Ro J Pediatr. 2023;72(4)
DOI: 10.37897/RJP.2023.4.6

Factors associated with biliary atresia in children with cholestasis in Surabaya

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ABSTRACT

Introduction. Biliary atresia (BA) represents a progressive and obstructive fibrotic cholangiopathy, serving as a leading cause of liver failure and pediatric liver transplantation worldwide. Identifying risk factors associated with BA incidence is crucial for expediting diagnosis; however, limited research has been conducted in Indonesia. This study aims to investigate the characteristics and analyze the factors associated with the occurrence of BA.

Methods. A retrospective observational analysis of medical records encompassing 523 cholestatic patients at the Pediatric Hepatology Outpatient Department and Pediatric Ward of "Dr. Soetomo" General Academic Hospital, Surabaya, from 2011 to 2022 was conducted. The data was segregated into BA and non-BA groups, and diagnostic chi-square tests were employed to determine interrelationships among variables. Subsequently, logistic regression analysis was performed to identify the most impactful variable.

Results. Notably, age at first time visiting specialist ($p = 0.003$), gestational age ($p < 0.001$), birth weight ($p = 0.039$), and domicile ($p = 0.028$) exhibited substantial associations with BA. Moreover, gestational age emerged as the most influential factor ($p = 0.004$). Infants with normal birth weight (≥2,500 grams) exhibited the strongest factor associated factor of BA cholestasis.

Conclusion. Initial specialist consultations were predominantly observed within the 0-6 months age range, while term infant and normal birth weight were linked to a higher likelihood of BA cholestasis. Residence in rural areas exhibited a significant correlation with BA incidence.

Keywords: cholestasis, biliary atresia, prematurity, low birth weight, rural

INTRODUCTION

Biliary atresia (BA) is a progressive obstructive fibrotic cholangiopathy involving both the extrahepatic and intrahepatic biliary systems. Progressive fibroinflammatory cholangiopathy in infancy is associated with the activation of innate and adaptive immune responses that target the bile duct [1]. The etiopathogenesis of BA is multifactorial and has been the subject of intensive investigation, considering several potential pathomechanisms. Generally, the causes of BA are classified as viral infections, toxins, genetic variations, immunogenic disorders, maternal microchimerism, vascular disorders, and morphogenesis failures [2]. The incidence rate of BA in the Asia-Pacific region (1.06 in 10,000 live births) is higher than that in the United States (US) (4.47 out of 100,000). Other research indicates that BA occurs in 1 in 15,000 births in the US, affecting all ethnicities, with a higher frequency among girls than boys [3,4]. In South Asia, the incidence of BA is approximately 1 in 3,000 births.

The etiological factors and risk factors contributing to the development of BA in children continue to be under investigation. This is of significant importance as the rise in case numbers is accompanied by a substantial allocation of the healthcare budget for disease treatment. Healthcare expenditures for all BA conditions are experiencing rapid growth. In 2011, the US spent a total of $2.7 billion on healthcare, marking a tenfold increase since 1980. Meanwhile, annual per capita healthcare spending exceeds $8,600 [5]. The treatment of Hepatoportoenterostomy (HPE) or Kasai procedures within the first 2-3 months of life is known to restore bile flow

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Article History:
Received: 24 December 2023
Accepted: 30 December 2023

and aid in preventing the progression of liver disease. However, a meta-analysis study reported that even after a successful procedure, 60.5% of post-Kasai patients continue to experience complications related to liver damage throughout their lives [6].

