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Pathological and Immunohistochemical Characteristics of the Pancreas in Infants who died from ARVI

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ABSTRACT

The purpose of the article was to make immunomorphological analysis of the pancreas endocrine apparatus structure in infants under one year old died from viral infections.

Material and methods. The results of 39 autopsies of children under one year of age who died from acute respiratory viral infection.

The work used case histories and autopsy results. Systemic morphological examination of organs was performed. Immunoperoxidase method for B cells determination (monoclonal antiserum NCL-JOVI 1, clone JOVI 1; working dilution 1: 100), A-cells (monoclonal antiserum NCL-DFB1, clone DF-B1; working dilution 1: 100),G cells (NCL-MCTRYP monoclonal antiserum, clone AA1; working dilution 1: 100) and serotonin (NCL-LN5 monoclonal antiserum, clone LN-5; working dilution 1:40) was used in the study.

Results and discussion. It was found that in viral infection, the lesion was of toxic and dyscirculatory nature and manifested mainly in plethora of the organ, laxity or sclerotic gland thickening and lymphohistiocytic infiltration, atrophy and dysplasia of endocrine parenchyma cells. Accumulation of a pinkish, amorphous type of secretion was observed in the lumen of most of the ducts. Inside the islet apparatus numerous cellular elements are located non-compactly.Voids and cell death in the pancreatic islets of Langerhans, an increase in the number of glucagon-producing cells, a decrease in the number of insulin-synthesizing cells and a lack of serotonin were found between the elements.

KEYWORDS

ARVI, Medical History, Pancreas, Immunohistochemistry, Autopsy Results.

Introduction

Decrease of perinatal mortality and morbidity, complications of pregnancy and childbirth, creating conditions for the normal development of newborns, timely identification of risk factors affecting the health of the future generation is an indispensable condition for ensuring the demographic status [1,2,3,6].

The morphogenesis of the pancreas in the physiological course of pregnancy and the minimum risk of developing perinatal pathology has been studied in details [5,7,9].

At present there are many markers allowing to determine not only the tissue identity of the cell, but also to identify its specific features, the nature and composition of the intracellular environment using immunohistochemical analysis [8,9,10].

Children are particularly vulnerable, especially the younger ones, in whom SARS accounts for 65% of all the registered diseases, and they are one of the main causes for hospitalization [4,6].

It should be noted that the incidence of respiratory infections is stably high, which is primarily due to the lack of specific immunoprophylaxis, ability of some viruses to latent persistence and chronic sensitization of the body. Up to 95% of respiratory infections are of viral nature. More than 200 viruses are known to cause respiratory tract damage. The most common causative agents of ARVI are: influenza viruses, parainfluenza, respiratory syncytial virus, adeno, market, flank, metapneumo-coronary, enteroviruses [11,12,18,20].

The listed means do not exhaust the etiological structure of acute respiratory viral infections in children, since today, even with the help of modern methods of laboratory diagnostics, no more than 70% of all registered acute respiratory diseases in children can be deciphered. It should be noted that the incidence of respiratory infections remains stably high, which is primarily due to the lack of specific immunoprophylaxis, the ability of some viruses to latent persistence and chronic sensitization of the body. Up to 95% of respiratory infections are viral in nature; more than 200 viruses are known to cause respiratory tract damage. The most frequent causative agents of ARVI are: influenza

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viruses, parainfluenza virus, respiratory syncytial virus, adeno-, rhino-, boca-, metapneumo-coronary and entero-viruses [11,12,18,20].

The mentioned means do not exhaust the etiological structure of acute respiratory viral infections in children, since today, even modern methods of laboratory diagnostics, reveal no more than 70% of all registered acute respiratory diseases in children.

Immunomorphological methods allow to perform more precise morphological studies and help to objectify the conclusion [6,13,14].

Many scientific studies devoted to viral infection showendocrine system involvement in the general body response to oxygen deficiency [9, 15, 18,21].

However, information on the functional pancreas state, depending on the duration of the course and severity of the viral infection, is scarce and contradictory [22].

Most researches are based on the results of biochemical analysis of hormones and sugar level in the blood. But the definition of even the entire spectrum of hormones does not always adequately reflect the state of the organ itself.

Different structural and metabolic state of the gland can be hidden in one and the same hormone level.

There are no morphological works dealing with this problem in the domestic and foreign literature.

There are no morphological studies reviling dependence of structural changes in pancreas endocrine apparatus in ARVI, considering the duration of the disease and the immediate cause of death.

