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SPATIO-TEMPORAL CHARACTERISTIC OF THE SPHINCTERS OF THE EXTRAHEPATIC BILE DUCTS DURING PRENATAL PERIOD OF HUMAN ONTOGENESIS

Key words: extrahepatic bile ducts, sphincteric apparatus, development, fetus, human being.

Abstract. *With the purpose to study peculiarities of development and blood supply of the sphincteric apparatus of the extrahepatic bile ducts during the prenatal period of human ontogenesis 104 series of histological sections of the specimens of human embryos, fetuses, fetuses and newborns measuring from 4,5 to 378,0 mm parietococcygeal length (PCL) (5-40 weeks of development) have been researched by means of complex of morphological methods (anthropometry, morphometry, vascular injections, macroscopy, microscopy, 3D-reconstructions, statistical analysis). It has been established that extrahepatic bile ducts are traced in the form of a well-defined tubular structure, starting from the 6th weeks of the development. The extrahepatic and intrahepatic bile ducts are isolated, but at the end of the embryonal period a tendency towards their fusion is traced. The forming of the intraorgan blood vessels of the common bile duct is detected at the end of the 7th week of the intrauterine development in fetuses measuring 18.0-19.0 mm PCL. The source of the vascularization of the common bile duct in its retroduodenal and pancreatic portions is the branches of the gastropancreatic artery, whereas in the intramural portion – of the inferior pancreaticoduodenal artery. The peculiarities of spatial structure of the arterial anastomosis around the coiled part of the cystic duct proved the existence of the locking device (sphincter) between the neck of gallbladder and cystic duct and play an important role in functioning vascular (arterial) component of it. The distinct character and angioarchitectonics differences in arterial and venous plexuses in the sphincter segments of the biliary system has been clearly observed at the end of the fetus period of development and in newborns, which may indicate an important functional role of the vessels in activity of sphincters, which provide biliodynamics.*

Introduction

Development of diagnostic methods and new non-invasive technologies in biliary surgery are impossible without exhaustive information concerning the embryogenesis and anatomy of the extrahepatic bile ducts [1, 3], including variants of blood supply and myeloarchitectonics of their sphincter apparatus [2, 7]. The blood supply of the extrahepatic bile ducts is notable for variability which is important to take into account during surgical interferences on the organs of the hepatobiliary system [5, 6]. Understanding the etiopathogenesis of congenital malformations of the system of the pancreatic and bile ducts requires detailed study of their embryonal development [4, 10]. But the modern reported scientific data are fragmentary and controversial. Publications of recent years concerning the formation of the blood channel of the derivatives of the intestinal tube during the in-

trauterine development (IUD) in human give only scrappy information [8, 9]. At the same time, ascertaining the specific characteristics of the formation of the blood vessels of the extrahepatic bile ducts (EBD) at an early stage of human ontogenesis will help to understand deeper the consistent patterns of the vascularization of the biliary tract [8, 9]. The comprehensive study of the prenatal development of the sphincter apparatus of EBD is morphological basis for improvement of surgical techniques [11].

The object of research

The goal of this study is establishing the sources of the anlage and the dynamics of spatio-temporal transformations of the sphincter apparatus of EBD, the specific features of the organization of the blood supply of the EBD in the prenatal period of human ontogenesis.

Material and Methods

The study was carried out on 104 series of histological sections of the specimens of human embryos, fetuses and newborns measuring 4.5-378.0 mm of parietococcygeal length (PCL) from the embryologic collection of Bukovyna State Medical University by means of the methods of anthropometry, morphometry, vascular injections, macroscopy, microscopy, 3D-reconstructions, statistical analysis. The IUD periods were systematized on the basis of the classification of Schmidt G.A. (1968). The obtained digital images of histologic specimens were studied using 3D-reconstruction method based on the computer program "Virtual Anatomist" (Kharkiv, Ukraine) [12].

