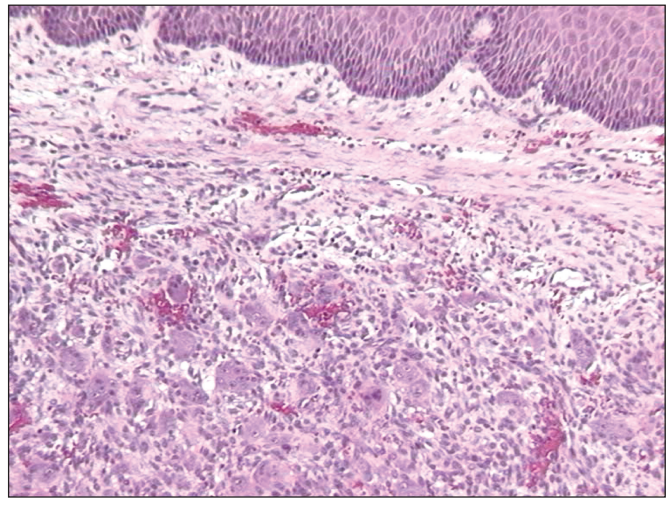

Fig. 2a. Case 2.

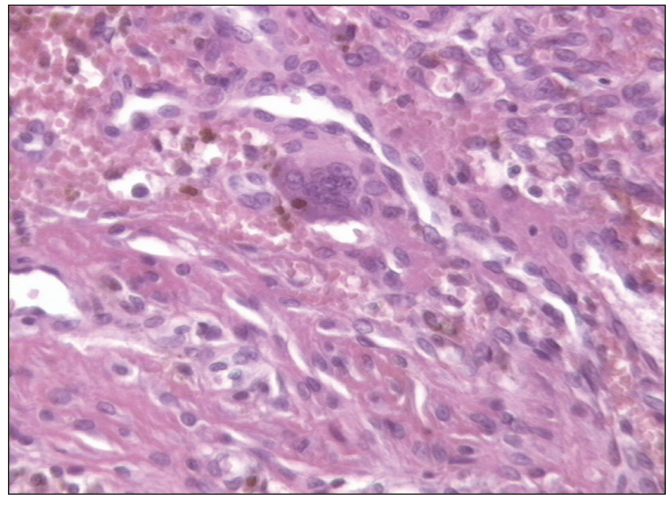

Fig. 2b. Case 2.

phages, monocyte and, in particular, in multinucleated giant cells (fig. 3a). Interestingly, in the periphery of the lesion these ones showed a moderate positivity of the blood vessels to CD34 related antigen (fig. 3b), reaction not evident deeper in the lesion within the aggregations of giant cells. The stromal cells and histiocytes were also positive for α-1 antitrypsin (fig. 3c).

DISCUSSION

Peripheral giant cell granuloma (PGCG) is a benign lesion characterized by a hyperplastic reaction to local injury or chronic trauma, developing only within the oral cavity (Flaitz CM 2000). The usual localization for PGCG is the gingival tissue in premolar region and the crest of the edentulous ridge. It is never found on mucosa that is not attached to bone (8). It is most common than central giant cell granuloma with a ratio of approximately 3:1, in fact Junquera and co-workers (10) mentioned the rarity of CGCG (0.4%-1.9%) in light of the related literature.

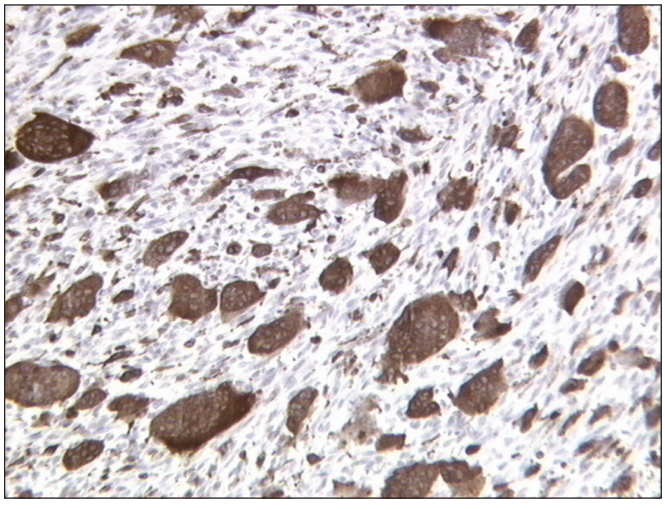

Fig. 3a. Analysis immunohistochemical of CD-68 (×100).

