

N.H. Aliyeva, B.M. Huseynov
Azerbaijan Medical University, Baku, Azerbaijan

**MORPHOLOGY AND AGE-RELATED, REGIONAL FEATURES
OF MORPHOMETRIC INDICATORS OF LYMPHOID STRUCTURES
OF HUMAN INTRAHEPATIC DUCTS IN NORM**

e-mail: medun91@mail.ru

The lack of reliable information on the morphology of the lymphoid structures of the intrahepatic bile ducts complicates the diagnosis and treatment of various liver pathologies associated with these structures. Therefore, intrahepatic bile ducts taken from 48 corpses of different ages were examined using microscopic and morphometric methods. The studies showed that intraepithelial lymphocytes are found in the walls of the intrahepatic ducts. In the right and left hepatic ducts, diffuse lymphoid tissue is additionally determined. The density of intraepithelial lymphocytes and diffuse lymphoid tissue cells of the intrahepatic bile ducts reaches a maximum in early childhood, and these indicators decrease at later ages. In all age groups, the density of intraepithelial lymphocytes increases from the interlobular duct to the right and left hepatic ducts. Except for newborns, at other ages, the density of diffuse lymphoid tissue cells in the right hepatic duct is higher than in the left.

Key words: intrahepatic ducts, intraepithelial lymphocytes, diffuse lymphoid tissue, size-quantitative indicators, regional features.

Н.Г. Алиєва, Б.М. Гусейнов

**МОРФОЛОГІЯ, ВІКОВІ ТА РЕГІОНАЛЬНІ ОСОБЛИВОСТІ МОРФОМЕТРИЧНИХ
ПОКАЗНИКІВ ЛІМФОЇДНИХ СТРУКТУР ВНУТРІШНЬОПЕЧІНКОВИХ ПРОТОК
ЛЮДИНИ В НОРМІ**

Відсутність достовірних відомостей про морфологію лімфоїдних структур внутрішньопечінкових жовчних проток ускладнює діагностику та лікування різних патологій печінки, пов'язаних із цими структурами. Тому мікроскопічними та морфометричними методами нами досліджено внутрішньопечінкові жовчні протоки, взятих від 48 трупів різного віку. Дослідження показали, що в стінках внутрішньопечінкових проток виявляються внутрішньоепітеліальні лімфоцити. У правій та лівій печінкових протоках додатково визначається дифузна лімфоїдна тканина. Щільність внутрішньоепітеліальних лімфоцитів і клітин дифузної лімфоїдної тканини внутрішньопечінкових жовчних проток, в ранньому дитячому віці досягає максимуму, а в старшому віці ці показники знижуються. У всіх вікових групах щільність внутрішньоепітеліальних лімфоцитів збільшується від міждолькової протоки до правої та лівої печінкових проток. Крім новонароджених в інших вікових групах щільність клітин дифузної лімфоїдної тканини в правому печінковому протоці вище, ніж у лівому.

Ключові слова: внутрішньопечінкові протоки, внутрішньоепітеліальні лімфоцити, дифузна лімфоїдна тканина, регіональні особливості.

According to epidemiological studies, the prevalence of functional disorders of the biliary tract is from 10 to 20 % of all gastrointestinal pathology with a tendency to progression in young patients. In Ukraine, the prevalence of gallstone disease per 100 thousand adults increased by 32.7 % over the period 2001-2010. The highest incidence rate is observed among residents of North America and Sweden, while in Iceland, Thailand and Singapore this figure is significantly lower [1].

Cholangiocarcinoma is increasing globally, currently accounting for 15% of all primary liver cancers and 3% of gastrointestinal malignancies. The silent presentation of these tumours combined with their highly aggressive nature and refractoriness to chemotherapy contribute to their alarming mortality, representing 2% of all cancer-related deaths worldwide yearly [2].

The involvement of the biliary tract in the pathophysiology of liver diseases and the increased attention to bile ducts in the bioconstruction of liver tissue for regenerative therapy have fueled intense research into the fundamental mechanisms of biliary development [9].

