

# A Comparative Immunohistochemical Study of CD105 Expression in Peripheral and Central Giant Cell Granuloma

Massoumeh Zargaran<sup>1</sup>, Fereshteh Baghai<sup>2</sup>

## ARTICLE INFO

**Article type:**  
Original Article

**Article history:**  
Received: Jan 1, 2017  
Accepted: Mar 3, 2017  
Available online:

<sup>1</sup>Associate Professor, Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Kurdistan University of Medical Sciences, Sanandaj, Iran.

<sup>2</sup>Associate professor, Dental Research Center, Dentistry Research Institute, Department of Oral & Maxillofacial Pathology, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

**Corresponding Author:**

Massoumeh Zargaran

**Address:**

Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Kurdistan University of Medical Sciences, Sanandaj, Iran.

**E-mail:** massoumehzargaran@gmail.com

**Telephone:** +988733668770

**Fax:** +988733668921

## Abstract

### Introduction:

Despite similar histopathological features, peripheral giant cell granuloma (PGCG) and central giant cell granuloma (CGCG) differ in their biological behavior. PGCG and CGCG are hemorrhagic/vascular lesions, clinically and microscopically. Angiogenesis is necessary for the growth and development of lesions and affects their clinical behavior. CD105 is a useful vascular marker for assessment of angiogenesis. This study aimed to assess the CD105 expression in PGCG and CGCG by immunohistochemistry in order to determine angiogenesis in these lesions.

### Materials and methods:

A total of 30 PGCG and 30 CGCG specimens were stained for CD105 marker. The expression of this marker and the angiogenic potential of lesions were determined by calculating the mean vascular density (MVD).

### Results:

All specimens revealed immunostaining of CD105 marker. MVD was  $26.56 \pm 11.03$  in PGCG and  $22.32 \pm 12.81$  in CGCG. Herein, the two groups did not differ significantly ( $P = 0.390$ ).

### Conclusion:

This study demonstrated neovascularization in PGCG and CGCG. The angiogenic potential between the two groups did not significantly differ. This finding may suggest that the distinct clinical behavior of PGCG and CGCG is independent of the number of vessels and angiogenesis.

### Key words:

•Neovascularization, Pathologic •Granuloma, Giant Cell •Immunohistochemistry •Endoglin

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Zargaran M, Baghai FA Comparative Immunohistochemical Study of CD105 Expression in Peripheral and Central Giant Cell Granuloma, 3DJ 2017;6(2):30-36.

## Introduction

The term angiogenesis/neovascularization defines the growth and development of new capillary vessels from the already existing blood vessels.<sup>(1)</sup> A cellular proliferation/pro-

liferative lesion requires angiogenesis for its survival and growth.<sup>(2)</sup>

CD105 (endoglin) is a homodimeric cell membrane glycoprotein, which is crucial in

blood vessel development.<sup>(3,4)</sup> Recent studies have suggested that endoglin may serve as a proliferation-associated marker in the endothelial cells. The endoglin antibody binds preferentially to the activated endothelial cells participating in angiogenesis in tumors and wound healing.<sup>(4)</sup> The ability of CD105 to quantitatively distinguish between the activated/proliferating endothelial cells and normal/quiescent endothelial cells may effectively assess the tumor angiogenesis and/or angiogenic potential.<sup>(5)</sup>