Immunomorphological studies of the pancreas in acute respiratory viral infections have not previously been carried out, and this was the trigger for the present research.

The aim of the study was an immunomorphological analysis of the pancreas structure the in infants under one year of age died from viral infections.

Materials and Methods

Retrospective and morphological analysis of 325-fatal cases for the period from 2015 to 2017, was carried out. In 39 cases IUI diagnosis was made on the basis of a morphological study. After studying the case histories and autopsy results, a systemic morphological examination of the organs was carried out. The material was fixed in 10% solution of neutral formalin. Tissue samples from various organs were taken for histological examination. Paraffin sections were stained with hematoxylin-eosin, picrofuchsin according to Van Gieson.

With professor Shabdarbayeva D.M. assistance glass preparations were made, stained to reveal apoptosis, cell proliferation (Ki 67) in the pancreas. In these studies, the immunoperoxidase method was used to determine B cells (monoclonal antiserum NCL-JOVI 1, clone JOVI 1; working dilution 1: 100), A-cells (monoclonal antiserum NCL-DFB1, clone DF-B1; working dilution 1: 100), G-cells (monoclonal antiserum NCL-MCTRYP, clone AA1; working dilution 1: 100) and serotonin (monoclonal antiserum NCL-LN5, clone LN-5; working dilution 1:40). All the used antisera were produced by the English company Novocastra Laboratories Ltd.

Apoptosis was revealed by immunohistochemical method using the ApoTaq kit (Oncor, Gaithersburg, MD) according to the company's instructions.

Immunohistochemical studies were performed under identical conditions according to a stereotype scheme, in which only antisera dilution was taken into account, and in some cases it was necessary to block the activity of nonspecific antigens, isolation in Permount with a cover glass overlay.

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To assess the overall histological picture, the classification of morphological changes in the pancreas Lazarus and Volk modified by G.M. Gorbunova [2007] was used, according to which the unchanged organ histostructure, lipomatosis, fibrosis, and cellular infiltration were isolated.

The pancreas materials from children of the same age died from diseases of different etiology unrelated to ARVI.

All antisera used were developed by the British company Novocastra Laboratories Ltd.

Apoptosis was determined by immunohistochemical method according to the instructions of the company ApoTaq (Oncor, Gaithersburg, MD).

Results and discussion: The obtained results of the retrospective and morphological study showed that according to the protocols of fetuses and newborns autopsies, there was an increase in the number of deaths from intrauterine infection of the fetus and newborns (IUI) - 6.4 and 6.8 times, respectively. This may be due to both an absolute increase in the number of cases and an improvement in the diagnosis of infectious diseases due to the introduction of various laboratory research methods. Of these, 52% were boys and 48% - girls.

The morphological picture of these cases coincides with the results of similar studies of domestic and foreign scientists [6; 8; 9].

In 2015 and 2016, the main etiological factors were infections, fetal and newborn lesions which were revealed in 41.6% and 45.7%, and in 2016 the number of viral infections decreased 1.8 times. By 2017, the relative number of double infections increased 3.5 times, the main etiological factor - 58.7%. There were no outbreaks of coinfection in 2015; in 2016 - 2.7%, in 2017 - 13.5%, 5 times increase (fig. 1).

