Original paper

Morphological features of non-parenchymal liver cells of full-term stillbirths from HIV-mono-infected mothers

Sergey Sherstiuk, Stanislav Panov, Igor Belozorov, Tetiana Liadova

Human Anatomy Department, Medical School, V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

Fifty-one liver tissue biopsies of full-term stillbirths from HIV-mono-infected mothers were investigated. All morphometric indices of non-parenchymal liver cells were calculated using the Avtandilov microscopic morphometric grid, which consisted of 100 equidistant points. It was inserted into the microscope ocular tube with a total × 200 microscope magnification. The number of points that were found in the corresponding types of non-parenchymal liver cells determined by immunohistochemistry was calculated. In each case, 10 random microscopic areas were selected and then all data were obtained, calculated and presented as percentages. Morphometric indices of non-parenchymal liver cells: Kupffer cells – 28.3±2.9% [control – 21.2 ±2.5], hepatic stellate cells – 14.1 ±1.4% [control – 9.8 ±1.2], LAL (liver-associated lymphocytes): CD4 – 29.2 ±2.2% [control – 43.3 ±4.9], CD8 – 25.3 ±1.9% [control – 23.1 ±1.7], dendritic cells – 3.9 ±0.3% [control – 2.6 ±0.3]. It was established that at the time of the birth of children from HIV-mono-infected mothers the Kupffer cells showed signs of their proliferation and liver-associated CD4 lymphocytes showed signs of their reduction; hepatic stellate cells showed signs of their proliferation and hyperactivation; dendritic liver cells showed signs of their proliferation.

Key words: HIV, liver, Kupffer cells, hepatic stellate cells, liver-associated lymphocytes.

Introduction

HIV infection is a very serious problem nowadays and it requires an enormous number of resources to resolve it in all countries around the world. Any scientific research and any scientific data about HIV infection attracts a lot of attention from the medical scientific community, the press, and governmental structures. HIV affects all levels of the population as well as people of any gender and any age, including fetuses and newborns from HIV-infected mothers.

Nowadays, according to the Official Bulletin of the Joint United Nations Programme on HIV and AIDS (UNAIDS) in 2018, there are 36.9 million (31.1-43.9 million) people living with HIV in the world; 35.1 million (29.6-41.7 million) are adults and 1.8 million (1.3-2.4 million) are children under the age of 15. The number of HIV-positive women over the age of 15 is 18.2 million (15.6-21.4 million). In 2018, there were 240 000 (230-260 000) HIV-positive people living in Ukraine, including 5000 (4500-5900) children under the age of 15. The number of HIV-positive women over the age of 15 is 110 thousand (100-120 thousand) [1, 2].

Considering the fact that adults are the predominant group among HIV-infected patients, there is a clear lack of data about newborns and children from HIV-infected mothers in the scientific literature.

Considering the specificity of the interaction between HIV and the body, researchers study the lympho-reticular system and opportunistic infections in HIV-infected children mostly [3]. The liver is a complex and multifaceted organ that performs many different tasks, which, as many researchers have shown in adult HIV-infected patients, is an important targeting structure for HIV [4, 5]. The scientific literature describes the development of hepatomegaly in fetuses and newborns from HIV-infected mothers [6], but with no detailed analysis of liver structures and how they are involved in the pathological process. Therefore, the study of the condition of non-parenchymal liver cells of stillbirths from HIV-infected mothers is an important problem that needs resolution.

The goal of this study is to investigate the morphological features of non-parenchymal liver cells of full-term stillbirths from HIV-mono-infected mothers with further provision of the data to the scientific community and practitioners to optimize the process of diagnosis of liver disease in children, and further, to establish whether the HIV infection in pregnancy has an impact on the fetal liver, creating a higher risk of chronic liver pathology in the post-natal period of life. The HIV-infected women have to take that fact into consideration while planning their future pregnancy. Moreover, it will give clinicians a reason to start the supportive therapy immediately after children's birth from HIV-mono-infected mothers with further significant improvement of the quality of life and prognosis.

