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TORCH serological marker on cholestasis with the occurrence of biliary atresia and its clinical manifestations

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ABSTRACT

Background and objectives. Biliary atresia occurs due to multifactorial etiology, and it was recently suggested that perinatal infection with TORCH, especially Cytomegalovirus (CMV), triggers inflammation of the bile ducts.
This study aims to evaluate the TORCH infection on cholestasis with the occurrence of biliary atresia and its clinical manifestations.

Materials and methods. A prospective single-center study was performed on 113 cholestatic infants and classified into two groups based on their positivity of immunoglobulin M and G (IgM and IgG). They were tested for CMV, Rubella, and Toxoplasmosis by a Gold Standard Diagnostics ELISA kit in our laboratory. Clinical conditions, laboratory tests (Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), and liver histopathology between the two groups were analyzed with $p < 0.05$, which was considered significant.

Results. Out of 113 infants of cholestasis, 94.7% (n = 107) were CMV IgG-positive, followed by Rubella IgG-positive (47.8%, n=54). Rubella and toxoplasma IgM antibodies were least commonly found in cholestasis (8% and 7.1%, respectively). CMV IgG-positive status was noted to be older at the onset of jaundice. Infants with Rubella IgG-positive had less incidence of extrahepatic cholestasis (37.7% vs 62.3%) and liver fibrosis (39% vs 61%) ($p<0.05$). There was no difference in sex, birth weight, gestational age, AST, ALT, GGT, coagulation hemostasis, and abdominal USG abnormality based on torch serological markers ($p>0.05$).

Conclusion: CMV infection mostly occurs in infants with cholestasis, followed by rubella infection. TORCH infection screening may be necessary in all infants with cholestasis.

Keywords: TORCH, CMV, infant, cholestasis

INTRODUCTION

Cholestatic jaundice is found in 1 in 2500 term babies born [1]. Most infant cholestasis is caused by extrahepatic biliary atresia [2,3]. Inflammation with extensive fibrosing of the intra- and extrahepatic bile ducts occurs in biliary atresia [4,5]. It causes severe cholangiopathy [5]. Obstruction of bile flow causes damage to liver cells, which will lead to cirrhosis and liver failure [4,5]. Biliary atresia can destroy the liver and cause portal hypertension to end-stage cirrhosis if treated late [4,6].

Some studies have implicated congenital infections, specifically TORCH (Toxoplasma, Rubella, Cytomegalovirus (CMV), and Herpes Simplex Virus (HSV) type 1 and type 2, as potential causes of neonatal cholestasis [7]. There is a lot of discussion surrounding the potential role of CMV in causing extrahepatic biliary atresia (EHBA) due to its known ability to cause damage to the intrahepatic bile ducts [8]. Since there has been ongoing debate and uncertainty surrounding the potential correlation between infectious agents and postnatal bile duct obstruction, this study aimed to evaluate the effects of TORCH infection on cholestasis, as well as the development of biliary atresia and its clinical symptoms.

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MATERIALS AND METHODS

This study is a prospective study conducted on cholestatic infants treated at Dr Soetomo General Academic Hospital, Surabaya, Indonesia. The inclusion criteria in this study were children with cholestasis who visited the pediatric hepatology department at Dr Soetomo General Academic Hospital, Surabaya, in 2020-2022. Cholestasis is indicated by an increase in serum direct bilirubin levels >1 mg/dl if total serum bilirubin levels were <5 mg/dl and serum direct bilirubin levels >20% if total serum bilirubin >5 mg/dl. Meanwhile, the exclusion criteria in this study were other congenital abnormalities. Clinical examination, liver function tests, imaging, and percutaneous liver biopsies were performed according to standard clinical guidelines. CMV, Rubella, and Toxoplasma IgG and IgM testing were performed by ELISA using a Gold Standard Diagnostics ELISA kit. Positive serology results for the following TORCH antibodies: IgM anti-Toxoplasma ≥0.6 index units; IgG anti-Toxoplasma ≥3 IU/mL; IgM anti-Rubella ≥1.6 index units; IgG anti-Rubella ≥10 IU/mL; IgM anti-CMV ≥1 index unit; IgG anti-CMV ≥6 AU/mL. Informed consent was obtained from parents before the study.

