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# CYTOGENETIC STUDY OF BUCCAL EPITELIUM IN PATIENTS WITH NEONATAL SEPSIS, WHOSE PARENTS LIVED PERMANENTLY IN DIFFERENT ENVIRONMENTAL CONDITIONS

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## Abstract

The micronucleus test is a generally accepted and available method for the cytogenetic assessment of the mutagenic effect of factors of various natures. Currently, this test is one of the non-invasive methods of surveying the population that are under the influence of environmental pollution.

Purpose of the study: to determine the features of the karyological indicators of the buccal epithelium in children with neonatal sepsis, whose parents lived permanently in different environmental conditions.

Material and methods. To achieve this goal, a comprehensive survey of 260 newborns suffering neonatal sepsis in 2016-2018 was carried out. The group-forming feature of a comprehensive assessment of the long-term burden on the body of newborns' parents in anthropogenic pollution of air, water and soil in the areas of the region became the proposed

coefficient of ecological risk (CER) taking into account the environmental situation in regional centers.

Thus, the first clinical group (main) included patients with neonatal sepsis, whose parents lived permanently in places with CER 2.0 and more and with unfavorable environmental characteristics of the regional center. The second group (comparison) was formed by newborns with sepsis, whose parents lived permanently in areas with a low risk of adverse effects of these environmental factors on their body (CER < 2.0). Micronuclear test of exfoliative buccal epithelium in patients with neonatal sepsis was performed and evaluated in the immunological laboratory of the Department of Pediatrics and Pediatric Infectious Diseases of BSMU according to the method of Kalaeva V.N.

Results and discussion. In the main group, the proportion of children with a high risk of cytogenetic disorders significantly prevailed with a slight decrease in the quota of patients with a low risk. So, in group I with a low risk of cytogenetic disorders there were 42.1% of cases, with an average risk of -5.3%, and with a high risk of -52.7% of cases. In the comparison group, there were 54.5%, respectively, with a low risk of cytogenetic disorders, (P> 0.05) cases, with an average risk -12.1%, (P> 0.05), and with a high risk -33.3% , (P = 0.05) cases.

Conclusions. The results of the karyological test of the exfoliatic buccal epithelium make it possible to note that cytogenetic disorders and, in some cases, disorders in cell kinetics in the form of proliferation and apoptosis were found significantly more often in the newborns of the main group compared with children in the comparison group. The index of accumulation of cytogenetic disorders was also significantly higher in patients of group I due to the predominance of patients with a high risk of cytogenetic disorders.

**Key words: neonatal sepsis; buccal epithelium; cytological examination; pollution.**

According to the concepts of biology of development and toxicology, the pathogenic effect of unfavorable environmental stimuli on the fetal development of a child is largely determined by the state of health of the father-mother and feto-placental interaction. Emerging developmental disorders is the result of biological-ecological interactions, in which the genetic factors of the parents play a significant role [1-2]. It should be noted that disorders of fetal development occur not only under the influence of pathogenic stimuli of the external environment on the mother's body, but also in the case when the father of the child falls under this influence. This is due to mutagenic or epigenetic mechanisms that affect sperm [3-4].

The above-mentioned pathogenic effect of environmental stimuli has been studied in relation to high-intensity eco-factors and, as a rule, one pollutant. However, at the beginning of the 21st century, the deepening of knowledge in the field of embryology, developmental biology and toxicology allowed to form the concept that the pathogenic effect of environmental pollutants has no threshold [5]. This idea not only dictates the need to reduce the intensity of adverse environmental incentives on the body of parents, but also stimulates further study of the impact of low-intensity environmental factors on the child's body, especially in sensitive periods of its development.

Cytogenetic monitoring is often used in studies of the environmental situation in adults and older children, but in newborns such studies are rare [12]. A common and available method of cytogenetic assessment of mutagenic effects of factors of various natures is the micronucleus test. Currently, this test is one of the non-invasive methods of the surveying the population, which are under the influence of environmental pollution [14].

**Purpose of the study**: to investigate the features of the karyological parameters of the buccal epithelium in children with neonatal sepsis, whose parents lived permanently in different environmental conditions.

**Material and methods.** To achieve this goal, a comprehensive survey of 260 newborns who suffered from neonatal sepsis in 2016-2018 was conducted. Examination of patients was carried out in the neonatal departments of the Municipal Medical Institution "Regional Children's Hospital" in Chernivtsi, as well as in the relevant departments of Khmelnytsky.

Diagnosis and treatment of neonatal sepsis was carried out according to the recommendations of leading neonatologists of Ukraine, taking into account international recommendations [6, 7]. The proposed environmental risk factor (CER) was a group-forming feature of a comprehensive assessment of the long-term body burden of newborns' parents of anthropogenic air, water and soil pollution in the regions, taking into account the environmental situation in regional centers.