Giant cell granulomas are among the common tumor-like lesions in the oral cavity<sup>(6,7)</sup> and are divided into two groups, namely central and peripheral lesions.<sup>(6)</sup> Peripheral giant cell granuloma (PGCG) is a reactive lesion caused by local irritation or trauma.<sup>(2)</sup> It has a slow growth and rarely causes underlying bone resorption.<sup>(7)</sup> Central giant cell granuloma (CGCG) is a benign intraosseous lesion,<sup>(6)</sup> which is more invasive than PGCG<sup>(8,9)</sup> and has a higher rate of growth and recurrence.<sup>(7)</sup> CGCG is an osteolytic lesion,<sup>(10)</sup> which can be painful.<sup>(11)</sup> It can cause root resorption and/or cortical perforation.<sup>(7)</sup> PGCG and CGCG differ in terms of biological and clinical behavior; however, their histopathological features remain the same.<sup>(12,13)</sup> This includes several multinucleated giant cells admixed with plump ovoid and spindle-shaped mesenchymal cells within a rich vascularized stroma containing extravasated erythrocytes, hemosiderin deposition, and blood filled pools.<sup>(2,6)</sup> It is suggested that endothelial cells may represent a significant cellular subpopulation in these lesions.<sup>(14)</sup> It is proposed that the blood vessels in these lesions may be responsible for some of their unique features.<sup>(15)</sup>

This study aimed to immunohistochemically evaluate the expression of CD105 in PGCG and CGCG.

## Materials and Methods

In this descriptive study, 30 cases of CGCG and 30 cases of PGCG were selected from the archive of Department of Oral Pathology, Faculty of Dentistry, Tehran University of Medical Sciences. Diagnosis was confirmed based on the pathological report and review of hematoxylin and eosin stained slides. Cases with extensive and diffuse hemorrhagic fields, abundant inflam-

mation, ulceration, and insufficient amounts of tissue for immunostaining, were excluded. Information including age, gender, location of lesions, and certain signs and symptoms (for CGCG) was obtained from the patient records.

### *Immunohistochemical methods*

4 µm thick sections were cut from the paraffin-embedded tissue blocks and were stained for endoglin by labeled streptavidin–biotin 2 system, horse radish peroxidase technique (LSAB2 system, HRP technique). Tissue sections were mounted on poly-L-lysine coated slides and were dried for 24 h at 37 °C. Sections were deparaffinized in xylene and rehydrated in descending ethanol series. Endogenous peroxidase activity was blocked with 3% H<sub>2</sub>O<sub>2</sub> for 5 min; thereafter, the slides were washed in distilled water. To unmask the hidden epitopes, sections were digested with proteinase K enzyme for 15 min and were then rinsed with Tris buffered saline and treated with a protein blocking agent for 5 min before staining to reduce the nonspecific antibody binding. The primary antibody used was mouse antihuman monoclonal antibody (SN6h, DAKO, Denmark) at a 1:10 dilution for 90 min; subsequently, biotinylated link was applied for 30 min. Furthermore, the sections were washed with Tris buffered saline for 10 min and were then incubated for 30 min with streptavidin-HRP and rinsed as aforementioned.

The antibody complex was visualized by a 3,3'-diaminobenzidine tetrahydrochloride (DAB) reaction. Sections were counterstained with hematoxylin and then were mounted. Negative controls included parallel sections from which the primary antibody was excluded. Sections of pyogenic granuloma tissue were prepared and stained as positive controls.

### *Assessment of CD105 immunoreactivity*

Immunostaining of sections was performed according to Saad et al.,<sup>(16)</sup> using a light microscope (BX40; Olympus) at low magnification (×40) for areas with the highest endoglin stained microvessel density (hot spots) and microvessels were then counted at ×400 magnification.

Additionally, CD105 microvessel density (MVD-CD105) was calculated in part of supportive peripheral stroma of PGCGs. Any brown stained endothelial cell or cluster that was clearly separated from the adjacent microvessels, tumor cells, and other connective tissue elements

was regarded as a single, countable microvessel.<sup>(4,17,18)</sup> No restriction was observed considering the size; however, vessels with muscular walls were excluded.<sup>(17)</sup> The mean vessel count from three fields was considered as MVD-CD105.

