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FEATURES OF MORPHOLOGICAL AND MORPHOMETRIC PARAMETERS OF LYMPHOID STRUCTURES IN BILIARY ATRESIA

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Currently, the morphology and morphometric indicators of the lymphoid structures of the bile ducts in biliary atresia have been little studied. Therefore, using total and histological preparations, we studied the lymphoid structures of the bile ducts obtained from the corpses of newborn children in whom the bile duct atresia was revealed during pathological examination. It has been established that the lymphoid apparatus of the walls of the bile ducts in biliary atresia is represented by diffuse lymphoid tissue and lymphoid nodules without a reproduction center. The number, area, and length of the lymphoid nodules are less than in the comparison group. The biggest difference is observed in the number of lymphoid nodules. The number of lymphocytes with this anomaly, in comparison with the standards, decreases, the macrophages and cells in a state of mitosis are not detected, the number of cells in a state of degeneration, on the contrary, increases.

Key words: bile ducts, lymphoid nodule, diffuse lymphoid tissue cells, size and quantitative indices, total preparations, histological preparations.

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ОСОБЛИВОСТІ МОРФОЛОГІЧНИХ І МОРФОМЕТРИЧНИХ ПОКАЗНИКІВ ЛІМФОЇДНИХ СТРУКТУР ПРИ АТРЕЗІЇ ЖОВЧНИХ ПРОТОКІВ

На сьогодні морфологія і морфометричні показники лімфоїдних структур жовчних проток при атрезії жовчних шляхів мало вивчені. Тому, з використанням тотальних та гістологічних препаратів, ми досліджували лімфоїдні структури жовчних проток, отриманих від трупів новонароджених, у яких при патологоанатомічному дослідженні виявлено атрезію жовчних проток. Встановлено, що лімфоїдний апарат стінок жовчних проток при атрезії жовчних шляхів представлений дифузною лімфоїдною тканиною та лімфоїдними вузликами без центру розмноження. Кількість, площа і довжина лімфоїдних вузликів були меншими, ніж у групі порівняння. Найбільша різниця спостерігається в кількості лімфоїдних вузликів. Кількість лімфоцитів при цій аномалії, порівняно зі стандартами зменшується, макрофагів і клітин у стані мітозу не виявляється, кількість клітин у стані дегенерації, навпаки, збільшується.

Ключові слова: жовчні протоки, лімфоїдний вузлик, дифузна лімфоїдна тканина, розмірно-кількісні показники, тотальні препарати, гістологічні препарати.

Biliary atresia is a blockage in the tubes (ducts) that carry bile from the liver to the gallbladder. This congenital condition occurs when the bile ducts inside or outside the liver do not develop normally [9].

Biliary atresia affects approximately 1:20,000 babies and is the most frequent cause of jaundice in children. It is the end result of a destructive inflammatory process of the bile ducts, with unclear origins. This anomaly is a condition seemingly unique to the neonatal period, characterized by obliteration of both intra and extrahepatic bile ducts [2].

The cause of biliary atresia is not fully understood and it is well possible that a number of factors may play a role, but especially maternal rotavirus infection during pregnancy and subsequent transmission of the virus to the child resulting in infection of the biliary epithelium and subsequent occluding fibrosis may be important in this respect. Some cases may relate to infection with other viruses (including COVID-19) and congenital cytomegalovirus infection as well [3].

Biliary atresia is a rare disease but remains the most common index for pediatric liver transplantation as there are no effective medical therapies to slow progression after diagnosis. Variable contribution of genetic, immune, and environmental factors contributes to disease heterogeneity among patients with biliary atresia. Developing a deeper understanding of the disease mechanism will help to develop targeted medical therapies and improve patient outcomes [1].