Materials and methods

The study investigated 51 liver tissue biopsies of full-term stillbirths from HIV-mono-infected mothers (group N0) and 45 liver tissue biopsies of healthy, full-term newborns from healthy mothers with physiological pregnancy (group K). To determine the sample size for each group and estimate a continuous average outcome variable in a single population the following formula was used: $n = (Z\sigma/E)^2$, where Z is the value from the standard normal distribution reflecting the confidence level that will be used ($Z = 1.96$ for 95%), σ is the standard deviation of the variable outcome ($\sigma = 6$) and E is the desired margin of error ($E = 2$). According to this formula the minimum number of subjects required to ensure that the margin of error in the confidence interval for μ does not exceed E is 35.

The gestation period in all cases was 37-40 weeks. In all cases, children died as a result of premature detachment of the normally located placenta during delivery or severe traumatic brain injury due to an abnormally quick and intensive delivery process (physical trauma during childbirth). To determine the HIV status of children from HIV-mono-infected mothers, a molecular genetic method for the determination of viral RNA by PCR is used only 6 weeks after the birth. As all the children in our study group from HIV-mono-infected mothers were stillborn, their HIV status was not determined. Examination of the autopsy material was carried out in accordance with the requirements of the "Instructions for autopsies" (Order of the Ministry of Health of Ukraine No. 6/17.01.1995), in accordance with the requirements, norms and standard provisions on ethics of the Ministry of Health of Ukraine No. 690/23.09.2009, and in accordance with the "Instructions for the removal of biological objects from the dead, whose bodies are the subject of forensic and pathological research for scientific purposes" (2018). The material had been collected during 1998-2018 period at Odessa and Dnipro regional pathological anatomy bureaus.

To avoid any misinterpretations of the obtained results and reduce the influence of confounding factors, we carefully selected healthy and HIV-mono-infected mothers analyzing the various data from the official medical documents with a standard questionnaire and measurements in them (form No. 096/o: "The history of pregnancy and childbirth"; Order of the Ministry of Health of Ukraine No. 29/21.01.2016). According to the data in the official medical documents, we carefully selected women who had almost identical age, socioeconomic background, lifestyle, habits, and health condition within their own study groups. The average age of healthy mothers was 29.0 ±3.9 (25.0-34.0). They were women of normal and stable socioeconomic background. They planned their pregnancy and attended doctors for routine check-up during pregnancy. They did not have any bad habits or health problems. The average age of HIV-mono-infected mothers was 26.0 ±4.1 (22.0-30.0). They were women of low socioeconomic background, who did not plan their pregnancy, had never been examined before, and had never attended any doctors during pregnancy. All women became aware of their HIV-positive status either shortly before or immediately after delivery and refused to receive antiretroviral therapy. All of those women received officially confirmed HIV mono-infection status without any clinical manifestations of AIDS ($CD_4 > 200$ cells/ml and VL [viral load] < 400 copies/ml in all cases) and without any other co-infections or comorbid health problems. During pregnancy and delivery, all women were not drug addicted, did not consume a large amount of alcohol and had a permanent residence.

For morphological investigation the liver pieces were fixed in 10% neutral formalin solution, and then the material was subjected to a standard procedure with alcohols of gradually increasing concentration, Nikiforov liquid (96% alcohol and diethyl ether in ratio of 1 : 1), chloroform, and then embedded in

paraffin blocks. Then numerous serial slices 3-5 mm thick were prepared, using the Microm HM-340 microtome. For general microscopic investigation, the slices were stained with hematoxylin and eosin and then were carefully studied using a BX43 optical microscope (Olympus Corporation, Tokyo, Japan).

Immunohistochemical data of non-parenchymal liver cells were collected using the indirect immunoperoxidase assay. The positive signals (positive expression in the form of a brown color) were measured using a microscopic Avtandilov microscopic morphometric grid, which consisted of 100 equidistant points. The grid was inserted into the microscope ocular tube with a total ×200 microscope magnification. The number of points that were found in the corresponding types of non-parenchymal liver cells was calculated. In each case, 10 random microscopic areas were selected and then all data were obtained, calculated and presented as percentages. Also, this standardized quantitative method made it possible to demonstrate the fact and estimate the level of cell proliferation/reduction, comparing the received data of non-parenchymal liver cells with each other in all study groups [7].

