ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Immunological Changes in Inflammatory Cysts.

**Oliver Dimitrovski[1, 2]*, Vancho Spirov[1], Blagoja Dastevski[3, 4], and Filip Koneski[2].**

[1]Department of Oral Surgery and Implantology, University Dental Clinical Center "St. Pantelejmon", Skopje.
[2]Department of Oral Surgery, Faculty of Dental Medicine, Ss. Cyril and Methodius University in Skopje.
[3]Department of Prosthodontics, University Dental Clinical Center "St. Pantelejmon", Skopje.
[4]Department of Prosthodontics, Faculty of Dental Medicine, Ss. Cyril and Methodius University in Skopje.

**ABSTRACT**

Cysts which appear in the orofacial region are represented as common pathological changes which often don't end with complications. In recent years, a dominant role in the pathogenesis of cysts belongs to the immunopathological reactions. It is assumed that the loss of bone in cysts is due to the presence of complementary cascades, prostaglandins synthesis and numerous neutrophil granulocytes. The undefined etiopathogenic mechanisms of the inflammatory cysts and the common complications from their presence in the mouth were the objective of this study, as well as the similarities and differences between the radicular, residual and the periodontal cysts from an immunological point of view. 150 patients with clinically diagnosed inflammatory cysts were examined, compared to the control group comprising 35 respondents. Immunoglobulins in serum i.e. IgA, IgG and IgM were determined with microelisa technique by Rook & Cameron, Engvall and Ulman. An appropriete conjugate Rubbitanihuman IgG, IgM or Iga HRP was used for each immunoglobulin. Standard values for IgA are from 0.90 to 4.50 g/L, for IgG from 8 to 18 g/L and for IgM from 0.60 to 2.65 g/L, while in saliva 8-12 mg/ml IgM and IgG in traces are used when tested with the same method. The difference of the basic values of the immunoglobulins before therapy and the basic values of the immunoglobulins in the control group was statistically significant only in the group of residual and periodontal cysts for IgG and IgM The difference of the average values of immunoglobulins (IgG, IgA and IgM) in the group with residual cysts before and one month after therapy is statistically significant (p=0.0000; p=0.0371; p=0.0276). A significant difference was registered in IgA among the three examined groups one month after surgical intervention. In defining the role and importance of the complex immune reactions among cysts in orofacial region, the parameters derived from the analysis of immunological findings may play an important role.

**Keywords**: inflammatory cysts; immunological changes; immunoglobulins

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

Cysts which appear in the orofacial region are represented as common pathological changes which often don't end with complications. For many years, scientists have been working on finding answers to many controversial issues and enigmas associated with the complicated and insufficiently clarified etiopathogenic changes in this pathological processes.

Cysts are common diseases that are often encountered in the clinical practice. The significance of these changes varies day by day, firstly because of the severity of the clinical picture, but also because of other reasons including: complications from the late and inadequate treatment, the pernicious effect on the general health, and the ability to represent potential focal seat of infection influencing other systems and organs in the organism. Cysts are usually a consequence of a more complex pathogenic mechanism, in which the carrier of the pathogenic effect and reduced immunological defense is mentioned, locally in the tissue and wider in the organism. There are numerous unresolved questions for the initiating stimulus that is still unexplained and for which there are numerous controversies.

In recent years, a dominant role in the pathogenesis of cysts belongs to the immunopathological reactions. Many authors, such as Piattelli[1], have proven that the appearance and development of the inflamantory cysts is conditioned by the immunopathological reactions. This insight does not rule out the possibility that the mechanisms of development in the orofacial cysts are similar, but as responsible factors there are other factors mentioned, some of which are different in every cyst.

In a narrower sense, immunological mechanisms are based on the influence of the specific and nonspecific immunity, through the action of humoral and cellular immune response.

There are numerous theories presented by many world-recognized researchers and related to the immunological changes in the periodontal-tissue complex. Immune components in the periapical lesions are the cause for bone destruction, as shown by some experimental evidence. [2] It is assumed that the loss of bone is due to the presence of complementary cascades, prostaglandins synthesis and numerous neutrophil granulocytes. Although the pathogenesis of chronic periodontal diseases is not clarified completely, it is still believed that the hydrolytic enzymes that facilitate destructive processes have their own impact on the pathogenesis.

Despite the evident role of the immune system in the pathogenesis of periodontal diseases, the importance of the humoral and cellular immune reactions is not exactly emphasized. It is suggested that the chronic periodontitis is a result of the joint influence by both immune reactions, but the difference in clinical manifestation is directly associated with the quality of the immune responses.

