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**Research Article**

## ENDOPERIODONTAL IMMUNE RESPONSE IN A CHRONIC LESION

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**Abstract:**

*The content and effect of CD-57 and VEGF markers in chronic pulp lesions were studied. CD57 is an additional marker of the immune system dysfunction in chronic diseases. CD-57 is expressed on NK-cells (natural killers), some T-lymphocytes, B-lymphocytes, monocytes. Increased expression on cytotoxic lymphocytes was shown to slow down T-cells proliferation. In other words, CD57 is a antigen to natural killers. Vascular endothelial growth factors (VEGF) are powerful angiogenic factors that are produced by macrophages, fibroblasts, hepatocytes, endothelial and other cells [1]. They are involved in activation, proliferation, migration and differentiation of endothelial cells in blood and lymphatic vessels, interacting with them through specific tyrosine kinase receptors. VEGF enhances the permeability of blood vessels and creates conditions for plasma proteins to penetrate into the extracellular tissue space, promoting the migration of endothelial cells, as well as participates in regulation of cell adhesion and modulation of immune properties. Timely and accurate identification and correct interpretation of ethiopathogenetic events in endoperiodontal region, knowledge of morphology creates a strong base to refine the diagnosis and to improve the treatment of patients with endoperiodontal inflammation.*

*The aim of the study was to assess the immunological pulp-periodontal status in patients with lesions of the endoperiodontal complex. The authors studied clinical and morphological markers CD-57 and VEGF and presented the characteristics for the expression of the markers under study. The results of the study provide morpho-functional data on the expression of these markers, which will give the opportunity to create a competent concept of the endoperiodontal complex immunological status and to contribute to improving early diagnosis and choosing an effective medical tactics.*

**Keywords:** chronic apical periodontitis, CD-57, VEGF, endoperiodontal lesions, NK-cells, immune properties, pulp, periodontium.

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**INTRODUCTION:**

Inflammation of the tooth pulp with subsequent involvement of periodontal tissues remains one of the unsolved problems of therapeutic dentistry [2]. Early diagnosis based on understanding immune and morphological changes in tissues and their influence on the outcome of the disease suggests the need to improve the complex of therapeutic and diagnostic measures for patients burdened with endo-periodontal lesions [3;4]. The state of nonspecific resistance and the course of the inflammatory process acquire an accentuated interdependent influence [5], which largely determines implementation of the pathological process spread and prognosis.

Inflammation of the pulp is known to be accompanied by local edema, increased intra-pulp pressure and cell death. The resulting local collapse of blood vessels with subsequent tissue hypoxia naturally results in local necrosis. Released chemical mediators, in turn, cause increased edema, create a vicious circle, which leads to advance of the process [6].

In accordance with modern concepts, the inflammatory process in periodontal tissues is considered as a typical immune response. The immune nature of the inflammatory response in chronic apical periodontitis is explained by the content of T- and B-lymphocytes in the cell clusters of the periapical focus. At this, most of the lymphocytes found in the inflamed periodontium belong to T-cells, which are known to mediate delayed allergic reactions.

**Purpose of research:**

to evaluate the immunological state of dental pulp tissue in patients with chronic inflammation of endoperiodontium.

**MATERIAL AND METHODS OF THE RESEARCH:**

we examined 40 patients (18 men and 22 women aged 18-50 years) who presented at the AI

"Republican Dental Polyclinic" of the Chuvash Public Health Ministry for planned treatment, after

receiving informed consent, they were divided into 3 groups:

Group 1, intact - with extirpation of the dental pulp due to prosthetic indications;

Group 2, control – with diagnosed chronic pulpitis (K04. 0);

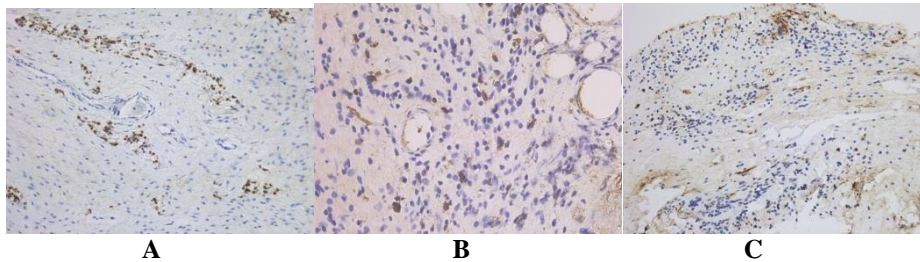
Group 3, experimental group - with diagnosed chronic apical periodontitis (K04. 5).

