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DIAGNOSTIC VALUE OF THE GINGIVAL CYTOGRAM IN SCHOOL-AGE CHILDREN SUFFERING FROM CHRONIC GASTRITIS AND DUODENITIS

Abstract. Diagnostic value of the gingival cytogram in school-age children suffering from chronic gastritis and duodenitis. Romanenko Ye.G., Komskyi M.P., Titov O.H., Bureha I.Y., Khotimska Yu.V., Lavreniuk Ya.V., Alieksieienko V.V., Holub A.V. In recent years, there has been an increase in the incidence of morbidity associated with digestive organ pathology in the pediatric population. The oral cavity is the digestive tract opening, sharing a common ectodermal origin with it. The changes in the cytogram of the oral mucous membranes can signal about exacerbation of pathological processes in the gastrointestinal tract. The aim of this work: to identify the features of gingival cellular composition in school-age patients with chronic gastritis and duodenitis in order to improve diagnostic methods at the disease stages. Examinations of the gingival cytogram in children aged 12-17 years with chronic gastritis

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Key words: cytogram, epitheliocytes, gingiva, diagnostics, chronic gastritis, duodenitis

Ключові слова: цитограма, епітеліоцити, язика, діагностика, хронічний гастрит, дуоденіт
The oral cavity is the digestive tract opening, sharing a common ectodermal origin with it. The changes in the cytogram of the oral mucous membranes can signal about exacerbation of pathological processes in the gastrointestinal tract. The aim of this work was to identify the features of gingival cellular composition in school-age patients with chronic gastritis and duodenitis in order to improve diagnostic methods at the disease stages.

Examinations of the gingival cytogram in children aged 12-17 years with chronic gastritis and duodenitis (27 individuals with the disease in acute stage, 30 – in remission) were carried out. The control group included 28 children who were not found to have any somatic pathology by the results of the examination. For cytological examination, imprint smears from the gingival vestibular surface of the upper and lower jaws were made. The smears were fixed and stained by the May-Grunwald-Pappenheim method, and then examined using a microscope with an immersion system. Children were found to have disorders in the gingival cellular composition with a predominance of dystrophic components, that was manifested by a decrease in the proportion of epithelial cells at terminal stages of differentiation, an increase in the proportion of cells with cytopathological phenomena, polymorphonuclear leukocytes and lymphocytes. Changes in the cytogram were especially expressed in the phase of disease exacerbation. Knowing the phases of the wound process, based on the timing and sequence of the cellular ensembles seen in the cytogram, it is possible to control the disease periods, and timely apply measures to prevent exacerbations. This diagnostic method is especially indicated for children who have relative and absolute contraindications for fibroesogastroduodenoscopy.
gastrointestinal diseases, although the use of fibresosgastroduodenoscopy (FEGDS) in children is limited by a number of absolute and relative contraindications. The oral cavity is the digestive tract opening, sharing a common ectodermal origin with it. The response of the oral epithelium and secretory glands to GIT diseases stems from the digestive apparatus morphofunctional unity. With exacerbation of the upper digestive tract diseases, patients experience GIT motor dysfunction (duodeno-gastric and gastroesophageal reflux). Chronic gastritis and duodenitis are acid-dependent diseases, the main link in the pathogenesis of which is the increased production of hydrochloric acid by the gastric glands. The oral mucosa is affected by the aggressive contents of the stomach and duodenum following involuntary reflux. In this case, the protective mechanisms of the oral cavity are implemented, including the epithelial barrier functions [4]. Therefore, changes in the cytogram of the oral mucous membranes can signal about exacerbation of pathological processes in the GIT. It is known that the cytogram of the oral mucous membranes in children is most commonly used to confirm dental diseases [5, 6], including those triggered by unhealthy habits [7, 8, 9, 10]. There are isolated references to a cytogram use in adults with peptic ulcer. This diagnostic method has not been used in children with gastroduodenal pathology. The advantages of the cytological method of examination include the technical simplicity of analyzing and taking a material, atraumaticity, as well as the possibility of multiple material sampling for the purpose of diagnosis and monitoring of treatment results.

The aim of this work: to identify the features of gingival cellular composition in school-age patients with chronic gastritis and duodenitis in order to improve diagnostic methods at the disease stages.

MATERIALS AND METHODS OF RESEARCH

For fulfillment of the task, 85 patients, who needed for medical assistance at the Regional Children's Hospital were examined. Among them, 39 were boys and 46 were girls aged 12 to 17 years.

