CYTOLOGICAL ANALYSIS OF GINGIVAL FLUID IN PRIMARY SCHOOL-AGED CHILDREN WITH CHRONIC LOCALIZED PERIODONTITIS WHO LIVE IN THE CRISIS ZONE OF ARAL SEA REGION

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Abstract

The relevance of the present study is explained by the fact that periodontal diseases appear in younger age and their aggressive forms develop in childhood, as well as high sensitivity of parodont structures in children to the impact of factors of the external and internal environment. The aim of the present study was to identify the peculiarities of cytological alterations in the parodont of primary school-aged children who live in the crisis zone of the Aral Sea region. The studies of morphology and functions of the organs of the dentoalveolar apparatus in the population of the crisis zone of the Aral Sea area in the age-related aspect were not performed. The leading approach to the investigation of this issue was a comparative analysis of a cytogram of gingival fluid in children with chronic periodontitis who live in the Aral Sea region and in an ecologically favorable region of Talgar city. It was established that, cytologically, chronic localized periodontitis in the examined children who live in the polluted zone of the Aral Sea region was characterized by a higher degree of the parodont damage than in the comparison group. The identified peculiarity of the parodont damage in children was explained by a complex influence of unfavorable factors in the Aral Sea region on a child organism (increased content of sulfates and chlorides, heavy metals and pesticides in the environment and food products). Materials of the article can be important for the diagnostics, therapy, and monitoring of inflammatory conditions of parodont and can be useful for dentists. Based on the obtained data, a program of the prevention of periodontal diseases in ecologically unfavorable regions of Kazakhstan will be developed.

Keywords: Cytogram; parodont; gingival fluid; chronic localized periodontitis; ecology.

I. Introduction

During the past years, the population in the Aral Sea region was characterized by the highest rates of general and childhood morbidity, as well as a decrease in the
life expectancy in comparison with other regions of Kazakhstan and Republics of Central Asia. High rates of mortality in childhood, infertility and congenital deformities are registered. A number of authors who studied children’s health revealed numerous abnormalities. A complex of deep alterations in the state of health of the growing generation was revealed that was characterized by the increase in the rate of morbidity in the respiratory, gastrointestinal, excretory tracts, and cardiovascular and immune systems, posture disturbances, delays in physical and sexual development (XIII, II, XXIII, XVI, XXV, VII). Annually, on average, 500 people get infected with TB (XVII). In the Aral Sea Region and Karakalpak Republic, a two-fold increase in the level of oncological diseased in children was observed (XXXIV).

One of the most important parameters of health status is the condition of dentoalveolar apparatus. However, the studies of the morphofunctional status of dentoalveolar apparatus in the population of the crisis zone of the Aral Sea area in the age-related aspect were not performed. Pathological alterations in the parodont tissue could be early signs of either an independent disease or manifestations of an organism’s systemic disease. In younger age, parodont tissues are characterized by high sensitivity to the factors of the external and internal medium due to the morphofunctional peculiarities of its organization (VIII), which is considered to be one of the reasons of diverse clinical manifestations of the pathological processes and their complicated diagnostics and treatment.

Still, there are no reliable and simple laboratory methods of diagnostics and monitoring of parodont diseases. There are no reliable methods of the evaluation of the therapy efficiency for gingivitis and periodontitis. Hence, the researchers develop new methods and modify the existing methods of their diagnostics and treatment (III, XXII, IV, XV, XLII, XL).

The cytological method is one of the most effective methods of parodont studies. Cytological diagnostics by the gingival fluid smears is significant for the study of a localized pathology and a number of somatic diseases (X, XLI, XXXIX).

Thus, the cytological analysis of gingival fluid in children who live in the Aral Sea region is important not only as an effective diagnostic test of the condition of dentoalveolar apparatus but also an indicator of general reaction of an organism of people who live in the extreme conditions of the Aral Sea region, which is necessary for the development of therapeutic and preventive measures.

The aim of the present study was to give a cytological evaluation of the status of parodont in primary school-aged children with chronic localized periodontitis who live in the crisis zone of the Aral Sea region.