Data Analysis

Descriptive analyses are described as Mean ± SD for regularly distributed values, median (min-max) range for non-normally distributed values, or number and percentage. The Kolmogorov-Smirnov test analyzes numerical data normality. Differences in quantitative data were compared using the Mann-Whitney test for abnormal distribution. Differences in qualitative data were compared using Pearson's chi-square, Fisher's Exact, and Continuity Correction. Significance is indicated by the P-value <0.05. Statistical analysis was performed using the SPSS statistical package version 21.

RESULTS

Out of 113 cholestatic infants, 75.3% had extrahepatic cholestasis with a median age of 10.30 (1.7-39.5) weeks and a mean age of onset jaundice of 2 (1-16) weeks. A total of 64 (56.5%) were boys, and 59 (72.8%) had liver fibrosis (Table 1).

In this study, there were 113 infants with cholestasis. This study found that as many as 107 (94.7%) cases of cholestasis had CMV IgG positive at presentation. Among the serological tests studied, CMV IgG was the most frequently found, followed by Rubella IgG, the most common (94.7% and 47.8%, respectively) in the study population. Rubella and toxoplasma IgM antibodies were the least common (8% and 7.1%, respectively) (Table 2).

TABLE 1. Basic characteristics of subjects

Characteristics	n (%)
Age, months	10,30 (1,7-39,5)
Onset of jaundice, week	2 (1-16)
Men, n (%)	64 (56,5)
Birth weight, gram	3000 (900-4200)
Gestational age, week	38 (28-41)
Cesarean Section	49 (43,4)
Hypertension portal	57 (50,4)
Ascites	25 (22,1)
Triangular cord sign/ Contracted gall bladder	53 (56,4)
Extrahepatic cholestasis	61 (75,3)
Fibrosis	59 (72,8)
Laboratory Measurement	**Mean ± SD Median (min – max)**
Hb	10,9 (3,4-21,5)
WBC	12,65 (4,45-31,94)
Platelet	354,56 ± 196,46
Albumin	3,57 (1,35-4,79)
Direct Bilirubin	7,50 (1,4-23,39)
Total Bilirubin	10,60 (2,2-34,06)
APTT	33,55 (11,4-70,6)
PPT	12 (9-55,7)
GGT	253 (21,36 – 2386,00)
AST	210 (29,9 -1642)
ALT	143 (22,3 – 560)

TABLE 2. TORCH serological examination in subject with cholestasis

	Reactive n (%)	Non-reactive n (%)
IgG CMV	107 (94,7%)	6 (5,3%)
IgM CMV	39 (34,5%)	74 (65,5%)
IgG Rubella	54 (47,8%)	59 (52,2%)
IgM Rubella	9 (8%)	104 (92%)
IgG Toxoplasma	27 (23,9%)	86 (76,1%)
IgM Toxoplasma	8 (7,1%)	105 (92,9%)

IgM: immunoglobulin M; IgG: immunoglobulin G; CMV: Cytomegalovirus

Out of 113 infants with cholestasis, 94.7% (n = 107) were CMV IgG positive, followed by Rubella IgG positive (47.8%, n=54). Rubella and toxoplasma IgM antibodies were least commonly found in cholestasis (8% and 7.1%, respectively). CMV IgG-positive status was noted to be older at the onset of jaundice. Infants with Rubella IgG-positive had less incidence of extrahepatic cholestasis (37.7% vs. 62.3%) and liver fibrosis (39% vs. 61%) (p<0.05). There was no difference in sex, birth weight, gestational age, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), coagulation hemostasis, and abdominal USG abnormality based on torch serological markers (Table 3).

TABLE 3. Differences in TORCH serology results based on supporting examinations

Variable		IgG anti CMV		IgM anti CMV		IgG anti Rubella		IgM anti Rubella		IgG anti Toxo		IgM anti Toxo	
		NR (%)	Reactive (%)	NR (%)	Reactive (%)	NR (%)	Reactive (%)	NR (%)	Reactive (%)	NR (%)	Reactive (%)	NR (%)	Reactive (%)
USG	Normal	7.3	92.7	58.5	41.5	46.3	53.7	87.8	12.2	80.5	19.5	95.1	4.9
	Abnormal	5.7	94.3	67.9	32.1	62.3	33.7	94.3	5.7	79.2	20.8	90.6	9.4
P value		1.000[c]		0.470[d]		0.183[d]		0.290[c]		1.000[d]		0.463[c]	
Liver fibrosis	Yes	5.1	94.9	55.9	44.1	61.0	39.0	94.9	5.1	83.1	16.9	94.9	5.1
	No	4.5	95.5	72.7	27.3	27.3	72.7	86.4	13.6	63.6	36.4	95.5	4.5
P value		1.000[c]		0.263[d]		0.014[d]*		0.337[c]		0.076[c]		1.000[c]	
Biopsy	Intrahepatic	10.0	90.0	70.0	30.0	20.0	80.0	100.0	0.0	65.0	35.0	100.0	0.0
	Extrahepatic	3.3	96.7	57.4	42.6	62.3	37.7	90.2	9/8	82.0	18.0	93.4	6.6
P value		0.354[c]		0.460[d]		0.002[d]*		0.328[c]		0.130[c]		0.567[c]	