Histologically, PGCG presents as a not-well circumscribed mass, constituted by fibrillar collagenous stroma containing two types of mononuclear cells (spindle and ovoid cells) and interspersed numerous multinucleated giant cells "osteoclasts-like" or larger than typical osteclasts, having rarely normal bone resorptive function. Sometimes these cells are also localized in the internal wall of vessels.

It is present a chronic and often acute inflammatory infiltrate and hemosiderin-laden macrophages surround areas of haemorrhage (16). It is characterized by rich vasculature, particularly in the peripheral areas, consisting mainly of thin walled, small sized vessels.

It contains numerous multinuclear giant cells, but when compared to giant cell tumour, it is more fibrous.

Its pathogenesis is not been thoroughly investigated. Several immunohistochemical studies have focused

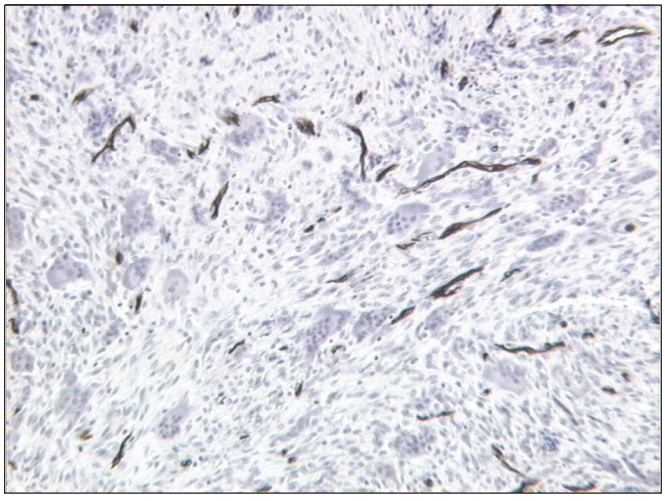

Fig. 3b. Analysis immunohistochemical of CD-34 (×100).

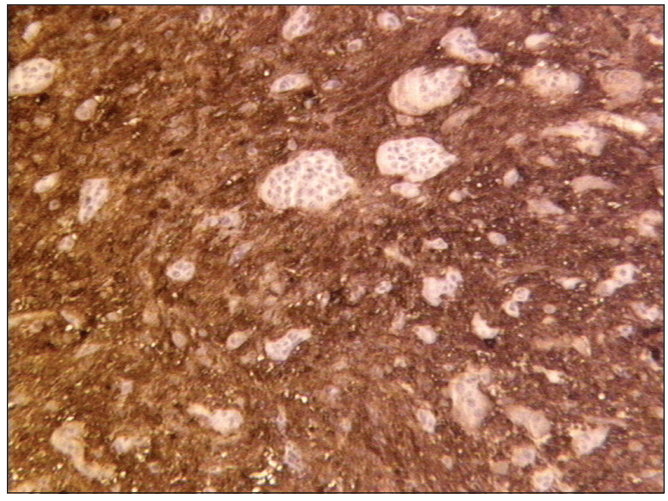

Fig. 3c. Analysis immunohistochemical of α-1 anti-tripsin (×250).

on identifying the nature and the interrelations between cellular components in the formation of GCG: the results have suggested that the mononuclear stromal cells may originate from fibroblasts and cells of histiocytic origin whereas the origin of giant cells has still been a source of controversy: in fact some authors suggest that they arise secondary to an alteration of the endothelial cells of the capillaries (Flaitz CM 2000), others as a consequence of a traumatic mechanism (some similarities to the osteoclasts) (17, 18).

