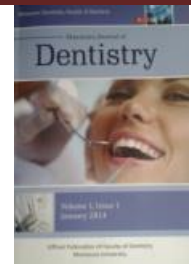




Assessment of Vascular Density, Myofibroblastic Activity and Cellular Cannibalism in Aggressive versus Non-Aggressive Central Giant Cell Granuloma of the Jaws



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Abstract:

Giant cell lesions in oral tissues occur as extraosseous lesions of soft tissues and intraosseous masses within the jaws. The CGCG was classified as reactive proliferative lesion and a neoplastic lesion due to its biological behavior and histopathological features. The study was carried out on forty paraffin blocks divided into aggressive and non-aggressive according to clinic-radiographical data. Hematoxylin and eosin staining was performed and then examined by light microscope for evaluation of the studied cases for confirmation of the diagnosis and for evaluation of cellular cannibalism. This study aimed to evaluate cellular cannibalism, angiogenicity and myofibroblastic activity in aggressive and non-aggressive CGCG lesions and assess the correlation between these parameters with the aggressiveness of these lesions. The results of the study showed a high level of significant difference between aggressive and non-aggressive CGCG lesions as regard cellular cannibalism, microvascular density using CD-34 and myofibroblastic activity using α -SMA. In addition, a high positive correlation was found between cellular cannibalism, CD-34 and α -SMA expression. From the current study, it can be concluded that: Findings as even distribution of giant cells with clear zones in most cases in addition to cellular cannibalism, which found in all cases might indicate a neoplastic nature of CGCG lesion rather a reactive lesion. In addition, cellular cannibalism can be used as a simple histopathological predictor of the behavior of CGCG lesions using H and E stain. There is a reciprocal relationship found between cellular cannibalism, angiogenicity and myofibroblastic differentiation where their high representation indicate aggressiveness of CGCG lesion. The higher prevalence of cellular cannibalism, angiogenicity and myofibroblastic activity might be used to anticipate the behavior of the lesion that make the follow up a mandatory issue in selected cases to give attention for recurrent cases.

Keywords

Giant cells, giant cell lesions, central giant cell granuloma, angiogenicity, CD-34, myofibroblasts, myofibroblastic activity, α -SMA, prognosis.

Introduction

The origin of giant cells has never been clearly established. Although they resemble osteoclasts in engulfing tissues, rarely they are viewed carrying a normal resorptive function like those cells. (1) There are many oral lesions that contain multinucleated giant cells (MNGCs) either extraosseous or intraosseous lesions. The central giant cell granuloma (CGCG) is a specific type of giant cell lesions that contains giant cells as a pathognomonic criterion. This lesion was considered as reactive proliferative lesion and a neoplastic lesion due to its clinical-radiographical behavior and histopathological features. It was classified according to its clinical and radiographical behavior into aggressive and non-aggressive CGCG. Where in aggressive lesions there are larger sizes, pain association and sometimes cortical perforation. In non-aggressive type, the sizes of the lesions are relatively smaller, no pain and cortical perforation never occur. Recurrence was also reported in some of the aggressive cases. (2, 3) Histopathologically lesions contain the stromal proliferation of fibroblasts and myofibroblasts, inflammatory cells, MNGCs, bone trabeculae and areas of hemorrhage may be present in several lesions. (3)

Cellular cannibalism is known incident of engulfment of a living cell to another living cell leading to its death "the internalized one". (4)

Vascular proliferation or angiogenesis is an important process in such tumor development and growth thus this criterion was assessed using vascular marker CD-34 which was used to measure how can the angiogenicity used as a prognostic marker of aggressiveness and development of the lesion. (5, 6)

Fibrosis of tissues may be a sign of healing but in cases of exaggerated fibrosis, it leads to excessive contraction and increased extra-cellular matrix formation of tissues. From the other side, the transformation of fibroblasts into myofibroblasts make the situation more complex. The α -SMA marker was used to assess the amount of transformed myofibroblasts to assess and predict the aggressiveness of CGCG lesions. (7)

Aim of the work

The present study aimed to evaluate the presence of cannibalistic cells in aggressive versus non-aggressive CGCGs of the jaws, investigate the vascular density & myofibroblastic activity in aggressive versus non-aggressive CGCGs of the jaws and correlate the expression of CD-34 & α -SMA as well as cellular cannibalism in CGCGs.

Materials and methods

The retrospective study was applied in forty paraffin blocks divided into two groups according to clinic-radiographical data into aggressive and non-aggressive CGCG lesions. There were 20 aggressive and 20 non-

aggressive lesions. Hematoxylin and eosin (H&E) stained slides were examined to confirm the diagnosis and to count the number of cannibal cells in each case.