Limited studies have focused on the factors associated for BA in children with cholestasis. A risk factor analysis of 85 children with BA identified maternal age, parity, low birth weight, and prematurity as significant risk factors [7]. Research at RSUD "Dr. Soetomo" stated that maternal age during pregnancy, parity, and gestational age are prenatal and postnatal risk factors that contribute to the incidence of biliary atresia but do not include environmental factors yet. Meanwhile, recent studies have demonstrated a connection with environmental factors; specifically, winter at conception and smoking during pregnancy are associated with a higher risk of BA occurrence [8]. Several studies have also linked exposure to toxins and cytomegalovirus (CMV) infection to the pathogenesis of BA. Additionally, conjugated hyperbilirubinemia can result from various causes, including TORCH infections (toxoplasmosis, rubella virus, CMV, and herpes simplex), genetic diseases (such as Alagille syndrome, α1-antitrypsin deficiency, and cystic fibrosis), metabolic disorders (like tyrosinemia, galactosemia, and hypothyroidism), choleduct cyst obstruction, and spontaneous bile duct perforation [9, 10]. Identifying risk factors based on the incidence of BA in Indonesia has not been extensively undertaken. Therefore, this study aims to investigate the characteristics and analyze the factors associated with the occurrence of BA.

METHODS

Study design: the research utilized secondary data extracted from the medical records of the Pediatric Hepatology Outpatient Department and Inpatient Department of "Dr. Soetomo" Regional Public Hospital, Surabaya, spanning from January 2011 to December 2022. The cross-sectional study design measures the risk factors that allowed the incidence of BA in children. The data was carefully reviewed for clarity and relevance to the research topic before initiating data collection. This study was carried out after was approved by the Health Research Ethics Committee of "Dr. Soetomo" General Public Hospital, Surabaya, Indonesia (No. 1202/LOE/301.4.2/1/2023).

Setting: this research set in the Pediatric Hepatology Outpatient Department and Inpatient Department of "Dr. Soetomo" Regional Public Hospital, Surabaya, Indonesia. Data collection of medical records was done by January 2023.

Participants: the cross-sectional procedure employed involved the eligibility criteria of patients both inclusion and exclusion. The inclusion criteria encompassed children aged 0-6 years with a diagnosed case of cholestasis and who received medical services at the Pediatric Hepatology Outpatient Department and Inpatient Department of "Dr. Soetomo" Regional Public Hospital, Surabaya, Indonesia. The diagnosis of BA was confirmed based on clinical manifestations, laboratory examinations, and histopathological analysis of liver biopsies, demonstrating ductular proliferation, periportal fibrosis, and bile plugs [11]. Medical record of patients with no cholestasis indication would be removed.

Variables: the data was categorized according to several independent and dependent variables. Independent variables included age at first time visiting specialist, birth weight, gestational age, sex, place of residence, results of anti-CMV, anti-rubella, and anti-toxoplasma serology tests, as well as cholestasis status presented as nominal data. Birth weight was documented as the infant's weight measured one hour after birth, categorized as low birth weight (LBW) (<2,500 grams) or normal (≥2,500 grams). Gestational age was classified as premature (<37 weeks) or at term (37-42 weeks). Sex included boys and girls with cholestasis. Residence was categorized as urban or rural. Rural settlements are outside urban areas with low population density and primarily based on agriculture and farming, while urban areas have non-agricultural activities and centralization of government services, social services, and economic activities. Rural areas are situated outside city boundaries and industries, while urban areas are centered and distributed. Anti-CMV serology results were divided into positive IgG (≥6 U/mL), positive IgM (≥0.9 U/mL), positive IgG and IgM, and negative IgG and IgM. Anti-rubella and anti-toxoplasma serology tests followed the same pattern as anti-CMV serology tests, but with thresholds of >10 U/mL (positive IgG) and ≥3 U/mL (positive IgM) for anti-rubella serology tests. Cholestasis was defined as direct bilirubin >2mg/dL when total bilirubin was <5mg/dL or 20% of total bilirubin when total bilirubin was >5mg/dL [11].

In addition to the independent variables, the dependent variable were the presence of BA, diagnosed based on clinical manifestations of jaundice and colicky stools, laboratory examination showing direct bilirubin >2mg/dL when total bilirubin was <5mg/dL or 20% of total bilirubin when total bilirubin was >5mg/dL, and histopathological examination of liver biopsies indicating ductular proliferation, periportal fibrosis, and bile plugs (Mack et al., 2012). The grouped data was presented in tables and accompanied by explanatory text.