There are 3 main variants of biliary atresia: Type 1 (5–10 %) is where obstruction to bile flow is at the level of common bile duct, and typically bile is found in the gallbladder. The proximal biliary tract is often cystic in these. In Type 2, the obstruction is at the level of the common hepatic duct and dissection within the porta hepatis will show two distinct, albeit thick-walled and abnormal, hepatic ducts. This is exceedingly rare in most series (1–2 %). By contrast, Type 3, is by far the most common (>90 %) with its obstruction level high within the porta hepatis and in these there are no visible macroscopic ductules present

– he transected porta presenting a relatively uniform bland appearance [4, 10]. Currently, increased attention is paid to the study of the immune structures of the walls of hollow organs in various congenital anomalies. [14].

Despite this, in the scientific literature, there is no data on the morphological features and morphometric parameters of lymphoid formations of the bile ducts in biliary atresia.

The purpose of the study was to establish the structural features and size-quantitative indices of lymphoid structures in bile duct atresia.

Material and methods. The object of the study was the lymphoid structures of the walls of the bile ducts obtained from the corpses of newborn children in whom the bile duct atresia was revealed during pathological anatomy research.

The material is divided into the following groups:

Group I – newborns with bile duct atresia (main group) (n=10).

Group II – newborns without this anomaly (comparison group) (n=10).

Three variants of biliary atresia were identified on corpses:

Group I – obstruction at the level of the common bile duct (n=3).

Group II – obstruction at the level of the common hepatic duct (n=1),

Group III – obstruction at the level of the porta hepatis (n=6).

Total preparations of lymphoid derivatives of the bile ducts were studied using the macromicroscopic method by T. Hellman. Harris hematoxylin (Avantor, Holland) was used to stain the lymphoid nodules. The colored preparation was placed in a 3 % acetic acid solution until lymphoid nodules appeared.

For histological research, pieces were taken from the left and right hepatic ducts, the common hepatic duct, and the common bile duct. Sections 5–7 μm thick were made from these pieces. Prepared sections were stained with hematoxylin-eosin, methylene blue, using the Van Gieson method.

Before staining with hematoxylin-eosin, paraffin sections were placed in a 96 % ethanol solution for 20–30 minutes. Dewaxed sections were stained with Ehrlich's hematoxylin solution (Penta Manufacturing, Poland). The preparations were covered with a coverslip and viewed under a microscope. [7].

For staining with methylene blue, the preparations were fixed in 96 % ethanol solution. The dried preparation was stained with a solution of methylene blue with 0.01 % potassium hydroxide (Penta Manufacturing, USA). After washing in water, the preparation was examined under an immersion microscope [11].

When staining according to Van Gieson, Weigert's iron hematoxylin solution and an acidic mixture of picrofuchsin according to Van Gieson (Avantor, Holland) were used. The prepared sections were placed in Weigert's hematoxylin solution for 2–5 minutes and washed with distilled water for 10 minutes. Then, it was placed in a picrofuchsin solution for 2–3 minutes. [6].

On preparations of the bile ducts, we determined the percentage of lymphoid nodules per unit area of the ocular reticle, the length, the area of the lymphoid node, the density of lymphoid cells in diffuse lymphoid tissue, in lymphoid nodes (the number of cells in a cross-sectional area of 880 μm^2), the composition and number of cells of lymphoid structures.

The digital data obtained during the research were subjected to statistical processing. Statistical analysis was performed using variation and dispersion methods in MS EXCEL-2019 and IBM Statistics SPSS-26 programs.

The mean values of the obtained samples M±m (M is the arithmetic mean, m is the standard error), minimum (min), maximum (max) values of the series are calculated. Comparisons were made between groups (P). For a preliminary assessment of the difference between the variation series, the parametric t-Student test was used. Next, to compare and determine the reliability of quantitative differences in groups and subgroups, the nonparametric Wilcoxon rank U test (Mann-Whitney) was used [12].

Results of the study and their discussion. As a result of the study, it was established that in the mucous membrane of the bile ducts without atresia (comparison group), all morphogenetic forms of lymphoid formations are determined – lymphocytes in the surface epithelium, diffuse lymphoid tissue, located predominantly subepithelial, as well as the lymphoid nodules with and without a reproduction center.