In the literature interpretations about the nature of the cysts are not consistent; they are contradictory and undefined. Therefore, a group of scientists has raised the issue of immune mechanisms as possible factors in the etiopathogenesis of these changes.

Suzuki [3] conducted immunohistochemical analysis on apoptosis-related factors in the epithelial lining of radicular cysts. The author starts from the fact that certain situations suggest that apoptosis-related factors are involved in the inflammatory processes of marginal periodontal lesions, which part in the periapical inflammatory lesions remains unclear.

The author questions the possible role of the apoptotic cell extinction in the periapical inflammatory lesions with immunohistochemical analysis.

The results from the research show that the gingival epithelium of radicular and residual cysts demonstrated expression of DNA in the unique DNA in the suprabasal and superficial epithelial cells and Ki 67 reactivity in the basal and parabasal cells. Expressions of Ki 67 and unique DNA in the radicular and residual cysts is slightly increased, unlike the values received from the gingival. Both, Ki 67 and the unique DNA reactivity are significantly increased in the radicular cysts with intensive inflammatory reaction or with dividing epithelial layer, than in the radicular cysts with less inflammatory reaction or with thinner inflammatory layer. The findings suggest that apoptosis-connecting factors are involved in the pathophysiological activities of

periapical inflammatory lesions. These factors can be affected by the structure of the epithelial lining and the degree of inflammatory changes.

In order to clarify the role of immune mechanisms in the pathogenetic changes of inflammatory cysts Piattelli [4] performed immunochemical and biological characterization of outer membrane proteins of Porphyromonas endodontalis.

The author indicated that the OMP-1 preparation contained numerous proteins with molecular mass with kDa 31. Unlike them, in the OMP 2 preparation, proteins with molecular mass from 14, 15, 25, 27 and 44 kDa were contained. The specific antigen immunoglobulins M(IgM), IgG and IgA from the secreting cells (SFC), enzymatically dissociated into single cells suspended from chronically inflamed periapical tissues. In patients with radicular cysts main isotopes of spontaneous SFC are IgG. In radicular cysts OMP-2 specific IgG are 0.13% of the total IgG. Parallel with these findings, the author confirms that none of these mononuclear cells produce antibodies specific to OMP-1, or liposaccharides for *Porphiromonas endodontalis*.

The undefined etiopathogenic mechanisms of the inflammatory cysts and the common complications from their presence in the mouth were the objective of this study, as well as the similarities and differences between the radicular, residual and the periodontal cysts from an immunological point of view.

## MATERIALS AND METHODS

For realization of the set goal, 150 patients with clinically diagnosed inflammatory cysts were examined. All of the patients who were included in this study were clinically monitored at the Clinic for Oral Surgery at the Dental Clinical Center St. Panteleimon in Skopje. All the examinations were made after getting a positive response from the ethical committee from the Faculty of Dentistry in Skopje and signed informed consent from the patients. Some of the examinations were carried out at the National Institute for Transfusion Medicine at the Faculty of Medicine in Skopje. To achieve the study objectives, the respondents were divided into two groups: an examined group consisting of 150 respondents and a control group comprising 35 respondents.

*Immunological research.* The immunological status in every participant from both examined groups and the three subgroups as well as the individuals examined was registered through quantitative and qualitative evaluation of the humoral immunity (in the blood).

The humoral immune response was monitored by determining immunoglobulins in the serum.

*Determining of immunoglobulins in serum.* Immunoglobulins in serum i.e. IgA, IgG and IgM were determined with microelisa technique by Rook & Cameron, Engvall and Ulman. For that purpose 96 microwell disks with flat bottom are used. It is proceeded through competitive type of microelisa technique where the concentration of immunoglobulins in serum is inversely proportional to the intensity of the enzymatic reaction (the coloring). The isolated human immunoglobulin binds to the solid phase of the microwell disk. The excess binding sites are blocked by a unreactive protein. After rinsing, the examined serum or saliva and the conjugated antibody with enzyme are added equally. They compete with each other for binding sites and at the same time the concentration of the present antibody is in inversecorrelation with the intensity of the enzymatic reaction. An appropriete conjugate Rubbitanihuman IgG, IgM or Iga HRP was used for each immunoglobulin. Standard values for IgA are from 0.90 to 4.50 g/L, for IgG from 8 to 18 g/L and for IgM from 0.60 to 2.65 g/L, while in saliva 8-12 mg/ml IgM and IgG in traces are used when tested with the same method.

