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## ORIGINAL ARTICLE

# Does Peripheral and Central Giant Cell Granuloma Express Different Profile of Ki67 and P27?

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**ABSTRACT**
*Objective(s): Evaluate and compare p27&Ki67 expression in peripheral and central giant cell granuloma (PGCG & CGCG). Study Design: This IHC study was performed on the 30 paraffin embedded blocks (15 PGCG, 15 CGCG). Labeling index was calculated. Statistical analysis was performed by SPSS 16 using Fissure exact and paired t tests. Results: Low &high expression of Ki67 and p27 was seen in 14(39.3%) &1(6.7%) and 13(86.7%) &2(13.3%) of PGCGs respectively. All CGCGs showed lowKi67 expression. 9(60%) &6(40%) of CGCGs showed low &high p27expression. Mean of p27&Ki67 expression were 14.95±13.45 &21±10.22 in peripheral and 12.96±5.40 &31.13±24.86 in central lesions. Conclusion(s): P27 and Ki67 showed low expression in both peripheral and central lesions. Correlation was found between expression of Ki67 &p27 in central lesions (p: 0.01). No correlation was found among these markers and age, sex, size, site and type of the lesions.*
***Keywords;*** *P27, Ki67, PGCG, CGCG, Giant cell lesions*

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## INTRODUCTION

The central giant cell granuloma (CGCG) of the jaws is a non-neoplastic and localized benign lesion. This lesion occurs in the mandible mostly as an expansile radiolucent lesion with variable clinical behavior with two growth pattern- nonaggressive and aggressive- and the latter is associated with teeth displacement, root resorption or bone cortical perforation . Local trauma, inflammation, intraosseous bleeding and genetic abnormalities have been considered as possible causes, but the pathogenesis has not explained clearly yet [1-3]. The peripheral giant cell granuloma (PGCG) is a peripheral giant cell lesion which exclusively occurs in the alveolar mucosa or gingiva and the lesion presents as a sessile or pedunculated purple-brownish mass. The exact etiology of PGCGs still remains uncertain. Developmental and inflammatory reactions in the periodontal ligament or the periosteum have been suggested as causative factors. Local irritations such as unsuitable or improper dental restorations and plaque or calculus accumulation can play a significant role in the development of a PGCG [2, 4].

Although CGCG and PGCG have the same histopathologic features which demonstrate numerous multinucleated giant cells in a spindle shaped mesenchymal cells background, their clinical behaviors are different [5]. Some studies have been evaluated cellular and biologic behaviors of stromal and giant cells in these lesions by studying morphological parameters or some cell cycle proteins or even cellular markers [6, 7]. Otherwise, dysregulation of the cell cycle proteins may contribute in the pathogenesis of giant cell granulomas and justify molecular changes and clinical behavior of these lesions, despite of having similar morphologic and histopathologic pattern. On the other hand, high proliferative activity could be consequence of down regulation of some tumor suppressor proteins or upregulation of some oncogenic proteins. For instance, Ki67 is expressed during cell proliferation and is used in pathology Lab

to predict the behavior of tumors. Some researchers have shown it is an independent predictive factor in many human neoplasms [10]. In contrast, P27 is a family member of Cyclin-Dependent Kinase (CDK) inhibitors which its reduced expression has been shown in some tumors. It has also been suggested that this marker is an indicative factor which shows cell maturation and even could be used to differentiate between benign and malignant tumors [11].

Although a group of researchers have suggested that the differences in the morphologic parameters of multinucleated giant cells could be justified as the reason of aggressive biological and clinical behavior of these lesions (6, 7), it has not proved yet. Moreover, vascular and stromal factors have also been investigated and different results have been achieved. For example, Falci revealed similar angiogenesis in both CGCGs and PGCGs (8) but Matos showed the increased expression of VEGF in PGCGs compared to those CGCGs [9].

However, Simultaneous evaluation of ki67 and p27 could be useful whereas evaluation of only one of them could be confusing, for example ki67 expression in medullary portion of adrenal gland is similar to neuroblastoma while p27 expression is so different in them. Therefore, assessment of coexpression of these markers has clinical importance and therapeutic value [12]. To our knowledge, there are a few studies in the literature about evaluation of ki67 in giant cell granulomas and there is no study about p27, even in PGCGs or CGCGs. Therefore, we assessed this study to evaluate the expression of ki67 and p27 in peripheral and central giant cell granulomas.

## MATERIAL AND METHODS

**Study group:** 15 PGCG and 15 CGCG paraffin-embedded blocks were selected. All clinicohistopathologic data (age, gender, anatomic location and size of the lesion) were extracted from pathology archive of Department of Oral and Maxillofacial Pathology, Dental Branch of Tehran, Islamic Azad University, Tehran, Iran. H &E slides were revised by two oral pathologists. In all the cases of CGCGs, laboratory tests values for serum calcium and phosphorus concentrations, alkaline phosphatase activity and parathyroid hormone level were done in order to rule out systemic disease like hyperparathyroidism.

