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EVALUATION OF THE DETERMINATION OF THE CHROMOGRANIN MARKER AS AN ADDITIONAL DIAGNOSTIC SIGN OF VARIOUS VARIANTS OF ESOPHAGEAL ATRESIA IN NEWBORNS

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ABSTRACT

When studying the immunohistochemical aspects of various clinical and morphological types of atresia, which is one of the developmental anomalies of the esophagus, the Chromogranin marker is used to identify secretory neurons that mark the neuromuscular synapses of neuroendocrine cells and to determine whether the synthesis of functional peptide bonds occurs. By its nature it is a glycoprotein marker, which makes it possible to assess the morphofunctional state by staining the vesicles of neuromuscular synapses in the muscle layer. The high sensitivity of this marker in very low titers demonstrates all the symptoms of APUD cells masking vesicles in neuromuscular synapses. This makes it possible to plan treatment based on important criteria for determining the treatment tactics for various types of esophageal atresia.

Key words: chromographin, immunohistochemical study, esophageal atresia, newborns.

INTRODUCTION

Relevance of the problem. Anomalies in the development of the esophagus are a process in the embryonic period that continues the development of the esophagus and its structural formations with anatomical and histological changes. Worldwide, 40 out of every 100,000 infants develop this pathology.The detection of this pathology in the early stages of pregnancy screening in the United States and European countries, based on strict recommendations for termination of

pregnancy, averages 4-8 cases per 100,000 babies. While the incidence of these pathologies in the CIS countries, including the Russian Federation, is 20-25 cases per 100,000, in the Republic of Uzbekistan this figure averages 8-10 cases per 1,000 infants, which is manifested by a high mortality rate of an average of 60-78% of cases within a month after the birth of infants. Currently, this issue is a problem for pediatricians and neonatologists, and the incidence rate in children born from a marriage between relatives with a high genetic predisposition is 2.5 times higher, which, as has been established on the basis of foreign literature and anamnestic collections, is multiple. It is the fact that the mortality rate for combined bronchial-esophageal adhesions of the esophageal malformation process exceeds 85%, which leads to the fact that infants die from aspiration pneumonia in the first days of the early neonatal period.

Materials and methods of research. Infants who died due to esophageal malformations, as well as autopsy materials for infants who underwent surgery and died without it, were used as materials. In these 18 cases, esophageal material was taken for immunohistochemical examination.

Aim. To study the results of immunohistochemical determination of chromogranin marker expression in clinical and morphological types of esophageal malformations.

The exam method. Biopsies containing paraffin using monoclonal antibodies were subjected to immunohistochemical examination using standard methods.4 μm thick sections are made from paraffin blocks, taken to the window of the item and dried for a day at room temperature. Before painting, the sections are placed in a thermostat for 60 minutes at a temperature of 55^0 C in a vertical position. After that, it is dewaxed in orthoxylene (for 10 minutes in each of the two capacitive batteries), rehydrated in ethyl alcohol in decreasing concentration (for 3 minutes in each of the three capacitive batteries) and washed with distilled water. The glass of the product from which the cutting is obtained is heated, placed in an unmasking chamber and placed in a water bath for 30-40 minutes at a temperature of 98^0C. The preparations are washed in a tris-buffer (pH=7.5) solution after cooling to room temperature. To block endogenous peroxidase, the incisions are treated with 3% H_2O_2 for 15 minutes. In an attempt to reduce at nospesific bonds and limit background staining, preparations are processed with protein Blosk (X0909) (Ventana Ultra) for 10 minutes. The incisions are converted into a special deliminating composition to save the reagents and get the front of the leakage before the addition of anti-agents. Incubation with primary at was carried out for 60-120 minutes at room temperature. As a visualization system, a universal LSAB2 KIT (Ventana Ultra) set was used for 40 minutes of minimal exposure.