## RESULTS

The immunological trials of the humoral immunity were made by blood analysis of the immunoglobulins before and one month after the surgical intervention.

The difference of the basic values of the immunoglobulins before therapy and the basic values of the immunoglobulins in the control group was statistically significant only in the group of residual and periodontal cysts for IgG and IgM (Table 1).

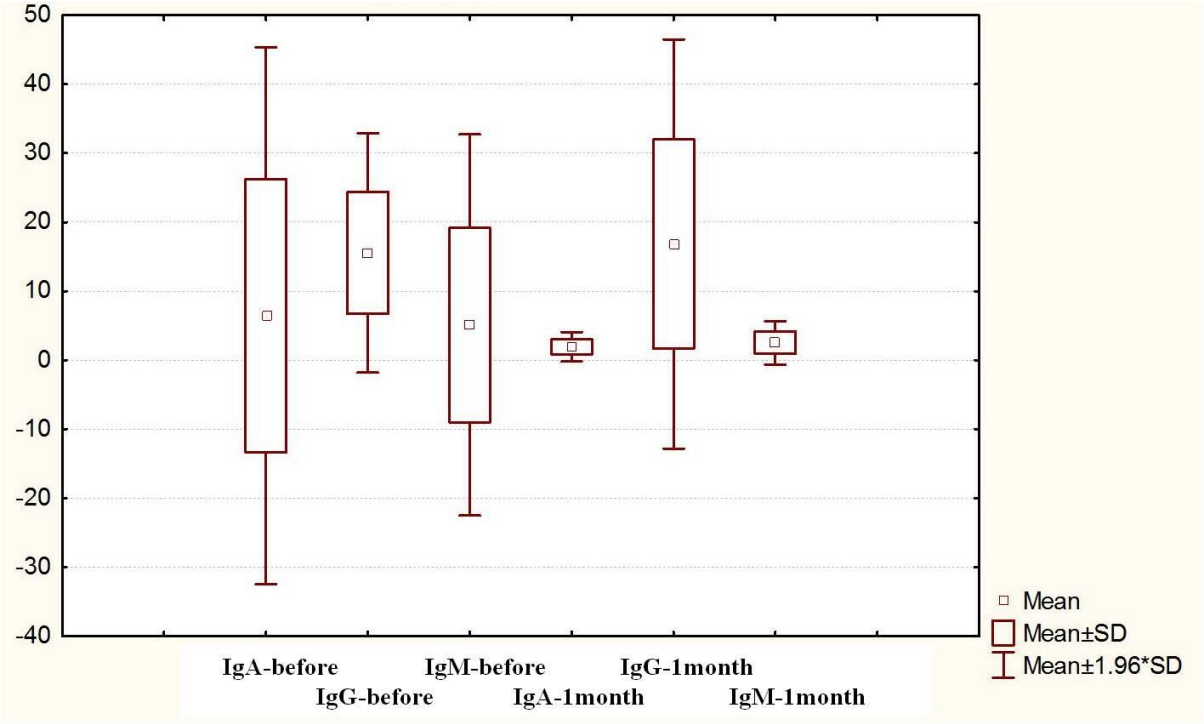
ISSN: 0975-8585

**Table 1. Average values of immunoglobulins before surgery in the three examined groups and in the control group**

| | Control group | | | Radicular cysts | | |
|---|---|---|---|---|---|---|
| | IgA | IgG | IgM | IgA | IgG | IgM |
| Average | 2.28 | 12.39 | 1.20 | 6.5 | 15.6 | 5.1 |
| SD | 1,019 | 3,96 | 0,35 | 19.8 | 8.8 | 14.1 |
| P= | | | | 0.2920 | 0.0872 | 0.1779 |
| | Control group | | | Residual cysts | | |
| | IgA | IgG | IgM | IgA | IgG | IgM |
| Average | 2..28 | 12..39 | 1.20 | 2.5 | 15.2 | 2.8 |
| SD | 1.019 | 3.96 | 0.35 | 0.9 | 1.4 | 1.3 |
| P= | | | | 0.3429 | 0.0000 | 0.0000 |
| | Control group | | | Periodontal cysts | | |
| | IgA | IgG | IgM | IgA | IgG | IgM |
| Average | 2.28 | 12.39 | 1.20 | 2.5 | 15.3 | 3.2 |
| SD | 1.019 | 3.96 | 0.35 | 1.1 | 7.0 | 1.7 |
| P= | | | | 0.4058 | 0.05 | 0.0000 |

The difference of the average values of immunoglobulins (IgG, IgA and IgM) in the group with radicular cyst before and after one month after therapy is statistically insignificant (p=0.1042; p=0.6284; p=0.1982); (Table 2 and Figure 1a).