Thus, the first clinical group (main) included patients with neonatal sepsis, whose parents lived permanently in places with CER 2.0 and more and with unfavorable environmental characteristics of the regional center. This clinical group will be defined as "basic". The second group (comparison) was formed by newborns with sepsis, whose parents lived permanently in areas with a low risk of adverse effects of these environmental factors on their body (CER <2.0). This clinical group will be referred to as the "comparison group".

Assessment of the ecological situation in the places of residence of parents was carried out in accordance with the methodological recommendations [8]. The source of data on soil, water and air pollution in Chernivtsi and Khmelnytsky regions was the data provided in the materials of the State Statistics Service [9, 10].

Micronuclear test of exfoliative buccal epithelium in 71 patients with neonatal sepsis was performed and evaluated in the immunological laboratory of the Department of Pediatrics and Pediatric Infectious Diseases of BSMU according to the method of V.N. Kalayev [11].

The buccal epithelium was taken with disposable cotton swabs from the inner surface of the buccal mucosa, the drugs were stained with Romanovsky-Gims dye [11]. At least 1000 cells were analyzed using an XSM-1.04 microscope at a magnification of 100 / 1.25. The presence of cells with micronuclei, protrusions such as "bubble" (Fig. 1) and "broken egg", nuclei of atypical shape, as well as indicators of proliferation (dinuclear cells) and indicators of apoptosis (vacuolation of the nucleus, chromatin condensation, karyopyknosis, karyolysis (2.2), karyorexis (Fig. 2) and apoptotic bodies) [13]; indicators of the early stage of nuclear destruction (nucleation of the nucleus, condensation of chromatin or the beginning of karyolysis) were evaluated.

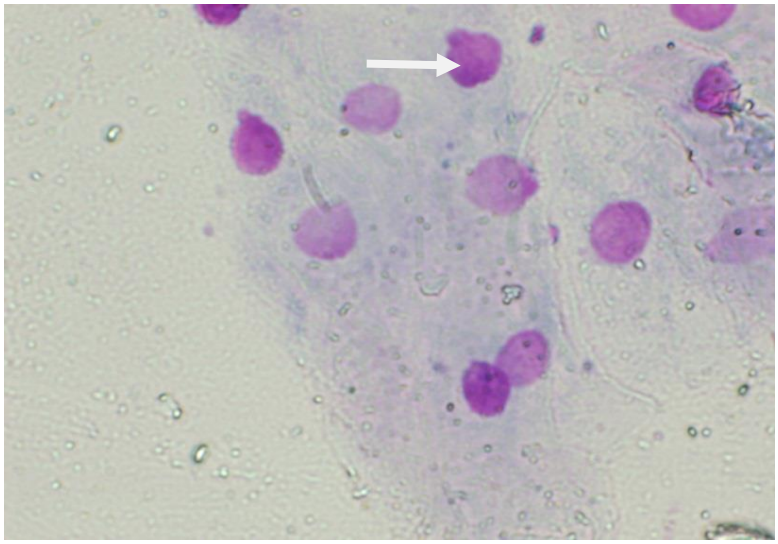

Fig. 1. - Photo. Exfoliative epithelial cells with protrusion of the vesicle type. Photomicrograph. Staining with hematoxylin and eosin. Coll .: x80. Symbols: 1 - protrusion of the bubble type;

In the early stage of destruction of the nucleus only condensation of chromatin (shrunken nucleus with dense chromatin) and the beginning of karyolysis, including

vacuolation of many nuclei were distinguished. Indicators of the late stage of nuclear destruction include karyopyknosis, karyorexis and complete karyolysis or the total amount of cells in apoptosis, taking into account the condensation of chromatin and the onset of karyolysis. Indicators of "perinuclear vacuole" and "nuclear membrane damage" are also considered as an indicator of nuclear destruction, but do not include it in the apoptotic index [12].

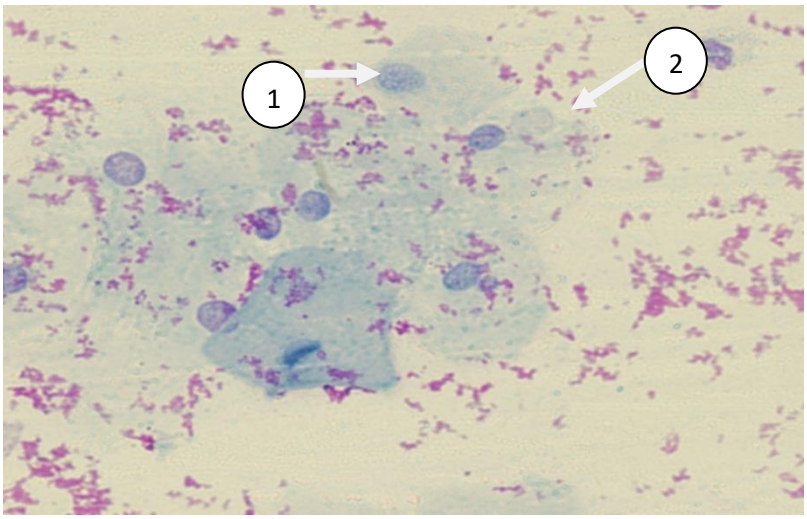


Fig. 2. - Photo. Exfoliative epithelial cells with karyorexis (1) and karyolysis (2). Photomicrograph. Staining with hematoxylin and eosin. Coll .: x80. Designations: 1 - karyorexis; 2 - karyolysis.