II. Materials and Methods

The present study was conducted at the Department of Histology at S.D. A sfendiyarov Kazakh National Medical University. The objects of the study were the smears of gingival fluid taken from the children during the period of the second childhood (primary school – 6-11 years old) (XVIII) who lived in the environmentally clear zone (Talgar city) and Aral Sea region (Aral city, Kyzyl-Orda city, Shiely city). Three groups of children were studied. The control group (clinically healthy patients) included 40 people (Talgar), the group of comparison (moderate chronic localized
periodontitis) – 45 people (Talgar) and the main group (moderate chronic localized periodontitis) – 64 people (Aral Sea region).

Preliminary, Oral Hygiene Index (Greene and Vermillion) was used for all the participants (IX). The gingival fluid was obtained with sterile threads (10 x 1 mm) made of gauze (IX). Before the introduction of sterile threads in a gingival sulcus or pocket, gingiva and teeth were carefully wiped with cotton swabs to remove dental deposit and saliva. Then, gingival sulcus or pocket was carefully isolated with cotton swabs from the saliva. Sterile threads were placed to the bottom of gingival sulcus or pocket with a probe in the area of incisors, premolars and first molar for 5-8 minutes. After that, gauze threads were removed and smears were prepared with rotary movements on the microslides. They were dried out, fixed in the spirit-acetone (1:1) solution for 5 minutes and May-Grünwald and Romanowsky-Giemsa stained (XXVI).

The smears were used to reveal epitheliocytes at different stages of differentiation, including dystrophically altered, invaded with neutrophils/mononuclear cells and contaminated with microorganisms. Besides, the authors revealed mononuclear cells with cytoplasm, bare nuclear mononuclear cells, segment nuclear neutrophils, erythrocytes, fibroblast-like cells, cocci, and rod-like bacteria. There were six stages of epitheliocytes differentiation defined for the cytogram count of mucous epithelium by the cytological parameters: basal (I stage), parabasal (II stage), intermediate cells of type I and II (III and IV stages), superficial cells with pyknotic nucleus (V stage), and nuclear-free cells (VI stages) (XL).

A number of indices were calculated by the results of cytogram. The index of cells differentiation ($IDif$) (V) is a sum of cells at the respective stages of differentiation (in percent) taking into account their numerical designations.

$$A = 1a + 2b + 3c + 4d + 5e + 6f,$$

where $A$ is an index of cells differentiation ($IDif$) of oral mucous epithelium in smears; $1, 2, 3, 4, 5, 6$ – numerical designations of the stages of cells differentiation; $a, b, c, d, e, f$ – percent of cells at the respective stage of differentiation. $IDif$ is an integral parameter of the correlation of the processes of proliferation and differentiation of oral mucus epithelium.

Left shift index ($LSI$) (X) is a sum of basal and parabasal (immature squamous epithelium of the deep sections of the spinous layer) to the general count of epithelial cells, which is expressed in percent.

$$LSI = \frac{\sum (I + II)}{n} \cdot 100,$$

where $\sum (I + II)$ is a sum of cells at I and II stages of differentiation; $n$ is a general count of epithelial cells. The increase in the $LSI$ reflects a general increase in the young epithelial cells, which is primarily associated with their enhanced proliferation.

Multicellular epithelial complexes index ($MECI$) (X) is a relation of the number of multicellular epithelial cells to the general number of epithelial cells, which is expressed in percent.

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MECI = \sum_{n}^{\text{MEC}} \cdot 100, \\
where \sum_{n}^{\text{MEC}} \text{is a number of multicellular epithelial complexes; } n \text{ – a general number of epithelial cells. It indicates the tendency of mucosa to desquamative manifestations. An increase in the index is usually observed in subjects with general loosening of the epithelial layer that is expressed by dystrophic changes in the epithelium with the damage of intercellular contacts.}

Index of the destruction of epithelial cells (\text{ID}) (XII) reflects the quantitative relation between the signs of cytopathology in epithelial cells and the form of pathological process in the oral mucosa.