To assess the level of expression of Kupffer cells, antibodies were used against human CD68 macrophages (1 : 100, DAKO Biotechnology Co., Ltd, Denmark). The blocks were heated for 60 minutes at 60°C, deparaffinized with xylene and washed in alcohols with gradually decreasing concentration. After extracting the antigen by heat (in a microwave oven with a citrate buffer for 20 minutes), trypsin (0.1% trypsin solution with phosphate buffer saline for 30 minutes at 37°C) and proteinase K (10 μg/ml proteinase K with Tris-HCl [pH 7.5] for 15 minutes), tissue slices were treated with 3% H_2O_2 solution with phosphate-buffered saline to block endogenous peroxidase and in 5% skim milk solution with phosphate-buffered saline to block nonspecific reactions. The slices were incubated with primary antibodies overnight at 4°C followed by secondary antibody treatment. Finally, the slices were stained using diaminobenzidine followed by hematoxylin contrast.

Anti-α-SMA antibodies (1 : 100, DAKO Biotechnology Co., Ltd, Denmark) were used to evaluate the level of hepatic stellate cell expression. The blocks were heated for 60 minutes at 60°C, then deparaffinized with xylene and washed in alcohols with gradually decreasing concentration. Exposure to antigenic sites was performed by incubation in a solution of citrate buffer with pH 6 in a microwave oven (3 cycles lasting 5 minutes each). A 0.3% solution of H_2O_2 with methanol was used to block endogenous peroxidase activity and then normal serum was used to block nonspecific antigens. The slices were incubated with primary antibodies diluted according to the manufacturer's recommendation (1 : 100) overnight at 4°C. Finally, the slices were stained using diaminobenzidine followed by hematoxylin contrast.

Appropriate monoclonal antibodies (CD4 clone OPD4 M834 and CD8 clone OPD8 M7103, DAKO Biotechnology Co., Ltd, Denmark) were used to assess the expression level of liver-associated T lymphocytes (LAL) CD4 and CD8. The blocks were heated for 60 minutes at 60°C, then deparaffinized with xylene and washed in alcohols with gradually decreasing concentration. Thereafter, 3% H_2O_2 solution was used to block endogenous peroxidase. Incubation with primary antibodies to CD4 and CD8 (dilution 1 : 1000 and 1 : 50 respectively) was performed overnight and then biotinized anti-mouse antibodies were added for 15 minutes at 37°C. Peroxidase-labeled streptavidin-biotin complex was then added for 15 minutes at 37ºC. Finally, the slices were stained using diaminobenzidine followed by hematoxylin contrast.

The monoclonal antibodies against CD1a (GM357104, clone 010, DAKO Biotechnology Co., Ltd, Denmark) were used to assess the expression level of dendritic cells. The blocks were heated for 60 minutes at 60°C, then deparaffinized with xylene and washed in alcohols with gradually decreasing concentration. Antigens were obtained using citrate buffer (pH 6.0) in a microwave oven for 5 minutes. Thereafter, 3% H_2O_2 solution was used to block endogenous peroxidase. Incubation with primary antibodies to CD1a (dilution 1 : 100) was performed overnight and then biotinized anti-mouse antibodies were added for 15 minutes at 37°C. Peroxidase-labeled streptavidin-biotin complex was then added for 15 minutes at 37°C. Finally, the slices were stained using diaminobenzidine followed by hematoxylin contrast.

The slides were studied using a BX43 optical microscope (Olympus Corporation, Tokyo, Japan). Statistical processing of the results was performed using the standard statistical software package STATISTICA 10.0 and MS Excel. The arithmetic mean (M) and standard error of the mean (m) were calculated from the obtained data. The Mann-Whitney U-test was used to assess the significance of differences between the groups.

Results

Microscopically, normal beam-radial structure, normal sinusoids and central veins, and few small foci of extra medullary hematopoiesis were registered in the liver of group K. Hepatocytes had a slightly granular eosinophilic cytoplasm and round basophilic nuclei.