Assessment of cytogenetic status was performed according to the formula L.P. Sychova [13] Iac = (Ic * Ip) / (Iapop) * 100, where Iac is the accumulation index of cytogenetic damage, Ic is the integrated index of cytogenetic action (sum of cells with micronuclei, nuclear protrusions and internuclear bridges in ppm), Ip is the integrated proliferation index (sum of cells with two or more nuclei in ppm -) and Iapo apoptotic index (the sum of all cells in apoptosis in ppm). In the event that the child has no cytogenetic abnormalities or binuclear cells, the zero value is replaced by one. Determining the index of accumulation of cytogenetic damage allows to distinguish 3 risk groups [13]: low (Iac ≤ 2), moderate (2 <Iac <4) and high (Iac ≥ 4) risk.

The results of the study were analyzed using the computer package "Statistica 6" StatSoft and ExcellXP for Windows on a personal computer using parametric and non-parametric calculation methods.

**Results of research and discussion**. Kariological test of exfoliative buccal epithelium was carried out on the 1st day of neonatal sepsis in the main clinical group and in the comparison group. The integrated cytogenetic index, the integrated proliferation index and the integrated apoptosis index were determined.

Indicators of cytogenetic changes were the presence in the epitheliocytes of protrusions such as "broken egg", "bubble". The sum of exfoliative cells of the buccal epithelium with these changes (‰) was an "integral cytogenetic indicator". The presence of epinuclear cells or binuclear cells in the epitheliocytes indicated a proliferative process. The sum of cells with similar findings was an "integral indicator of proliferation." Changes in cells such as chromatin condensation, karyopyknosis, karyolysis, karyorexis and apoptosis indicated different stages of nuclear destruction.

The sum of cells with these disorders was an "integral indicator of apoptosis." The ratio of the product of the first two indices to the "integral index of apoptosis" multiplied by 100, determined the "index of accumulation of cytogenetic disorders." The value of this index less than 2.0 indicates a low risk of environmental pressure. At values of the index of accumulation of cytogenetic disorders ≥2.0, though <4.0, the presence of an average degree of risk of ecological effect is assumed, and at values of this index ≥4.0 - there is a high degree of risk of ecological influences.

Table 1 shows the average values of kariological parameters of exfoliative cells of the buccal epithelium in children of the comparison groups.

Table 1 - Kariological indicators of exfoliative buccal epithelium in newborns of comparison groups

| Clinical groups | Quantity of patients | Indicators (M±m) | | | |
|---|---|---|---|---|---|
| | | Ic,‰* | Ip, ‰** | Iapop,‰ *** | Iac **** |
| I | 38 | 0,17±0,05 | 0,19±0,06 | 0,34±0,07 | 9,12±0,07 |
| II | 33 | 0,04±0,003 | 0,38±0,08 | 0,59±0,08 | 2,48±0,39 |
| P | | <0,05 | =0,05 | <0,05 | <0,01 |

Note: * Integrated indicator of cytogenetic action
** integral proliferation index
*** apoptotic index
**** index of accumulation of cytogenetic damage

These data allow us to conclude that in newborns of the I clinical group cytogenetic disorders of the buccal epithelium in the form of nuclear protrusions prevailed, and in patients of the comparison group - proliferation and apoptosis. In general, based on the value of the

index of accumulation of cytogenetic disorders, the risk of environmental impact was much more significant in newborns of the main group than in patients of the comparison group. It should be noted that in the main group the share of children at high risk of cytogenetic disorders with a slight decrease in the quota of low-risk patients statistically prevailed. Thus, in group I with a low risk of cytogenetic disorders there were 16 children (42.1%), with a medium risk - 2 children (5.3%), and with a high risk - 20 patients (52.7%). In the comparison group, according to the low risk of cytogenetic disorders, there were 18 children (54.5%, P> 0.05), with a medium risk - 4 patients (12.1%, P> 0.05), and with a high risk - 11 newborns (33.3%, P = 0.05).

Thus, the results of the karyological test of the exfoliatic buccal epithelium allow us to note that cytogenetic disorders and, in some cases, abnormalities in cell kinetics in the form of proliferation and apoptosis were found significantly more often in the newborns of the main group compared to children in the comparison group. The index of accumulation of cytogenetic disorders was also significantly higher in patients of group I due to the prevalence of patients with a high risk of cytogenetic disorders.

**Prospects for further research.** In the future, we consider it expedient to create a mathematical model to establish correlations between the accumulation of cytocenetic disorders in children with neonatal sepsis, whose parents live in places with unfavorable environmental characteristics.

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