Paraffin 57 os in the incisions was dissolved and removed in a thermostat using xylol, then we used immunogystochemical markers to study the general histological condition of esophageal tissue. This means that the prepared cubes are placed in a glass with a special adhesive preparation, and the material is passed through a microtome. Then the hematoxylin dye is kept for 2 minutes. On special automated equipment (VentanaUltra), the selected markers are applied in drops to the surface of the sliding window, which is equipped with special stickers with a QR code. After 20 minutes, the marker is washed off with distilled water. The surfaces of the painted preparations surfaces are covered with protective glass. In the study, the expressed cells are light yellow, orange in color, mainly focusing the esophagus on the muscular, muscular mucous membrane range, masked pores on the mucous membranes. To check if our work is correct, when the drug glass is seen in a microscope, we see gold yellow-brown cells in bioptates in reactions with the above markers. So our way of working will be done correctly. The above markers are seen as having an expression level "+" output to the change in color indicator when reacting with a specific protein in the lymphocyte membrane. In this case, we evaluate markers as expression by name.

Discussion and results. Subsequent immunogystochemical investigations of the Chromogranine marker are also used to label neuroendocrine cells secretory neurons that mark nerve muscle synapses and to determine whether functional peptide bond synthesis is occurring. It is a glycoproteid by marker nature, providing an opportunity to assess morphofunctional status by staining vesicles containing nerve muscle synapses on the muscle floor. This marker is highly sensitive to APUD cells at very low titers and exhibits all its symptoms by masking vesicles in neuromuscular synapses. It is through the chromagranin marker that it makes it possible to eliminate clinical morphological signs by analyzing the degree of maturation of the apud cells contained in the mucous membrane in the esophagus wall, assessing their morpho-functional characteristics, forecasting morphological signs of maturation.

The fact that APUD supports, albeit partially, cell control over neuromuscular synapses allows us to evaluate in advance the aspects of evaluating expected results in the diagnosis of esophageal atresia and planning treatment using a conservative or surgical method.

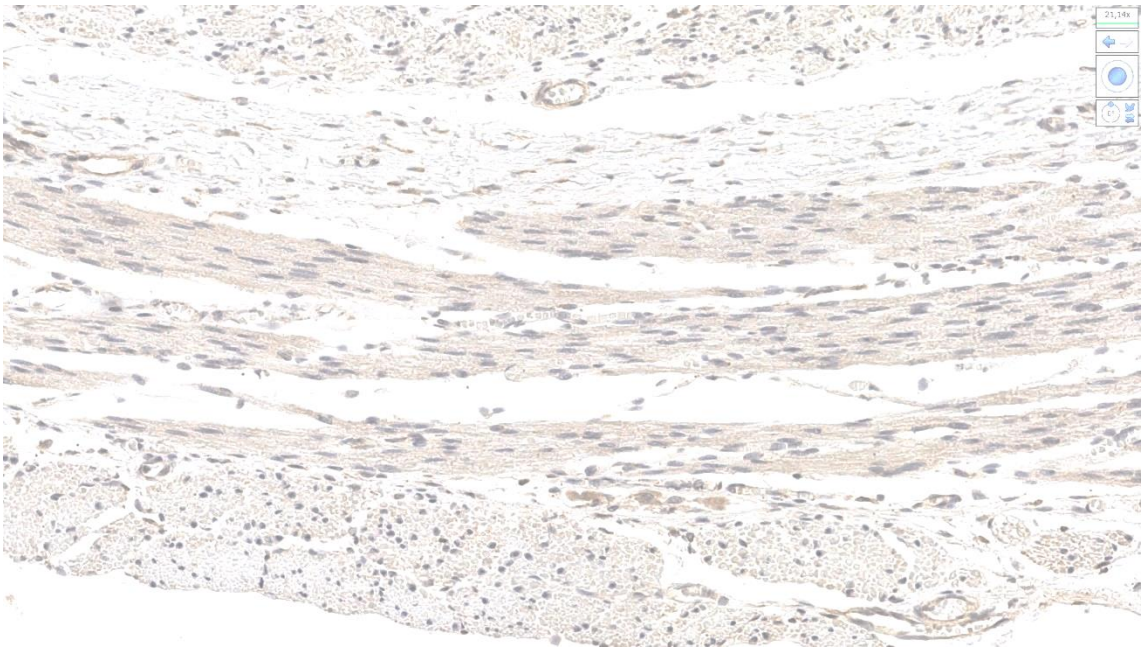

Figure 1. Congenital atresia of the esophagus, 1st variant, reveals a weak positive reaction to the chromogranin marker, while neuroendocrine cells between the muscle floor are found as traces in focus, indicating that improvement lingers in this place. The staining smear is chromogenic. Size 10x10.