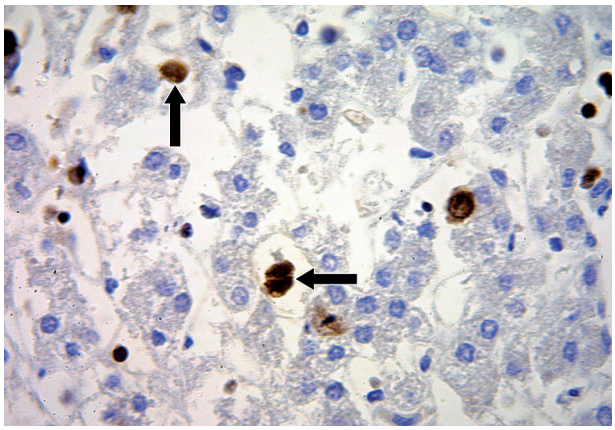

Fig. 1. Liver of a child (group K). Kupffer cells (CD68) in the sinusoids with round shapes and large round nuclei (arrows). Indirect immunoperoxidase assay, magnification 400×

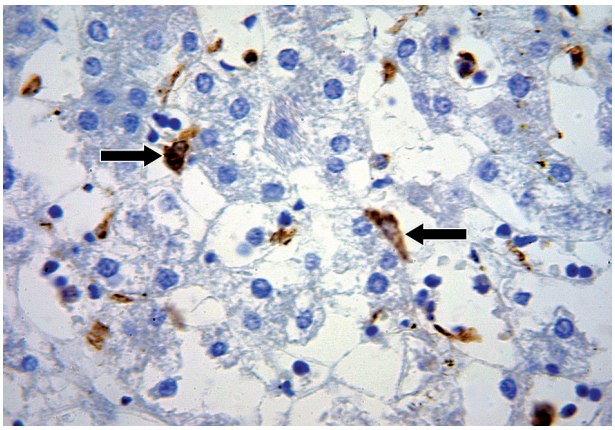

Fig. 2. Liver of a child (group K). Hepatic stellate cells (α-SMA) in the Disse spaces along sinusoids with elongated shapes and oval nuclei (arrows). Indirect immunoperoxidase assay, magnification 400×

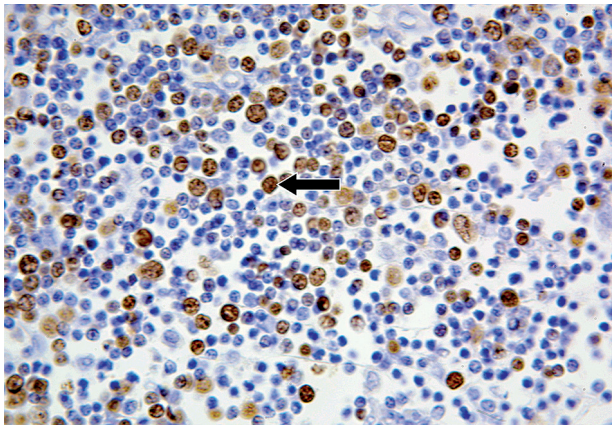

Fig. 3. Liver of a child (group K). Numerous liver-associated CD4 lymphocytes in the portal tract (arrow). Indirect immunoperoxidase assay, magnification 400×

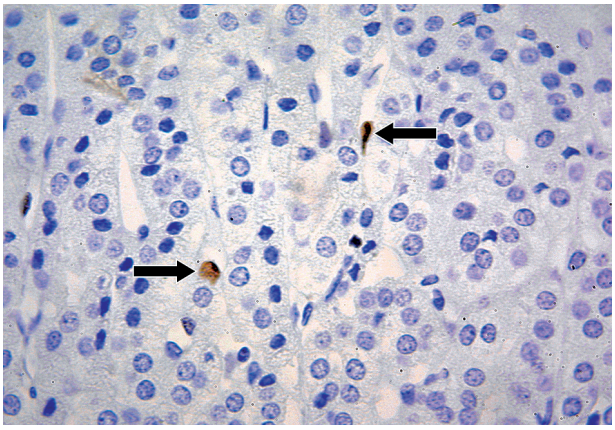

Fig. 4. Liver of a child (group K). Single dendritic cells (CD1a) in the sinusoids (arrows). Indirect immunoperoxidase assay, magnification 400×