**Figure 1a: Mean values of immunoglobulins before and one month after the treatment of radicular cysts**



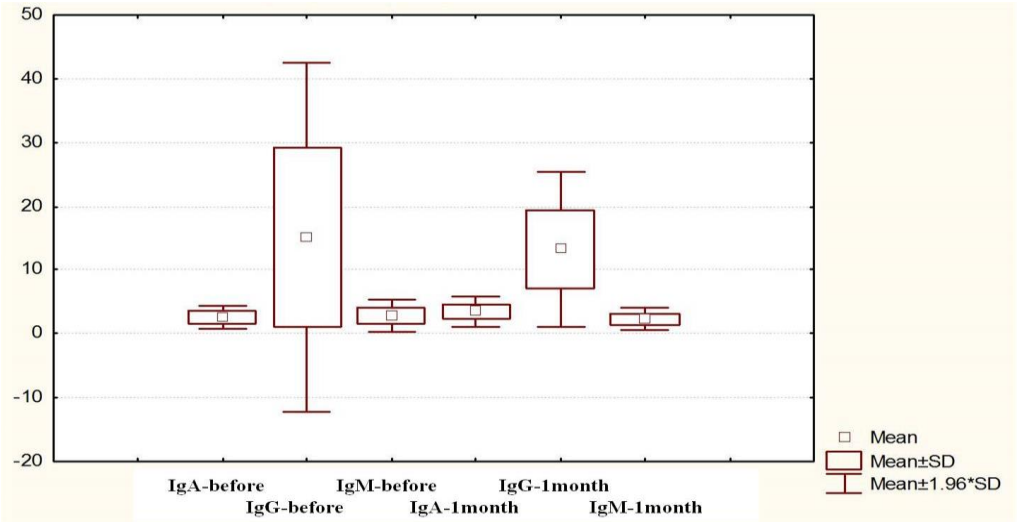
ISSN: 0975-8585

**Table 2. Average values of immunoglobulins before and one month after therapy in the three examined groups**

| | Number | Average | Minimum | Maximum | SD |
|---|---|---|---|---|---|
| **Radicular cysts** | | | | | |
| IgA-before | 50 | 6.44960 | 0.250000 | 103.0000 | 19.83398 |
| IgG- before | 50 | 15.56420 | 1.600000 | 45.7300 | 8.84192 |
| IgM- before | 50 | 5.10900 | 0.500000 | 102.0000 | 14.09074 |
| IgA-after 1 m | 50 | 1.95300 | 0.100000 | 4.7500 | 1.08098 |
| IgG- after 1 m | 50 | 14.96700 | 1.800000 | 42.7100 | 8.90236 |
| IgM- after 1 m | 50 | 2.51220 | 0.200000 | 7.8000 | 1.61098 |
| **Residual cysts** | | | | | |
| IgA- before | 50 | 2.55100 | 1.140000 | 4.6000 | 0.93140 |
| IgG- before | 50 | 15.15440 | 4.600000 | 102.0000 | 13.96158 |
| IgM- before | 50 | 2.86360 | 1.200000 | 6.6000 | 1.29485 |
| IgA- after 1 m | 50 | 3.52120 | 1.800000 | 8.9000 | 1.19220 |
| IgG- after 1 m | 50 | 13.27040 | 2.100000 | 33.2000 | 6.17416 |
| IgM- after 1 m | 50 | 2.29600 | 0.700000 | 4.6000 | 0.89768 |
| **Periodontal cysts** | | | | | |
| IgA- before | 50 | 2.54780 | 0.200000 | 4.7000 | 1.08287 |
| IgG- before | 50 | 15.35840 | 1.500000 | 35.9000 | 7.04143 |
| IgM- before | 50 | 3.20920 | 0.400000 | 7.9000 | 1.71278 |
| IgA- after 1 m | 50 | 2.06000 | 0.100000 | 4.7000 | 1.04335 |
| IgG- after 1 m | 50 | 13.39200 | 1.600000 | 41.6000 | 7.85264 |
| IgM- after 1 m | 50 | 2.26400 | 0.200000 | 6.8000 | 1.26568 |

The difference of the average values of immunoglobulins (IgG, IgA and IgM) in the group with residual cysts before and one month after therapy is statistically significant (p=0.0000; p=0.0371; p=0.0276); (Table 2 and Figure 1b).