\text{ID} = \frac{F1X1 + F2X2 + F3X3}{n} \cdot 100, \\
where F_i \text{ - coefficient of contingency } i = 1,2,3; \ n \text{ – a general number of epithelial cells; } X_i, X_2, X_3 \text{ – ascending order of the values in percent from the number of epithelial cells with basophilia of cytoplasm (cells of irritation), dystrophy, and intracellular invasion of leukocytes/mononuclear cells.}

Inflammatory-destructive index (\text{IDI})(XII) is a relation of leukocyte count, bare nuclear mononuclear cells, and fibroblast-like (epithelial-like) cells to the number of unaltered mononuclear cells with cytoplasm per 1000 cells.

\text{IDI} = \frac{\sum (L + G + F)}{M}, \\
where L \text{ – a number of leukocytes (criterion of the intensity of inflammation); } G \text{ – a number of bare nuclear mononuclear cells (criterion of destruction); } F \text{ – number of fibroblasts (epithelial-like cells) (criterion of granulation); } M \text{ –thenumber of undamaged mononuclear cells (criterion of the inflammation termination). It is directly associated with inflammation and is calculated based on the number of connective tissue cells that is found in the composition of inflammatory infiltrate when inflammation develops. Their number varies depending on the character and expression of the inflammatory process and partly on the alteration of the cells in the inflammatory infiltrate.}

The imaging was performed with a morphodensitometric complex Leica: microscope DM 1000 and digital camera DFC-320. This complex was used to take pictures of the cytogram cells in TIFF format.

\text{Statistical analysis.} The analysis of the obtained data and the evaluation of the significance of the differences were performed with a Student’s t-test and professional statistical software package SPSS (version 21.0 SPSS Inc.). The changes were significant at \( P<0.05. \)

\text{Approval of the local ethical committee.} The study was approved by the local ethical committee of S.D. Asfendiyarov Kazakh National Medical University (protocol of approval № 9 (60) dated September 22, 2017). The protocol of the study complies with the principles of the Helsinki Declaration (1975). All the patients
included in the study signed a form of informed consent and permission for the use of their data and materials in the study.

III. Results

The examination of the children from the control group revealed a satisfactory condition of the oral cavity, the average OHIS index was 1.27 ± 0.12. However, the children from the group of comparison and the main group had an unsatisfactory condition of the oral cavity: the average OHIS index was 1.68 ± 0.14 and 2.11 ± 0.04, respectively. Clinically, such patients had cyanotic hyperemia and bleeding of interdental and marginal gingiva, edema of gingival papillae, and dental plaquerevealed.

The results of the gingival fluid cytological analysis taken from the children with chronic localized periodontitis in the main and comparison groups revealed a significant amount of segmentonuclearneutrophils, primarily damaged (Figures 1,2,3,4,5,6,7,8), and active vacuolated neutrophils (Figure 3). The content of neutrophils in the cytogram of gingival fluid in children from the main group was significantly higher than in children from the group of comparison and healthy children (P< 0.05)(Table 1). The children from the main group had bare nuclear monocytes and undamaged monocytes (Figures 1, 4, 5). However, undamaged monocytes prevailed in the group of comparison (Table 1). In the smears of gingival fluid, the majority of children from the main group had a high erythrocyte count (Figures 2,9). In the groups of comparison and control, such cases were rare or were not observed.

![Fig. 1: The smear of gingival fluid of 8-year-old patient D. Chronic localized periodontitis. Group of comparison. Segmentonuclearneutrophils, mononuclear cells. Threads of mucin. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.](image-url)
**Fig. 2:** The smear of gingival fluid of 8-year-old patient T. Chronic localized periodontitis. Main group. Erythrocytes and segmentonuclear neutrophils prevail, primarily destructed. Invasion of segmentonuclear neutrophils to intermediate epitheliocytes of I type. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.

**Fig. 3:** The smear of gingival fluid of 9-year-old patient B. Chronic localized periodontitis. Group of comparison. Segmentonuclear neutrophils prevail, primarily destructed. Active neutrophils with vacuolated cytoplasm are observed. Superficial epitheliocytes with pyknotic nuclei. Cocci and rod-like bacteria that form thread-like structures are seen. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.
Fig. 4: A smear of gingival fluid of 7-year-old patient Zh. Chronic localized periodontitis. Main group. Destructed segmentonuclear neutrophils. Mononuclear cell. Contamination of epitheliocytes with cocci microorganisms. Major clusters of coccimicroflora. May-Grünewald and Romanowsky-Giemsa stained. Magnification x 400.

