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HUMORAL IMMUNE RESPONSE BEFORE AND AFTER SURGICAL THERAPY IN PATIENTS WITH ODONTOGENIC INFLAMMATORY CYSTS

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Abstract

Odontogenic inflammatory cysts are pathological lesions that are often represented in clinical practice and they presented potential focal points with an impact on other organs and systems in the body

The aim of this research is to compare the patients' humoral immune response by verifying the level of immunoglobulins IgA, IgG and IgM in the serum before and one month after surgical therapy in patients with odontogenic inflammatory cysts.

44 male and female patients were undergone surgical enucleation of the cysts in toto - Cystectomio (Partch II). The biopsy material was sent at the Institute of Pathological Anatomy-Skopje for histopathological verification. Patients diagnosed with odontogenic inflammatory cysts were divided into three groups. The values of IgA, IgM and IgG in serum were examined before and one month after the surgical therapy in Cobas 6000 model c501 (Roche, Germany).

The level of IgA did not indicate a significant difference for p=0.2716 vs. p=0.2898. A significant difference, was also not found in terms of the IgG level for p=0.2692 vs. p=0.3614. The comparison of the three groups of patients regarding the level of IgM in serum indicated a significantly higher value of this parameter before the intervention (p=0.0067) and 1 month after the intervention (p=0.0263).

The surgical removal generate a decrease in the levels of immunoglobulins' level. These findings suggest that IgA, IgG and IgM may play an important role in the occurrence, development and persistence of cystic lesions.

Keywords: Odontogenic inflammatory cysts, immunological analysis, humoral immune response, immunoglobulins IgA IgG and IgM.

Introduction

Odontogenic inflammatory cysts are pathological changes that are often represented in clinical practice and often end with complications due to untimely or inadequate treatment, the harmful impact on the general health condition, as well as the possibility of representing potential focal hotspots with an impact on other organs and systems in the body [1].

Cysts are usually result of more complex pathogenetic mechanisms in which the influence of the pathogenetic effect of the causative agent is often mentioned and the reduced immune defense locally in the tissue, but also in the whole organism[2].

There is no doubt that the basic prerequisite for the appearance of inflammatory cysts is the previous presence of an epithelium at the site of cyst development and an inflammatory stimulus. In recent years it has been considered a dominant role in the pathogenesis of cysts occupy immunopathological reactions. In a narrower sense, immune mechanisms are based on the action of nonspecific and specific immunity through the activity of the humoral and cellular immune responses.

Piatelli et al., believe that the appearance and development of inflammatory cysts is conditioned by immunopathological reactions[3].

In a narrower sense, immune mechanisms are based on the action of non-specific and specific immunity through the activity of the humoral and cellular immune response. There are numerous theories that arise from many researches that deal precisely with the immunological interactions in the periodontal tissue complex.

Bernardi et al., suggest that for the formation and growth of apical cysts many factors are responsible, involving numerous interactions in the cyst epithelium and stroma [4].

In addition, it has been emphasized that some systemic diseases and conditions may affect their outcome from the treatment of cystic lesions, suggesting that these factors may also have an impact on their growth and development[5].

Odontogenic inflammatory cysts are characterized by a wide variety, resulting in different morphological characteristics between them[6].

According to the World Health Organization (WHO), Classification of odontogenic inflammatory cysts from 1992, they are divided into the following groups;

- Radicular cysts with their subclassifications (apical, lateral),
- residual and
- periodontal.

Radicular cysts are the most common cysts in the jaws and their inflammatory origin is associated with presence of an avital tooth. This group also includes residual cysts left in the jaws after extraction of affected teeth and lateral radicular cysts associated with a lateral root canal of the teeth.

The etiopathogenesis of these pathological lesions is still insufficiently known. Modern knowledge about the nature of immune reactions is huge, but it is still insufficient to define their principles completely.

Namely, the organism can respond to the stimulus from foreign antigens by creating B lymphocytes through specific antibodies (humoral immune response) or by activating sensitized T lymphocytes (cellular immune response).