This marker was characterized only by a low positive expression of esophageal atresia only in variant 1, which led to a low and negative reaction in the remaining variants of the group. This is characterized by the fact that it is with all types of atresia isolating the esophagus that the developmental abnormalities are extremely serious, while the developmental abnormalities manifest themselves in a combined form, the number of APUD cells in the esophageal wall is very low, and the number of functional spots is low. This protein marker is also glycoproteidally complex, providing a positive reaction by staining the membrane of neurosecretory cells. This, in turn, is detected in light yellow in functionally active members.

If the dark liver is expressed in color, the high concentration of the glycoproteid protein means that high activity and tumor processes have occurred in neurosecretory cells. In our research work, it was found in variant 1 at medium intensity.

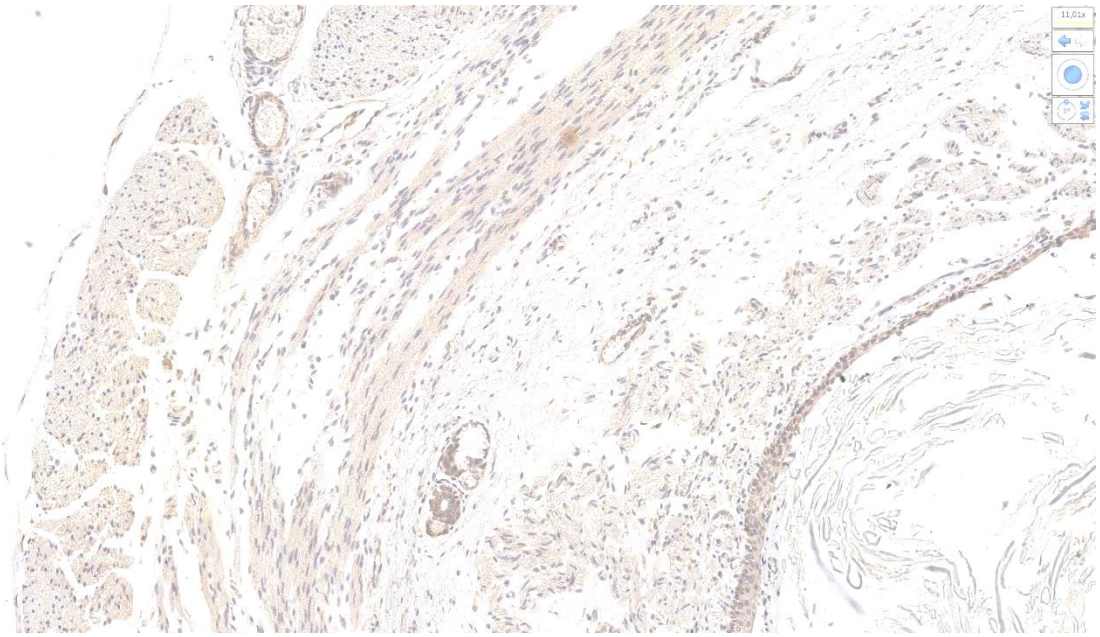

Figure 2. Congenital atresia of the esophagus of the 2nd variant, a negative reaction to the chromogranin marker is detected, while neuroendocrine cells between the muscle layers form very slowly, which indicates a lag in deep development. The staining smear is chromogenic. Size 10x10.