Cytogram of smears of gingival fluid taken from the children from all the groups revealed epitheliocytes at different stages of differentiation. In children with chronic localized periodontitis from the main group, a significant increase was observed in the ratio of basal, parabasal and intermediate epitheliocytes of type I (Figure 6) due to the decrease in the number of intermediate epitheliocytes of type II, superficial epitheliocytes with pyknotic nucleus, and nucleus free epitheliocytes in both children from the main group and from the group of comparison. The content of basal, parabasal and intermediate epitheliocytes of type I was significantly higher in children from the main group than in children from the groups of comparison and control (P< 0.05)(Table 1). Smears from the patients from the main group and the group of comparison revealed fibroblast-like cells (Figure 5).

Fig. 5: A smear of gingival fluid of 10-year-old patient D. Chronic localized periodontitis. Group of comparison. Fibroblast-like cells, mainly destructed segmentonuclear neutrophils, mononuclear cells. Cocci and rod-like bacteria. May-Grünewald and Romanowsky-Giemsa stained. Magnification x 400.

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Fig. 6: A smear of gingival fluid of 7-year-old patient K. Chronic localized periodontitis. Main group. Groups of basal and parabasalepitheliocytes, intermediate epitheliocytes of I type, clusters of coccimicroflora and rod-like bacteria. Segmentonuclear neutrophils, primarily destructed. Erythrocytes. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.

Epitheliocytes contaminated with diplococci were revealed (Figure 4, 7).

Fig. 7: A smear of gingival fluid of 10-year-old patient C. Chronic localized periodontitis. Main group. Contamination of the superficial epitheliocytes with pyknotic nucleus diplococci. Primarily destructed segmentonuclear neutrophils. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.

Dystrophically altered epitheliocytes with signs of hydropic degeneration were observed (Figure 8, 9). An invasion of neutrophils and monocytes to epitheliocytes was revealed Figure 2, 8). The content of dystrophically altered
epitheliocytes and neutrophil and monocyte invaded epitheliocytes was significantly higher in children from the main group than in children from the group of comparison (P<0.05) (Table 1). Threads of mucin (Figure 1, 8) and clusters of coccimicroflora and rod-like bacteria were seen (Figure 3, 4, 5, 6, 8).

**Fig. 8:** A smear of gingival fluid of 9-year-old patient С. Chronic localized periodontitis. Main group. Primarily destructed segmentonuclear neutrophils prevail. Invasion of neutrophils to epitheliocytes, hydropic degeneration of epitheliocytes at the IV stage of differentiation (intermediate cell of II type), and mucin threads were seen. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.

**Fig. 9:** A smear of gingival fluid of 9-year-old patient Zh. Chronic localized periodontitis. Main group. Erythrocytes, epitheliocytes at the II stage of differentiation – parabasal cells, hydropic degeneration of epitheliocytes at the II stage of differentiation. May-Grünwald and Romanowsky-Giemsa stained. Magnification x 400.

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Table 1: Cytogram of gingival fluid in healthy children (Talgar city) and in children with chronic localized periodontitis in the second childhood who live in Talgar city and crisis zone of the Aral Sea region