Immunohistochemical reactions

Paraffin-embedded sections were obtained from each case with about 4µm thickness, the sections were mounted on silanized slides, the slides were dewaxed, remoisturized and then treated with hydrogen peroxide in methanol for 30 minutes then washed in phosphate buffer solution (PBS) for 5 minutes. Section are pretreated after that by immersion in boiling buffer 1 mm EDTA for about 10 minutes then cooled at room temperature and finally washed with water. Antibodies of both CD-34 and α-SMA are then applied on the slides without dilution, as both are ready to use. Section are then incubated in secondary antibodies for 30 minutes at room temperature and an Avidin-Biotin complex peroxidase solution was applied. Finally, the slides are incubated for 15 minutes after application of DAB for colored reaction representation after that they are counterstained using Mayer's Haematoxylin, dehydrated and coverslips were put.

Evaluation of staining

All slides were examined and analyzed using an optical microscope.

Scoring for cellular cannibalism

The H&E stained sections were examined by two independent observers to evaluate the number of cannibal cells per cases at magnification x40 battlefield method for counting. Scores were given as grade 1: <5 cells present, grade 2: 5-20 cells present, grade 3> 20 cells present.

(4, 8)

Evaluation and scoring of immunohistochemical markers

The positivity and intensity of Immunohistochemical staining of CD-34 and α-SMA were examined, evaluated and scored in different CGCGs on all slides. Immunostaining was evaluated for both markers using a semi-quantitative analysis where the percentage of stained cells and intensity of staining were taken into account. The percentage of stained cells (incidence) 0: total absence of positively stained cells, 1: less than 25% of cells are positively stained, 2: 25-50% of cells are positively stained, 3: 50-75% of cells are positively stained and 4: more than 75% of cells are positively stained. Where the percentage of stained cells (incidence) Score 0: Negative, 1: Faint, 2: moderate and 3: Intense. Then the sum of the percentage of stained cells and intensity scores were calculated and categorized as the final score for each case as the following: Absence of staining: 0, Weak staining: 1-4, and Strong staining: 5-7. Only the final scores were used to perform the correlation tests for the markers. (9)

Statistical Analysis:

Statistical analysis of data made by using statistical package for social science (SPSS) version 17.0. The data were expressed using measures of central tendency. For comparison between two groups, the Mann-Whitney test and students T-test were used. In addition, for testing the relationship between categorical variables chi-square test was used. A significant difference was considered when the

P value is < 0.05. Spearman's correlation was used to evaluate the correlations between different variables.

Results

Cellular cannibalism was examined via H&E stained section. Cellular cannibalism appeared in all cases. Cannibalistic MNGCs showed either partial or complete cellular cannibalism of stromal cells of the lesion. (Fig 1 A, B) Cellular cannibalism scored in non-aggressive CGCGs as the following: 17 cases (85%) was graded as grade 1 and 3 cases (15%) was graded as grade 2, where no cases were found in grade 3. On the other hand, in aggressive CGCGs 3 cases found to as in grade 2 (15%) and 17 cases (85%) found to be in grade 3, where no cases found in grade 1.

There was a high level of significance found to be between aggressive and non-aggressive cases using chi-square if we come to cellular cannibalism.

CD-34 was used as a cytoplasmic membrane marker of the endothelial cells of blood vessels. The vascular density score occurred in non-aggressive cases the score mean±SD was 3.35±0.8127, the minimum score was 2 and the maximum was 5, the median was 3 and range from 2 to 3. On the other hand, in aggressive cases the score mean±SD was 5.85±0.6708, the minimum score was 5 and the maximum was 7, the median of the scores was 6 and ranged from 5 to 6. there was a high level of difference of significance between non-aggressive and aggressive cases regarding the cd-34 final score. (Fig.1 C)

The α-SMA marker was used as a cytoplasmic marker of myofibroblasts stromal cells. The final marker score expression in non-aggressive cases the score mean±SD was 3.05±0.887, the minimum score was 2 the maximum score was 4, the median was 3 and range from 2.0 to 4.0. Otherwise, non-aggressive cases, in aggressive cases the score mean±SD was 6±0.9733, the minimum was 4, the maximum was 7, and the median was 6.0 and range from 4.0 to 7.0. There was a high level of difference of significance between non-aggressive and aggressive cases regarding the CD-34 final score. (Fig.1 D)

Among all cases, there was a high positive correlation between cellular cannibalism, CD-34, and α-SMA expression. Also a high level of significance between them. There was also a high positive correlation between CD-34 and α-SMA expression, and a high level of significance between their expressions among cases. (Table 1)

Table(1): Correlations and Difference of Significance between CD-34, α-SMA and cellular cannibalism in CGCG studied cases:

			CD34 score	SMA score
All cases	cannibalism grade	r	.869	.869
		P	<0.001*	<0.001*
	CD34 score	r		.905
		P		<0.001*

r: Spearman's correlation coefficient P: Probability *: significance<0.05

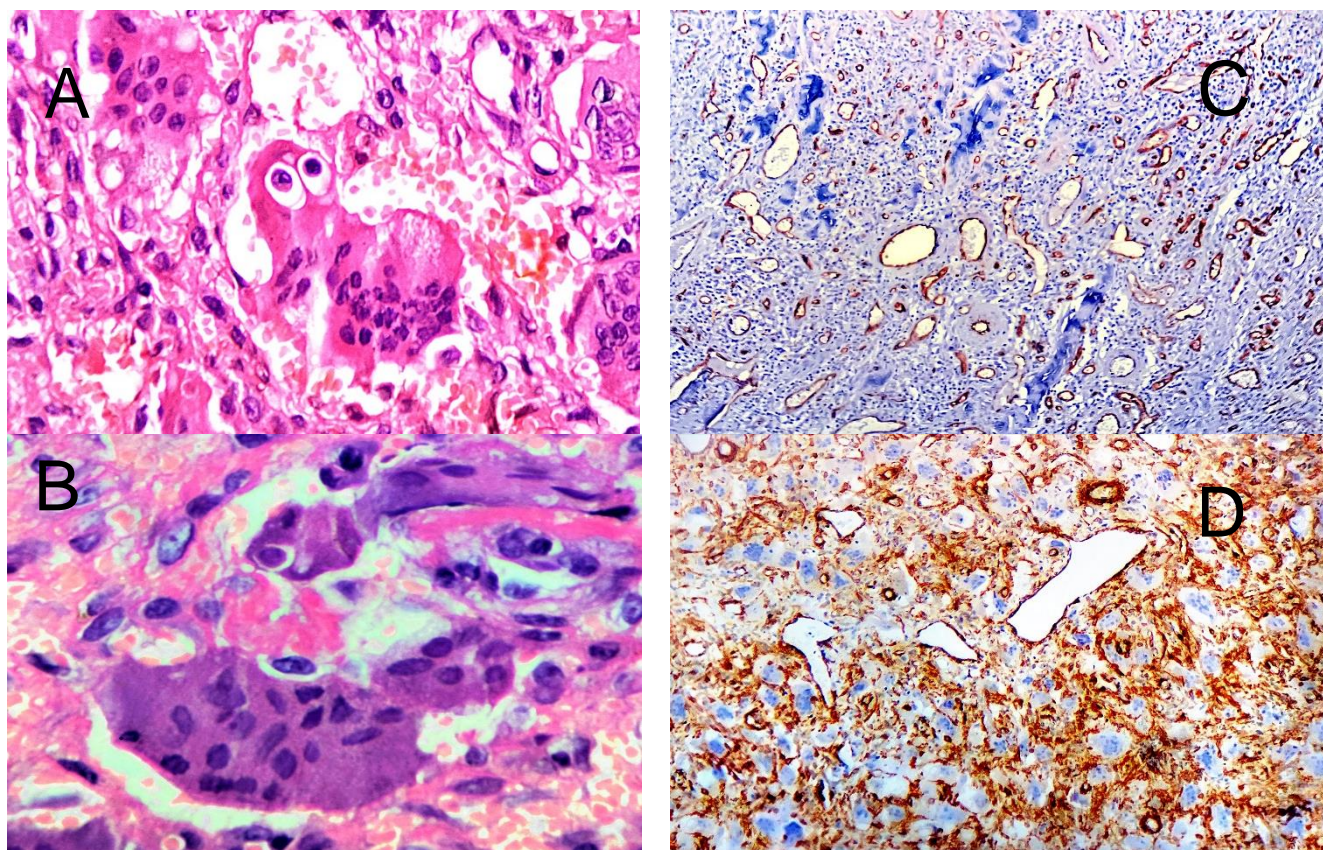


Figure 1: A: H&E stain showing complete cellular cannibalism, B: H&E stain showing partial cellular cannibalism, C: immunohistochemical staining showing CD-34 vascular expression score 7, D: immunohistochemical staining showing α -SMA staining score 6.