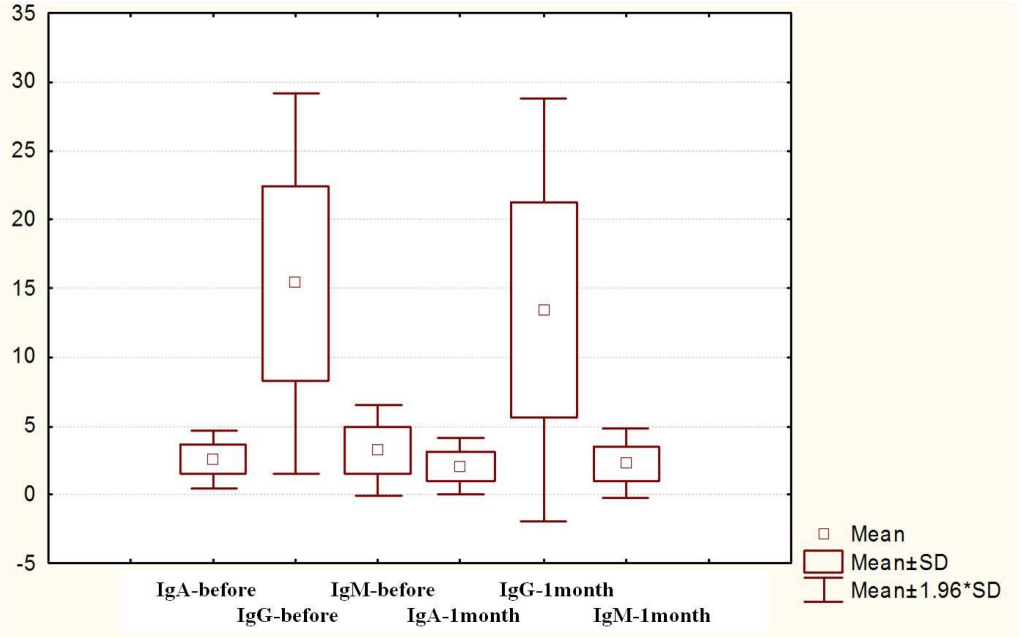
**Figure 1b: Mean values of immunoglobulins before and one month after the treatment of residual cysts**



ISSN: 0975-8585

The difference of the average values of immunoglobulins (IgG, IgA and IgM) in the group with periodontal cysts before and one month after therapy was statistically insignificant (p=0.0647; p=0.1966; p=0.1237); (Table 2 and Figure 1c).

**Figure 1c: Mean values of immunoglobulins before and one month after the treatment of periodontal cysts**




The difference between the average values of immunoglobulins before therapy and the average values of immunoglobulins in the control group was statistically significant only in the group with residual and periodontal cysts for IgG and IgM (Table 3).

**Table 3: Difference among IgA, IgG and IgM prior to and after surgical intervention**

| | SS | df | MS | SS | df | MS | F | p |
|---|---|---|---|---|---|---|---|---|
| IgA-before | 507.0523 | 2 | 253.5261 | 19375.91 | 147 | 131.8089 | 1.92344 | 0.149763 |
| IgG-before | 4.1984 | 2 | 2.0992 | 15811.66 | 147 | 107.5623 | 0.01952 | 0.980675 |
| IgM-before | 146.1750 | 2 | 73.0875 | 9954.81 | 147 | 67.7198 | 1.07926 | 0.342523 |
| IgA-1m | 76.7634 | 2 | 38.3817 | 180.24 | 147 | 1.2261 | 31.30288 | 0.000000 |
| IgG-1m | 89.5644 | 2 | 44.7822 | 8772.77 | 147 | 59.6787 | 0.75039 | 0.473983 |
| Igm-1m | 1.8228 | 2 | 0.9114 | 245.15 | 147 | 1.6677 | 0.54652 | 0.580134 |

The difference registered between the average values of immunoglobulins in the examined groups was statistically insignificant. A significant difference was registered in IgA among the three examined groups one month after surgical intervention (Table 3).