Data sources: each of variables extracted from medical report of patients with BA during January 2011 to December 2022 from the Pediatric Hepatol-

ogy Outpatient Department and Inpatient Department of "Dr. Soetomo" Regional Public Hospital, Surabaya, Indonesia. The data was carefully reviewed for clarity and relevance to the research topic before initiating data collection. Medical records with unclear and irrelevant identity would be excluded from screening.

Medical record data from the Pediatric Hepatology Outpatient Department and Pediatric Ward of "Dr. Soetomo" General Academic Hospital, Surabaya, spanning from January 2011 to December 2022, was meticulously reviewed for clarity and relevance to the research topic prior to commencing data collection.

Bias: the data was carefully reviewed for clarity and relevance to the research topic before initiating data collection. Medical records with unclear and irrelevant identity would be excluded from screening. After data was collected and screened, data would be divided into two groups consisting BA and non-BA patients before tested with logistic regression analysis.

Study size: the data was carefully reviewed for clarity and relevance to the research topic before initiating data collection. Medical records with unclear and irrelevant identity would be excluded from screening. After data was collected and screen, data would be divided into two groups consisting BA and non-BA patients before tested with logistic regression analysis.

Population of this study was the cholestasis-diagnosed children patient's medical records from Outpatient Department and Inpatient Department of "Dr. Soetomo" Regional Public Hospital, Surabaya, Indonesia, spanning from January 2011 to December 2022. The sample consist child patients with inclusion criteria. It was diagnosed based on clinical manifestations of jaundice and colicky stools, laboratory examination showing direct bilirubin >2mg/dL when total bilirubin was <5mg/dL or 20% of total bilirubin when total bilirubin was >5mg/dL, and histopathological examination of liver biopsies indicating ductular proliferation, periportal fibrosis, and bile plugs [11].

Quantitative variables: all quantitative analysis was conducted at IBM SPSS version 26 (IBM SPSS Corporation, New York, USA). Each variable was classified to several categories and compared to others with chi-square bivariate and logistic regression for multivariate analysis.

Statistical methods: each variable collected was subjected to bivariate analysis using the Chi-square test to establish the relationship between independent and dependent variables. Significant variables ($p < 0.05$) were further examined through calculation of the prevalence ratio (PR) and subjected to logistic regression analysis to determine their impact on the dependent variables. All variable analyzed using IBM SPSS version 26 (IBM SPSS Corporation, New York, USA).

RESULTS

Participants: the research encompassed medical record data of pediatric patients aged 0-6 years diagnosed with cholestasis treated at the Pediatric Hepatology Outpatient Department and Pediatric Ward of "Dr. Soetomo" General Academic Hospital, Surabaya, from January 2011 to December 2022. A total of 523 data sets from pediatric patients with cholestasis were collected over a 12-year period.

Descriptive data: among data on Table 1, 231 (44%) patients were diagnosed with BA, while 292 (56%) patients were from non-BA cases. In BA group, normal BW and at term gestational age was dominated than non-BA group. Aside, LBW contributed the dominant case of infant's BW and both gestational age shares equal proportion (50.0%). Other variables were predominated with same categories in two groups (Table 1). These patients, both diagnosed with and without BA, were analyzed based on several variables, including age at first time visiting specialist, sex, gestational age, birth weight (BBL), residence, as well as anti-CMV, anti-rubella, and anti-toxoplasma serology test results. The distribution of the study subjects across various variables was described descriptively (Table 1).

Outcome data: table 1 shows that decreasing trend of patient's age at first admission on both BA and non-BA groups and resulting significances ($p = 0.003$). On the other hand, birth weight predominately with LBW (<2,500 grams) infants. However, non-BA demonstrated opposite result. Both lower and normal BW support fundamentally differences for BA and non-BA classes ($p = 0.039$). Gestational age portrayed significance outcome with at term pregnancy age ($p<0.001$). In addition, domicile and anti-CMV serology test result with the rural and IgG positive majority ($p = 0.028$ and $p = 0.049$).