Results

In this study we found that the anlage of the bile ducts and liver is a hepatic diverticulum which can be easily seen in a 4.5 mm PCL embryo (Figure. 1). This hepatic bud is a diverticulum of endoblastic epithelium of the ventral wall of the upper portion of the foregut (the future duodenum) into the transverse septum that is a mesodermal lamina between the pericardial cavity and the peduncle of the yolk sac. The cells of the bud rapidly proliferate and grow into the caudal portion of the mesoderm of the transverse membrane between the right and left cardinal veins, into the so-called hepatic mesoderm. The hepatic diverticulum grows in the surrounding cellular layers and rapidly divides onto the cranial and caudal por-



Figure. 1. Sagittal section of human embryo of 4.5 mm PCL. Hematoxylin and eosin staining. Lens: x8; ocular: x7: 1 – anlage of duodenum; 2 – anlage of liver; 3 – anlage of gallbladder; 4 – anlage of common bile duct; 5 – anlage of portal vein

tions. At the end of the fourth week, the shape of the liver bud gradually changes and the head (in the cranial larger portion) and the neck (in the caudal lesser portion) of the liver diverticulum can be seen very distinctively. The cranial part of diverticulum is a liver anlage and has dimensions of 500x315 μm . The caudal portion reaches 250x125 μm in size.

The formation of extrahepatic part of the bile duct is complete somewhere between the end of the fourth and the beginning of the fifth week of the intrauterine development. Its formation takes place due to elongation of the caudal portion of the liver diverticulum. The caudal portion of the liver diverticulum (the cystic diverticulum), turns into the gallbladder and its "neck" forms the cystic duct. It has been traced that cells, forming the gallbladder and the cystic duct originate from a histologically distinct population of endodermal cells. The stalk of the hepatic diverticulum between the primitive gut, which is differentiated into the duodenum and the cystic diverticulum, transforms into the common bile duct. Thus, intensive processes of a transformation of the liver diverticulum and the formation of the human biliary system during the fourth week take place. Therefore, any unfavourable factors of the internal or external environment may bring about the appearance of anatomical variants or congenital malformations of the gallbladder and EBD.

The intensive proliferation of the hepatic cells and the elongation of the extrahepatic bile ducts take place during the fifth week of IUD. At this time, the intestinal tube starts closing and forming the duodenum. In the end of the fifth week, lumen of these tubular structures is filled with epithelial cells. During this period the cystic diverticulum can be easily traced. Its dimensions reach 250x130 μm . The anlage of the gallbladder is surrounded by a mesenchymal layer. Its muscular and connective tissue membranes are formed by the hepatic tissue. The dorsal pancreatic diverticulum is formed from the dorsal wall of the duodenum and is positioned against the place of the origination of the liver diverticulum and shortly, the ventral pancreatic diverticulum arises from the anlage of the bile duct, more caudally from the primordium of the gallbladder. ## Although the hepatic diverticulum arose from the ventral wall of the foregut, the processes of growth and rotation of the duodenum cause a shift of the place of the confluence of the bile duct and the ventral pancreatic primordium on the dorsal wall of the intestine and their location within the bounds of the dorsal mesentery.

At the beginning of the sixth week of IUD, the size of the hepatic anlage continues to grow intensively. At this time, its transverse size is about 900 μm , the dorsoventral size is about 400 μm , and the craniocaudal one is about 455 μm . It occupies the