Patients of group 1 with chronic gastritis and duodenitis in the acute stage (27 children – 13 girls (48.2%) and 14 boys (51.9%) aged 14.0±0.3 years were treated at the Gastroenterological Department of the hospital. Group 2 included 30 children with chronic gastritis and duodenitis in remission stage – 14 girls (40.7%) and 16 boys (59.3%). The average age of the patients reached 13.6±0.3 years. A diagnosis of gastroduodenal pathology was verified after clinical endoscopic and ultrasound examination. The disease duration in the examined groups of patients ranged from 1 to 6 years according to an anamnesis (3.3±0.3 years in the first group, 3.3±0.2 years in the second group). Group 3 – control, included 28 children – 16 girls (57.1%) and 12 boys (42.9%), aged 14.0±0.3 years, who did not have any somatic pathology after the examination. The groups of subjects did not differ in age and gender (p>0.05).

The research was conducted in accordance with the principles of bioethics set out in the WMA’s Declaration of Helsinki – “Ethical principles for medical research involving human subjects” and “Universal Declaration on Bioethics and Human Rights” (UNESCO), minutes No. 3 of the meeting of the Bioethics Committee of the DMI of TNM dated 10.03.23. A written agreement was signed with the parents whose children agreed to participate in the study.

The groups of patients were formed based on the aim and objectives of the study. Criteria for inclusion in the study groups:

1. Patients of both sexes.
2. The age of patients from 12 to 17 years.
3. The diagnosis of chronic gastritis and duodenitis, confirmed by FEGDS for the groups of patients 1 and 2.

The exclusion criterion was a refusal of patients or their parents to undergo the examination.

For cytological examination, imprints from the gingival vestibular surface of the upper and lower jaws were made using a sterile tapered fragment of a rubber band. The imprints were taken from the studied areas by lightly pressing the rubber band; the material was placed to a glass slide and a smear was made. The imprints were fixed and stained by the May-Grunwald-Pappenheim method, and then examined using a microscope with an immersion system. Microphotographs of the smears were recorded in an Axioplan 2 microscope (“Carl Zeiss”, Germany) using a digital camera Camedia C5060WZ Olympus (Japan). The percentage of epithelial cells and connective tissue cells – polymorphonuclear leukocytes (PMNL) and lymphocytes was calculated per 100 cells. The number of pathologically altered epithelial cells was determined: with vacuolated cytoplasm, with nucleus deformation [6].

Statistical processing of the results was performed on a personal computer in the program Statistica® for Windows 13.0 (StatSoft Inc., USA, license No. JPZ80438230ARCN10-J) [11]. When performing statistical processing of the data obtained, we used checking the normality of the distribution of quantitative data using the Shapiro-Wilk test (p>0.05); calculation of the arithmetic mean and its standard error (M±m); assessment of the probability of the difference in the results obtained in the compared groups using the Student's t test(t).

RESULTS AND DISCUSSION

In the gingival cellular composition in the control group children (Table), keratinized cells (43.96±0.66%)
and nucleated squamous epithelial cells (37.61±0.88%), predominated, which was considered a variant of the norm. The number of pathologically altered epithelial cells reached 0.27%; basal, fibroblast-like cells were not detected.

In the imprints of children with chronic gastritis and duodenitis in the acute stage, cells at the final stages of differentiation – epithelial superficial cells and keratinized cells were significantly less common (p<0.01) than in cytograms of the control group children. This state was attributed to an increased activity of mechanisms impeding the final cell maturation.

**Gingival cellular composition in children (M±m)**

<table>
<thead>
<tr>
<th>Cellular elements (%)</th>
<th>Group 1 (n=27)</th>
<th>Group 2 (n=30)</th>
<th>Group 3 (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal and parabasal epithelial cells</td>
<td>0.34±0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nucleated epithelial cells</td>
<td>23.70±1.02 p&lt;0.01</td>
<td>30.67±1.51 p&lt;0.01</td>
<td>37.61±0.88</td>
</tr>
<tr>
<td>Keratinized cells</td>
<td>25.15±0.95 p&lt;0.01</td>
<td>32.23±0.67 p&lt;0.01</td>
<td>43.96±0.66</td>
</tr>
<tr>
<td>Cytopathologically altered epithelial cells:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with vacuolated cytoplasm</td>
<td>11.52±1.02 p&lt;0.01</td>
<td>3.17±0.29 p&lt;0.01</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>with nucleus deformation</td>
<td>9.92±0.47 p&lt;0.01</td>
<td>1.26±0.17 p&lt;0.01</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>PMNL</td>
<td>93.96±3.67 p&lt;0.01</td>
<td>25.57±0.88 p&lt;0.01</td>
<td>9.79±0.47</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>23.63±1.61 p&lt;0.01</td>
<td>2.12±0.22 p&lt;0.01</td>
<td>0.10±0.05</td>
</tr>
</tbody>
</table>