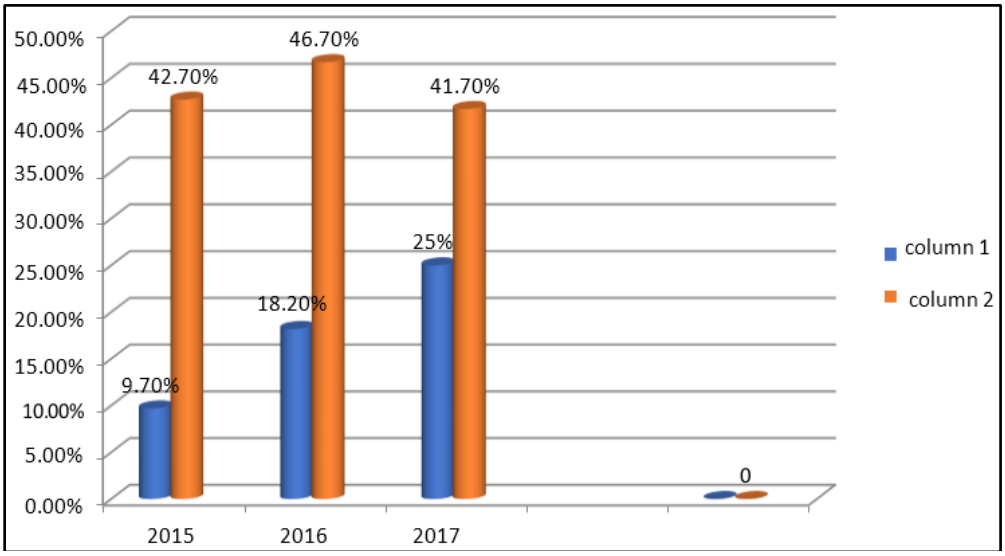

Fig. 1. The relative number of mothers, fetuses and newborns examined for IUI during the period from 2015 to 2017

In 2015, 2016, there was a decrease in the relative number of infant mortalities by 2.7 times compared to 2016, but there was a negative tendency - an increase in intrauterine mortality (during childbirth) in 2017 by 4.6 times compared to 2016. In the years analyzed, the main number deaths were in the early and late neonatal periods - about 70%.

Many cellular elements were not uniformly concentrated in the islets of the gland. In diameter, the elements of the cell consisted of rounded nuclei of various sizes and shapes. Small granules were found in individual cells. The diameter of the cell element was of different size, distinct, with rounded nuclei., Fine-point granularity was revealed in individual cells. The interstitium between the lobules was moderately unevenly expanded due to edema and small focal inflammatory infiltration with leukocytes. The vessels were dilated, desolate or full-blooded with erythrocyte sludge, moderate intravascular leukocytosis, their endothelium was partially desquamated [fig. 2,3].

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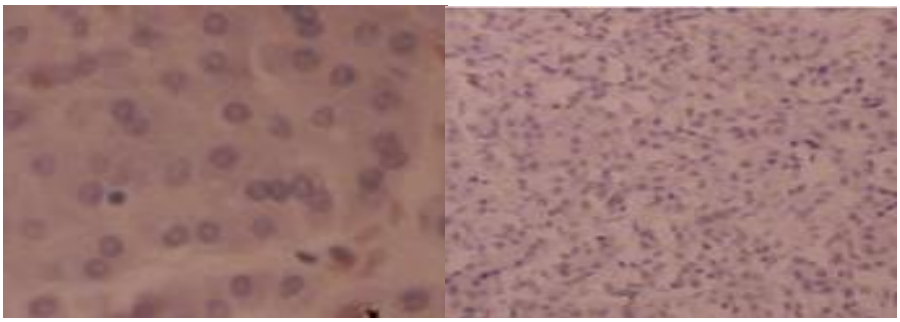

Figure 2-3. The state of the pancreas acidic tissue using immunohistochemical methods for serotonin determination. a - serotonin in the exocrine part.x 140; b - absence of serotonin in the exocrine part, x 140; immunohistochemical dyes

In some cases, plethora of the organ, laxity or sclerotic thickening of the gland, and lymphohistiocytic infiltration, atrophy and dysplasia of the endocrine parenchyma cells were noted. In the lumen of most of the ducts, an accumulation of a pinkish, amorphous type of secretion was observed. Inside the islet apparatus numerous cellular elements were located non-compactly; voids between the elements were found.

In generalized intrauterine infections in combination with developmental abnormalities, formed lobules of the externally secretory part of the gland, consisting of densely located acini, were observed.

Periductal and perivascular accumulations of a large amount of dense coarse fibrous connective tissue were found indicating mesenchymosis [6; 8; 9.17]

Immunohistochemical studies revealed differences in the number of A cells in the pancreas and differences in the causes of death. Thus, changes in the islets of Langerhans in the pancreas in cases of intrauterine viral infection were shown to be much more common with A-cell death than in somatic diseases (Figure 4, 5, 6).

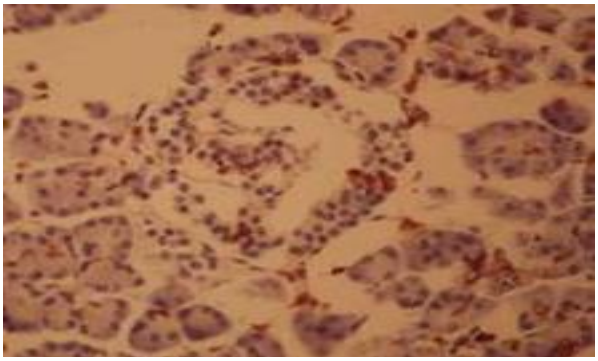

Fig. 4. Apoptotic cells in the islet and acinar tissue. x 140. Immunohistochemical staining.