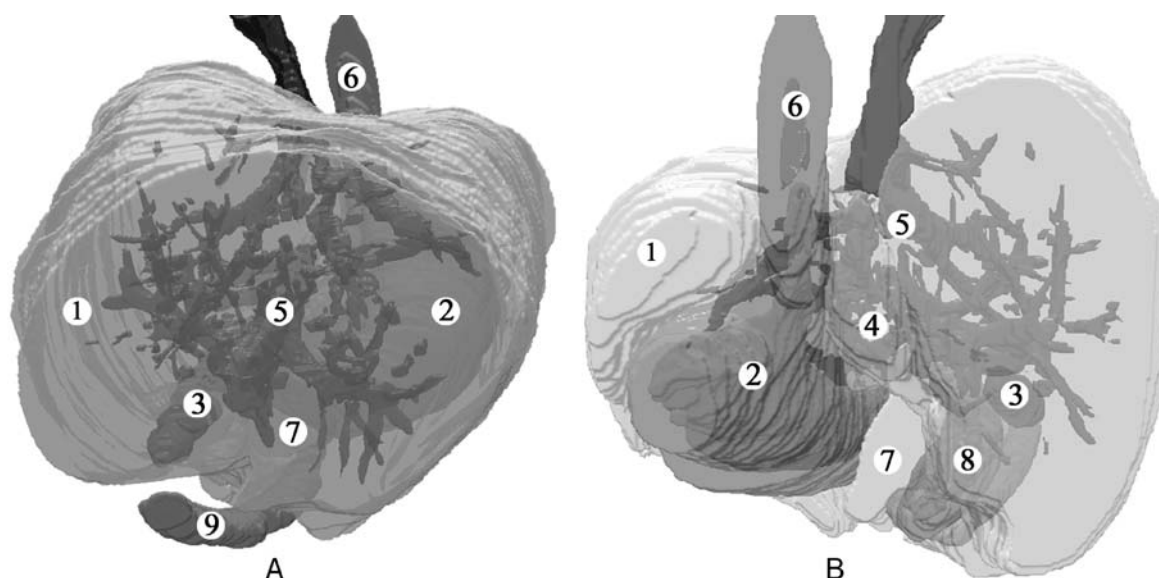


Figure 2. 3D-reconstruction of serial frontal histotopographic sections of abdominal organs of the fetus of 21.0 mm PCL. A – anterior view, B – posterior view: 1 – liver; 2 – stomach; 3 – gallbladder; 4 – cystic duct; 5 – hepatic veins; 6 – esophagus; 7 – pancreas; 8 – duodenum; 9 – jejunum

cranioventral section of the abdominal cavity, and its right segment exceeds in size its left one reaching out the posterior wall of the abdominal cavity. The anlage of the organ starts gradually separating itself more distinctively from the diaphragmatic part of the transversal septum and becomes a true abdominal structure located between the leaves of the ventral mesentery which were formed owing to the closure of the gut and the abdominal wall. The hepatic ligaments, and the lesser omentum, in particular, are formed from the remnants of the transversal and the ventral mesenteries. The ventral mesentery provides a special framework to the bile ducts and vessels which go to the porta hepatic. It degenerates in a caudal direction from them. The lesser omentum, and the hepatoduodenal ligament in particular, consist of the portal vein, the hepatic artery and the common bile duct which are called a portal triad. The formation of topographic variants and malformations of the hepatic vessels during this period is possible.

Throughout the sixth week of the development, vacuolization and recanalization of the lumen of the tubular structures begin. These processes start in the duodenal end of the intestinal tube. An abnormality of the normal processes of recanalization, namely incomplete recanalization, may induce the appearance of the membranous common bile duct with further atresia at this stage of development. For example, due to viral inflammatory infections, it may result in sclerotic changes of the bile ducts. At the beginning of the sixth week of development, the ventral and dorsal pancreatic anlages become contiguous between themselves within the bounds of the dorsal mesentery. In the end of the week their complete fusion takes place with the formation of the definitive pancreas.

We found that the hepatic duct develops from the superior cranial portion of the hepatic diverticulum. The caudal parts of the right and left hepatic ducts arise from the extrahepatic ducts and are well-defined by the end of the sixth week of development. A reconstruction of the extrahepatic bile ducts demonstrated their independent development from the intrahepatic ductal system. Both bile systems are separated since the time of their anlage, but at the end of the embryonal period a tendency towards their union was detected (Figure. 2).

It was found that at the end of the seventh week of the IUD when the fetuses PCL is 18.0-19.0 mm, CBD is located in the thickness of the mesenchyma of the ventral mesogastrium behind the superior portion of the duodenum. It is connected with the duct of the ventral anlage of the pancreas on the concave surface of the descending portion of the intestine. The layer of the mesenchymal cells adjacent to the CBD walls becomes separated from the neighboring cells of the surrounding mesenchyma in a caudal direction assuming a clear-cut circular orientation.

Isolated lumens of the blood vessels of the capillary type are detected in the mentioned above mesenchymal layer, primarily on the left and caudally from CBD. This means the beginning of formation of its intraorgan blood channels during this period. In the eighth week of IUD (fetuses 23.0-29.0 mm in PCL), one can differentiate 3 portions in the CBD: the retroduodenal section located behind the superior part of the duodenum, the pancreatic segment between the pancreatic head and the medial wall of the descending portion of the duodenum, and the intramural one – in the thickness of the medial wall of the organ. The duodenal branch of the gastroduodenal artery is located on the left and in front at a distance of 150 μ m from

the retroduodenal portion of the CBD. The pancreatic section of the CBD is accompanied by the superior posterior pancreaticoduodenal artery in a descending direction (Figure. 3). The inferior pancreaticoduodenal artery branch approaches the terminal portions of the CBD and the pancreas caudally.