**Inclusion criteria:** microscopic diagnosis of PGCG/CGCG by two oral pathologists.

**Exclusion criteria:** 1-Not enough sample in the paraffin-embedded block. 2- Insufficient clinical data. 3-Any other disease after serum evaluation of calcium, phosphorous and alkaline phosphatase which done for all CGCGs.

19 cases were females (60% of PGCGs and 66.7 of CGCGs). The mean age of patients with PGCG and CGCG was 43.33±17.78 years and 29.87±12.57 years, respectively.

### Immunohistochemistry (IHC):

The standard streptavidin-biotin peroxidase method was used for IHC. In brief, the three micrometer sections from the paraffin embedded blocks were dewaxed by xylene and dehydrated. The sections were autoclaved in citrate buffer (10mM) for 10 minutes at 121 degree of Centigrade, then placed in 0.3 percent H2o2 containing methanol for 15minutes to inactivate endogenous peroxidase. Then they were incubated with monoclonal antibody of ki67 (MIB1, ready to use, Dako, Denmark) and p27 (kip1, 1:50 dillution, Dako, Denmark) for 60 minutes at room temperature. They were rinsed with PBS and incubated in streptavidin-biotin peroxidase. Afterwards, the sections were incubated in DAB and counterstained with hematoxylin for 3 minutes. As negative control, the primary antibody was omitted and as positive control, breast cancer was used for ki67 and adenoid tissue was used for p27. The nuclei stained brown was considered as positive immunoreactivity.

The samples were evaluated by two individual pathologists in a blind condition under light microscopy who counted stained cells in 1000 cells in 10HPF determined as Labelling Index (LI) and then classified to two groups: For ki67: LI≤48% and LI>48 was considered as low and high expression respectively. For p27: LI≤25% and LI>25 was considered as low and high expression, respectively (13, 14).

**Statistical analysis:** Statistical analysis was performed by SPSS 16.0 using Fisher exact and paired T tests. The differences were considered significant if $p<0.05$.

## RESULTS

### Clinical findings:

Out of 30 cases, 11 (36.7%) and 19(63.3%) cases were in male and female, respectively. 13 cases were in maxilla and 17 cases were found in the mandible.

**PGCG:** Out of 15 cases, 6 (40%) and 9(60%) cases were in male and female, respectively. 8 cases were in maxilla and 7 cases were found in the mandible.

**CGCG:** Out of 15 cases, 5 (33.3%) and 10(66.7%) cases were in male and female, respectively. All cases were in the mandible. 3 and 12 lesions were aggressive and nonaggressive type respectively.

**Histopathological findings:**

**Ki67 (results summarized in table1):**

**PGCG:** 14 (93.3%) and 1(6.7%) cases showed weak and strong staining, respectively.

**CGCG:** All cases showed weak staining.

Mean of ki67 expression was 14.95± 13.45(percent) in PGCGs and 12.96± 5.40 (percent) in CGCGs. Fisher exact test revealed no statistical differences between expression of ki67 in peripheral and central lesions (P: 0.50)

**P27 (results summarized in table2):**

**PGCG:** 13 (86.7%) and 2(13.3%) cases showed weak and strong staining, respectively.

**CGCG:** 9 (60%) and 6(40%) cases showed weak and strong staining, respectively.

Mean of p27 expression was 21± 10.22(percent) in PGCGs and 31.13± 24.86 (percent) in CGCGs. Fisher exact test revealed no statistical differences between expression of p27 in peripheral and central lesions (P: 0.107)

Paired T test showed no correlation between ki67 and p27 expression in PGCGs but revealed significant correlation between these two markers in CGCGs (P: 0.01)

Frequency and distribution of expression of these markers are summarized in table 3 and 4. Fisher exact test showed no correlation between each marker and age, sex, size, location and the type of the lesion.

**Staining pattern:**

**Ki67:** The stromal ovoid to spindle cells showed more immunoreactivity with ki67 (Figure 1). Basal cells stained intensely while there was mucosa in the sample.

**P27:** Multinucleated giant cells showed more immunoreactivity with p27 (Figure 2). Suprabasal cells stained intensely while there was mucosa in the sample.