Other parameters like sex, anti-rubella and anti-toxoplasma serology test results demonstrate insignificant chi-square results ($p = 0.409$, $p = 0.440$, and $p = 0.159$). Those parameters are dominated by male as well as IgM and IgG negative for both groups. Only parameters with the significance chi-square results will be continually tested by logistic regression analysis.

Main result: an analysis of bivariate factors using the chi-square test was conducted to identify the associated factors influencing the incidence of BA in children. The results of the analysis are presented in Table 2. In this study, age at first time visiting specialist, birth weight, gestational age, and domicile were found to be significantly associated with the

TABLE 1. Characteristics of study subjects children's cholestasis coverage in Dr. Soetomo General Public Hospital in 2011-2022

Variable	Category	Group		p
		BA n (%)	Non-BA n (%)	
Age at first time visiting specialist	0-6 months	196 (44.4%)	245 (55.6%)	0.003
	>6 months-1 year	22 (64.7%)	12 (35.3%)	
	>1-3 year	7 (30.4%)	16 (69.65)	
	>3-6 year	4 (18.2%)	18 (81.85)	
Sex	Male	118 (43.1%)	156 (56.9%)	0.409
	Female	102 (46.8%)	116 (53.2%)	
Birth weight	<2,500 grams	95 (40.6%)	139 (59.4%)	0.039
	≥2,500 grams	100 (51.0%)	96 (49.0%)	
Gestational age	Premature	10 (25.6%)	29 (74.4%)	<0.001
	At term	60 (67.4%)	29 (32.6%)	
Domicile	Rural	139 (47.8%)	152 (52.2%)	0.028
	Urban	65 (36.9%)	111 (63.1%)	
Anti-CMV serology test result	IgM positive	4 (25%)	12 (75%)	0.049
	IgG positive	43 (50.6%)	42 (49.4%)	
	IgM and IgG positive	19 (32.2%)	40 (67.8%)	
	IgM and IgG negative	37 (48.7%)	39 (51.3%)	
Anti-rubella serology test result	IgM positive	7 (77.8%)	2 (22.2%)	0.440
	IgG positive	23 (46%)	27 (54%)	
	IgM and IgG positive	1 (11.1%)	8 (88.9%)	
	IgM and IgG negative	72 (45.9%)	85 (54.1%)	
Anti-toxoplasma serology test result	IgM positive	3 (37.5%)	5 (62.5%)	0.159
	IgG positive	7 (29.2%)	17 (70.8%)	
	IgM and IgG positive	1 (16.7%)	5 (83.3%)	
	IgM and IgG negative	89 (48.1%)	96 (51.9%)	

incidence of BA in children with cholestasis (p <0.05). Conversely, sex, anti-CMV serology test results, anti-toxoplasma serology test results, and anti-rubella serology test results did not show a significant relationship. Furthermore, all variables were analyzed to determine the most significant factors affecting the incidence of BA in children with cholestasis.

An analysis of multivariate factors was conducted to identify the strongest associated factors influencing the incidence of BA in children. All variables with p-values <0.25 were included in the analysis. The logistic regression test was employed for this purpose. The findings of the analysis revealed that the most impactful variable was low birth weight (p = 0.004). Specifically, infants with a normal birth weight (≥2,500 grams) were most associated factor developing BA cholestasis compared to non-BA cholestasis (Table 3).

Other analysis: bivariate analysis illustrates that premature BW infants has prevalence 1.524 higher than normal BW infants in BA incidence with cholestasis. Premature pregnancy and rural domicile also show the fundamental outcome of prevalence with 6.00 and 0.064 times higher associated with incidence of BA in children with cholestasis than the children with at term pregnancies and urban residencies (Table 2). On the other hand, BW considers the most impactful risk factor of BA case in infants. It is known that LBW or premature infants expected to have 7.4 times higher risk to be affected with non-BA cholestasis than the non-BA one (Table 3).