Palacios et co-workers suggested that giant cell formation to be a fusion of hystiocytes, endothelial cells and fibroblasts (18).

Our immunohistochemical evaluation revealed a diffuse presence of CD68 (antigen most widely distributed in monocyte/macrophages lineage at various differentiation stages as well dendritic cells and osteoclasts) in a fraction of round mononuclear stromal cells and in mononuclear giant cells. This result confirms that these latter may derive from osteoclasts, according to previous study (19).

In addition, it is interesting to show the staining pattern of the blood vessels to CD34 related antigen in the peripheral giant cell granuloma: the capillaries on the periphery of the lesions were strongly positive for this antibody (fig. 3b), reaction product not evident in the lesion within the aggregations of multinucleate giant cells. This data may suggest that multinucleate giant cell not arise from endothelial cells of the capillaries.

The present case study was performed also to evaluate the role of the alpha1-antitrypsin (α_1-AT) in patients with PGCG. α_1-AT is a physiological inhibitor of activated protein C and therefore decreases activated protein C activity. α_1-AT, a 52,000 D glycoprotein, is secreted mostly by hepatocytes, lung epithelial cells and phagocytes. α_1-AT inhibits a variety of serine proteinases by its active site (Met358-Ser359), but its preferential target is human neutrophil elastase (HNE) as demonstrated by the high association rate constant (K_{ass}) for this proteinase. The immunohistochemical analysis showed a diffuse, strong immunopositive of mononuclear stromal cells ad only focally for multinuclear giant cells for α-1 antitrypsin (fig. 3c). These data pointed out that this antigen is able to inhibit the activity of human neutrophil elastase (fig. 3c).

The differential diagnosis of PGCG particularly involves giant cell tumour (Chaparro-Avendano AV 2005): nonossifying fibroma which differs from PGCG lesions in consistency and colour; pyogenic granuloma which is difficult to distinguish from PCGC lesions; CGCG which is an expansive and destructive intraosseous lesion that can perforate the cortex, mimicking PGCG; chondroblastoma which, localized in the gum, may provoke irregular bone destruction below the exophytic lesion; odontogenic cyst; parulis, which is frequently associated with a necrotic tooth or with periodontal disorder; haemangioma cavernosum, which is distinguished from PGCG lesions by their pulsatile nature; fissured epulis (9).

The treatment of choice is surgical excision with the suppression of the underlying etiologic factors (5). The periosteum must be included in the excision to prevent recurrences; in fact recurrence is frequent and is observed in 5% and 11% of cases according to Eversole (22) and Mighell (23) respectively. Curettage in addition to the excision to remove the base of the lesion also has been suggested. The recurrence rate of PCGC has been reported to range from 5-70,6%. This wide variation may be attributed to the surgical technique used in excision (8-10).

In conclusion our immunohistochemical study suggests, according with previous immunohistochemical study, that multinucleated giant cell shows an osteoclast phenotype and that probably derive from monocyte/macrophage lineage and that giant cells do not derive from the endothelial cells of the capillary.

In second instance an early and precise diagnosis of PGCG, based on the clinical, radiological findings and histological study, allows conservative management with a lower risk for the teeth and adjacent bone. In all cases described here, the treatment applied is that reported in the literature (5, 7), consisting of surgical excision and subsequent

curettage to remove the base of the lesion and all associated irritant factors (9).

REFERENCES

1. Shafer WG, Hine MK, Levi BM. Tratado de patologìa Bucal. eds. 4th. Rio de Janeiro: Guanabara-Koogan; 1987. p.143-5.

2. Reichart PA, Philipsen H. Atlas de Patologìa Oral. eds.1 Masson; 2000. p. 164.

3. Wolfson L, Tal H, Covo S. Peripheral giant cell granuloma during orthodontic treatment. Am J Orthod Dentofac Orthop 96: 519-23.

4. Bagàn Sebastiàn JV. Atlas de enfermedales de la mucosa oral. eds. 5th Barcelona; 1995. p. 186.

5. Flaitz CM. Peripheral giant cell granuloma: a potentially aggressive lesion in children. Pediatr Dent 2000; 22: 232-3.