Tiny duodenal branches of the gastroduodenal artery approach the left wall of the retroduodenal portion of the CBD, primarily, at the front and behind in a longitudinal direction in the ninth week of the IUD in prefetuses of 32.0-40.0 mm PCL. The branches in the pancreatic portion of CBD come from the superior pancreaticoduodenal artery from the right and normally to their axes.

Tiny blood vessels, passing mainly from the inferior pancreaticoduodenal artery, are located cranially and on the left between the circular and longitudinal fascicles of myoblasts, surrounding the intramural portion of the CBD and the hepaticopancreatic junction.

At the end of the tenth week of IUD (prefetuses PCL is 45.0-52.0 mm), lumens of the blood vessels are well identified. Their walls are covered with endothelium and surrounded by a circular layer of mesenchymal cells. Vessels are located around the hepaticopancreatic junction and between the muscular fascicles of Oddi's sphincter (Figure. 4). All this is the indication of the formation of the subepithelial and intramuscular vascular plexuses of the major duodenal papilla. The branches of the inferior pancreaticoduodenal artery are the sources of its visualization. The same concerns the intramural CBD portion.

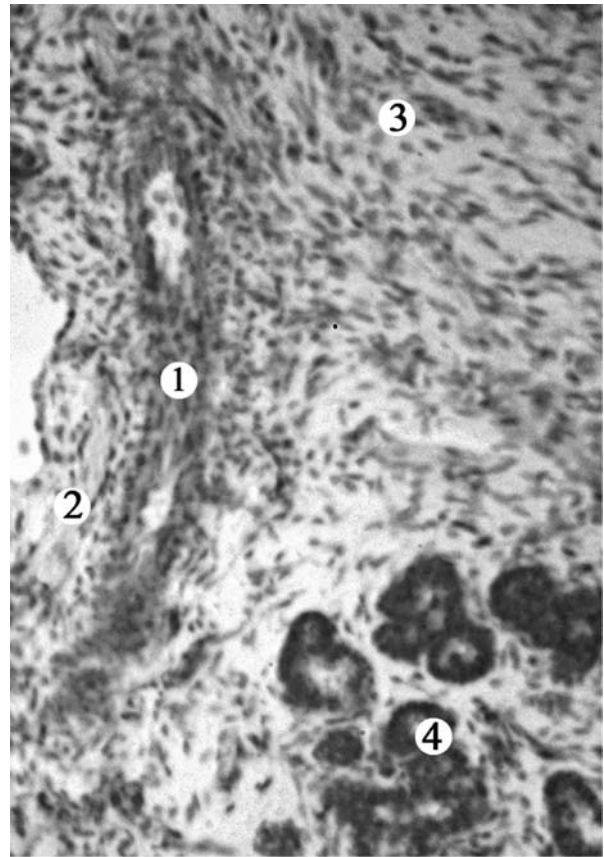


Figure 3. Frontal section of terminal portion of common bile duct of the prefetus of 27.0 mm PCL. Hematoxylin and eosin staining. Lens: x8; ocular: x7: 1 – common bile duct; 2 – superior posterior pancreaticoduodenal artery; 3 – mesenchymal cells; 4 – pancreas