DISCUSSION

The patients' age at first admission to specialists considered pertinent to the diagnosis of BA. In this study, the majority of cholestatic patients sought treatment between 0-6 months, accounting for 44.4% in the BA group and 55.6% in the non-BA group. Another study investigating treatment timing and the ethnicity of BA patients found that white infants exhibited shorter time spans to treatment (p = 0.007) compared to Black and Hispanic infants (p = 0.004) [12].

Accelerating the diagnosis of BA has been correlated with reduced delays and a decreased need for liver transplantation. However, challenges arise as infants are often admitted after the initial 60 days of life. Hence, expediting the diagnosis and treatment of BA, which directly impacts diagnostic time, becomes imperative. Another study highlights strategies adopted by various countries to encourage ear-

TABLE 2. Analysis of bivariate to BA cases in children with cholestasis in Dr. Soetomo General Public Hospital between in 2011-2022

Variable	Case N (%)	PR	CI 95%	p
Age at first time visiting specialist		-	-	0.006
0–6 months	196			
>6 months–1 year	22			
>1–3 years	7			
>3–6 years	4			
Sex		-	-	0.409
Male	118			
Female	102			
Birth weight		1.524	1.039-2.235	0.031*
<2,500 grams	95			
≥2,500 gram	100			
Gestational age		6.00	2.579-13.960	<0.001*
Premature	10			
At term	60			
Domicile		0.064	0.437-0.939	0.023*
Rural	139			
Urban	65			
Anti-CMV serology test result		-	-	0.054
IgM positive	4			
IgG positive	43			
IgM and IgG positive	19			
IgM and IgG negative	37			
Anti-rubella serology test result		-	-	0.074
IgM positive	7			
IgG positive	23			
IgM and IgG positive	1			
IgM and IgG negative	72			
Anti-toxoplasma serology test result		-	-	0.165
IgM positive	3			
IgG positive	7			
IgM and IgG positive	1			
IgM and IgG negative	89			

PR = prevalence ratio; CI 95% = confidence interval of 95%; and * = statistically significant (p <0.05)

TABLE 3. Analysis of multivariate factors related to BA cases in children cholestasis in Dr. Soetomo General Public Hospital in 2011-2022

Risk Factor	Exp. (B)	CI 95%	p
Age at first time visiting specialist	-	-	0.859
Gestational age	-	-	0.799
Anti-CMV serology test result	-	-	0.382
Sex	-	-	0.111
Birth weight	7.429	1.907–28.936	0.004*
Constant			

Exp. (B) = exponential of the coefficient B; CI 95% = confidence interval of 95%; and * = statistically significant (p <0.05)

lier visits for BA patients, as outlined in a technical report by the American Academy of Pediatrics. The most prevalent strategy involves the use of stool color cards [13,14]. These programs rely on primary healthcare providers and/or parents to identify infants who may exhibit BA symptoms based on pale stools over time. Another approach involves screening newborns for direct or conjugated bilirubin levels [15, 16]. Additionally, the BA patients' age at first admission correlates with the age at which the BA patients undergo the Kasai procedure. While liver fibrosis significantly depends on time, the relationship between porto-enterostomy and surgical outcomes doesn't exhibit a linear correlation solely with the patients' age at first admission. This underscores that, in isolated BA cases, porto-enterostomy outcomes cannot be solely determined by age up to approximately 90 days old [17,18]. Notably, in Indonesia, particularly at "Dr. Soetomo" Regional Public Hospital, Surabaya, early detection programs based on stool color cards have been actively promoted.

These initiatives aim to boost the number of early visits for BA patients, ultimately leading to improved outcomes, cost savings, and efficiency.

