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The Levels of Serum Immunoglobulin A, G and M in Oral Inflammatory Cysts before and after Surgical Therapy

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SUMMARY

Background/Aim*: Cysts which appear in the orofacial region are represented as common pathological changes which underlying mechanism of development is still not fully clear. 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 main objective was to determine the levels of Ig G, A and M in serum and saliva of patients with radicular, residual and periodontal cysts before and after the surgical treatment.* ***Material and Methods:*** *The study included 185 patients, of which 150 patients were diagnosed with inflammatory cysts (radicular, periodontal and residual), while the control group consisted of 35 patients without presence of inflammatory cyst. The immunoglobulins were determined prior to the surgical removal of the cyst and one month after the procedure, when complete clinical wound healing was observed. The levels of these immunological markers were compared to each other before and after the cyst extirpation, taking into consideration the different cyst types. A comparison was also made between both examination and control group.* ***Results****: 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. ***Conclusions****: The levels of serum immunoglobulins in patients with inflammatory cysts were elevated before the treatment and dropped after the cyst removal. This study suggests that the IgA, IgG and IgM may play an important role in the occurrence, development and persistence of the cystic lesions.*

Key words: Periodontal Cyst, Radicular Cyst, Residual Cyst, Inflammation, Pathogenesis, Immunoglobulins, Surgical Treatment

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Introduction

Cysts which appear in the orofacial region are common pathological changes with no clear underlying mechanism of development. 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.

It is believed that the 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. Piattelli et al. showed that the appearance and development of the inflammatory cysts is conditioned by the imunopathological reactions[1]. 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.

Bernardi et al. suggest that for the sustenance and growth of apical cysts are responsible a number and combination of many factors involving an epithelial-stromal interactions[2]. Moreover, it was suggested that some systemic diseases and conditions may affect the outcomes of the treatment of the cytic lesions, suggesting that these factors may also have influence on their development[3].

Immune components in the periapical lesions may be the cause for bone destruction, as shown by some experimental evidence[4]. It is assumed that the loss of bone is due to the presence of complementary cascades, prostaglandins synthesis and numerous neutrophil granulocytes. Moreover, changes in the CRP levels, immunoglobulin levels, interleukins, tumor necrosis factors, transforming growth factors and acute phase proteins were found to be elevated in patients with periapical pathology in previous studies. These findings suggest that the periapical lesions lead to increased systemic inflammation response[5,6]. 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.

Marton and Kiss found that several regulation mechanisms overlap during the formation of periapical inflammation and further, in the development of the inflammatory cysts. These pathways consist of complex immunological changes of reparation and destruction[7]. In another review study of Marton et al. it is pointed out that the immune response of the host to the bacteria arising from the tooth root canal system plays an important role in the development of such lesions[8].

Meyle et al. summarized that the innate immunity is primary responsible for the rapid molecular and cellular response of the host in the inflammatory processes[9]. These mechanisms were also investigated in another study, which points out that the complex interactions between numerous cytokines and other inflammatory mediators stimulate the tissue breakdown[10].

Piattelli et al. performed immunochemical and biological characterization of outer membrane proteins of Porphyromonas endodontalis. The authors indicated that the OMP-1 preparation contained numerous proteins with molecular mass with kDa 31[11].

The main objective of this study is to determine the levels of Ig G, A and M in serum and saliva of patients with radicular, residual and periodontal cysts before and after the surgical removal.

Material and Methods

Study design and sample collection

The study included 185 patients, of which 150 comprised the examination group with clinically and radiogically diagnosed with inflammatory cysts in the orofacial region, and a control group consisted of 35 patients, without cystic lesions, confirmed with panoramic x-ray. The examination group was subdivided into three groups, depending on the type of the cyst: radicular, residual and periodontal cysts. 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. Pantelejmon 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. The inclusion criteria for the examination group was a presence of one of the mentioned inflammatory cysts in the jaws (radicular, periodontal or residual). Both groups included patients at 20-60 years of age. The exclusion criteria for both groups represented presence of other inflammatory conditions in the jaw not classified as cyst, as well as taking anti-inflammatory or antibiotic medication. Pregnant women and patients with chronic and uncontrolled chronic disease were also excluded from the study. The laboratory analysis were performed in blood collected from the cubital vein from all of the patients. In the examination group, the blood collections were made right before the surgical treatment of the cyst and one month afterwards.