Notes: p< – significant differences compared to the control group indicators; p< – significant differences compared to group 1 and group 2 indicators.

A shift in the cytograms towards less mature epithelial cells in children with gastroduodenal pathology compared with the indicators in the group of healthy individuals indicated an increase in the proliferative activity of cambial cells. In 23 children of group 1 (85.2%), gingival imprint smears showed the presence of cells at the early stages of differentiation and dystrophic epithelium, which combined with the accumulation of leukocytes and fibrin strands indicated a damage to the gingival epithelial cell layer (erosions) in patients with chronic gastritis and duodenitis in the acute stage (Fig. 1, 2, 3, 4). Fragments of leukocyte destruction formed debris without clear contours or a definite structure.

**Fig. 1. Gingival cytogram of a group 1 patient. The presence of basal cells, macrophages, leukocytic debris. Hematoxylin and eosin staining. Magnification х 200**
Fig. 2. Gingival cytogram of a group 1 patient. Fibrin strands. Hematoxylin and eosin staining. Magnification x 200

Fig. 3. Gingival cytogram of a group 1 patient. In the center – several basal cells with hyperchromic nuclei surrounded by flora, keratinized cells, dystrophic epithelium. Hematoxylin and eosin staining. Magnification x 200

Fig. 4. Gingival cytogram of a group 1 patient. Neutrophils, single eosinophils, histiocytes over the entire field of view. Hematoxylin and eosin staining. Magnification x 200
The presence of macrophages in the gingival cytograms was a favorable sign indicating the body protective reaction activity, and the subsequent appearance of fibroblasts (Fig. 5) indicated the healing of an erosion. Before healing, erosion was cleaned of leukocytes.

![Gingival cytogram of a group 1 patient. Fibroblasts. Hematoxylin and eosin staining. Magnification x 200](image)

Cytograms of gingival imprints in group 1 children revealed a significant increase in the number of pathologically altered epithelial cells: with vacuolated cytoplasm – by 82.3 times, with nucleus deformation – by 76.3 times (p<0.01). The number of PMNL and lymphocytes was also found to be significantly increased (p<0.01) indicating the activated cellular protection of the epithelial layer. In group 2, a significant decrease in the number of nucleated and anucleated cornified epithelial cells (p<0.01) was detected.

At the same time, group 2 was characterized by the significantly increased number of pathologically altered epithelial cells: with vacuolated cytoplasm – by 22.6 times, with nucleus deformation – by 9.7 times (p<0.01). In comparison with the indicators of children with gastritis and duodenitis in the exacerbation stage, group 2 demonstrated 3.7 times (p<0.01) decreased number of PMNL, which was indicative of a reduction in the gingival inflammatory reaction. Basal and parabasal epithelial cells were not found in the cytograms of group 2 children.

So, when analyzing gingival cytograms in patients of studied groups 1 and 2, the following general trends were identified in comparison with healthy children:
- decreased number of cells at the terminal stages of differentiation;
- increased number of cells related to the connective tissue population;
- altered intact – to – damaged epitheliocytes ratio.

The cells of this population provide humoral immunity (production of antibodies) and cell-mediated immunity (contact interaction with victim cells) [12].

An inverse ratio of epitheliocytes was observed in the specimens of healthy children: undamaged epithelial cells predominated over damaged ones. An increase in the proportion of pathologically altered and immature cells in the cytograms of the examined children with chronic gastritis and duodenitis was the cause of a disturbed epithelial desquamation and decreased gingival barrier properties.

Gingival imprint smears of children with exacerbation of gastroduodenal pathology indicated the presence of damage to the epithelial layer. It should be noted that in 20 children of group 1, when examining with the help of FEGDS, single erosions were seen in the gastric antrum mucosa. This means that 74% of patients had a combined mucous membrane lesion of the gingiva and stomach.