c: Fisher's Exact Test; d: Continuity Correction; p significant < 0,05

DISCUSSION

Congenital or perinatal viral infections are associated with neonatal cholestasis [9]. Most neonatal cholestasis is caused by biliary atresia [10]. In this study, 61 (75.3%) had extrahepatic cholestasis as a sign of biliary atresia, and 59 (72.8%) had liver fibrosis. Several studies suggest that biliary atresia is not a congenital anomaly. Still, it occurs due to an inflammatory mechanism triggered by an infection that triggers an autoimmune response that targets the bile duct, which results in chronic fibrosclerotic injury [11]. Recent studies have shown that activation of the immune response leading to bile duct injury plays an important role in promoting the process of fibrogenesis in the bile ducts [5]. Immunological studies show a dysregulation of the immune system after viral infection involving the role of helper T cells, B cells, and natural killer cells, which cause the mechanism of bile epithelial damage [11]. Patients with biliary atresia had been tested for multiple viruses in previous studies. Cytomegalovirus (CMV) is the most common virus associated with biliary atresia [12]. A high incidence of CMV infection was found in neonatal cholestasis [13]. Previous studies have also shown that the prognosis of biliary atresia is poor in infants with CMV [14,15].

An emerging theory regarding the pathogenesis of biliary atresia is that viral infection initiates biliary tract damage through the induction of an abnormal autoimmune response, even after the virus has been eliminated [13,15]. Therefore, the mechanism of acquired or perinatal biliary atresia is a progressive inflammatory injury to the bile ducts, leading to fibrosis and destruction of the extrahepatic and intrahepatic bile ducts due to the viral infection [14,16]. Studies in experimental animals and humans show the influence of the environment, especially CMV, on damage to the biliary system [17].

In this study, out of 113 infants of cholestasis, 94.7% (n = 107) were CMV IgG positive. This study is consistent with pre-existing studies. CMV is a double-stranded DNA virus from the Herpesviridae family most commonly found within the bile duct epithelia of biliary atresia patients [13,18]. Studies in China show that the majority of biliary atresia patients are infected with CMV [19]. Th-17 cells infiltrate the liver and are found predominately in CMV-associated biliary atresia [20]. CMV causes induction of CD8+ lymphocyte infiltration [21]. Another study found a higher deposit of immunoglobulin M (IgM) in the hepatocellular canaliculi membrane in patients with biliary atresia with CMV infection than in biliary atresia patients who were not infected with CMV [22]. TORCH infection occurred in 22% of cases of neonatal cholestasis, which was CMV-dominated. In cases of cholestasis, IgG TORCH infection, which was a transplacental transfer from the mother, was obtained [23].

Rubella IgG was this study's next most common antibody type, accounting for 47.8% (n = 54). Various viruses, such as CMV and rubella, have been suggested as potential causes of hepatobiliary injury in animals. However, no definitive causative agent has been identified. Studies indicate that the coexistence of various infections, including syphilis, rubella, toxoplasmosis, and herpes virus, can result in neonatal cholestasis, coagulopathy, and growth retardation. Usually, newborns with these infections present with jaundice within the first 24 hours of life [1].

According to this study, the least common antibodies in cases of cholestasis were toxoplasma and IgM rubella (8% and 7.1%, respectively). Infants who tested positive for Rubella IgG showed a lower occurrence of extrahepatic cholestasis (37.7% vs 62.3%) and liver fibrosis (39% vs 61%) ($p < 0.05$). This is consistent with another study that indicated TORCH infection as the primary cause of intrahepatic cholestasis [24].