Their examination was carried out according to the standard scheme with the use of basic and additional methods, radiovisiography and CBCT-study **were used as radiation diagnostic methods.** The material for immunohistochemical study was pulp extirpate, which was sampled during planned endodontic treatment. To study the morphological composition of the pulp and to conduct morphometry, the material was subjected to standard procedures of staining, fixation, histologic diagnosis on a Leica ASP 200 tissue histoprocessor, after which paraffin sections were made and staining with hematoxylin and eosin was performed. Immunohistochemical studies (George L. Kumar, LarsRudbeck, 2011) were performed in accordance with the standard Protocol and using autostainers AUTOSTAINER-3 60 (THERMO, UK) and Leica BOND-MAX (Germany) using imaging systems En-vision (DAKO, Denmark) and NovoLinkpolymer (NovoCastr, UK). Monoclonal antibodies to VEGF and CD-57 were used in the study. Computer morphometry was carried out using the archiving system based on Leica DM4000B microscope using a color camera and a licensed program. Photomicrographs for morphometric measurements were obtained at magnifications X200 and X400. Linear morphometric measurements were performed using the licensed program LeicaApplicationSuite 3.6.0. Quantitative morphometric measurements were performed using the licensed program "Micro-Analysis", as well as the demo version of the program SigmaScanPro. Statistical processing of digital data was carried out on the computer IntelCore 2 Duo with the help of the licensed

package MicrosoftOffice 2003 (Word and Excel), as well as G-Stat and "Biostatistics" programs,

reliability was determined by the Student's criterion (t).

## RESULTS AND DISCUSSION:



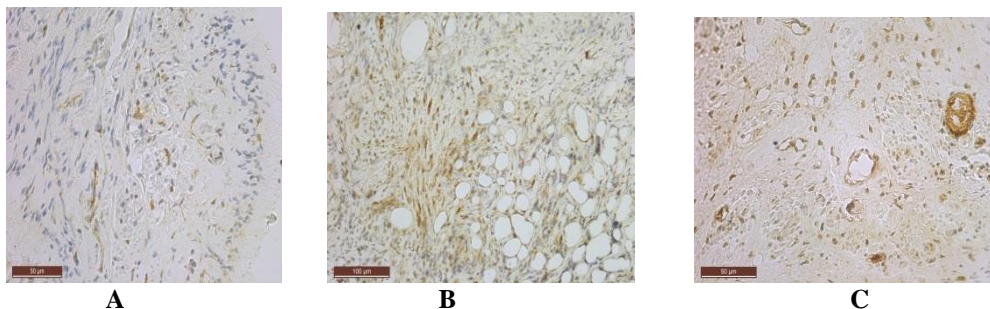
**Figure 1: Immunohistochemical method for detecting CD-57 marker. Dental pulp. Intact group (A), pulpitis group (B), periodontitis group (C). CD-57-positive NK cells.**

NK cells giving a positive response to CD-57 in a normal pulp tissue form compact perivascular clusters. More often they are arranged in chains of 20-30 cells. In an inflammatory process in the pulp, the nature of NK cells distribution significantly changes. Their discrete arrangement among stroma cells without perivascular clusters forming is noted. Advance of the inflammatory process in the periodontal tissue is characterized by even more pronounced changes in distribution of these cells. Formation of compact clusters of NK cells among lymphocytic infiltrates without perivascular location is noted. With the development of a full-scale morphological picture of inflammation, an increase in the number of NK cells is noted (table 1).

**Table 1: Comparison of the number of NK-cells in the dental pulp tissue with an increase of X400.**

	the norm	pulpitis	periodontitis
<b>Number of NK cells</b>	15±3,31*	29±4,56*	46±3,9*

\*- P<0.01



**Figure 2: Immunohistochemical method for determining VEGF. Dental pulp. Intact group (A), pulpitis group (B), periodontitis group (C).**

VEGF distribution in intact pulp is characterized by expression in microvascular endothelial cells as well as in few perivascular cells. Pulp inflammation is manifested by a bright positive cytoplasmic reaction of stroma cells to VEGF. The cells are arranged evenly, sometimes forming clusters of 5-10. The number of VEGF-expressing cells exceeds 100 in the field of view at magnification X400. Periodontitis is characterized by a pronounced positive reaction with VEGF, at this cytoplasmic reaction in stroma cells is characteristic, while the reaction in vascular endothelial cells is reduced.

The number of pulp cells expressing VEGF in individual fields reaches 30-40 in the field of view at magnification of X400.

## CONCLUSIONS:

1. A clear interdependency between the development of pulp inflammation and changes in NK cells localization was revealed.

2. A guaranteed increase in the number of NK-cells was determined when the periodontium was involved in the inflammatory process.

3. Pulp inflammation is manifested by a bright reaction of stroma cells to VEGF. Attention is drawn to a large amount of cells expressing to VEGF in the amount of more than a hundred in the field of vision.

4. When periodontium is involved in the process, there is a clear decrease in the number of VEGF-positive cells along with a decrease in the reaction of vascular endothelial cells.

5. A significant immune response of pulp cells to the inflammatory process was obtained.

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