### Statistical analysis

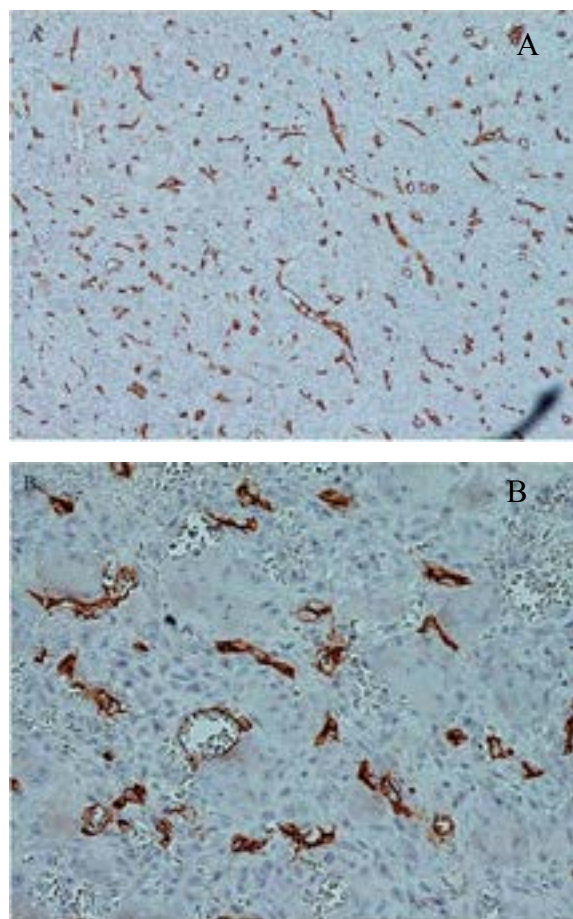
Data were analyzed by the SPSS software version 11.5 using two-way ANOVA analysis, Pearson correlation coefficient, t-test, and paired t-test with  $P < 0.05$  as the limit of significance.

## Results

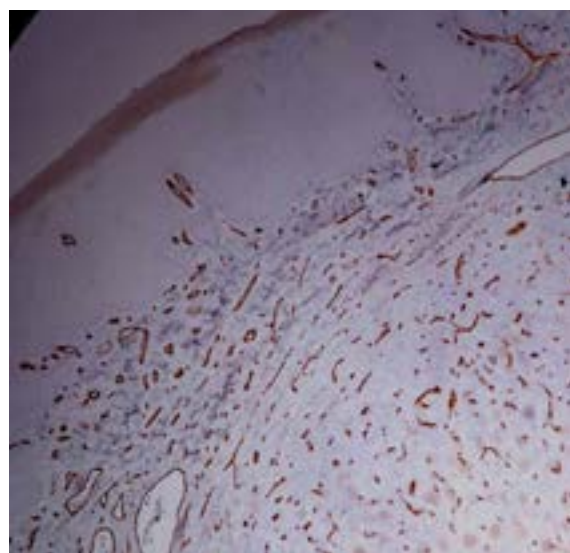
Table 1 presents the case distribution based on gender, age, and lesion location. All cases of PGCG and CGCG revealed immunoreactivity with the antibody tested (Figures 1 and 2). Table 2 presents the MVD data of the two groups. CD105 expression did not differ significantly for the total mass of lesions in the CGCG and PGCG groups ( $P = 0.390$ ). In the PGCG group, no significant difference was observed between the total mass of lesions and the supportive peripheral stroma ( $P = 0.402$ ).

No significant correlation was observed between the CD105 expression and gender (PCGCG = 0.927, PPGCG = 0.460), lesion location (PCGCG = 0.625, PPGCG = 0.934), or age (PCGCG = 0.099, PPGCG = 0.705) in either of the two groups. The patients in the CGCG group were further divided into three categories based on the information obtained from the patient records. Here in 21 cases were symptomatic with one or more signs or symptoms, including tooth mobility or displacement, pain, lip paresthesia, root divergence or resorption, and recurrence after treatment; 4 cases were asymptomatic; and 5 cases were classified as unknown, since no data was available regarding the signs and symptoms from the medical records.

Moreover, the 30 cases studied (in CGCG group) were subclassified based on the cortical perforation: 10 cases revealed cortical perforation, 15 did not reveal perforation, and no data was available for remaining 5 cases. No significant correlation was observed between the CD105 expression and presence of symptoms ( $P = 0.317$ ) or cortical perforation ( $P = 0.434$ ).