Lymphoid structures are considered to be the source of a number of malignant and benign tumors (lymphoma, cholangiocarcinoma, papillary neoplasms of the bile ducts, etc.) [14].

Intraductal papillary neoplasms of the bile ducts (IPNB) are a rare variant of biliary tumors characterized by papillary growth within the lumen of the bile ducts. Bile duct neoplasms typically evolve through a multistep process involving various precursor pathways, ranging from an initial lesion detectable only microscopically, i.e., biliary intraepithelial neoplasia, to the distinctive grades of IPNB and to the final stage represented by invasive cholangiocarcinoma. Sophisticated and extensive studies are needed to establish the correct diagnosis and to determine adequate bile duct mapping that supports proper treatment. The recently introduced subclassifications of types 1 and 2 highlight the histopathological and clinical aspects of IPNB as well as their natural evolution with special emphasis on prognosis and survival [10].

The potential role of immune cells in the mechanisms of bile duct restoration is particularly emphasized [8].

Representatives of the scientific school of Honored Scientist, Professor V.B. Shadlinski conducted research devoted to the study of lymphoid structures located in the walls of internal organs [6, 12].

However, the lymphoid structures of the bile ducts remain a poorly studied area of morphology. The morphology of the lymphoid structures of the intrahepatic bile ducts is particularly poorly studied. The proportion of studies performed on autopsy material is significantly lower.

The purpose of the study was to assess the microscopic anatomy and age-related, regional features of the size-quantitative indicators of the lymphoid structures of human intrahepatic bile ducts in norm.

Materials and methods. The object of the study was the lymphoid structures of the intrahepatic bile ducts taken from 48 corpses of different ages (newborns; early childhood; adolescence, mature age; elderly age; old age). The study material included corpses of people who died because of wounds, suffocation, and various types of poisoning. The selection did not include corpses in which pathological examination revealed diseases of the immune system and liver. The material was collected in the morgues of the Association of Forensic Medicine and Pathological Anatomy of the Ministry of Health of the Republic of Azerbaijan and the Department of Human Anatomy and Medical Terminology of Azerbaijan Medical University. The material is divided into groups according to age.

For histological examination, pieces were taken from the intrahepatic bile ducts. From these pieces, sections of 5–7 μm thickness were made.

To study the lymphoid structures of the intrahepatic bile ducts, microscopic preparations were stained with hematoxylin-eosin, methylene blue, and Van Gieson.

Before staining with hematoxylin-eosin, paraffin sections were placed in a 96 % alcohol 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 [5].

For staining with methylene blue, the preparations were fixed in 96% ethyl alcohol. 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 [4].

Using these methods, the morphological structure of the lymphoid structures was studied, and morphometric indices were determined (the density of intraepithelial lymphocytes and lymphoid cells located in diffuse lymphoid tissue).

The preparations were examined under a light-optical microscope “MicroOptix (Germany)”, equipped with a video system “Topica TP1002DS”.

The quantitative and qualitative indicators obtained during the study were analyzed using modern biostatistics methods. Statistical analysis was performed using the dispersion and dispersion methods in MS Excel 2019 and IBM Statistics SPSS-26 programs.

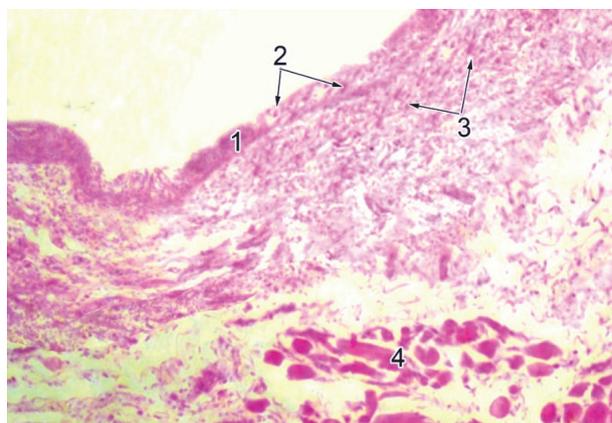


Fig. 1. Lymphoid structures located in the wall of the right hepatic duct. Hematoxylin and eosin staining. Magnification X200. 1. Epithelial layer; 2. Intraepithelial lymphocytes; 3. Cells of diffuse lymphoid tissue; 4. Muscular membrane.