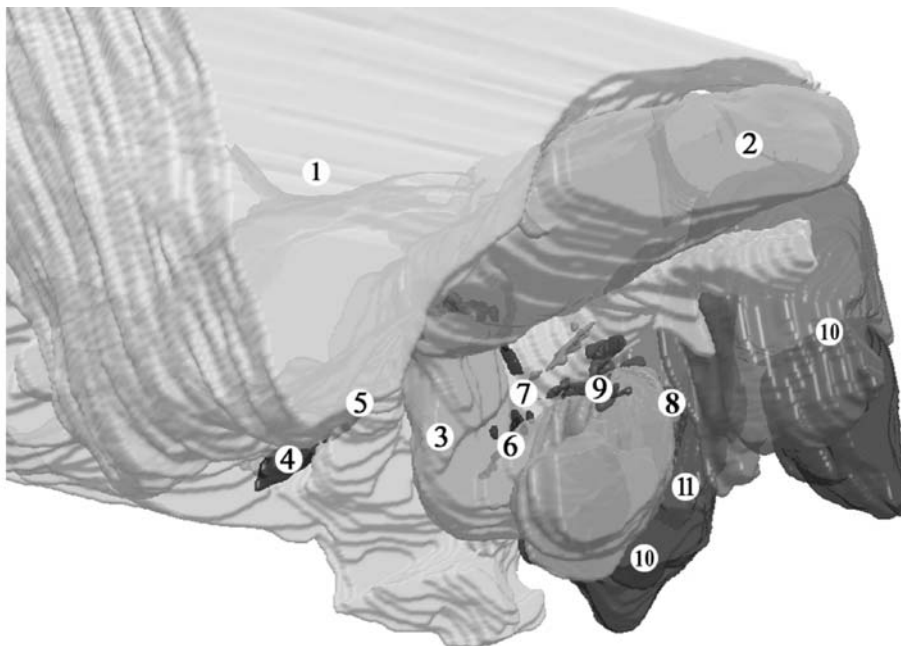


Figure 4. 3D-reconstruction of serial sagittal histotopographic sections of abdominal organs of the male prefetus of 48.0 mm PCL. Left antero-inferior view: 1 – liver; 2 – stomach; 3 – duodenum; 4 – common bile duct gallbladder; 5 – cystic duct; 6 – terminal portion of common bile duct; 7 – main pancreatic duct; 8 – superior mesenteric artery; 9 – inferior (common) pancreaticoduodenal artery; 10 – kidney and suprarenal gland; 11 – aorta

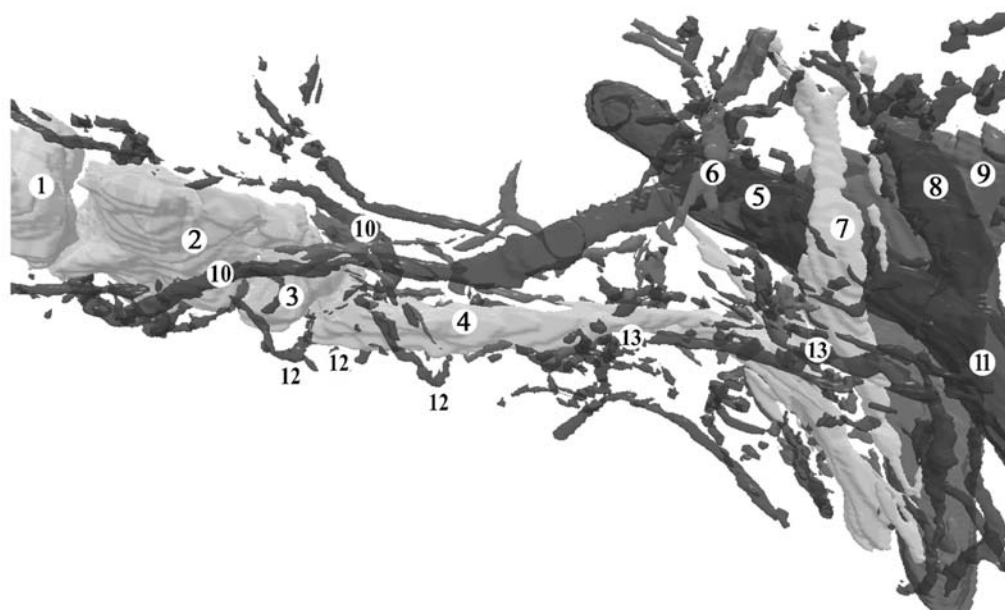


Figure 5. 3D-reconstruction of serial frontal histotopographic sections of the female fetus of 130.0 mm PCL. Anterior view: 1 – mucous membrane of the fundus of gallbladder, 2 – mucous membrane of the body of gallbladder, 3 – Hartmann's pouch and the neck of gallbladder, 4 – cystic duct, 5 – right branch of the proper hepatic artery, 6 – cystic artery and vein, 7 – common hepatic duct, 8 – left branch of the proper hepatic artery, 9 – portal vein, 10 – branch of the cystic artery, 11 – proper hepatic artery, 12 – spiral arteries of the cystic duct, 13 – anastomoses (arterial circle) of the cystic duct