The current study has unveiled a significant association between gestational age and BA ($p < 0.001$). Notably, infants born with a term infant to exhibit higher rates of BA cholestasis compared to non-BA. This outcome differs from the findings of other studies, which indicate that BA prevalence is higher in premature infants compared to infants born at term (OR: 1.65) [18]. In line with the present study, it has been reported that the occurrence of BA is more pronounced in premature infants (96.3%) compared to infants born at term. Furthermore, this research highlights the impact of prematurity ($p < 0.05$) on the incidence of BA, suggesting that prematurely born infants face a 1.65-fold higher risk of BA in comparison to those born at term [19]. Additional studies present mixed results, indicating that premature infants with BA may experience outcomes similar to infants born at term, albeit with delayed diagnoses and a higher occurrence of syndrome forms. These outcomes may be attributed to various factors, such as impaired bile duct development either in isolation or in conjunction with other anomalies, bile duct ischemia, or bile duct injury stemming from a virus-triggered immune response [20,21].

The onset of such injuries can span from early stages in the first trimester during embryonic organogenesis (e.g., biliary atresia associated with splenic malformation) to the perinatal period (e.g., BA-associated CMV infections). Much of the recent research relies on insights derived from diverse animal models of virus-induced BA, with limited human evidence available. While cholestatic jaundice is more prevalent in the premature population, instances of BA have been observed in infants born at term with normal birth weights. Interestingly, this is also applicable to patients with isolated BA (IBA) or embryonic origin [22]. Based on the combined findings from these studies, the results of the current study align with the theory that preterm birth is more prevalent in the non-BA cholestasis group, which is primarily characterized by perinatal infections such as neonatal CMV infection, hepatitis B, and congenital abnormalities like gallbladder hypoplasia.

This study demonstrates a significant relationship between birth weight and BA ($p < 0.001$). This is attributed to the fact that infants with normal birth weight are inclined to have a higher incidence of BA cholestasis as opposed to non-BA. The outcomes from the multivariate analysis underscore the potency of this factor, revealing that infants with LBW (<2,500 grams) face an elevated risk of non-BA cholestasis compared to BA cholestasis ($p = 0.004$). This finding diverges from earlier studies that indicated LBW was not notably linked to the incidence of BA, but rather pointed to premature gestational age as the variable of concern [7] . Numerous other studies have reported contrasting outcomes regarding the association between LBW and abbreviated gestational age with the emergence of BA. A case-control study from New York, USA, underscored an escalated BA risk in LBW and LBW premature infants (OR: 2.92 and 2.36) in comparison to normally weighted infants [23]. A parallel connection was identified in Atlanta, USA, wherein LBW infants born at term exhibited a heightened risk compared to normally weighted infants (OR: 3.52) [24].

Furthermore, a cohort study from Sweden, utilizing the Swedish Medical Birth Register to appraise BA risk, revealed an incremental likelihood of BA associated with gestational age and preterm status (SGA). SGA infants exhibited an RR of 5.75; infants born at gestational ages of 22-32 weeks had an RR of 6.75; and infants born at gestational ages of 33-36 exhibited an RR of 2.48 compared to infants born at term. This finding is bolstered by the prevalence of viral infections in utero linked with intrauterine growth retardation or premature birth. Jaundice occurring in both premature and term infants arise from an amplified bilirubin load within hepatocytes, diminished hepatic uptake of bilirubin from plasma, and/or impaired bilirubin conjugation. Hyperbilirubinemia is more frequent, severe, and prolonged in premature infants compared to term infants. The conditions of prematurity, LBW, as well as the underdeveloped metabolism of bile acids and enterohepatic circulation contributing to cholestasis in LBW infants are often associated with prematurity in contrast to LBW resulting from intrauterine growth restriction (IUGR) [7].