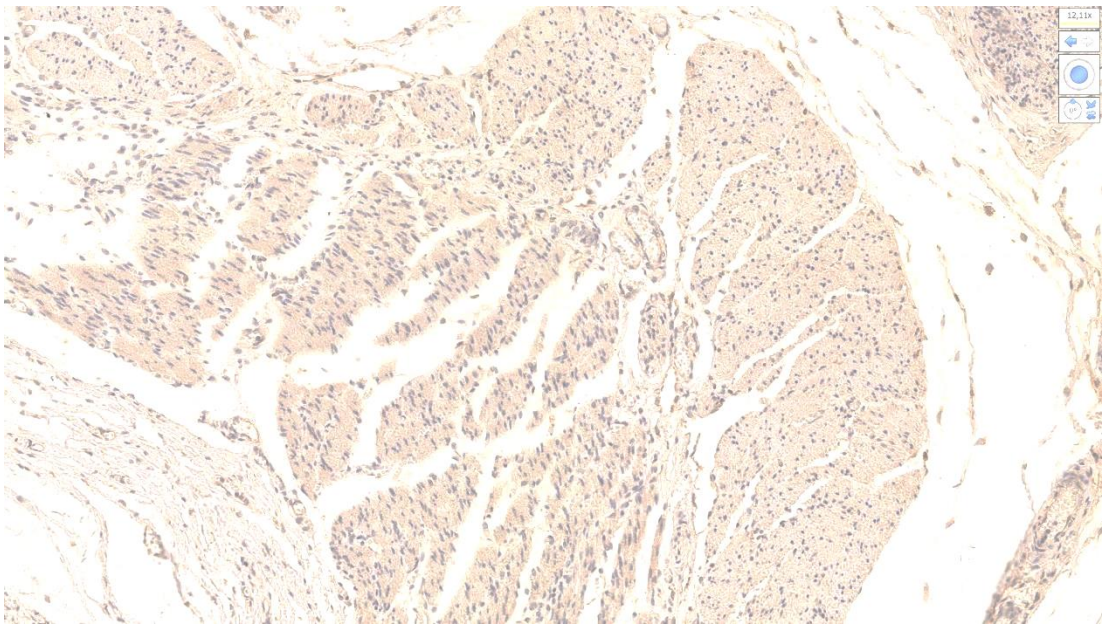

Figure 3. Congenital atresia of the esophagus of the 3rd variant, a negative reaction to the chromogranin marker is detected, while neuroendocrine cells between the muscle layers form very slowly, which indicates a lag in development. The staining smear is chromogenic. Size 10x10.

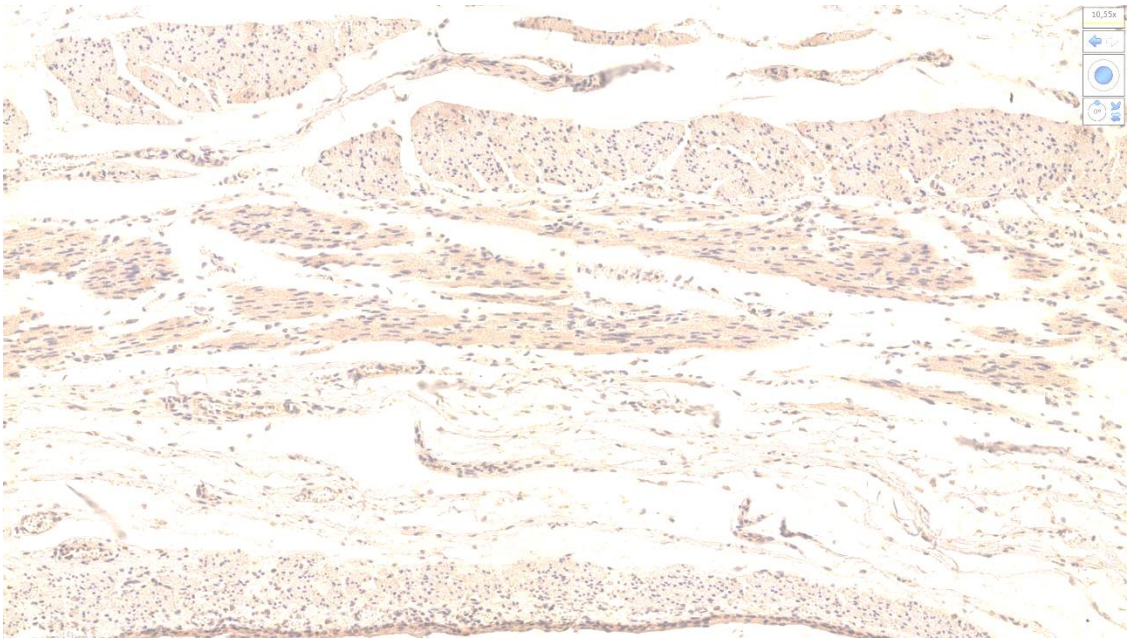

Figure 4. Congenital atresia of the esophagus of the 4th variant, a negative reaction to the chromogranin marker is detected, while neuroendocrine cells form very slowly between the muscle layers, which indicates a deep developmental delay. The staining smear is chromogenic. Size 10x10.