The aim of this research is to compare the humoral immune response by verifying the level of immunoglobulins IgA, IgG and IgM in the serum before and one month after surgical therapy in patients with odontogenic inflammatory cysts.

Materials and methods

This cohort study included a total of 44 male and female patients with clinically diagnosed odontogenic inflammatory cysts.

The study group of patients was divided into three subgroups:

- Group 1 - patients with presence of radicular cysts
- Group 2 - patients with residual cysts
- Group 3 - patients with periodontal cysts

Inclusion criteria in the studied group were presence of an inflammatory cyst in the jaw (radicular, residual or periodontal) in patients of good general health of the both genders, with no acute diseases. Patients were fully aware of the meaning and course of intervention itself and all agreed to respond to the following control examinations.

Exclusion criteria for entering the study group were patients with a diagnosis of odontogenic inflammatory cysts underwent on anti-inflammatory and antibiotic therapy 4-6 weeks before the surgical intervention, patients with cardiovascular diseases, liver disorders, malignancies, patients on bisphosphonate therapy, chemotherapy, pregnant women and women in lactation.

Participants from the study group signed an Informed Consent before the start of the study. Clinical and paraclinical examinations were performed individually on all subjects.

Clinical investigations represented a well-recorded medical history and clinical examination. The paraclinical investigations included radiological (panoramic and retoalveolar X-ray), pathohistological, immunohistochemical and immunological (laboratory) examinations.

Immediately before the surgical intervention, the patients' immune status was evaluated by testing the humoral immunity in the blood. A sample of venous blood (9 ml) was taken at the Clinic for Clinical Biochemistry-Skopje by Vacutainer venipuncture method, after which the presence of the humoral immune response was observed by determining the values of immunoglobulins IgA, IgM and IgG in serum in Cobas 6000 device, model c501 (Roche, Germany) [6].

Cobas 6000 device, model c501 (fig.12) is a biochemical analyzer of spectrophotometric, immunoturbodimetric and ion selective method of determining the concentrations of biochemical analytes.

Figure 1. Cobas 6000 device, model c501

The values of IgA, IgM and IgG are based on the principle of immune agglutination using specific antibodies - Anti-IgA, Anti-IgM and Anti-IgG that react with the antigen in the sample and form an antigen-antibody complex, which follows agglutination that is measured turbidimetrically along with the use of polyethylene glycol (PEG) which increases and improves the sensitivity, accuracy and precision of the method[9].

Figure 2. Set for determining the values of immunoglobulins A, M, G

Standard values for IgA according to CRM 470 Protein Standardization are 0.74 g/L; 4.38- 25.0 µmol/L; 70-400 mg/dL;

Standard values for IgM according to CRM 470 Protein Standardization are 0.4-2.3 g/L; 0.4-2.4 µmol/L; 40-230 mg/dL;

Standard values for IgG according to CRM 470 Protein Standardization are 7-16 g/L; 46.7-107 µmol/L; 700-1600 mg/dL.

The surgical protocol included application of local anesthesia, creating a mucoperiosteal flap, osteotomy, exposure of the cystic sacus and enucleation of the cysts in toto – the method of cystectomy (Partsch II). The bioptic material of the cysts was sent to the Institute of Pathological Anatomy - Skopje for examination and histopathological verification of the cysts and establishing a diagnosis by their typing.

The values of immunoglobulins in the serum of the patients were also examined one month after the surgical therapy when complete clinical healing of the wound occurred with no postoperative complications or early relapses.

Statistical analysis

The data obtained with the research were processed in SPSS software package, version 22.0 for Windows. Qualitative series were analyzed with relationship coefficients, proportions and rates, and quantitative series with measures of central tendency (average, median, minimum and maximum values), as well as measures of dispersion (standard deviation).

The Shapiro-Wilk W test was used to determine the correctness of the distribution frequency of the investigated variables, which resulted with their irregular distribution.