The lymphoid apparatus of the walls of the bile ducts in biliary atresia is represented by diffuse lymphoid tissue and lymphoid nodules without a reproduction center. Lymphocytes are not found in the surface epithelium (Fig. 1).

We analyzed the morphometric characteristics of the lymphoid structures of the bile ducts in biliary atresia. The characteristics of these structures in the absence of this anomaly were considered as a comparison group.

The percentage of lymphoid nodules in biliary atresia in the left and right hepatic ducts is 1.8 times (P<0.001), in the common hepatic duct is 2.1 times (P<0.001) and in the common bile duct is 2.4 times (P< 0.001) less than that of the comparison group.

The area of lymphoid nodules in biliary atresia in the left and right hepatic ducts is 1.7 times (P<0.001), in the common hepatic duct is 1.9 times (P<0.001) and in the common bile duct is 2.1 times (P<0.001) less than that of the comparison group (Table 1).

Table 1

Dimensional-quantitative indicators of lymphoid nodules of the bile ducts of newborn children with biliary atresia

Groups	Bile ducts			
	Common bile duct	Common hepatic duct	Left hepatic duct	Right hepatic duct
Number of lymphoid nodules per unit area of the ocular reticle (in %)				
Main group	3.9±1.6*** 1.5–5.7	3.4±0.5*** 1–5	3.1±0.4*** 0–3	2.8±0.3*** 0–3
Comparison group	9.5±3.5 5.1–14.7	7.2±1.7 4.6–9.3	5.4±1.0 3.3–7.8	5.1±0.5 3.2–8.4
Area of lymphoid nodules (mm^2 10^{-4})				
Main group	35.3±2.1*** 25.8–41.2	33.2±2.3*** 26.3–43.3	31.3±2.2*** 26.3–43.3	30.9±2.2*** 25.2–42.2
Comparison group	73.1±1.4 43.2–56.4	63.8±1.4 32.3–54.8	52..6±1.1 49.6–65.3	51.9±0.9 40.2–48.4
Length of lymphoid nodules (in microns)				
Main group	48.6±3.3*** 37–62	46.3±3.2*** 36–60	42.2±3.2*** 32–56	42.4±2.9*** 37–59
Comparison group	98.8±2.4 88.0–112.3	86.9±3.3 62.5–95.7	60.8±2.6 50.2–76.7	60.5±3.5 63.0–97.7

Note: *** – high level of statistical difference between groups (P < 0.001)

The length of lymphoid nodules in biliary atresia in the left and right hepatic ducts is 1.4 times (P<0.001), in the common hepatic duct is 1.9 times (P<0.001) and in the common bile duct is 2.0 times (P<0.001) less, than the comparison group.

Thus, in biliary atresia, the number of lymphoid nodules and their length and width decreases. The number of lymphoid nodules shows a significant difference between the indices of the main group and the comparison group. A decrease in the considered parameters is observed in biliary atresia and in the comparison group, from the common bile duct to the left and right hepatic ducts.

The density of lymphoid cells in diffuse lymphoid tissue in biliary atresia in the common bile duct is 1.6 times (P<0.001), in the common hepatic duct is 1.7 times (P<0.001), in the left hepatic duct is 2.2 times (P<0.001) and in the right hepatic duct is 2.1 times (P<0.001) less than in the comparison group (Table 2).

Table 2

Density of lymphoid cells in the lymphoid structures of the bile ducts of newborn children with biliary atresia

Groups	Bile ducts			
	Common bile duct	Common hepatic duct	Left hepatic duct	Right hepatic duct
Density of lymphoid cells in diffuse lymphoid tissue				
Main group	12.2±0.9*** 8-15	14.2±1.1*** 8–16	15.1±2.4*** 9–27	15.8±1.5*** 8–19
Comparison group	20.3±0.6 18–24	24.7±1.1 20–29	32.5±0.9 26–34	33.8±0.9 22–28
Density of lymphoid cells in lymphoid nodules				
Main group	14.6±0*** 10–17	17.2±1.3*** 13–23	20.1±1.5*** 14–25	20.7±1.5*** 13–21.5
Comparison group	23.5±0.6 20–26	29.8±1.1 23–32	35.5±0.9 30–38	35.9±0.9 26–32

Note: *** – high level of statistical difference between groups (P < 0.001)

The density of lymphoid cells in the composition of lymphoid nodules in biliary atresia in the common bile duct is 1.6 times (P<0.001), in the common hepatic duct is 1.7 times (P<0.001), in the left

hepatic duct is 1.8 times (P<0.001) and in the right hepatic duct is 1.8 times (P<0.001) less than in the comparison group.