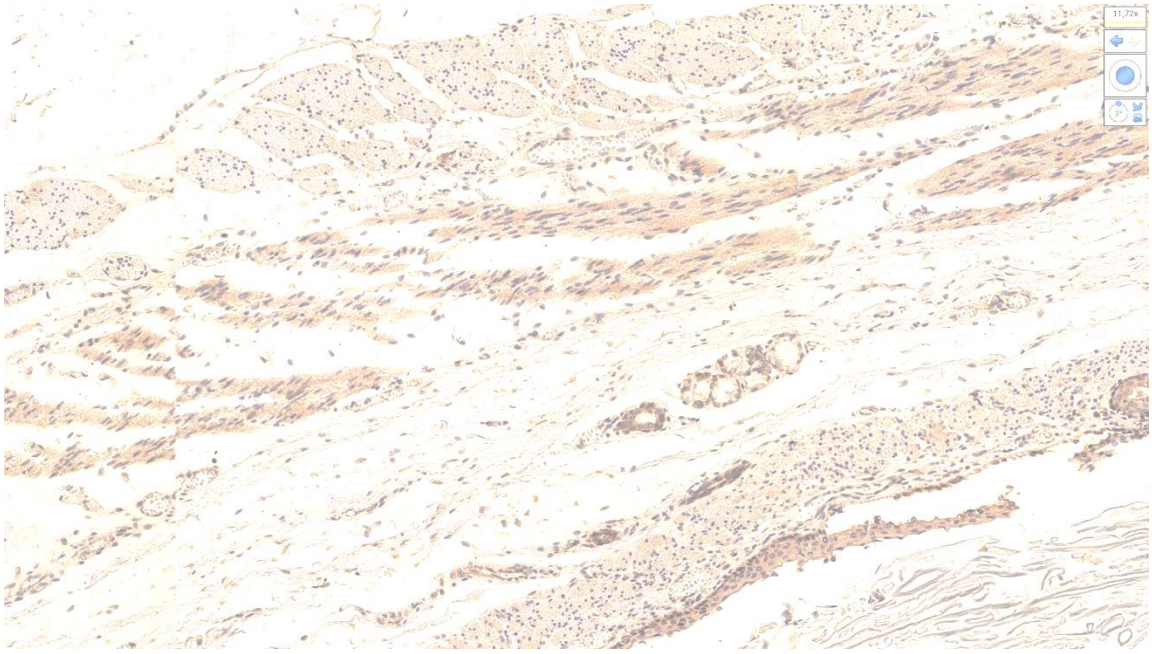

Figure 5. Congenital atresia of the esophagus of the 5th variant, a negative reaction to chromogranin, a new marker, is detected, while neuroendocrine cells form very slowly between the muscle layers, which indicates a lag in deep development. The staining smear is chromogenic. Size 10x10.

In 15 out of 113 cases of low expression of the chromagranin marker, low levels were detected in 13.3% of cases, which led to serious developmental

abnormalities in these examined parts of the esophagus. It is the low position of the chromogranin marker that is explained by the increase in the majority of fibroblasts and histiocytes around the components that are fully formed during the development of neuromuscular synapses, and the appearance of a complex of muscular fibrous tissue. This, in turn, means that the preliminary assessment and treatment of esophageal atresia require a clear period of time, and the manifestation of clinical and morphological signs that threaten the lives of infants and the failure to take timely non-selective therapeutic measures in most cases results in a fatal outcome of up to 90%.

Conclusion.

The low response of the chromogranin marker was caused by a lack of biologically active substances produced by neuromuscular synapses and APUD cells, which clinically morphologically disrupted the connection between muscle and nerve in various types of esophageal atresia.

This makes it possible to prolong the patient's life by 50-92% as a result of treatment, in order to ensure the excitability of the neuromuscular synapse when offering a treatment algorithm.

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