When analyzing the indicators to describe the variation series, the averages were calculated (M – average, $\pm m$ – standard error, confidence interval – 95 % lower limit, 95 % upper limit) and average structural (Me – average, $Q1$ - $Q3$ – quartiles, min – minimum, max – maximum values) indicators. For the initial comparison of ranks, variance analysis was used. The difference between the indicators grouped by factors was estimated using Fisher's F-test. The results obtained were tested using the nonparametric Kruskal-Wallis H-test. If the statistical significance of the difference is $p < 0.050$, the null hypothesis is rejected. In cases where hypothesis “0” was rejected, the indicators of the groups were compared pairwise using the Student-Bonferroni test and it was determined between which groups a difference was found [7].

The final results obtained from the measurements were recorded, and the results of the obtained measurement indicators were collected in specially prepared statistical maps.

Results of the study and their discussion. In all the studied preparations of intrahepatic bile ducts, we conducted anatomical and morphometric studies of the lymphoid structures.

Studies have shown that intraepithelial lymphocytes are normally found in the walls of the interlobular, segmental, right and left hepatic ducts; in addition to these structures, diffuse lymphoid tissue is determined in the right and left hepatic ducts (Fig. 1).

Using the morphometric method, age-related features of the density of intraepithelial lymphocytes in the intrahepatic bile ducts, that is, the number of lymphocytes per 100 epithelial cells were studied (Table 1).

Table 1

Density of intraepithelial lymphocytes in the intrahepatic bile ducts in people of different ages (number of lymphocytes per 100 epithelial cells), Me (M) [Q1 – Q3]

Age (years)	n	Intrahepatic bile ducts			P _H
		Interlobular duct	Segmental duct	Right and left hepatic duct	
Newborns (1–10 days)	8	24.5 (24.5) [23.0–26.5]	20.5 (20.9) [18.5–23.5]	16.5 (16.9) [15.5–18.5]	0.001
Early childhood (1–3 years)	6	30.5 (32.3) [29.0–37.0]	31.0 (30.5) [29.0–32.0]	26.5 (26.3) [24.0–29.0]	
P ₀		0.014*	0.005*	0.002*	0.136
Childhood (4–7 years)	4	29.0 (30.0) [27.0–33.0]	27.0 (27.5) [23.0–32.0]	22.5 (22.3) [19.0–25.5]	
P		0.520	0.392	0.201	
P ₀		0.026*	0.050	0.059	
P _m		0.520	0.392	0.201	0.154
Adolescence (16–20 years)	4	26.0 (26.8) [24.5–29.0]	24.0 (23.8) [22.0–25.5]	18.5 (18.8) [16.5–21.0]	
P		1.000	0.885	0.770	
P ₀		0.303	0.147	0.262	
P _m		0.163	0.042*	0.019*	0.024
Mature (22–55 years)	6	24.5 (25.2) [20.0–30.0]	20.5 (20.3) [19.0–22.0]	16.5 (16.2) [12.0–19.0]	
P		0.520	0.054	0.391	
P ₀		1.000	0.795	0.794	
P _m		0.127	0.005*	0.006*	0.020
Elderly (56–74 years)	10	19.5 (18.5) [18.0–20.0]	15.5 (16.3) [13.0–19.0]	13.0 (12.4) [11.0–14.0]	
P		0.127	0.139	0.038*	
P ₀		0.002*	0.023*	0.002*	
P _m		0.001*	0.002*	0.001*	0.005
Old (75–90 years)	10	13.5 (14.4) [12.0–15.0]	12.5 (12.6) [10.0–14.0]	9.0 (9.0) [7.0–10.0]	
P		0.027*	0.040*	0.003*	
P ₀		0.001*	0.001*	0.000*	
P _m		0.001*	0.001*	0.001*	0.001

Note: Me – median, M – mean, Q1 – first quartile; Q3 – third quartile. Reliability of the difference between the group indicators: P – with the indicators of the previous age group (according to the Mann-Whitney U-test); P₀ – with the indicators of newborns (according to the Mann-Whitney U-test); P_m – with maximum values (according to the Mann-Whitney U-test); P_H – between the bile ducts (according to the H- Kruskal–Wallis test); * – Hypothesis “0” is rejected.