Surgical treatment

The surgical treatment of the cysts in the examination group was performed under local anesthesia. The mucoperiostal design was planned and performed depending on the localization and dimension of the lesion. A complete removal of the cystic lesion was done with combined sharp and blunt dissection. It was ensured that the cysts were enucleated in toto, i.e. with

their whole epithelial lining and capsule. In cases with radicular and periodontal cysts, the teeth from which the cysts were arising were extracted. The bony walls of the cystic defect were further checked for consistency and absence of any granulation or epithelial tissue. The confirmation of the clinical and radiological diagnosis was made with a histological evaluation. The presence of chronic epithelium surrounding the cystic cavity and cholesterol crystals were clear signs for setting the final diagnosis. The second blood collection from the patients from the examination group was made one month after the procedure, after the complete clinical wound healing was observed and in conditions of absence of postoperative complications or early recidives.

Laboratory analyses

The laboratory analyses were done in the National Institute for Transfusion Medicine in the Faculty of Medicine in Skopje. The immunological status in every participant from both examined and control groups was registered through quantitative and qualitative evaluation of the humoral immunity. The humoral immune response was monitored by determining immunoglobulins in the serum from the collected blood, as previously described. Immunoglobulins IgA, IgG and IgM in the serum were determined with microelisa technique by Rook & Cameron, Engvall and Ulman[12, 13]. 96 microwell disks with flat bottom were used for implementing the competitive type of microelisa technique. The concentration of immunoglobulins in serum in this technique is inversely proportional to the intensity of the enzymatic reaction by determining the optical density on basis of the level of coloring. The isolated human immunoglobulin binds to the solid phase of the microwell disk and the excess binding sites are blocked by the non-reactive protein. The examined serum or saliva and the conjugated antibody with enzyme were added equally to the plates after previous rinsing. They compete with each other for binding sites in the same time. The concentration of the present antibody is in inverse correlation with the intensity of the enzymatic reaction, i.e. the higher concentration of the antibody means the lower enzymatic reaction and lower coloring. An appropriete conjugate Rubbitanihuman IgG, IgM or IgA HRP was used for determining each immunoglobulin. The normal values for IgA range 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.

Statistical analyses

The carried data was statistilcally analyzed in the software program Statistica version 7 for Microsoft Windows. The distribution was checked with Colmogorov-Smirnof test. The differences between the parameters in the groups were tested with analysis of the variance (ANOVA test) for dependent variables. The significance was set for p-value lower than 0,05.

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).

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).

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). 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).

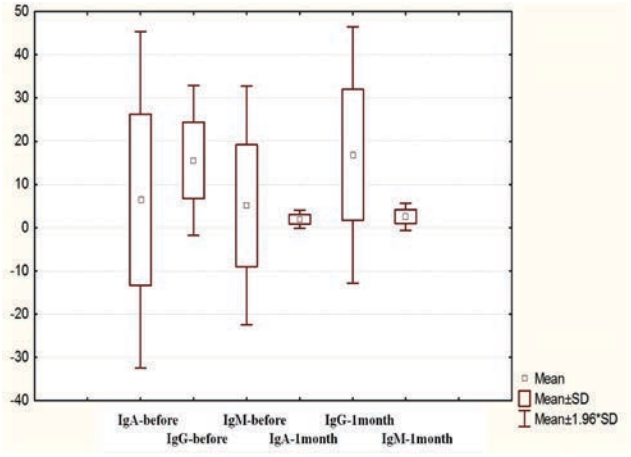

Figure 1a. Mean values of immunoglobulins before and one month after the treatment of radicular cysts