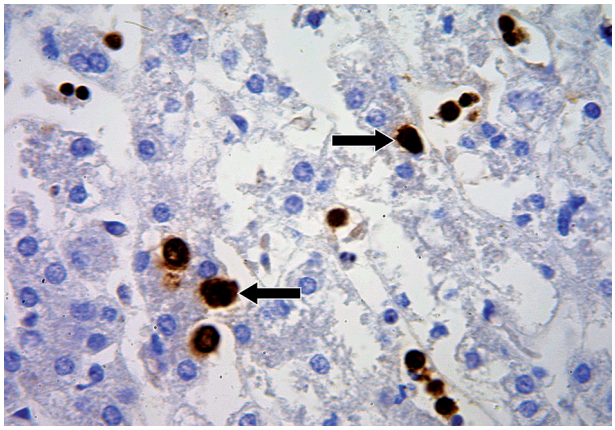

Fig. 5. Liver of a child (group N0). Increased number of large Kupffer cells (CD68) in the sinusoids (arrows). Indirect immunoperoxidase assay, magnification 400×

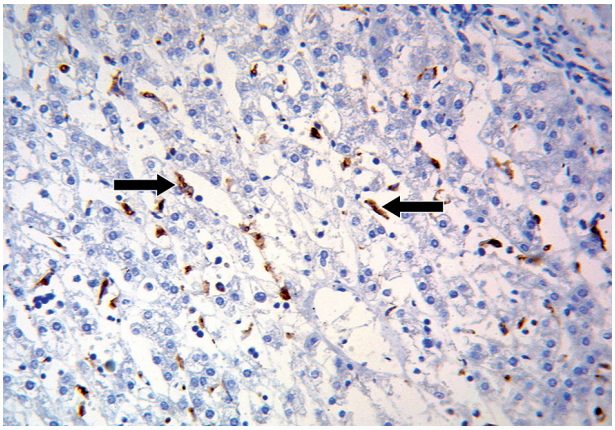

Fig. 6. Liver of a child (group N0). Multiple activated hepatic stellate cells (α-SMA) in the Disse spaces along sinusoids with elongated shapes and oval nuclei (arrows). Indirect immunoperoxidase assay, magnification 200×

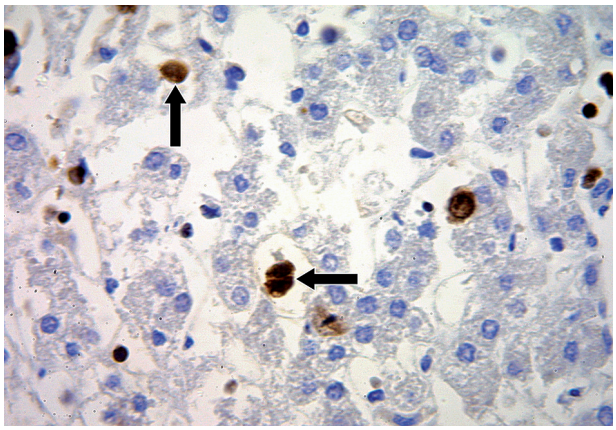

Fig. 7. Liver of a child (group N0). Liver-associated CD4 lymphocytes in the portal tract with large nuclei (arrow). Indirect immunoperoxidase assay, magnification 400×

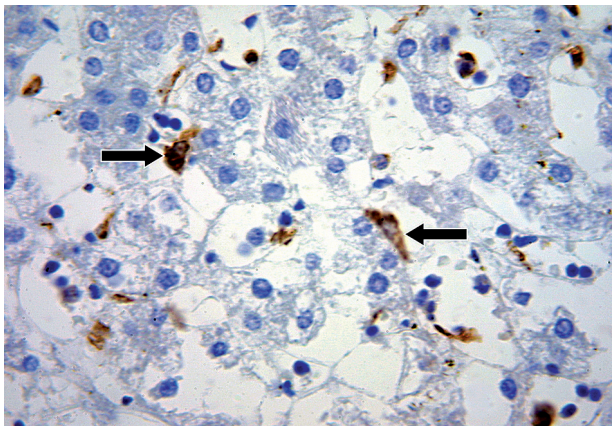

Fig. 8. Liver of a child (group N0). Single dendritic cells (CD1a) in the sinusoids with hyperchromic nuclei (arrows). Indirect immunoperoxidase assay, magnification 400×