**Figure 1.** Microvessels positive by immunohistochemical staining of CD105 (endoglin) in CGCG: A  $\times 100$ ; B  $\times 400$



**Figure 1.** Microvessels positive by immunohistochemical staining of CD105(endoglin) in supportive stroma and mass of PGCG,  $\times 100$

**Table 1.** Distribution of cases by gender, age, and location

| Clinical Information |   | CGCG          | PGCG          |
|----------------------|---|---------------|---------------|
| Gender               | Male                                      | 11            | 10            |
|                      | Female                                    | 19            | 20            |
|                      | Total                                     | 30            | 30            |
| Age                  | Male                                      | 31.72 ± 23.37 | 27.20 ± 19.46 |
|                      | Female                                    | 24.52 ± 15.06 | 28.85 ± 14.41 |
|                      | Total                                     | 27.60 ± 18.48 | 28.30 ± 15.95 |
| Location             |   |               |               |
| Maxilla              | Anterior                                  | 8 (66.7%)     | 6 (66.7%)     |
|                      | Posterior                                 | 3 (25%)       | 3 (33.3%)     |
|                      | Anterior & Posterior unilateral           | 1 (8.3%)      | -             |
|                      | Anterior bilateral & Posterior unilateral | -             | -             |
|                      | Anterior bilateral                        | -             | -             |
|                      | Total                                     | 12 (100%)     | 9 (100%)      |
| Mandible             | Anterior                                  | 5 (27.8%)     | 6 (28.5%)     |
|                      | Posterior                                 | 9 (50%)       | 14 (66.7%)    |
|                      | Anterior & Posterior unilateral           | 1 (5.5%)      | 1 (4.8%)      |
|                      | Anterior bilateral & Posterior unilateral | 2 (11.2%)     | -             |
|                      | Anterior bilateral                        | 1 (5.5%)      | -             |
|                      | Total                                     | 18(100%)      | 21 (100%)     |

**Table 2.** Microvessel density in two studied groups

| Group | location  | No | Mean  | ±SD    | Minimum | Maximum |
|-------|-----------|----|-------|--------|---------|---------|
| CGCG  | Periphery | -  | -     | -      | -       | -       |
|       | Mass      | 30 | 22.32 | ±12.81 | 1.67    | 49.33   |
| PGCG  | Periphery | 30 | 24.78 | ±12.52 | 3       | 55      |
|       | Mass      | 30 | 26.56 | ±11.03 | 5.33    | 47      |

## Discussion

In this study, all cases of CGCG and PGCG revealed immunoreactivity for the antibody; similarly, in a study by Falci et al.,<sup>(2)</sup> this immunoreactivity was 92% and 93% for CGCG and PGCG, respectively.<sup>(2)</sup> This study detected CD105 expression in two lesions and in the supportive peripheral stroma of PGCG. These results did not support the findings of previous studies.<sup>(14,15,19)</sup> el-Mofty and Osdoby,<sup>(19)</sup> Lim et al.,<sup>(15)</sup> and O'mally et al.<sup>(14)</sup> assessed the immunoreactivity of factor VIII-related antigen and CD34 markers in CGCG and PGCG lesions.<sup>(14,15,19)</sup> Their findings revealed that the tumor-supporting blood vessels on the periphery of the lesions were stained with these markers.<sup>(14,15,19)</sup> Moreover, lack of reactivity of blood vessels was observed in the deep parts of the lesions and among the multinucle-

ated giant cells aggregations.<sup>(14,15)</sup> Lim et al.<sup>(15)</sup> reported that these findings may be the result of the difference in the microcirculation on the periphery and mass of the lesions and may indicate the absence of mature functional microvasculature in the deeper areas.<sup>(15)</sup> Hence, we hypothesized that the discrepancy between the former investigations and our study may be due to the characteristics of the vascular markers used. Presumably, immunostaining results using different vascular markers may vary depending on the degree of differentiation of the vascular endothelial cells and degree of maturation of the vessels. In our study, in contrast to others,<sup>(14,15)</sup> CD105 was used to identify the newly formed vessels, which were observed in the mass of these lesions.<sup>(20)</sup>