Figure 6. 3D-reconstruction of serial frontal histotopographic sections of the male fetus of 290.0 mm PCL. Anterior view: 1 – mucous membrane of the gallbladder, 2 – common bile duct, 3 – tunica muscularis of the medial wall of descend part of the duodenum, 4 – proper hepatic artery, 5 – portal vein, 6 – superior mesenteric artery, 7 – pancreatic duct (Wirsung's duct) 8 – right gastroepiploic vein, 9 – gastroduodenal artery, 10 – anterior superior pancreaticoduodenal vein, 11 – posterior superior pancreaticoduodenal vein, 12 – venous plexus of the duodenum

The centers of blood vessels formation were found in the seventh week of development. At the end of twelfth week of IUD, all branches of celiac trunk and superior mesenteric artery are well traced. It was found that definitive structure of the arterial system of EBD starts from the beginning of fetus period of human ontogenesis. Three types of arterial anastomoses were found on the surface of EBD. Namely, we found the arterial network, a chain of longitudinal anastomoses, and the arterial circle. The peculiarities of spatial structure of the arterial anastomoses around the coiled part of the cystic duct proved the existence of the locking device (sphincter) between the neck of gallbladder and cystic duct. The sphincter plays an important role in functioning of vascular (arterial) component of blood supply system (Figure. 5).

The structure of the vascular system of 4-month-old fetuses starts demonstrating characteristics which are similar to its definitive topography. Topography and a structure of the venous plexus differ from those for arterial structures in areas of an extra- hepatic bile duct (EBD) sphincter (around the cystic duct and the terminal part of CBD). The arteries accompanying CDB develop anastomoses in the form of a chain of longitudinal vessels close to walls of a duct. These anastomotic branches resemble the arterial arches that connect the upper and lower pancreatic-duodenal artery. In turn, the same name veins form a plexus around EBD. That mesh follows arteries laterally. The anatomical architecture changes in the internal wall of EBD and in the wall of duodenum. The ter-

minal branches of arterial vessels are perpendicular to the direction of duodenum's axis and wrap it around starting from the medial edge and giving twigs in all layers of the body. Veins form a plexus in the whole wall of duodenum. A longitudinal to the medial wall of the descending part of the duodenum direction of venous plexuses can be seen on the 3D image reconstruction (Figure. 6).

Thus, during the fetal period of IUD, the changes in structural complexity and spatial transformation of the vasculature of EBD take place. Structural differences in arterial and venous plexuses of the sphincter segment of biliary system between the late fetal period and in newborns are clearly seen. Therefore, we may assume that they play an important role in the function of sphincters, which provide the dynamics of bile.

Conclusions

1. Intensive processes of a transformation of the hepatic diverticulum and form-building of the bile system throughout the 4th week of the development may be regarded as a critical period.
2. The extrahepatic bile ducts are traced in the form of a well-defined tubular structure, starting from the sixth month of the development, whereas the intrahepatic bile ducts are represented by a primitive tubular plate. The extrahepatic and intrahepatic bile ducts are isolated, but at the end of the embryonal period a tendency towards their fusion is traced.
3. The forming of the intraorgan blood vessels of the common bile duct is detected at the end of the seventh week of the intrauterine development in prefetuses measuring 18.0-19.0 mm PCL.
4. The source of the vascularization of the common bile duct in its retroduodenal and pancreatic portions is the branches of the gastroduodenal artery, whereas in the intramural portion – of the inferior pancreaticoduodenal artery.
5. The peculiarities of spatial structure of the arterial anastomoses around the coiled part of the cystic duct proved the existence of the locking device (sphincter) between the neck of gallbladder and cystic duct and play an important role in functioning of vascular (arterial) component of it.
6. The distinct character and angioarchitectonics differences in arterial and venous plexuses in the sphincter segments of the biliary system has been clearly observed at the end of the fetus period of development and in newborns, which may indicate an important functional role of the vessels in activity of sphincters, which provide biliodynamics.

Outlooks of the scientific inquiry

In order to detect possible variants of the blood supply of the common bile duct it is expedient to in-

vestigate the specific characteristics of the formation of its blood channel in human fetuses and newborns.