Microscopically, fuzzy beam-radial structure, and significant blood congestion in the sinusoids and central veins were registered in the liver of group N0. Also, multiple foci of extramedullary hematopoiesis were detected throughout the liver parenchyma and small focal lymphoid infiltrates near sinusoids. Hepatocytes had foamy eosinophilic cytoplasm and hyperchromic nuclei. Also, multiple binucleated hepatocytes were revealed in all fields of view. The periportal hepatocytes contained numerous small droplets of fat vacuoles.

Along the sinusoids in the liver of group K, mainly in the periportal zones, a small amount of Kupffer cells (CD68) that had round shapes and round nuclei was detected (Fig. 1). Also, in the Disse spaces a few hepatic stellate cells (α-SMA) that had elongated shape were detected (Fig. 2). Numerous liver-associated CD4 and CD8 lymphocytes were detected in the portal tracts (Fig. 3) and single dendritic cells (CD1a) were detected along the sinusoids (Fig. 4).

Along the sinusoids in the liver of group N0 numerous small Kupffer cells (CD68) were found throughout the whole liver tissue that had round shapes and were situated either as single cells or as small groups around hepatocytes, which showed signs of cell death (Fig. 5). Also, pronounced proliferation of activated hepatic stellate cells (α-SMA) was registered throughout the whole liver tissue in the Disse spaces along the sinusoids. The hepatic stellate cells were large, elongated in length and had multiple processes, and often formed small focal clusters that were seen more distinctively when using microscope magnification 200× (Fig. 6). In the portal tracts a few liver-associated CD4 (Fig. 7) and CD8 lymphocytes and a few dendritic cells (CD1a) along the sinusoids (Fig. 8) were found.

Table I. Morphometric indices of non-parenchymal liver cells of children from HIV-infected mothers and children of the control group (M ±m)

Cells	Study group	
	K	N0
Kupffer cells (CD_{68}), %	21.2 ±2.5	28.3 ±2.9*
Hepatic stellate cells (α-SMA), %	9.8 ±1.2	14.1 ±1.4*
LAL:		
CD4, %	43.3 ±4.9	29.2 ±2.2*
CD8, %	23.1 ±2.7	25.3 ±1.9
Dendritic cells, %	2.6 ±0.3	3.1 ±0.2

$p < 0.05$ compared to similar indices of group K.

Morphometric indices of non-parenchymal liver cells of children from HIV-infected mothers and children of the control group are presented in Table I.

Discussion

The non-parenchymal liver cells, such as Kupffer cells, hepatic stellate cells, dendritic cells and liver-associated lymphocytes, are involved in immunological surveillance within sinusoids, control of the inflammatory response and regulation of fibrogenesis.

Numerous studies of the liver of HIV-infected adult patients showed that the number of Kupffer cells significantly decreases in the liver over time, which significantly reduces the ability of the liver to excrete microbial translocation products from portal blood flow [8]. Moreover, HIV-infected macrophages/monocytes secrete an increased amount of transforming growth factor-beta (TGF-β), which in turn activates the hepatic stellate cells with the subsequent development of liver fibrosis [9]. In this study, as can be seen in Table I, it was found that at the time of birth

of children from HIV-infected mothers (group N0) Kupfer cells in the liver increased by 7.1% compared to controls ($p < 0.05$), indicating the presence of liver damage on the one hand and good cellular immunity as a compensatory reaction on the other hand. It also may indicate that increased numbers of macrophages in the liver of children from HIV-mono-infected mothers might increase the risk of liver fibrosis by increasing the number of activated hepatic stellate cells, which in turn activate fibrogenesis.