6. Nedir R, Lombardi T, Samson J. Recurrent peripheral giant cell granuloma associated with cervical resorption. J Periodontol 1997; 67:381-4.

7. Pandolfi PJ, Felefli S, Flaitz CM, Johnson JV.An aggressive peripheral giant cell granuloma in a child. J Clin Pediatr Dent 1999; 23: 353-5.

8. Breault LG, Fowler EB, Wolfgang MJ, Lewis DM. Peripheral giant cell granuloma: a case report. Gen Dent 2000; 48:716-9.

9. Gandara-Rey JM, Pacheco Martins Carneiro JL, Gandara-Vila P, Blanco-Carrion A, Garcia-Garcia A, Madrinan-Grana P, Martin MS. Peripheral giant cell granuloma. Review of 13 cases. Med Oral 2002;7:254-9.

10. Junquera LM, Lupi E, Lombardia E, Fresno MF. Multiple and synchronous peripheral giant cell granulomas of the gums. Ann Otol Rhinol Laryngol 2002; 111: 751-3.

11. Ceballos-Salobrena A. Tumores benignos de la mucosa oral. En: Bagàn-Sebastian JV, Ceballos-Salobrena A, Bermejo-Fenoll A, Aguirre-Urizar JV, Penarrocha-Diago M. Medicina Oral; 1995. p.182-3.

12. Kfir Y, Buchner A, Hansen LS. Reactive lesions of the gingiva. A clinicopathological study of 741 cases. J Periodontol 1980; 51: 655-61.

13. Regezi JA, Zarbo RJ, Lloyd RV. Muramidase, alpha-1 antitrypsin, alpha-1 antichymotrypsin, and S-100 protein immunoreactivity in giant cell lesions. Cancer. 1987 Jan 1;59(1):64-8

14. Regezi JA, Nickoloff BJ, Headington JT Oral submucosal dendrocytes: factor XIIIa+ and CD34+ dendritic cell populations in normal tissue and fibrovascular lesions. J Cutan Pathol. 1992 Oct;19(5):398-406.

15. Panico L, Passeretti U, De Rosa N, D'Antonio A, De Rosa G. Giant cell reparative granuloma of the distal skeletal bones. A report of five cases with immunohistochemical findings. Virchows Arch. 1994;425(3):315-20.

16. Wood NK. Diagnòstico diferencial de las lesiones orales y maxilofaciales. Madrid: Harcourt Brace de Espana SA;1998. p. 141-2.

17. Matsumura T, Sugahara T, Wada T, Kawakatsu K. Recurrent giant-cell reparative granuloma: report of case and histochemical patterns. J Oral Surg 1971; 29: 212-6.

18. Sapp JP. Ultrastructure and histogenesis of peripheral giant cell reparative granuloma of the jaws. Cancer 1972; 30: 1119-29.

19. Palacios E, Valvassori G. Giant cell reparative granuloma. Ear Nose Throat J 2000; 79: 688.

20. Burghaus B, Langer C, Thedieck S, Nowak-Gottl U. Elevated alpha1-antitrypsin is a risk factor for arterial ischemic stroke in childhood. Acta Haematol. 2006;115(3-4):186-91

21. Chaparro-Avendano AV, Berini-Aytes L, Gay-Escoda C. Peripheral giant cell granuloma. A report of five cases and review of the literature. Med Oral Patol Oral Cir Bucal. 2005; 10(1): 53-7.

22. Eversole LF, Rovin S. Reactive lesions of the gingiva. J Oral Pathol 1972; 1: 30-8.

23. Mighell AJ, Robinson PA, Hume WJ. Peripheral giant cell granuloma: a clinical study of 77 cases from 62 patients, and literature review. Oral Dis1995;1: 12-9.

CORRESPONDENCE

Prof. Lorenzo Lo Muzio
Via Carelli 28
71100 Foggia – Italia.
Phone nunber and Fax 0039 0881 685809
Correo electrónico: *lomuziol@tin.it* o *llomuzio@tin.it*