This study showed no difference in sex, birth weight, gestational age, AST, ALT, GGT, coagulation hemostasis, and abdominal USG abnormality based on torch serological markers. Previous studies have shown that increased levels of transaminases in the blood (AST, ALT) may be present, suggesting potential damage to the liver cells, although not conclusively. Alkaline phosphatase levels may increase in cholestasis, similar to its occurrence during bone growth. Increased levels of GGT suggest a possible biliary obstruction [25]. A different study found that extrahepatic cholestasis leads to a significant rise in GGT and alkaline phosphate (ALP) levels [26]. Serum GGT is important for distinguishing extrahepatic biliary atresia from other causes of cholestasis. Early abdominal ultrasonography for all cases with infantile cholestasis and training on identifying the triangular cord sign to help diagnose infants with extrahepatic biliary atresia early and early surgical intervention [27]. Abdominal ultrasonography is the most helpful imaging test to assess cholestasis [28]. Although it is operator-dependent, abdominal ultrasonography shows a high degree of accuracy in identifying extrahepatic obstruction and distinguishing it from intrahepatic biliary dilatation [29].

Studies show evidence of complex innate immunity, such as oxidative stress, metabolic changes, and induction of epigenetic changes, including the AGE-RAGE pathway, with macrophages that have a central role in modulating the inflammatory response in biliary atresia, enabling non-surgical therapy [30]. A high prevalence of autoantibodies is found in biliary atresia, including ANA and ANCA [31]. Another study stated that IgM autoantibodies were also found to predominate in cholangiocytes in the first year after Kasai Porto-enterostomy in patients with biliary atresia, such as anti-CHI3L1, anti-DLL-4, and anti-SFTPD IgM autoantibodies suggesting that autoimmunity plays a role in bile duct injury [32]. In biliary atresia, hypo-inflammatory macrophages, expanded cytotoxic T cells, TNF-α, IFN-γ-activated genes (STAT-1), Th1 cytokines (TNF-α, lymphotactin, IL-12p40, and MIP −1γ), impaired Kupffer cells, deficiency of natural killer (NK) and Treg cells were found [18,33]. In biliary atresia with reactive CMV-IgM, there is a decrease in Tregs compared to non-CMV due to autoantibodies and activated hepatic T-lymphocytes [15,33]. Tregs that are deficient in number and function lead to an exaggerated inflammatory response, leading to "bystander" bile duct injury and increasing trends of autoimmunity targeted to subsequent bile ducts. Conditions mediated by autoimmune mechanisms predispose to excessive inflammation that injures the bile ducts [33].

Currently, the treatment of biliary atresia is still carried out with Kasai porto-enterostomy (KPE); however, in almost 50% of patients with KPE, it fails to improve bile flow and causes intrahepatic cholangiopathy [34]. Although in some countries, biliary atresia currently has a life expectancy of up to 89%, and 30% of patients with biliary atresia do not need liver transplantation into adulthood, good outcomes are not always achieved in all countries, especially in limited resources for liver transplantation [35]. By understanding the mechanism of biliary atresia, which is based on immune dysregulation, it is hoped that the possibility of forming new therapeutic targets can be studied further to suppress the occurrence of fibrosis in the bile ducts and eliminate the need for liver transplantation.

CONCLUSION

CMV infection mostly occurs in infants with cholestasis, followed by rubella infection. TORCH infection screening may be necessary in infants with cholestasis. By thoroughly examining the maternal history and consulting with the obstetrician and neonatal intensive care team, valuable insights can be gained regarding placental abnormalities, which can guide the investigation of potential infections.