The results show that the density of intraepithelial lymphocytes in the interlobular duct in early childhood increases by 1.3 times (P₀=0.014) compared to newborns, reaching a maximum (32.3±2.8). The value of this indicator compared to this age in childhood is 1.1 times (P_m=0.520), in adolescence - 1.2 times (P_m = 0.163), in mature age – 1.3 times (P_m=0.127), in elderly age – 1.7 times (P_m=0.001), in old age – 2.2 times (P_m=0.001) decreases.

The density of intraepithelial lymphocytes in the segmental duct increases by 1.5 times (P₀=0.005) compared to newborns in early childhood and reaches a maximum (30.5±1.9). With age, the density of intraepithelial lymphocytes decreases. Thus, compared to early childhood, this indicator decreases in childhood by 1.1 times (P_m=0.392), in adolescence – by 1.3 times (P_m=0.042), in mature age – by 1.5 times (P_m=0.005), in elderly age – by 1.9 times (P_m=0.002), in old age – by 2.4 times (P_m=0.001).

The density of intraepithelial lymphocytes in the right and left hepatic ducts increases by 1.6 times (P₀=0.002) compared to newborns, reaching a maximum (26.3±1.5) in early childhood. Then, compared to

this indicator in early childhood, it decreases by 1.2 times ($P_m=0.201$), in childhood by 1.3 times ($P_m=0.054$), by 1.6 times ($P_m=0.006$) in adolescence, by 1.7 times ($P_m=0.004$) in mature age, by 2.1 times ($P_m=0.001$) in elderly age, by 3.0 times ($P_m=0.001$) in old age.

We analyzed regional features of the density of intraepithelial lymphocytes in the intrahepatic bile ducts.

According to the obtained data, the density of intraepithelial lymphocytes in the interlobular duct of newborns is 1.2 times ($P_H=0.001$) higher than the value in the segmental duct, and 1.5 times ($P_H=0.001$) higher than the value in the right and left hepatic ducts. In the interlobular duct of early childhood, the density of intraepithelial lymphocytes is 1.2 times ($P_H=0.100$) higher than in the segmental duct, and 1.3 times ($P_H=0.100$) higher than in the right and left hepatic ducts. In early childhood, the density of intraepithelial lymphocytes in the interlobular duct is 1.1 times ($P_H=0.136$) higher than in the segmental duct, and 1.2 times ($P_H=0.136$) higher than in the right and left hepatic ducts. In childhood, the density of intraepithelial lymphocytes in the interlobular duct is 1.1 times ($P_H=0.154$) higher than that in the segmental duct, and 1.3 times ($P_H=0.154$) higher in the right and left hepatic ducts. In adolescence, the density of intraepithelial lymphocytes in the interlobular duct is 1.1 times higher ($P_H=0.024$) than that in the segmental duct, and 1.4 times ($P_H=0.024$) higher than that in the right and left hepatic ducts. In mature age, the density of intraepithelial lymphocytes in the interlobular duct is 1.1 times ($P_H=0.020$) higher than that in the segmental duct, and 1.5 times ($P_H=0.020$) higher than that in the right and left hepatic ducts. In elderly people, the density of intraepithelial lymphocytes in the interlobular duct is 1.1 times ($P_H=0.005$) higher than in the segmental duct, and 1.3 times ($P_H=0.005$) higher than in the right and left hepatic ducts. In old age, the density of intraepithelial lymphocytes in the interlobular duct is 1.1 times ($P_H=0.001$) higher than in the segmental duct, and 1.4 times ($P_H=0.001$) higher than in the right and left hepatic ducts.