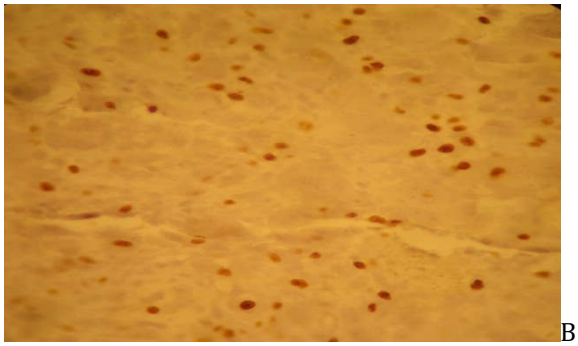
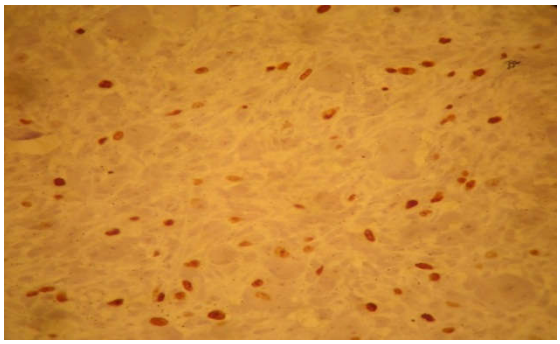
A  B 

Figure1: Demonstrating mononuclear stromal cells immunostained by anti-ki67 antigen in peripheral (A) and central (B) giant cell granulomas. ×400

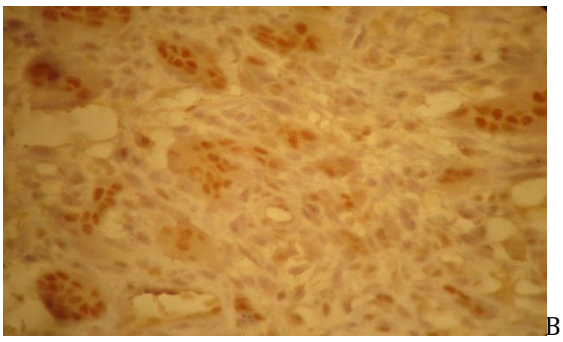
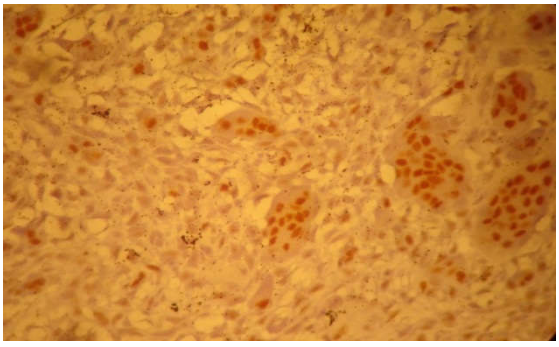
A  B 

Figure2: Demonstrating multinucleated giant cells immunostained by anti-p27 antigen in peripheral (A) and central (B) giant cell granulomas. ×400

Table1: Frequency of ki67 expression in PGCGs and CGCGs

| Ki67expression | PGCG<br>N (%) | CGCG<br>N (%) |
|---|---|---|
| Low expression<br>≤48 | 14(93.3) | 15(100) |
| High expression<br>>48 | 1(6.7) | 0(0) |
| Total | 15(100) | 15(100) |

Table2: Frequency of P27 expression in PGCGs and CGCGs

| Ki67expression | PGCG<br>N (%) | CGCG<br>N (%) |
|---|---|---|
| Low expression<br>≤25 | 13(68.7) | 9(60) |
| High expression<br>>25 | 2(13.3) | 6(40) |
| Total | 15(100) | 15(100) |

Table3: Frequency of ki67 and p27 expression in PGCGs according to age, gender, location and size of the lesion

| IHC expression<br>Clinicopathologic factors | | Ki67 expression | | P27 expression | |
|---|---|---|---|---|---|
| | | ≤ 25<br>N (%) | >25<br>N (%) | ≤ 48<br>N (%) | >48<br>N (%) |
| Age | $> Mean$ | 8 (61.5) | 2 (100) | 9(64.3) | 1(100) |
| | $< Mean$ | 5(38.5) | 0(0) | 5(35.8) | 0(0) |
| Gender | Male | 6(46.2) | 2 (100) | 6(42.9) | 0(0) |
| | Female | 7(53.8) | 0(0) | 8(57.1) | 1(100) |
| Location | Maxilla | 5(38.5) | 0(0) | 8(57.1) | 0(0) |
| | Mandible | 8(61.5) | 2 (100) | 6(42.9) | 1(100) |
| Size | <2cm | 8(61.5) | 2 (100) | 9(64.3) | 1(100) |
| | $> 2cm$ | 5(38.5) | 0(0) | 5(35.7) | 0(0) |
| Total | | 13(100) | 2 (100) | 14(100) | 1(100) |