## DISCUSSION AND CONCLUSION

There are still concerns about the possible etiopathogenetic mechanisms responsible for the emergence and development of cysts, which are a common appearance in the oral cavity. In theory, many potential etiological factors are mentioned, including microbiological allergologic, immunological and others. Authors say that all these factors, in their own and unique way, participate in the expression and development

of cysts, despite their nature, but not in a very convincing way. Contemporary knowledge about the nature of immune responses is much bigger and complex, but still it is impossible to fully define their principles. Namely, with the stimulus of the foreign antigens the organism may respond by creating specific antibodies (humoral immune response) or by activation of the sensitized T-lymphocytes (cellular immune response).

In the humoral immune response the antibodies which are the final product of plasma cells mediate and represent a final form in the differentiation of B-cells. These antibodies belong to the group of immunoglobilins which structural characteristic is a specifically bi-functional molecule. The main function of the immunoglobulins is not destruction of the agent, but preventing the attack and removal of these substances from the immune system. Intermediaries in the humoral immune response are the group of proteins that react with each other. This group of proteins, with this type of composition, is called complementary system.

The complementary system belongs to the category of amplification systems, and is activated by classical and alternative way. Experimentally it has been proven that by activation of the complement different biological changes happen, which include: cell lysis, immune adherence, neutralization of viruses etc. [5]

Unlike humoral immunity, carriers of cellular immune response are T-lymphocytes and their role is manifested through the ability of a specific immune response to foreign antigens in the organism. Antigenic stimulation causes blast transformation of T-lymphocytes that create true offspring of different T-cells. It is proven that for different activities of T-lymphocytes special populations exist. The breakthrough of monoclonal antibodies has serious participation in the discovery of some specific subclasses of T-cells: cytotoxic, helper, suppressor, killer etc.

Our examination of the condition of immunoglobulins in the three examined groups has shown certain changes. Before prescribing therapy, elevated IgA values were observed in participants with radicular cysts, IgG were increased in all groups, while IgM were elevated in participants with radicular cysts. However, statistically significant findings were only those for IgG and IgM in radicular and periodontal cysts.

After prescribed therapy and evaluation of the difference of average values between the level of immunoglobulins before and one month of therapy there were statistically significant values in the group with radicular cysts for the three classes of immunoglobulins.

The analysis of variance between the three groups one month after prescribed therapy showed no significant difference in the values of IgA and IgM, but they did for IgA. Other authors came to similar results, such as Kubota [6], who found the highest values of immunoglobulins in radicular cysts (IgA- 488.9 mg/100 ml, IgG – 2535.4 mg/100 ml, IgM – 135.6 mg/100 ml), unlike the follicular (IgA -2308.4 mg/100ml, IgG – 1618.2 mg/100 ml, IgM – 155.6 mg/100 ml) and especially the odontogenic keratocysts (IgA – 135.6 mg/100 ml, IgG – 491.9 mg/100 ml, IgM – 54.1 mg/100 ml).

In defining the role and importance of the complex immune reactions among these diseases can help the parameters derived from the analysis of the inflammatory cell infiltrate in the periapical lesions and its surroundings [6]. Although there are different findings in the literature, certain immunological parameters can play a role in the etiopathogensis of inflammatory cysts.

Sometimes the presence of B-cells prevails over the presence of T-lymphocytes. In some studies the data is contradictory, and T-cell population is more dominant. These, and similar findings open new fields for research in these still unclear fields.

## REFERENCES

[1] Piattelli A, Rubini C, Iezzi G, Fioroni M. CD1a-positive cells in odontogenic cysts. J Endod. 2002;28(4):267-8.

[2] Tay JY, Bay BH, Yeo JF, Harris M, Meghji S, Dheen ST. Identification of RANKL in osteolytic lesions of the facial skeleton. J Dent Res. 2004;83(4):349-53.

[3] Suzuki T, Kumamoto H, Kunimori K, Ooya K. Immunohistochemical analysis of apoptosis-related factors in lining epithelium of radicular cysts. J Oral Pathol Med. 2005;34(1):46-52.
[4] Piattelli A, Fioroni M, Santinelli A, Rubini C. P53 protein expression in odontogenic cysts. J Endod. 2001;27(7):459-61.
[5] Takeda Y, Oikawa Y, Furuya I, Satoh M, Yamamoto H. Mucous and ciliated cell metaplasia in epithelial linings of odontogenic inflammatory and developmental cysts. J Oral Sci. 2005;47(2):77-81.
[6] Kubota Y, Ninomiya T, Oka S, Takenoshita Y, Shirasuna K. Interleukin-1alpha-dependent regulation of matrix metalloproteinase-9(MMP-9) secretion and activation in the epithelial cells of odontogenic jaw cysts. J Dent Res. 2000;79(6):1423-30.