We studied the characteristics of the density of lymphoid cell (the number of cells per 1 mm² of organ wall) in diffuse lymphoid tissue located in the walls of the right and left hepatic ducts at different ages (Fig. 2).

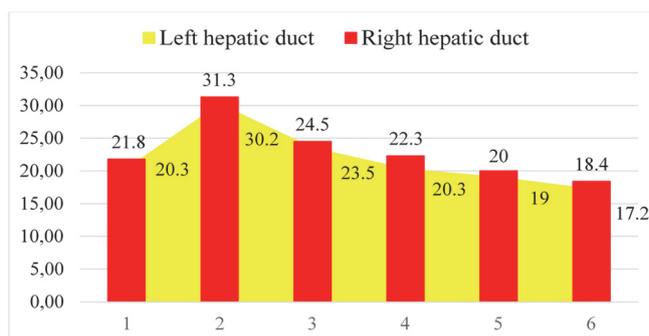


Fig.2. Density of lymphoid cells located in the diffuse lymphoid tissue of the right and left hepatic ducts in people of different ages. Note: 1. Newborns; 2. Early childhood; 3. Adolescence; 4. Mature age; 5. Elderly age; 6. Old age.

It was found that the cell density in the diffuse lymphoid tissue of the right hepatic duct in early childhood compared to newborns increases by 1.5 times ($P_0=0.002$) and decreases in later age periods. Thus, compared to early childhood, its value decreases by 1.1 times ($P_m=0.198$) in childhood, by 1.2 times ($P_m=0.088$) in adolescence, by 1.4 times ($P_m=0.013$) in mature age, by 1.6 times ($P=0.002$) in elderly age and by 1.7 times ($P_m=0.001$) in old age.

The cell density in the diffuse lymphoid tissue of the left hepatic duct in early childhood compared to newborns increases by 1.4 times ($P_0=0.109$), reaching a maximum (30.2 ± 1.8), and decreases at later ages. Thus, compared to early childhood, its value decreases by 1.1 times ($P_m=0.522$) in childhood, by 1.3 times ($P_m=0.088$) in adolescence, by 1.5 times ($P_m=0.016$) in mature age, by 1.7 times ($P_m=0.005$) in old age and by 1.8 times ($P_m=0.003$) in the elderly age.

We compared the density of diffuse lymphoid tissue cells in the right and left hepatic ducts (Diagram). According to the data obtained, the density of diffuse lymphoid tissue cells in the right hepatic duct is 1.0 times ($P_H>0.001$) higher than the same indicator in the left hepatic duct in early childhood, childhood, and adolescence, and 1.1 times ($P_H>0.001$) higher in mature, elderly and old ages. In newborns, on the contrary, the density of lymphoid tissue cells in the left hepatic duct is 1.1 times ($P_H=0.290$) higher than the indicator in the right hepatic duct.

The location of lymphoid structures in the mucous membrane of the organ is explained by their protective function (barrier, screen), associated with the creation of local immune protection, which, it should be emphasized, is very important and significant [3].

The results of our study indicate that the greatest number of lymphoid nodules in the walls of the hepatic ducts are observed in early childhood. In subsequent age periods, a gradual age-related involution of the lymphoid structures occurs. The data we obtained correspond to previously identified general patterns of age-related transformations of the organs of the immune system [13].

Analysis of digital data made it possible to identify the presence of regional features of the size and quantitative indicators of lymphoid structures in the walls of intrahepatic ducts, observed throughout postnatal ontogenesis.

It should be noted that the presence of regional features of the size and quantitative indicators of the lymphoid structures of the mucous membranes is also typical for other internal organs: urinary bladder [6], vestibule of the vagina [12], etc.

Conclusions

1. Intraepithelial lymphocytes are normally found in the walls of the interlobular, segmental, right and left hepatic ducts; in addition to these structures, diffuse lymphoid tissue is determined in the right and left hepatic ducts.