Activation of hepatic stellate cells is a key point in the process of initiation of liver fibrogenesis. Hepatic stellate cells can be activated by cellular components released from damaged hepatocytes; increased accumulation of lipids; secretion of reactive oxygen species produced by macrophages; exposure to various cytokines produced by macrophages, lymphocytes and endothelial cells (TNF-α, PDGF, IL-1β, osteopontin, EGF, TGF-β) [10, 11, 12]. When the liver is damaged, the hepatic stellate cells become rapidly activated and trans-differentiated into proliferative, contractile and myofibroblast-like cells. Moreover, activated hepatic stellate cells are able to proliferate, migrate, initiate the process of fibrogenesis and, if the pathogenic stimuli persist, maintain themselves in a permanently activated state [13]. This study presented the morphometric analysis of α isotype of smooth muscle actin (α-SMA), which is expressed by activated hepatic stellate cells of children from HIV-infected mothers [14]. As can be seen from Table I, by the time of birth of children from HIV-infected mothers (group N0), the number of activated hepatic stellate cells increased by 4.3% compared to the control group ($p < 0.05$). The activated hepatic stellate cells produce a lot of pro-inflammatory and pro-fibrogenesis cytokines [15], which significantly increases the risk of liver fibrosis in the future. In addition, studies have shown that, like dendritic cells, HIV-infected stellate cells can serve as a reservoir and transmit the virus to CD4 lymphocytes. The stellate cells localize beneath the fenestrated endothelium of the sinusoidal capillaries of the liver and interact directly with T lymphocytes [16]. Also, HIV-infected stellate cells can infect adjacent hepatocytes, thereby initiating their damage and apoptosis [17].

The main liver-associated lymphocytes (LAL) in the stroma of the liver are CD4 and CD8 lymphocytes, which perform immunological surveillance in the liver [18]. As in the blood plasma of HIV-infected people, the number of CD4 lymphocytes in the liver becomes progressively low. In the children of group N0, the number of CD4 lymphocytes in the liver decreased in comparison to the control by 14.1%. However, the number of CD8 lymphocytes in the liver increased, albeit non-significantly, in comparison to the control ($p > 0.05$). Thus, progressive HIV-induced death of CD4 lymphocytes occurs not only in the blood plasma but also in the lymphoid organs, which can also be attributed to the liver, thereby leading to "immune paralysis" of the liver, which creates the conditions for its further damage. HIV-infected CD4 lymphocytes may also play a role in the development of liver fibrosis. Patients with HIV/hepatitis C co-infection have been shown to have hepatitis C- and HIV-infected CD4 lymphocytes in the liver. At the same time, HIV-infected CD4 lymphocytes were more functionally active than hepatitis C-infected CD4 lymphocytes, which suggested that they could be more actively involved in the activation of hepatic stellate cells and the promotion of liver fibrosis [19].

Dendritic cells of the liver can serve as reservoirs for HIV infection, because they express the targeting receptors for CCR5 and CXCR4 on their surfaces, and can infect adjacent CD4 lymphocytes [20, 21]. In this study the number of dendritic cells increased non-significantly at the time of birth of children from HIV-infected mothers (group N0; $p>0.05$). Considering the fact that the dendritic cells are antigen-presenting cells, their increasing number (proliferation) in the liver of children from HIV-infected mothers may indirectly indicate the presence of effective HIV infection.

Conclusions

1. At the moment of birth of children from HIV-infected mothers the Kupffer cells showed signs of their proliferation (Kupffer cells: [K – 21.2 ±2.5%; N0 – 28.3 ±2.9] and the liver-associated CD4 lymphocytes showed signs of their reduction: [K – 43.3 ±4.9%; N0 – 29.2 ±2.2]).
2. At the moment of birth of children from HIV-infected mothers, hepatic stellate cells showed signs of their proliferation and hyperactivation ([K – 9.8 ±1.2%; N0 – 14.1 ±1.4]).
3. At the moment of birth of children from HIV-infected mothers, dendritic liver cells showed signs of their proliferation ([K – 2.6 ±0.3%; N0 – 3.1 ±0.2]).

The prospect of further research. Since non-parenchymal liver cells, such as hepatic stellate cells, Kupffer cells, and dendritic cells, are directly related to the initiation of liver fibrosis, further investigation of the indices in the liver stromal component of children from HIV-infected mothers will allow us to understand more deeply the pathogenesis of liver fibrosis that is induced by HIV infection.

The authors declare no conflict of interest.