Table4: Frequency of ki67 and p27 expression in CGCGs according to age, gender, location and size of the lesion

| IHC expression<br>----------<br>Clinicopathologic factors | | Ki67 expression | | P27 expression |
|---|---|---|---|---|
| | | ≤ 25<br>N (%) | >25<br>N (%) | ≤ 48<br>N (%) |
| Age | $> Mean$ | 3(33.3) | 2 (33.3) | 5(33.3) |
| | $< Mean$ | 6(66.7) | 4(66.7) | 10(66.7) |
| Gender | Male | 3(33.3) | 2 (33.3) | 5(33.3) |
| | Female | 6(66.7) | 4(66.7) | 10(66.7) |
| Location | Maxilla | 3(33.3) | 2 (33.3) | 5(33.3) |
| | Mandible | 6(66.7) | 4(66.7) | 10(66.7) |
| Size | <2cm | 7(77.8) | 6(100) | 13(86.7) |
| | $> 2cm$ | 2(22.2) | 0(0) | 2(13.3) |
| Total | | 9(100) | 6(100) | 15(100) |

**DISCUSSION**

To our knowledge this is the first study regarding simultaneous determination of ki67 and p27 in PGCGs and CGCGs of the jaws. Our study showed that ki67 is expressed weakly in most cases of PGCGs and CGCGs. Mean of ki67 expression was 14.95± 13.45(percent) in PGCGs and 12.96± 5.40 (percent) in CGCGs. Moreover, the mean of ki67 was not statistically significant between peripheral and central lesions (p: 0.50) Low expression of ki67 is indicative of lower growth and it might justify non tumoral behavior of these lesion. On the other hand, these lesions are reactive rather than tumoral. Unfortunately, we could not evaluate the possible difference of ki67 expression between aggressive and non-aggressive type of CGCGs because of low sample size. O'Malley M and colleagues showed that only mononuclear cells stained with ki67, and they found no differences between expression of ki67 in aggressive and non-aggressive

tumors. They concluded that cellular phenotypes and numbers of cells in cell cycle are similar in both aggressive and non-aggressive tumors[13].
De Souza PE evaluated the immunohistochemical expression of p53, MDM2, Ki-67 and PCNA in CGCG and GCT (Giant Cell Tumor). They demonstrated that the percentage of Ki-67 and PCNA-positive cells in CGCG was statistically higher than positive cells in GCT and they concluded that CGCG has a higher proliferative activity comparing to GCT. [14]
Our study showed that p27 is expressed weakly in most cases of PGCGs and CGCGs. The mean of p27 was not statistically significant between peripheral and central lesions. Mean expression of p27 was 21% in PGCGs while it was higher (31%) in CGCGs. Low expression of p27 is indicative of cell proliferation and if we consider only this marker we could conclude that the central lesions might have lower proliferative activity comparing to peripheral lesions.
High expression of ki67 is supportive of cell propagation while low expression of p27 is in favor of cell proliferation. We found a negative correlation between expression of ki67 and p27 in CGCGs (P: 0.01) which showed low expression of ki67 is associated with high expression of p27 and vice versa. Furthermore, in our study these markers might balance proliferation activity resulting reactive behavior of our samples. In addition, we could not found any correlation between ki67 and p27 in peripheral lesions (P: 0.08). Therefore, Simultaneous evaluation of ki67 and p27 is important to interpret proliferative activity. Although many cell cycle proteins could play important role in this way, we evaluate just these two markers which show non tumoral behavior of both lesions. Fisher exact test showed no correlation between each marker and age, sex, size, location and the type of the lesion.

**STAINING PATTERN**

Staining pattern of ki67 which showed more immunoreactivity with mononuclear stromal cells was the same as Kauzman A1 and O'Malley M expressed [13, 15]. We found that Ki67 was also positive in basal cells comparing to p27 which was positive in suprabasal layers.
P27 expression was observed mostly in multinucleated giant cells than that of stromal cells. As p27 have been shown as an indicative marker of differentiation [11, 16], it could be justified that these cells are more differentiated compared to stromal mononuclear cells. Regezi also speculated that the subset of CGCGs that show a locally aggressive behavior may develop from a reactive lesion through an epigenetic event occurring in spindle-shaped mesenchymal cells which escape from cell cycle controls and differentiate into multinucleated giant cells [17, 18]. The pattern of staining in these samples is also more in favor of high differentiation in multinucleated giant cells compared to spindle cell mesenchymal cells.

**CONCLUSION:**

Low expression of ki67 and p27 in both peripheral and central giant cell granulomas may confirm non tumoral and reactive behavior of these lesions. Increased expression of ki67 was found simultaneously with decreased expression of p27 and vice versa in central giant cell granulomas which could be concluded that these markers balance each other, resulting reactive behavior of the studied samples in the clinic. Further studies with larger samples size and evaluation of other cell cycle proteins are recommended.

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