Basal and parabasal cells were revealed in the gingival imprints of group 1 children, but no such appearance was identified in the gingival cytograms of groups 2 and 3 patients. The presence of basal cells, macrophages, and leukocytic debris in the cytograms indicated the erosion depth. The epithelial cells were abundantly contaminated with microorganisms.

It is known that an important role in damage to the epithelial layer is played by neutrophils, which predominate in inflammatory sites until the day 3-4 [13, 14]. These cells start to migrate in the first hours of inflammation and reach a maximum by the 1 or day 2, progressively declining from day 5-6 [15]. Neutrophils are responsible for phagocytosis against invading microorganisms, release antibacterial substances, lysozyme, lysosomal acid hydrolases, collagenase, and elastase [16, 17]. The environment is...
especially enriched with these substances during neutrophil degradation by apoptosis starting from the day 2. This process helps to clean the area of inflammation and prepare the next stage of reparative regeneration [18]. It is worth mentioning that at this stage, the leukocyte and other cell nucleolar products of degradation are distinguished by the absence of debris character, but are elements with clearly defined contours, stained well with nuclear stains in dark purple.

The next stages of regeneration are greater influenced by macrophages, which start to migrate into damaged areas of the gingiva reaching the maximum level only from day 3 and decreasing after the day 6-7 [15]. The effect of macrophages arises not just from their function of cleaning-up the inflammation zone through phagocytizing leukocytic debris and fibrin, but also from releasing specific substances that activate fibroblast proliferation [19].

The direct effect of lymphocytes (T cells) on wound healing has recently been identified by a number of studies [20]. Infiltrating T cells produce a range of cytokines and growth factors that control immune responses and wound healing [21]. Humans have been reported to have an influx of T cells into the oral mucosa 3 days following injury. By the day 6, the number of lymphocytes decreases markedly [22]. In our opinion, the presence of gingival erosions is associated with impaired processes of epithelial differentiation in children with gastroduodenal pathology, as well as with a violation of the oral fluid acidity due to the activation of gastrointestinal motility and regurgitation of stomach acidic contents into the oral cavity (reflux), which is observed in patients at the active phase of the disease.

Since the oral cavity is the beginning of the digestive tract, changes in the gingival mucosa may correlate with processes occurring in the gastric and duodenal mucosa. For instance, the detection of microerosions (not visible to the naked eye) on the gingival mucosa corresponded to erosive lesions of the upper digestive tract mucous membranes in 75% of cases. Knowing the phases of the wound process, based on the timing and sequence of the cellular ensembles seen in the cytogram, it is possible to control the disease periods, and timely apply measures to prevent exacerbations. This diagnostic method is especially indicated for children who have relative and absolute contraindications for FEGDS.

**CONCLUSIONS**

1. In children with chronic gastritis and duodenitis in the acute phase, the gingival cellular composition is disturbed with the dystrophic component predominance, which is manifested by a decrease in the number of nucleated epithelial cells by 23.7%, keratinized cells by 25.2%, an increase in the proportion of cells with cytopathological phenomena by 21.4%, polymorphonuclear leukocytes by 94% and lymphocytes by 23.6% (p1 < 0.01).

2. In 23 (85.2%) of children with chronic gastritis and duodenitis in the acute phase, the presence of cells at the early stages of differentiation is observed, which combined with the accumulation of leukocytes and fibrin strands indicated erosive lesions of the gingival epithelial layer.

3. Gingival cytograms of the children with chronic gastritis and duodenitis in the remission phase demonstrated the decrease in the number of nucleated epithelial cells by 30.7%, keratinized cells by 32.2%, the increase in the proportion of cells with cytopathological phenomena by 4.4%, polymorphonuclear leukocytes by 25.6% and lymphocytes by 2.1% (p1 > 0.01).

4. Gingival cytogram indicators in children with gastroduodenal pathology should be used to monitor the disease in order to prevent seasonal exacerbations.

**Contributors:**

Romanenko Ye.G. – data collection, methodology;
Komskyi M.P. – conceptualization, data analysis;
Titov O.H. – methodology;
Bureha I.Y. – design;
Khotimska Yu.V. – methodology;
Lavreniuk Ya.V. – design;
Alieksieienko V.V. – design;
Holub A.V. – methodology.

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