For the analysis of immune response parameters (IgA, IgG and IgM) between the two measurements (before surgery and 1 month after) for each of the three studied groups, the Wilcoxon Signed Ranks test was used for two dependent variables.

The comparison between the three groups regarding the level of IgA, IgG and IgM before and one month after the intervention was made with the Kruskal-Wallis H test for multiple independent variables. A two-sided analysis with a significance level of $p<0.05$ was used to determine statistical significance.

Results

After the obtained histopathological findings, the diagnoses for odontogenic radicular cysts (23), residual cysts (10) and periodontal cysts (11) were confirmed.

The research included a total of 44 patients divided into three groups according to the type of inflammatory cyst in the mouth, namely:

a) Group I - 23 patients with radicular cyst;

b) Group II - 10 patients with residual cyst; and

c) Group III - 11 patients with periodontal cyst.

In every patient, the immune response was monitored through three parameters of humoral immunity IgA, IgG and IgM in serum twice, before and 1 month after the surgical intervention.

In Group I of patients with radicular cyst (n=23), the analysis indicated that in all three investigated parameters of humoral immunity (IgA, IgG and IgM in serum) there is a significant difference in a decrease of the value 1 month after the surgical intervention compared to the condition before the intervention.

It was observed that IgA (p=0.0001), IgG (p=0.00004) and IgM (p=0.0093). In 50% of patients with radicular cyst: a) the level of IgA in serum before the intervention and compared 1 month later was higher than the consequent 2.1 vs. 2.0 g/L; b) the level of IgG in serum before the intervention compared to 1 month was higher than the consequent 11.4 vs. 11.2 g/L; and c) the level of IgM in serum before the intervention compared to 1 month later was higher than the consequent 0.9 vs. 0.8 g/L (Table 1).

Table 1. Comparison of immune response parameters in patients with radicular cyst before and one month after surgical intervention .

Parameters	**Patients with radicular cyst**				**P**
	Number (n)	**Mean± SD**	**Min/Max**	**Median (IQR)**	
IgA – g/L					
Before operation	23	2,25±1,06	0,8/5,5	2,1 (1,7-2,6)	Z=3,723; p=0,0001*
After 1 month	23	2,07±1,04	0,6/5,4	2,0 (1,6-2,4)	
IgG – g/L					
Before operation	23	11,86±2,49	7,6/17	11,4 (9,9-13,8)	Z=4,107; p=0,00004*
After 1 month	23	11,05±2,38	7,3/16,0	11,2 (9,8-13,6)	
IgM – g/L					
Before operation	23	1,11±0,55	0,4/2,7	0,9 (0,8-1,3)	Z=2,600; p=0,0093*
After 1 month	23	1,09±0,58	0,3/2,5	0,8 (0,7-1,3)	
***Significant for p<0,05**					

In Group II of patients with a residual cyst (n=10), a significant difference was observed in all three investigated parameters of humoral immunity (IgA, IgG and IgM in serum) with decrease of their values 1 month after the surgical intervention compared with results before the intervention - IgA (p =0.0144), IgG (p=0.0051) and IgM (p=0.0117).

In 50% of the patients with a residual cyst the following results were obtain: a) the level of IgA in serum before the intervention compared to 1 month later was higher than the consequent 2.0 vs. 1.9 g/L; b) the level of IgG in serum before the intervention compared to 1 month was higher than the

consequent 12.8 vs. 12.4 g/L; and c) the level of IgM in serum before the intervention compared to 1 month later was higher than the consequent 0.8 vs. 0.7 g/L (Table 2).

Table 2. Comparison of immune response parameters in patients with residual cyst before and one month after the surgical intervention.