Thus, compared with the norm, the density of lymphoid cells within lymphoid structures in biliary atresia decreases to a greater extent in the left and right hepatic ducts.

The study of the cellular composition of lymphoid structures showed that the lymphoid structures of the walls of the bile ducts of newborn children without anomalies (comparison group) include lymphocytes, lymphoblasts, plasma macrophages and reticular cells. In the lymphoid structures of these organs, there are always cells with signs of mitosis, which reflects the processes of lymphocytopoiesis, and a few mast cells; cells are in a state of degeneration. Lymphocytes make up 50–70 % of all lymphoid cells.

In contrast to the comparison group, the cellular composition of the lymphoid structures of the mucous membrane of the bile ducts during atresia is mainly represented by lymphocytes, plasma cells, cells in a state of degeneration, and other cells of the lymphoid series (Fig. 2).

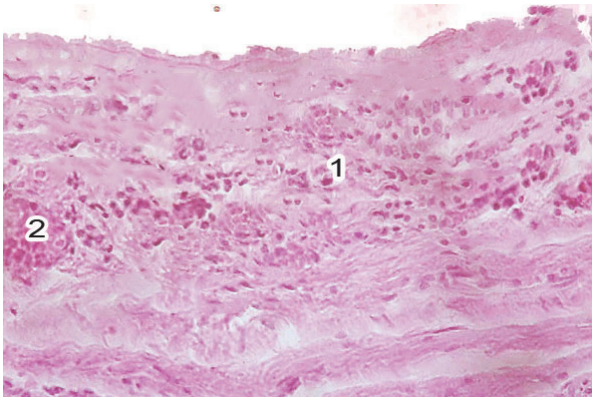

Fig. 1. Lymphoid structures of the mucous membrane of the common hepatic duct of a newborn with biliary atresia of this organ. Hematoxylin-eosin staining. Zoom rate X250. 1. Diffuse lymphoid tissue; 2. Lymphoid nodule.

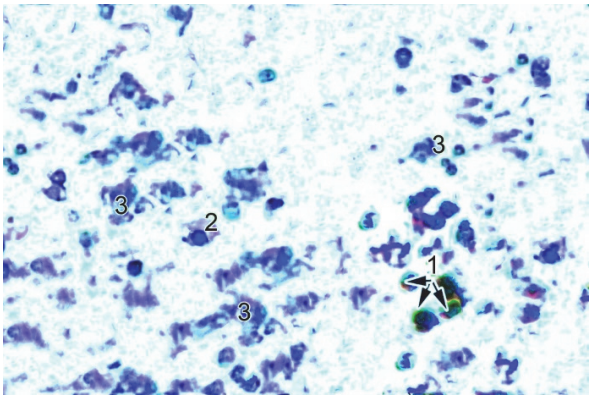

Fig. 2. Cellular composition of the lymphoid structures of the common bile duct. Staining with methylene blue. Zoom rate X600. 1. Lymphocytes; 2. Plasmocytes; 3. Cells in a state of degeneration.

On histological preparations of the bile ducts, in contrast to the comparison group, plasmacytic–lymphocytic and macrophage–lymphocytic complexes are not detected.

Using the morphometric method of microscopic sections of the biliary tract, we analyzed the cellular composition of diffuse lymphoid tissue in biliary atresia of newborns, comparing the obtained digital data with the comparison group.