This work is a fragment of a comprehensive research program of the Human Anatomy Department of the V.N. Karazin Kharkiv National University: "The impact of maternal pathology on the fetal and newborn organism development". State Registration Number: 0117U004838. This work was funded according to the plan of that program. The authors did not receive any financial support from the manufacturers of medical instruments and drugs.

References

1. UNAIDS. Joint United Nations. Programme on HIV/AIDS. Geneva 2018; 370.
2. Stover J, Bollinger L, Izazola JA, et al. What is required to end the AIDS epidemic as a public health threat by 2030? The cost and impact of the fast-track approach. PLoS One 2016; 11: e0154893.
3. Ravichandra KR. Opportunistic infections in HIV infected children and its correlation with CD4 count. Int J Contemp Pediatrics 2017; 4: 1743-1747.
4. Penton PK, Blackard JT. Analysis of HIV quasispecies suggests compartmentalization in the liver. AIDS Res Hum Retroviruse 2014; 30: 394-402.
5. Joshi D, O'Grady J, Dieterich D, et al. Increasing burden of liver disease in patients with HIV infection. Lancet 2011; 377: 1198-1209.
6. Jindal AK, Ahluwalia J, Sharma A, et al. HIV-infected children with hepatomegaly and ascites: is there something more than an infection? AIDS 2017; 31: 870-872.
7. Avtandilov GG. Osnovy kolichestvennoy patologicheskoy anatomii {The basics of quantitative pathological Anatomy} [in Russian]. Medicine, Moscow 2002.
8. Ahsan MH, Gill AF, Alvarez X, et al. Kinetics of liver macrophages (Kupffer cells) in SIV-infected macaques. Virology 2013; 446: 77-85.
9. Patel P, Khan N, Rani M, et al. The expression of HIV-1 Vpu in monocytes causes increased secretion of TGF-β that activates profibrogenic genes in hepatic stellate cells. PLoS One 2014; 9: e88934.
10. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Ann Rev 2011; 6: 425-456.
11. Gandhi CR. Hepatic stellate cell activation and pro-fibrogenic signals. J Hepatol 2017; 67: 1104-1105.
12. Fabre T, Kared H, Friedman SL, et al. IL-17A Enhances the expression of profibrotic genes through upregulation of the TGF-β receptor on hepatic stellate cells in a JNK-dependent manner. J Immunol 2014; 193: 3925-3933.
13. Renga B, Francisci D, Schiaroli E, et al. The HIV matrix protein p17 promotes the activation of human hepatic stellate cells through interactions with CXCR2 and syndecan-2. PLoS One 2014; 9: e94798.
14. Carpino G, Morini S, Ginanni Corradini S, et al. Alpha-SMA expression in hepatic stellate cells and quantitative analysis of hepatic fibrosis in cirrhosis and in recurrent chronic hepatitis after liver transplantation. Dig Liver Dis 2005; 37: 349-356.
15. Mastroianni CM, Lichtner M, Mascia C, et al. Molecular mechanisms of liver fibrosis in HIV/HCV coinfection. Int J Mol Sci 2014; 15: 9184-9208.
16. Muhanna N, Horani A, Doron S, Safadi R. Lymphocyte-hepatic stellate cell proximity suggests a direct interaction. Clin Exp Immunol 2007; 148: 338-347.
17. Ganesan M, Poluektova LY, Kharbanda KK, et al. Liver as a target of human immunodeficiency virus infection. World J Gastroenterol 2018; 24: 4728-4737.
18. Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol 2016; 13: 267-276.
19. Vali B, Yue FY, Jones RB, et al. HIV-specific T-cells accumulate in the liver in HCV/HIV co-infection. PLoS One 2008; 3: e3454.
20. Kijewski SD, Gummuluru S. A mechanistic overview of dendritic cell-mediated HIV-1 trans infection: the story so far. Future Virol 2015; 10: 257-269.
21. Manches O, Frleta D, Bhardwaj N. Dendritic cells in progression and pathology of HIV infection. Trends Immunol 2014; 35: 114-122.

Address for correspondence

Stanislav Panov
Human Anatomy Department
Medical School
V.N. Karazin Kharkiv National University
6 Svobody Sq
Kharkiv, 61022, Ukraine
e-mail: stanislavpanov@karazin.ua