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ПРОСТОРОВО-ЧАСОВА ХАРАКТЕРИСТИКА СФІНКТЕРНОГО АПАРАТУ ПОЗАПЕЧІНКОВИХ ЖОВЧНИХ ПРОТОК У ПРЕНАТАЛЬНОМУ ПЕРІОДІ ОНТОГЕНЕЗУ ЛЮДИНИ

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Резюме. З метою вивчення особливостей розвитку і кровопостачання сфінктерного апарату позапечінкових жовчних проток упродовж пренатального періоду онтогенезу людини досліджено 104 серії гістологічних зрізів препаратів ембріонів, передплідів, плодів та новонароджених людини 4,5-378,0 мм тім'яно-куприкової довжина (ТКД) (5-40 тижнів розвитку) за допомогою комплексу морфологічних методів (антропометрія, морфометрія, ін'єкція судин, макро- і мікроскопія, 3D-реконструювання, статистичний аналіз). Встановлено, що позапечінкові жовчні протоки простежуються у формі чіткої трубчастої структури, починаючи з 6-го тижня внутрішньоутробного розвитку. Поза- та внутрішньо-печінкові жовчні протоки спочатку ізольовані, але наприкінці зародкового періоду простежується тенденція до їх сполучення. Формування внутрішньоорганних кровосносних судин спільної жовчної протоки виявлено наприкінці 7-го тижня внутрішньоутробного розвитку в передплідів 18,0-19,0 мм ТКД. Джерелами васкуляризації спільної жовчної протоки в її позадванадцятипалокишковому та підшлунковому відділах є гілки шлунково-дванадцятипалокишкової артерії, в інтрамуральному відділі – нижньої підшлунково-дванадцятипалокишкової артерії. Особливості просторової будови артеріального анастомозу навколо звивистої частини міхурової протоки свідчать про існування замикального пристрою (сфінктера) між шийкою жовчного міхура та міхуровою протокою і відіграють важливу роль у функціонуванні судинного (артеріального) його компонента. Особливості будови та відмінності ангіоархітектоніки артеріальних і венозних спле-

ть у сфинктерних сегментах жовчної системи чітко простежуються наприкінці плодового періоду розвитку та в новонароджених, і можуть вказувати на важливу функціональну роль судин в діяльності сфинктерів, які забезпечують білідинаміку.

Ключові слова: позапечінкові жовчні протоки, сфинктерний апарат, розвиток, плід, людина.

**ПРОСТРАНСТВЕННО-ВРЕМЕННАЯ
ХАРАКТЕРИСТИКА СФИНКТЕРНОГО АППАРАТА
ВНЕПЕЧЕНОЧНЫХ ЖЕЛЧНЫХ ПРОТОКОВ В
ПРЕНАТАЛЬНОМ ПЕРИОДЕ ОНТОГЕНЕЗА
ЧЕЛОВЕКА**

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Резюме. С целью изучения особенностей развития и кровоснабжения сфинктерного аппарата внепеченочных желчных протоков в течение пренатального периода онтогенеза человека исследовано 104 серии гистологических срезов препаратов эмбрионов, предплодов, плодов и новорожденных человека 4,5-378,0 мм теменно-копчиковой длины (ТКД) (5-40 недели развития) с помощью комплекса морфологических методов (антропометрия, морфометрия, инъекция сосудов, макро- и микроскопия, 3D-реконструирования, статистический анализ). Установлено, что внепеченочные желчные протоки прослеживаются в форме четкой трубчатой структуры, начиная с 6-й недели внутриутробного развития. Вне- и внутривнутрипеченочные желчные протоки сначала изолированы, но в конце зародышевого периода прослеживается тенденция к их соединению. Формирова-

ние внутриорганных кровеносных сосудов общего желчного протока выявлено в конце 7-й недели внутриутробного развития у предплодов 18,0-19,0 мм ТКД. Источниками васкуляризации общего желчного протока в ее позадидванадцатиперстном и поджелудочном отделах являются ветви желудочно-дванадцатиперстной артерии, в интрамуральном отделе – нижней поджелудочно-дванадцатиперстной артерии. Особенности пространственного строения артериального анастомоза вокруг извилистой части желчного протока свидетельствует о существовании запирающего устройства (сфинктера) между шейкой желчного пузыря и желчным протоком, и играют важную роль в функционировании сосудистого (артериального) его компонента. Особенности строения и отличия ангиоархитектоники артериальных и венозных сплетений в сфинктерных сегментах желчной системы четко прослеживаются в конце плодного периода развития и у новорожденных, и могут указывать на важную функциональную роль сосудов в деятельности сфинктеров, которые обеспечивают биодинамику.

Ключевые слова: внепеченочные желчные протоки, сфинктерный аппарат, развитие, плод, человек.

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