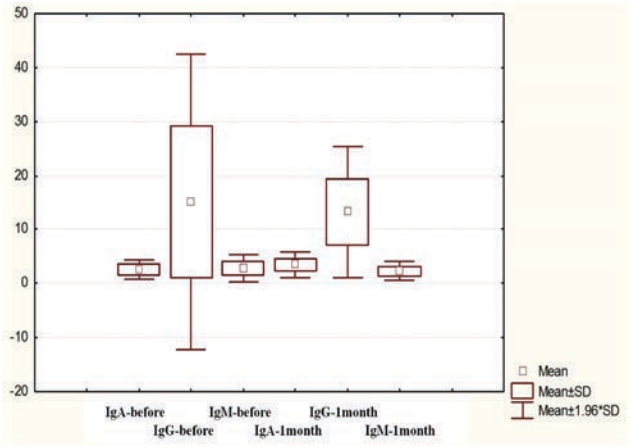

Figure 1b. Mean values of immunoglobulins before and one month after the treatment of residual cysts

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

	N°	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

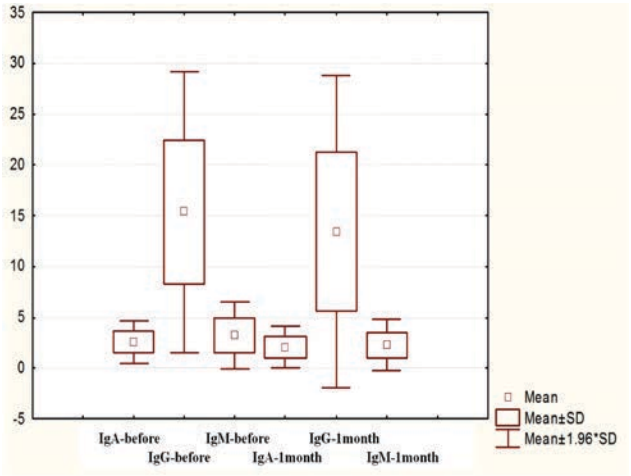

Figure 1c. Mean values of immunoglobulins before and one month after the treatment of periodontal cysts

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 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). The difference registered between the average values of immunoglobulins in the examined groups was not statistically significant. Significant differences were observed in IgA values among the three examined groups one month after surgical intervention, compared to the levels before the procedure (Table 3).

Discussion

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. According to the literature data, all these factors participate in the expression and development of cysts, despite their nature. However, the present data are insufficient to support this statement strongly. The contemporary knowledge about the nature of immune responses is more complex, but still it is impossible to fully define the detailed mechanisms. Namely, when stimulated by foreign antigens, the organism may respond by creating specific antibodies (humoral immune response) or by activation of the sensitized T-lymphocytes (cellular immune response).

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[14].

Unlike the humoral immunity, carriers of cellular immune response are the 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. The mechanisms and pathways of the immune response are very complex, with a lot of different cytokines and other inflammatory mediators being involved in the development of apical and periodontal pathological conditions[7,8,9,10]. Our examination of the level of immunoglobulins in the three examined groups has shown certain changes. Before the surgical removal of the cysts, different levels were obtained. Elevated IgA values were observed in participants with radicular cysts, IgG were increased in all groups, while IgM levels were elevated in participants with radicular cysts. However, statistically significant findings were found only in those for IgG and IgM in radicular and periodontal cysts. After the cysts surgical removal 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 determined similar results, such as Kubota[15], 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).

Piattelli found that the specific antigens IgM, IgG and IgA from the secreting cells (SFC) were enzymatically dissociated into single cells suspended from chronically inflamed periapical tissues[11]. In patients with radicular cysts main isotopes of spontaneous SFC are IgG. These findings are in accordance with the results obtained in our study. In the mentioned study from Piattelli et al., the radicular cysts OMP-2 specific IgG were 0.13% of the total IgG. This finding is in accordance with the values gathered in our study, because the main immunoglobulin subdivision in the radicular cysts was the IgM. Parallel with these findings, the author confirmed that none of these mononuclear cells produce antibodies specific to OMP-1, or liposaccharides for *Porphiromonas endodontalis*.

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[11]. 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.

Conclusions

The levels of immunoglobulins in the patients with inflammatory cysts in the orofacial region before the surgical treatment were elevated, depending on the type of the cyst. The surgical removal of the cysts influenced the levels of the immunoglobulins which significantly dropped. These findings suggest that the IgA, IgG and IgM may play an important role in the occurrence, development and persistence of the cystic lesions. Future studies are necessary to point out if these markers are associated with other systemic responses and factors and if their detection may be of importance in completely understanding the process of early formation of the cystic lesions.

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