| Parameters                                             | Control, % (Talgar city) | Chronic localized periodontitis, % | P         |
|--------------------------------------------------------|--------------------------|-----------------------------------|-----------|
|                                                        | Group of comparison, %    | Main group (%) (Aral Sea region)  |           |
| Basal epitheliocytes, %                                | 0.1±0.04                 | 2.0±0.12                          | 3.8±0.21  | \(P_{1,2}<0.01\) \(P_{2,3}<0.05\) \(P_{1,3}<0.05\) |
| Parabasalepitheliocytes, %                            | 0.12±0.05                | 2.2±0.11                          | 4.2±0.17  | \(P_{1,2}<0.01\) \(P_{2,3}<0.05\) \(P_{1,3}<0.05\) |
| Intermediate epitheliocytes of type I, %               | 5.2±0.4                  | 7.0±0.3                           | 8.3±0.5   | \(P_{1,2}<0.05\) \(P_{2,3}<0.05\) \(P_{1,3}<0.05\) |
| Intermediate epitheliocytes of type II, %              | 22.0±0.7                 | 16.4±0.6                          | 9.2±0.72  | \(P_{1,2}<0.01\) \(P_{2,3}<0.01\) \(P_{1,3}<0.01\) |
| Superficial epitheliocytes with pyknotic nucleus, %    | 62.82±3.6                | 24.52±1.2                         | 10.8±0.2  | \(P_{1,2}<0.01\) \(P_{2,3}<0.01\) \(P_{1,3}<0.01\) |
| Nucleus free epitheliocytes, %                         | 1.29±0.17                | 1.38±0.9                          | 1.12±0.8  | \(P_{1,2}<0.01\) \(P_{2,3}<0.05\) \(P_{1,3}<0.05\) |
| S/nuclear neutrophils, %                              | 6.47±1.22                | 42.0±2.9                          | 56.1±3.4  | \(P_{1,2}<0.01\) \(P_{2,3}<0.05\) \(P_{1,3}<0.05\) |
| Undamaged mononuclear cells, %                         | 2.0±0.1                  | 4.1±0.13                          | 3.9±0.16  | \(P_{1,2}<0.05\) \(P_{2,3}>0.05\) \(P_{1,3}<0.05\) |
| Bare nucleus mononuclear cells, %                      | 0                        | 0                                 | 2.54±0.13 | \(P_{1,2}>0.05\) |
### Table 1: Parameters of cellular changes in gingival fluid

| Parameter                                      | Healthy Children | Children with Chronic Localized Periodontitis in Group of Comparison | Children with Chronic Localized Periodontitis in Main Group | P<sub>1,2</sub> | P<sub>2,3</sub> | P<sub>1,3</sub> |
|-----------------------------------------------|------------------|----------------------------------------------------------------------|------------------------------------------------------------|----------------|----------------|----------------|
| Dystrophically altered epitheliocytes, %      | 0                | 2.6±0.12                                                             | 4.7±0.4                                                   | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.01 | P<sub>1,3</sub><0.05 |
| Epitheliocytes with neutrophil and monocyte invasion, % | 0                  | 9.5±0.21                                                             | 16.24±1.4                                                 | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.01 | P<sub>1,3</sub><0.05 |
| ID<sub>Dif</sub> (index of differentiation)  | 465.18±9.1       | 417.95±10.4                                                          | 360.2±9.2                                                 | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.05 | P<sub>1,3</sub><0.05 |
| LSI (Left shift index)                        | 0.24±0.06        | 7.85±0.26                                                            | 21.36±2.91                                                | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.01 | P<sub>1,3</sub><0.01 |
| ID<sub>ID</sub> (index of destruction of epithelial cells) | 0                  | 62.0±4.9                                                             | 155.2±6.4                                                 | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.01 | P<sub>1,3</sub><0.05 |
| ID<sub>IDI</sub> (inflammatory-destructive index) | 3.24±0.22        | 10.5±1.15                                                            | 15.6±1.13                                                 | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.01 | P<sub>1,3</sub><0.01 |
| IMEC (index of multicellular epithelial complexes) | 1.8±0.11         | 3.7±0.12                                                             | 16.8±0.13                                                 | P<sub>1,2</sub><0.01 | P<sub>2,3</sub><0.01 | P<sub>1,3</sub><0.01 |

**Note:** P<sub>1,2</sub> – the significance of difference in the parameters in healthy children and children with chronic localized periodontitis in the group of comparison. P<sub>2,3</sub> – the significance of difference in the parameters in children with chronic localized periodontitis between the group of comparison and the main group. P<sub>1,3</sub> – the significance of difference in healthy children and children with chronic localized periodontitis in the main group.

### IV. Discussion

The revealed content of erythrocytes in the smears of gingival fluid in patients from the main group (in comparison with the groups of comparison and control) indicated the enhanced permeability of gingiva vessels and was considered to be a specific cytological marker of the damaging influence of the unfavorable factors of the Aral Sea region. A significant increase in the segment nuclear neutrophils, including active vacuolated and primarily destructed, and the appearance of mononuclear cells in the cytogram of gingival fluid indicated the development of

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inflammatory-destructive reactions. Inflammatory-destructive processes in parodont are indicated by high **IDI** values in the main group and the groups of comparison and control (P<0.01)(Table 1). The appearance of bare nuclear cells (cytoplasm free mononuclear cells and fibroblast-like cells) in the cytogram of gingival fluid taken from the children from the main group and the group of comparison indicate a high degree of alteration in the focus of damage.