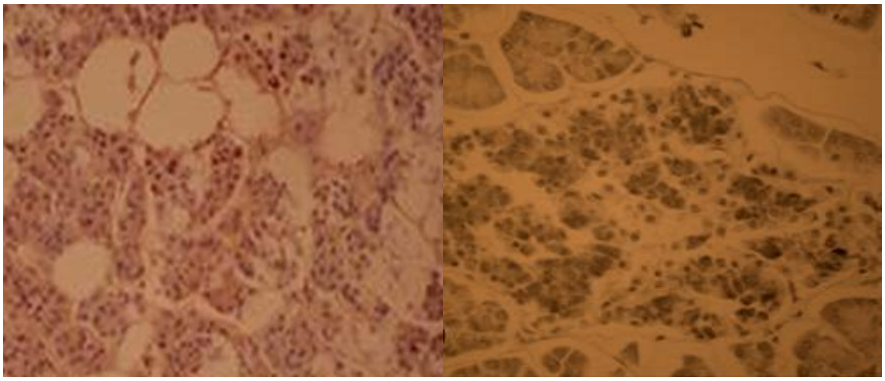

Fig. 5-6. Apoptotic cells and B cells in lipomatosis. x 140. Immunohistochemical staining.

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Pathogenetic mechanisms of the pathology of the pancreas endocrine apparatus were extremely complex and largely remained unclear. One of the main factors in the development of the disease is the activation of pancreatic enzymes in the ducts and parenchyma of the gland, which leads to edema, necrosis and subsequent fibrosis with exocrine and endocrine insufficiency. The development of hyperenzymemia is facilitated by factors leading to a disorder in the outflow of pancreatic juice; a significant role is played by microcirculation disorders, which results ischemia, edema, impaired permeability of cell membranes, and destruction of acinar cells develop. Pancreatic enzymes and other biologically active substances, in particular vasoactive amines, enter the systemic circulation, which disrupts microcirculation outside the pancreas and causes damage to other organs and systems. Pathogenic influences at this time often cause developmental disorders, contributing to the formation of functional failure of the body systems, and the digestive system in particular in the postnatal period[3,9,12,19,22].

The figure shows a pronounced programmed cell death, increase in the number of cells producing glucagon, decrease in cells synthesizing insulin, and lack of serotonin in the islet of Langerhans in case of death from an acute respiratory viral infection [8,16,18,21].

A number of works prove the relationship between the localization of serotonin and insulin in the B cells of the islets of Langerhans and serotonin role in insulin secretion [5,8,10].

Respiratory stress syndrome was morphologically assessed by alveolar damage. All deaths were diagnosed due to diffuse alveolar lesions and multiple organ changes.

Sputum microflora and antibiotic susceptibility were studied in clinical studies and increase was shown in combination with the following bacteria (Streptococcus mitis, Staphylococcus aureus, Candida albicans in Haemophilus influenzae). Our data are consistent with previously published studies that show that the bacterial microflora of the upper respiratory tract is increased due to the predominance of the influenza virus through specific antibacterial protective factors [5,8,13,14].

According to the macroscopic autopsy results, the lungs were enlarged by 100%, tightly elastic, smooth, with a small amount of hemorrhagic fluid, which indicates viral-bacterial pneumonia. The lower respiratory tract is moderately hyperemic, full-blooded. Changes in cerebral small blood vessels showed thickening of the walls, endothelial edema. According to the above immunohistochemical data, in infants who died from a viral infection, cell death in the islets of Langerhans, increase in the number of glucagon-producing cells, decrease in the number of insulin-synthesizing cells and lack of serotonin are clearly programmed.

Conclusion

1. As the effect and duration of the course of the viral infection, atrophic and sclerotic processes of the islet apparatus and the phenomenon of cell death, increase in the number of cells that produce glucagon, decrease in cells synthesizing insulin, and lack of serotonin are observed.
2. Organ plethora, laxity or sclerotic thickening of the gland, as well as lymphohistiocytic infiltration, atrophy and dysplasia of endocrine pair cells and inflammatory cell infiltrates are influenced by viral infections;
3. Within 5-7 days of the disease, insulin deficiency develops due to the destruction of serotonin, and with a decrease in the number of insulin-producing cells from the endocrine pancreas, an increase in the number of cells synthesizing glucagon occurs.

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