According to our data, the percentage of lymphocytes during atresia in the diffuse lymphoid tissue of the common bile duct, common hepatic duct, left and right hepatic duct is 1.1 times (P<0.05) less than in the comparison group.

The number of plasma cells in the diffuse lymphoid tissue of the common bile duct, common hepatic duct, left and right hepatic ducts does not differ significantly in biliary atresia and in the comparison group (relative norm).

Macrophages and cells in a state of mitosis in newborns with atresia of all bile ducts in diffuse lymphoid tissue are rare or absent.

The percentage of cells in a state of degeneration in atresia in the diffuse lymphoid tissue of the common bile duct is 6.5 times (P<0.001), the common hepatic duct is 6.6 times (P<0.001), the left and right hepatic ducts is 8.5 times (P<0.001) more than in the comparison group.

The significance of our study is determined by the fact that in recent years, targeted examinations using modern diagnostic methods in clinical practice have often revealed various anomalies of internal organs. One such anomaly is bile duct atresia [8]. Therefore, we conducted microanatomical and morphometric studies of the lymphoid structures of the wall of the bile ducts of newborn children with atresia of these organs.

The results of the study of these structures in organ walls in atresia were compared with similar indices obtained from newborns and children without this anomaly (comparison group).

We have established that changes in the lymphoid apparatus of bile duct atresia are systemic in nature. Morphometric methods have proven a decrease in the number and size of lymphoid structures (number, length, area of lymphoid nodules) in the walls of these organs.

According to our data, the percentage of lymphocytes with this anomaly, compared with the standards, in diffuse lymphoid tissue is less than in the comparison group. The percentage of plasma cells does not differ significantly in biliary atresia and in the comparison group (relative norm). In newborns

with this anomaly the macrophages and cells in a state of mitosis in the diffuse lymphoid tissue are rare or absent. In biliary atresia, the number of lymphoid cells with signs of destruction in all lymphoid formations increases significantly.

Our data are consistent with the results of S.V. Shadlinskaya, who, having studied the lymphoid apparatus of the vaginal vestibule in some anomalies of the internal female genital organs, came to the conclusion that in anomalies, the quantitative changes are accompanied by qualitative changes in the cellular composition of the lymphoid formations of the vaginal vestibule [14].

According to L. Fabris et al. the biliary tree is the target of atresia. After damage to the biliary epithelium, the inflammatory changes stimulate a reparative response with the proliferation of cholangiocytes and restoration of the architecture of the biliary tract. These changes will ultimately lead to pathological recovery with the formation of biliary fibrosis and clinical progression of the disease [5].

Conclusions

1. The lymphoid apparatus of the walls of the bile ducts in biliary atresia is represented by diffuse lymphoid tissue and lymphoid nodules without a reproduction center. Lymphocytes in the surface epithelium and the intercellular complexes are not found.

2. The number of lymphoid nodules in biliary atresia in the hepatic ducts is on average 2.0 times ($P<0.001$), the area of lymphoid nodules is on average 1.9 times ($P<0.001$), the length of lymphoid nodes is on average 1.7 times ($P<0.001$), the density of lymphoid cells in diffuse lymphoid tissue is on average 1.9 times ($P<0.001$), and in lymphoid nodules is 1.7 times ($P<0.001$) less than in the comparison group. Thus, the greatest difference between the indices of the bile ducts in biliary atresia and similar indicators of the comparison group is noted in the number of lymphoid nodules.

3. The percentage of lymphocytes in this anomaly, compared with the standards, in diffuse lymphoid tissue is 1.1 times ($P<0.05$) less than in the comparison group. The number of plasma cells does not differ significantly in biliary atresia and in the comparison group. Macrophages and cells in a state of mitosis in newborns with this anomaly are absent in the diffuse lymphoid tissue, the percentage of cells in a state of degeneration in biliary atresia in diffuse lymphoid tissue on average is 7.2 times higher ($P<0.001$) than in comparison group.

4. The data obtained can be used both for a better understanding of the mechanisms of occurrence of this anomaly, and for the subsequent rehabilitation of patients after successful surgery.

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