In the present study, MVD-CD105 was calculated for PGCG and CGCG and revealed that angiogenesis was not significantly different in the two groups; although it was slightly higher in PGCG than in CGCG group. This finding was similar to the findings of Falci et al.,<sup>(2)</sup> regarding the expression of CD105 in PGCG and CGCG.<sup>(2)</sup> Angiogenesis is the result of an imbalance between the positive and negative angiogenic factors.<sup>(21)</sup> Several angiogenic factors such as growth factors and cytokines<sup>(1)</sup> are produced by multinucleated giant cells, mononuclear stromal cells,<sup>(1)</sup> and inflammatory cells<sup>(6)</sup> in giant cell granulomas.<sup>(1,6)</sup> Alternatively, loss of inhibitors of this process such as wild type P53 can also induce angiogenesis.<sup>(22)</sup> No significant difference in calculated MVDs-CD105 in current study can suggest a balance between positive and negative angiogenic factors in PGCG and CGCG (also, the balance of these factors between part of supportive peripheral stroma and mass of PGCG). Moreover, the lack of expression of P53 has been reported in PGCG and CGCG.<sup>(23)</sup> Thus, presumably, angiogenesis in the two groups in our study was adjusted by the function of wild type P53, reduction in production of angiogenic factors, and induction of antiangiogenic molecules; however, higher MVD in PGCG can be attributed to the stronger inflammatory response in this group compared to CGCG, although inflammation was considered as a confounding factor in this study and we ensured selection of specimens with minimal inflammation.

Similar to our study, Kashyap et al. found no significant difference between PGCG and CGCG in MVD (in H & E stained slides).<sup>(24)</sup> Matos et al. indicated that the calculated MVD (using vWF marker) in PGCG was higher than in CGCG although they reported a significant difference between the CGCG and PGCG groups.<sup>(13)</sup> Falci et al.<sup>(2)</sup> also reported that MVD in PGCG was higher than CGCG using CD34 marker, whereas the difference between the two groups was significant.<sup>(2)</sup> Some studies have assessed the expression of influential factors on angiogenesis in these lesions (but not with the use of MVD) and reported controversial results.<sup>(12, 13, 25)</sup> Several factors can affect the results of studies such as the method of assessment, sample size, heterogeneity of tissues, paraffin blocks storage condition, materials and methods used for tissue fixation,

preparation of blocks, different methods of immunohistochemical staining, and type of antibody used. The biological behavior of CGCG ranges from non-aggressive to aggressive. In general, it is difficult to reliably predict the aggressive or nonaggressive behavior of CGCG based on the histological findings during a routine histological examination.<sup>(14,26)</sup>

In the present study, we tried to subclassify the CGCG group according to signs, symptoms, and cortical perforation; however, we could not find any significant correlation between CD105 expression and signs/symptoms or cortical perforation. This may be explained, at least partly, by the small sample size. Moreover, in our study, information regarding these parameters was extracted from the patient files and can be influenced by the incompleteness of medical records. Essentially, this finding may indicate the independence of these properties (signs, symptoms or cortical perforation) from angiogenesis in CGCG, according to the present study.

## Conclusion

This study reported neovascularization in PGCG and CGCG. Both lesions revealed angiogenic potential; however, it did not differ significantly between them. This finding may suggest that different clinical behavior of PGCG and CGCG is independent of the number of vessels and angiogenesis.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgments

The authors acknowledge the financial support provided by the Medical sciences/University of Tehran (grant number 3791-69-03-85). The authors are also grateful to Dr. Maryam Kalili and Dr. Mohammad Javad Kharazi for their precious scientific guidance and statistical analysis assistance, respectively.

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