Author's contributions

Conceptualization, R.A.P., B.S. and S.A.; methodology, R.A.P., S.M.O., B.S., and S.A.; software, R.A.P. and S.M.O.; validation, R.A.P., S.M.O., B.S., and S.A.; formal analysis, R.A.P., S.M.O., B.S., and S.A.; investigation, R.A.P., S.M.O., B.S, and S.A.; resources, R.A.P., S.M.O., B.S., and S.A.; data curation, R.A.P and B.S.; writing–original draft preparation, R.A.P., S.M.O., and B.S.; writing–review and editing, R.A.P., S.M.O., and B.S.; visualization, R.A.P. and B.S.; supervision, B.S. and S.A.; project administration, R.A.P. and B.S.; funding acquisition, R.A.P. and B.S. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Fawaz R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL et al. Guideline for the Evaluation of Cholestatic Jaundice in Infants: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr.* 2017;64:154-68. doi: 10.1097/MPG.0000000000001334
2. Dehghani SM, Efazati N, Shahramian I, Haghighat M, Imanieh MH. Evaluation of cholestasis in Iranian infants less than three months of age. *Gastroenterol Hepatol Bed Bench.* 2015;8:42-8. PMID: 25584175; PMCID: PMC4285931
3. Gottesman LE, Del Vecchio MT, Aronoff SC. Etiologies of conjugated hyperbilirubinemia in infancy: a systematic review of 1692 subjects. *BMC Pediatr.* 2015;15:192. doi: 10.1186/s12887-015-0506-5
4. Asai A, Miethke A, Bezerra JA. Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes. *Nat Rev Gastroenterol Hepatol.* 2015;12:342-52. doi: 10.1038/nrgastro.2015.74
5. Chusilp S, Balsamo F, Li B, Vejchapipat P, Pierro A. Development of liver inflammatory injury in biliary atresia: from basic to clinical research. *Pediatr Surg Int.* 2023;39:207. doi: 10.1007/s00383-023-05489-9
6. Shneider BL, Abel B, Haber B, Karpen SJ, Magee JC, Romero R et al. Portal Hypertension in Children and Young Adults With Biliary Atresia. *J Pediatr Gastroenterol Nutr.* 2012;55:567–73. doi: 10.1097/MPG.0b013e31826eb0cf
7. Suchy FJ. Neonatal cholestasis. *Pediatr Rev.* 2004;25:388-96.
8. Mohanty S, Shah I, Bhatnagar S. Evolving biliary atresia with cytomegalovirus. *Indian Pediatr.* 2011;48:644-6.
9. Landing BH. Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst--the concept of infantile obstructive cholangiopathy. *Prog Pediatr Surg.* 1974;6:113-39.
10. Mack CL. What Causes Biliary Atresia? Unique Aspects of the Neonatal Immune System Provide Clues to Disease Pathogenesis. *Cell Mol Gastroenterol Hepatol.* 2015;1:267-74. doi: 10.1016/j.jcmgh.2015.04.001
11. Zagory JA, Nguyen MV, Wang KS. Recent advances in the pathogenesis and management of biliary atresia. *Curr Op Pediatr.* 2015;27:389-94. doi: 10.1097/MOP.0000000000000214
12. Zhao D, Long X-D, Xia Q. Recent Advances in Etiology of Biliary Atresia. *Clin Pediatr (Phila).* 2015;54:723-31. doi: 10.1177/0009922814548841
13. Zhao D, Gong X, Li Y, Sun X, Chen Y, Deng Z et al. Effects of cytomegalovirus infection on the differential diagnosis between biliary atresia and intrahepatic cholestasis in a Chinese large cohort study. *Ann of Hepatol.* 2021;23:100286. doi: 10.1016/j.aohep.2020.100286
14. Kodo K, Sakamoto K, Imai T, Ota T, Miyachi M, Mori J et al. Cytomegalovirus-associated biliary atresia. *J Pediatr Surg Case Rep.* 2018;35:17–20. doi: 10.1016/j.epsc.2018.02.002
15. Zhao Y, Xu X, Liu G, Yang F, Zhan J. Prognosis of Biliary Atresia Associated With Cytomegalovirus: A Meta-Analysis. *Front Pediatr.* 2021;9:710450. doi: 10.3389/fped.2021.710450
16. Feldman AG, Mack CL. Biliary atresia: cellular dynamics and immune dysregulation. *Seminars Pediatr Surg.* 2012;21:192-200. doi: 10.1053/j.sempedsurg.2012.05.003
17. Bezerra JA, Wells RG, Mack CL, Karpen SJ, Hoofnagle JH, Doo E et al. Biliary Atresia: Clinical and Research Challenges for the Twenty-First Century. *Hepatol.* 2018;68:1163-73. doi: 10.1002/hep.29905