Discussion

Cellular cannibalism presence in all cases suggests the lesion as a neoplasm rather than a reactive lesion. That agrees with S.C. Sarode and G.S. sarode (2017), and S.C. Sarode et. al. (2014).^(4, 8) The existence of cellular cannibalism still a debate, most probably it is essential to fulfill nutritional needs, nutritional stresses as well as hunger of tumor cells. Also, it increases with increased acidity of the lesion. Studies have shown that metastatic tumors tend to this phenomenon in case of low nutritional supplies and thus this property offers them a survival benefit. Descriptions in pieces of literature of cellular cannibalism are still rare in benign tumors. Fernandez florez et. al. (2012) have shown images in a benign condition of localized giant cell tumor of the tendon sheath that have shown internalized cells that displayed apoptotic appearance by routine H&E stains with loss of nuclei and increased cytoplasmic density that resembles the finding found in CGCG. A recent study by G.S. sarode, and S.C. Sarode et. al. (2017) reported also mean cannibalistic cells in aggressive CGCG was higher than that of non-aggressive CGCG and mean of giant cell tumor was higher than that of aggressive CGCG. The MNGCs in CGCG are most probably derived from monocyte-macrophage lineage and resembles osteoclasts so, have that inherited property of engulfment, which is

responsible for cellular cannibalism of stromal cells. Cellular cannibalism may represent a higher metabolic activity of

MNGCs and that can be correlated to aggressiveness of lesions. Thus, this phenomenon can be used as a biological prognostic marker.^(4, 10, 11)

CD-34 angiogenic marker that used to identify vascular activity found to be with a high level of significant difference between non-aggressive and aggressive CGCG lesions. Those results agreed with many pieces of research.⁽¹²⁻¹⁴⁾ These findings can suggest that increase CD-34 expression as indicating increased angiogenesis of the lesion, it can be inductive for the blood vessels and its cells (monocytes/macrophages) to differentiate into osteoclasts and osteoclast-like giant cells which in return increase the growth rate, osteolysis, more destructive effect on bone and of course enlargement of the lesion.⁽¹⁵⁾

The myofibroblastic activity indicative marker used α -SMA showed results of a high level of significant difference between aggressive and non-aggressive lesions.^(12, 16) The myofibroblasts are involved in wound healing normally because of their component alpha-smooth muscle actin, act for matrix contraction, but after that process, myofibroblasts supposed to disappear by apoptosis. Their persistence causes a dysfunction in the repair mechanism leading to excessive contraction and increased extra-cellular matrix formation

leading to fibrosis. In addition, it was found that α -SMA positive myofibroblasts represent additional property and contribute to complex tumor milieu as it facilitates tumor local invasion and suppresses host immune response. (17-19)

As far as we know in English literature, there was no study made to explain this relation between cannibalism, angiogenicity and myofibroblastic activity. To explain this relation we can start to understand the relation between angiogenesis and myofibroblastic differentiation and activity. Myofibroblasts may have many different origins but their development has a definite sequence of events. It may be developed from fibroblasts with excessive expression of SMA but some authors proposed it may be developed by an endothelial-mesenchymal transition (End MT). (20-22) The unique tumor microenvironment such as broad variety of cellular differentiation and high level of reactive oxygen species (ROS) promotes tumor-associated fibroblasts (myofibroblasts) functions which lead to chronic repair response and chronic ECM deposition (fibrosis), which resulting in increased interstitial fluid pressure due to remodeling and contraction of stroma stimulating more vascular proliferation. Also increased hypoxia state due to stresses condition because of extra oxygen consumption. Therefore, increased vascular activity and vascular proliferation can give more chance for myofibroblastic differentiation in tumor stroma. Another explanation can be found through understanding another circulating cell in the blood that can be affected by tumor microenvironment to be differentiated into myofibroblasts, Fibrocytes. Fibrocytes are bone marrow-derived cells that are circulating in the bloodstream and express CD-34 which have the ability to migrate to areas of tissue injury or tumors, secreting matrix metalloproteases helping in matrix remodeling and differentiate into pro-myofibroblasts and

finally mature fibroblasts. (23-25) As mentioned before, the unique tumorous microenvironment contains a high level of ROS resulting in oxidative stress, which in result causes macromolecular damage as cancers and tumors. As it has known that cellular cannibalism is believed to be caused due to such causes as nutritional stresses and hypoxia so it may be a cause of direct relation between increased cellular cannibalism because of ROS and other results of increased ROS byproducts as increased myofibroblastic activity and so increased vascular activity. (25, 26)

Conclusion

The results of this study might indicate that the cellular cannibalism can be used as a simple histopathological predictor of the behavior of CGCG lesions using H&E stains, in which high prevalence of cellular cannibalism may indicate aggressiveness of the lesion. Also, there is a reciprocal relationship between cellular cannibalism, angiogenicity and myofibroblastic differentiation where indicate aggressiveness of CGCG lesion. Using these measures might anticipate the behavior of the lesion that makes the follow up a mandatory issue in selected cases to give attention to recurrent cases.

Recommendation

Treatment approaches targeting vascularity and myofibroblastic differentiation pathways may be helpful to limit the enlargement and destruction resulting from CGCG lesion, especially aggressive ones. Further researches are needed for more understanding of the relationship between vascularity and myofibroblastic differentiation in CGCG and such lesions. Further molecular biology researches are recommended for a better understanding of the nature of CGCG as a neoplastic or a reactive lesion.

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