Parameters	Patients with residual cyst				P
	Number (n)	Mean± SD	Min/Max	Median (IQR)	
IgA – g/L					
Before operation	10	2,40±1,31	0,8/5,4	2,0 (1,7-3,2)	Z=2,446; p=0,0144*
After 1 month	10	2,29±1,28	0,7/5,3	1,9 (1,7-3,0)	
IgG – g/L					
Before operation	10	13,26±2,47	10,3/19,5	12,8 (11,8-13,8)	Z=2,803; p=0,0051*
After 1 month	10	12,72±2,31	10,0/18,4	12,4 (11,0-13,4)	
IgM – g/L					
Before operation	10	0,98±0,74	0,2/2,6	0,8 (0,5-0,9)	Z=2,520; p=0,0117*
After 1 month	10	0,95±0,71	0,2/2,5	0,7 (0,5-0,9)	
***Significant for p<0,05**					

In all three investigated parameters of humoral immunity (IgA, IgG and IgM in serum), in Group III of patients with periodontal cyst (n=11), a significant difference was observed in addition to decrease in their values 1 month after the surgical intervention compared to before the intervention - IgA (p=0.0033), IgG (p=0.0051) and IgM (p=0.0033).

In 50% of patients with periodontal cyst the following results were obtain: a) the level of IgA in serum before the intervention compared to 1 month later was higher than the consequent 1.7 vs. 1.5 g/L; b) the level of IgG in serum before the intervention compared to 1 month was higher than the consequent 12.0 vs. 11.2 g/L; and c) the level of IgM in serum before the intervention compared to 1 month later was higher than the consequent 1.9 vs. 1.8 g/L (Table 3).

Table 3. Comparison of immune response parameters in patients with periodontal cyst before and one month after the surgical intervention.

Parameters	Patients with periodontal cyst				P
	Number (n)	Mean± SD	Min/Max	Median (IQR)	
IgA – g/L					
Before operation	11	1,76±0,96	0,7/3,8	1,7 (1,0-2,6)	Z=2,934; p=0,0033*
After 1 month	11	1,59±0,88	0,6/3,2	1,5 (0,9-2,5)	
IgG – g/L					
Before operation	11	12,53±2,07	10,2/17,0	12,0 (11,0-13,2)	Z=2,803; p=0,0051*
After 1 month	11	11,99±1,96	9,1/15,9	11,2 (11,0-12,9)	
IgM – g/L					
Before operation	11	2,10±1,33	0,8/5,5	1,9 (1,2-2,0)	Z=2,934; p=0,0033*
After 1 month	11	1,89±1,26	0,8/5,1	1,8 (0,9-1,9)	
***Significant for p<0,05**					

In addition, the comparison of the IgA level before and 1 month after the intervention between the three groups of patients with radicular, residual and periodontal cyst in the mouth did not indicate a significant difference for p=0.2716 vs. p=0.2898.

A significant difference between the three groups of patients, before and 1 month after the operation, was also not found in terms of the IgG level for p=0.2692 vs p=0.3614.

The comparison of the three groups of patients regarding the level of IgM in serum indicated a significantly lower value of this parameter in patients with periodontal cyst (p=0,0033) compared to radicular cysts (p=0,0093) and higher value compared to the residual cyst (p=0,0117) before the intervention and 1 month after the intervention.

Discussion

Contemporary understanding about the nature of immune reactions in the pathogenesis of odontogenic inflammatory cysts is widely elaborated, but it is still impossible to define their principles completely. Namely, the body can respond to the stimulus from foreign antigens by creating specific antibodies (humoral immune response) or by activating sensitized T lymphocytes (cellular immune response).

The humoral immune response is mediated by antibodies, which are the final product of plasma cells, and represent the final form in the differentiation of B-lymphocytes.

These antibodies belong to the group of immunoglobulins whose structural feature is a specific bifunctional molecule. Their main function is not to destroy the agent, but to prevent their breakthrough and remove these substances from the immune system.

The humoral immunity participates in protection against extracellular microorganisms through the neutralization of microorganisms and their toxins. Mediators in the humoral immune response are the group of proteins that belong to the complement system. Carriers of the cellular immune response are the T lymphocytes. Their role is manifested through the ability of the body's specific immune response to foreign antigens. Cellular immunity participates in the body's defense against intracellular microorganisms.