18. Wang J, Xu Y, Chen Z, Liang J, Lin Z, Liang H et al. Liver Immune Profiling Reveals Pathogenesis and Therapeutics for Biliary Atresia. *Cell.* 2020;183:1867-1883.e26. doi: 10.1016/j.cell.2020.10.048
19. Xu Y, Yu J, Zhang R, Yin Y, Ye J, Tan L et al. The Perinatal Infection of Cytomegalovirus Is an Important Etiology for Biliary Atresia in China. *Clin Pediatr (Phila).* 2012;51:109-13. doi: 10.1177/0009922811406264
20. Hill R, Quaglia A, Hussain M, Hadzic N, Mieli-Vergani G, Vergani D et al. Th-17 cells infiltrate the liver in human biliary atresia and are related to surgical outcome. *J Pediatr Surg.* 2015;50:1297-303. doi: 10.1016/j.jpedsurg.2015.02.005
21. Janowska M, Bierła JB, Kaleta M, Wierzbicka-Rucińska A, Czubkowski P, Kanarek E et al. The Impact of a CMV Infection on the Expression of Selected Immunological Parameters in Liver Tissue in Children with Biliary Atresia. *JCM.* 2022;11:7269. doi: 10.3390/jcm11247269
22. Fischler B, Woxenius S, Nemeth A, Papadogiannakis N. Immunoglobulin deposits in liver tissue from infants with biliary atresia and the correlation to cytomegalovirus infection. *J Pediatr Surg.* 2005;40:541-6. doi: 10.1016/j.jpedsurg.2004.11.035
23. Silasi M, Cardenas I, Kwon J, Racicot K, Aldo P, Mor G. Viral Infections During Pregnancy. *Am J Rep Immunol.* 2015;73:199-213. doi: 10.1111/aji.12355
24. Mahmud S, Gulshan J, Parvez M, Tasneem F, Ahmed SS. Etiology and outcome of neonatal cholestasis: an experience in a tertiary center of Bangladesh. *Egypt Liver J.* 2022;12:1. doi: 10.1186/s43066-021-00168-7
25. Dani C, Pratesi S, Raimondi F, Romagnoli C. Italian guidelines for the management and treatment of neonatal cholestasis. *Ital J Pediatr.* 2015;41:69. doi: 10.1186/s13052-015-0178-7
26. Urganci N, Cetinkaya F, Kalyoncu D, Papatya Cakir E, Yilmaz B. Infants With Cholestasis: Diagnosis, Management And Outcome. *Marmara Med J.* 2012;25:83-6. doi: 10.5472/MMJ.2012.01947.3
27. Donia A, Ibrahim S, Kader M, Saleh A, El-Hakim M, El-Shorbagy M, et al. Predictive Value of Assessment of Different Modalities in the Diagnosis of Infantile Cholestasis. *J Int Med Res.* 2010;38:2100-16. doi: 10.1177/147323001003800626
28. Iorio R, Liccardo D, Di Dato F, Puoti M, Spagnuolo M, Alberti D et al. Ultrasound Scanning in Infants with Biliary Atresia: The Different Implications of Biliary Tract Features and Liver Echostructure. *Ultraschall in Med.* 2013;34:463-7. doi: 10.1055/s-0033-1335455
29. Modha K. Clinical Approach to Patients With Obstructive Jaundice. *Techniq Vascul Intervent Radiol.* 2015;18:197-200. doi: 10.1053/j.tvir.2015.07.002
30. Ortiz-Perez A, Donnelly B, Temple H, Tiao G, Bansal R, Mohanty SK. Innate Immunity and Pathogenesis of Biliary Atresia. *Front Immunol.* 2020;11:329. doi: 10.3389/fimmu.2020.00329
31. Pang S-Y, Dai Y-M, Zhang R-Z, Chen Y-H, Peng X-F, Fu J et al. Autoimmune liver disease-related autoantibodies in patients with biliary atresia. *WJG.* 2018;24:387-96. doi: 10.3748/wjg.v24.i3.387
32. Luo Y, Brigham D, Bednarek J, Torres R, Wang D, Ahmad S et al. Unique Cholangiocyte-Targeted IgM Autoantibodies Correlate With Poor Outcome in Biliary Atresia. *Hepatol.* 2021;73:1855-67. doi: 10.1002/hep.31504
33. Brindley SM, Lanham AM, Karrer FM, Tucker RM, Fontenot AP, Mack CL. Cytomegalovirus-specific T-cell reactivity in biliary atresia at the time of diagnosis is associated with deficits in regulatory T cells. Hepatol. 2012;55:1130–8. doi: 10.1002/hep.24807.
34. Lendahl U, Lui VCH, Chung PHY, Tam PKH. Biliary Atresia – emerging diagnostic and therapy opportunities. *eBioMedicine.* 2021;74:103689. doi: 10.1016/j.ebiom.2021.103689
35. Chardot C, Buet C, Serinet M-O, Golmard J-L, Lachaux A, Roquelaure B et al. Improving outcomes of biliary atresia: French national series 1986-2009. *J Hepatol.* 2013;58:1209-17. doi: 10.1016/j.jhep.2013.01.040