2. The density of lymphocytes located in the epithelial layer of the mucous membrane of the walls of the intrahepatic bile ducts reaches a maximum in early childhood (32.3 ± 2.8), and in later years the value of these indicators decreases by 1.1–1.7 times ($P_H > 0.001$). In all age groups, this indicator increases from the interlobular duct to the right and left hepatic ducts.

3. The density of diffuse lymphoid tissue cells located in the mucous membrane of the walls of both the right and left hepatic ducts is highest in early childhood (30.2 ± 1.8), while at a later age the value of this indicator decreases by 1.1–1.7 times ($P_H > 0.001$). In the right hepatic duct this indicator is higher than in the left hepatic duct in early childhood, adolescence, mature, elderly and old ages, while in newborns, on the contrary, the density of diffuse lymphoid tissue cells is higher in the left hepatic duct.

References

1. Hoyda SM. Tendentsiyi poshyrenosti zhovchnokamyanoi khvoroby sered naselennya Ukrayiny. Ukrayinskyy medychnyy chasopys. 2011; 4 (84): 112–113.
2. Banales JM, Marin JG, Lamarca A, Rodrigues PM, Khan SA, Lewis R Roberts LR et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol*. 2020; Sep;17(9):557-588. doi: 10.1038/s41575-020-0310-z.
3. Bar-Ephraim Y, Mebius R. Innate lymphoid cells in secondary lymphoid organs. *Immunol Rev*. 2016;271(1):185–199.
4. Halabi CM, Robert P. Van Gieson's Stain. [Internet]. Switzerland: Springer; 2018. [updated 2018]. Available from: <https://www.sciencedirect.com/topics/medicine-and-dentistry/van-giesons-stain#chapters-articles>.
5. Haldar A. Notes on Histological Techniques. 1th ed. Ahmedabad, India: Sara book publication. 2019. Chapter 1. Types of Eosin and steps of Eosin preparation; p. 10–23
6. Huseynova GA. The micro-anatomical features of the lymphoid structures of the urinary bladder. *Journal of Morphology and Anatomy*. 125:2021; 1-4.
7. Kafarov IA. Biostatistics . Baku : Muallim; 2021:238.
8. Lan T, Qian S, Tang C, Gao J. Role of Immune Cells in Biliary Repair *Front Immunol*. 2022 Mar 30;13:866040. doi: 10.3389/fimmu.2022.866040. eCollection 2022.
9. Lemaigre FP. Development of the Intrahepatic and Extrahepatic Biliary Tract: A Framework for Understanding Congenital Diseases. *Annu Rev Pathol*. 2020, Jan 24;15:1-22. doi: 10.1146/annurev-pathmechdis-012418-013013.
10. Mocchegiani F, Vincenzi P, Conte G, Nicolini D, Rossi R, Cacciaguerra AB et al. Intraductal papillary neoplasm of the bile duct: The new frontier of biliary pathology. *World Journal of Gastroenterology, World J Gastroenterol* 2023. Oct 14;29(38):5361–5373. doi: 10.3748/wjg.v29.i38.5361.
11. Prakash M. Methylene Blue staining: [Internet]. Stanford, Jul 25, 2016. URL: <https://www.protocols.io/view/Methylene-Blue-staining-q26g7y69gwz1/v1>.
12. Shadlinskaya SV, Aliyeva NH, Huseynov BM. Macromicroscopic anatomy of the lymphoid formations of the vaginal vestibule and intrahepatic bile ducts and human. 2022. 5(3):8–11. doi: 10.31080/ASGIS.2022.05.0475.
13. Thompson HL, Smithey MJ, Surh CD, Nikolich-Zugich J. Functional and Homeostatic Impact of Age-Related Changes in Lymph Node Stroma. *Front Immunol*. 2017 Jun 14;8:706. doi: 10.3389/fimmu.2017.00706.
14. Vithayathil M, Khan SA Current epidemiology of cholangiocarcinoma in Western countries. *J. Hepatol*. 2022. Dec;77(6):1690–1698. doi: 10.1016/j.jhep.2022.07.022.

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