A significant increase in **LSI** and decrease in **Idif** of the epithelial cells (P<0.05)(Table 1) is typical for the process of inflammation and indicates the increase in the content of young epithelial cells associated with their enhanced proliferation. The results of the present study agree with the data of the mitotic index of gingival epithelium in patients with gingivitis and periodontitis depending on the age and sex of a patient (36). The mitotic index of oral mucous epithelium increases depending on the degree of the inflammation and age of a patient (32, 33, 34, 35, 36).

A significant increase in the **IMEC** index (P<0.05)(Table 1) resulted from the loosening of the epithelial layer because of destructive alterations in epitheliocytes and raptures of intercellular contacts and indicated the enhancement of desquamation processes. Dystrophically altered epithelial cells were primarily characterized by minor and major vacuolization of the cytoplasm and basophil inclusions. These alterations in epithelial cells were typical for the damaging effect of pathological process in the oral mucosa associated with the expressed structural changes in its tissue components, which is confirmed by a significant increase in **ID** values (P<0.05)(Table 1).

Both in the group of comparison and the main group (in comparison with the control group), there was a significant increase in the number of epitheliocytes invaded with neutrophils or mononuclear cells at the VI, V, IV, III and even II stage of differentiation. Their cytoplasm included one or several nuclei of segmentonuclear neutrophils or mononuclear cells. The appearance of epitheliocytes invaded with neutrophils and mononuclear cells indicate high activity of the inflammation process and aggressiveness of the cells of infiltrate (XXXVII).

The increase in the number of contaminated epithelial cells at different stages of differentiation, including parabasal, was observed. The cytoplasm of epitheliocytes and their surface have different cocci and rod-like bacteria that formed thread-like structures (XIX-XXI). This indicates the loosening of epithelial layer associated with the weakening of intercellular contacts and destructive alterations in the gingival epithelium.

Thus, despite the consistency of clinical observations, the revealed cytological alterations were more significant in patients who lived in the crisis zone of the Aral Sea region than in patients from the group of comparison. Probably, this fact is associated with high sensitivity of parodont structures in childhood to the influence of harmful environmental factors (VIII), and in particular, a complex of harmful factors typical for the crisis zone of Aral Sea region.

Thus, during the past 50 years, the area of aquatorium of the Aral Sea region reduced by more than 7 times, the water volume reduced by 13 times. The concentration of salts in the western area of the sea reached 110–112 g/L, and in the eastern part – 280 g/L. The Sea headed out from the shore 120-200 km (38).
Annually, the air from the seabed raises and moves up to 75 million tons of salt and dust containing sand. The major part of this sand contains sulfates and chlorides that have a negative impact on human health, pollute water and reduce the soil fertility (VII, XXIX).

Besides, lately, the researchers revealed an increased content of heavy metals (hydrargyrum, cadmium, lead, zinc, copper, and iron) and pesticides in the air, water and soil. This migration resulted in a manifold increase in the content of heavy metals in the most consumable food products in the region: meat and meat products, milk and milk products, bread, confectionary, vegetables, and fruit (XXXIII, XXXVI, XXXV, XIV, I).

Thus, the performed cytological analysis of the gingival fluid showed that a complex of harmful factors of the Aral Sea region significantly enhanced the damage of parodont in children with chronic localized periodontitis during the period of the second childhood.

V. Conclusions

Chronic localized periodontitis in children during the period of the second childhood who live in the crisis zone of the Aral Sea region was characterized by a higher degree of parodont damage in comparison with the group of comparison because of high sensitivity of parodont structures in childhood to the harmful environmental factors. The revealed peculiarity of the parodont damage in children is explained by a complex influence of unfavorable factors of the Aral Sea region (including an increased content of sulfates and chlorides, heavy metals, and pesticides that pollute environment and food) on a child organism.

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