Antigenic stimulation causes a blast transformation of T lymphocytes that give rise to a progeny of different T lymphocytes. It has been proven that there are special subpopulations of T lymphocytes for different activities.

The specific occurrence, the independent and progressive growth and the complexity of the events in the cystic shell are the subject of numerous clinical, microbiological and immunopathological investigations that contribute to the understanding of some regularities of the occurrence and growth of these lesions. On the other hand, the use of a greater number of new technological possibilities and the development of biochemical methods contribute to proposing new hypotheses about the appearance and evolution of cystic lesions [7].

For a definitive answer about the role and importance of complex immune reactions in these diseases, the parameters resulting from the analysis and distribution of the cells present in the inflammatory infiltrate in the periapical lesions and its immediate surroundings are important[8].

There is new evidence in the literature that confirms how in the presence of apical periodontitis the systemic levels of inflammatory markers in patients can be modified, eg. high-sensitivity C-reactive protein (hs-CRP), interleukin-1β (IL-1β) , IL-6, IL-12, IL-10, tumor necrosis factor (TNF-α), matrix metalloproteinases (MMP-8 and MMP-9), soluble vascular cell adhesion molecule 1 (sVCAM-1), endothelial leukocyte adhesion (E-selectin and intercellular adhesion molecule (ICAM)), immunoglobulins IgA, IgM, IgG, asymmetric dimethylarginine (ADMA) and complement-C3 levels[9].

This is significant, not only for the presence of apical periodontitis with symptoms and teeth with unsuccessfully treated root canals, but also to highlight the potential negative impact of asymptomatic apical periodontitis on the systemic immune response of the body.

The impact of odontogenic inflammatory cysts on the immune system is the aim of the work of Rahnama et al., which through blood and saliva using flow cytometry prove a significantly higher number of T lymphocytes in radicular cysts compared to follicular cysts[10].

In the study, they proved the presence and role of B cells in the process of creating odontogenic cysts and indicate how important is the rapid detection and treatment of cysts that cause not only local, but also systemic complications. Their results confirm the significant impact of the presence of

odontogenic cysts on the body's systemic immune system and also they are in accordance with the results obtained in our study.

The immunological analyzes obtained in our research correspond with the results obtained in the study of Dimitrovski et al., in which were performed blood analyzes of immunoglobulins prior the surgical intervention and one month after it[11].

The level of serum immunoglobulins IgG and IgM was elevated in periodontal and residual cysts before surgical therapy and significantly decreased one month after enucleation of the cyst. This research emphasizes the important role of immunoglobulins in the process of creation, development and persistence of the cystic lesion.

Kubota et al., in their study presented the highest values of immunoglobulins in radicular cysts (IgA- 488.9 mg/100ml, IgG – 2535.4 mg/100 ml, IgM – 135.6 mg/100 ml), in contrast to follicular (IgA -2308.4 mg/100 ml, IgG – 1618.2 mg/100 ml, IgM – 155.6 mg/100 ml) and especially on odontogenic keratocysts (IgA – 135.6 mg/100 ml, IgG –491.9 mg /100 ml, IgM – 54.1 mg/100 ml). [12].

These findings suggest that IgA, IgG, and IgM may play an important role in the occurrence, development, and persistence of cystic lesions.

Conclusion

Immunoglobulin levels in patients with odontogenic inflammatory cysts in the orofacial region before surgical treatment were significantly elevated, depending on the type of the cyst. Surgical removal of the odontogenic inflammatoty cysts resulted in a decrease of immunoglobulin levels in serum.

This confirmed the importance of early detection of inflammatory cysts and the effectiveness of the therapeutic intervention to the body's immune defense capacity. Future studies are needed to indicate whether these markers are related to other systemic responses and factors and whether their detection can be important for a complete understanding of the process of early formation of cystic lesions.

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