The results underscore the significance of domicile concerning BA ($p < 0.001$), where rural domiciles exhibit a 1.5–fold increased tendency toward BA cholestasis. Other studies align with these outcomes, suggesting that non-BA cholestasis is more pronounced in rural areas than in urban areas due to higher rates of TORCH infections among women residing in rural regions [25]. Viewing this from a health management perspective, women of childbearing age laying the groundwork for healthy pregnancies is of paramount importance. The uneven development of health facilities in underdeveloped regions, coupled with substantial mobility among the young rural population in recent years, limited access to education, and extensive distances to healthcare facilities, has contributed to these discrepancies. A study assessing the knowledge levels of TORCH infections in women of childbearing age indicated that urban women had a distribution of moderate (68%), poor (27%), and good (5%) knowledge. In contrast, rural women's knowledge levels

were divided into poor (80%), average (19%), and good (1%). Consequently, rural women exhibit lower levels of knowledge concerning TORCH infections compared to their urban counterparts [26]. Thus, the higher prevalence of prematurity and LBW within the non-BA group can be attributed to these factors. However, BA predominates among patients in rural areas, prompting consideration of additional theories beyond perinatal infections to elucidate the pathogenesis of isolated BA (IBA) disorders. This finding is in alignment with an IBA study indicating a 68% higher incidence in rural areas (0.67 per 10,000) compared to urban areas (0.40 per 10,000) ($p = 0.02$).

A significant association between anti-CMV serology test results and BA was observed ($p = 0.049$), with positive anti-CMV serology test result being more prevalent in the non-BA cholestasis group. Nevertheless, the subsequent analysis for calculating prevalence ratios (PR) through logistic regression yielded nonsignificant results ($p = 0.054$). A study conducted in 31 infants with neonatal cholestasis reported a weak correlation between positive serology test results and CMV detection in liver tissue. This could be attributed to the fact that anti-CMV IgM is not a reliable indicator for detecting CMV in liver tissue [27]. The anti-CMV serology test of IgG and IgM is not recommended for diagnosing CMV infection in infants. A positive anti-CMV IgG result reflects IgG antibodies transmitted from the mother via the placenta and does not serve as a diagnostic criterion for CMV infection. Maternal IgG antibodies persist in the infant's bloodstream up to 18 months of age and cannot distinguish between congenital, neonatal, or postnatal infections [28]. The utility of anti-CMV IgM antibody testing for primary infection diagnosis is hampered by low sensitivity and specificity, as IgM can yield positive results in cases of reactivated CMV infection [29].

Instances of patients presenting positive CMV IgM results but negative DNA PCR outcomes might be attributed to false-positive IgM readings. CMV infection can actively replicate in hepatocytes and cholangiocytes, directly leading to damage to the liver, the biliary duct system, and the immune cells within infected cells. Notably, inclusion bodies are observed in hepatocytes and vascular endothelial cells along the biliary duct epithelial cells. This phenomenon can result in the thickening of the biliary duct epithelium, causing obstruction of biliary flow, ultimately culminating in intrahepatic and extrahepatic cholestasis [30]. However, the findings of this study diverge from investigations suggesting a linkage between BA and CMV infection, wherein active CMV infection causes enhanced infiltration of CD8+ cells, which may play a role in BA immunopathogenesis [31]. In another study encompassing 74 patients with hepatobiliary diseases spanning from 2000 to 2011, 39 patients were diagnosed with BA, while the remaining 35 cases were non-BA. Within the BA cohort, 21 patients (78%) tested positive for CMV IgM/IgG [32]. In Sri Lanka, 3 BA cases were reported based on liver biopsy results indicating positive CMV DNA PCR findings. CMV infection has also been implicated as a potential cause of extrahepatic BA, often associated with a poor prognosis post Kasai surgery [33] .

Cytomegalovirus (CMV) infection can occur before, during, and after birth, as mounting evidence suggests that patients with BA exhibit cholestasis from birth, and the underlying disorder likely originates during fetal development. Retrospective analysis of CMV DNA from Guthrie cards, which are preserved for newborn metabolic disease screening, has been explored as a means to detect these diseases. A study focusing on Guthrie cards collected within three days of birth found that only one out of 11 BA patients tested positive for CMV during the Kasai portoenterostomy (KPE) procedure. Consequently, certain patients may be categorized as congenitally infected, while the timing of infection for the remaining patients remains uncertain [34] . This hypothesis is reinforced by the discovery that 56% of BA patients exhibit significant elevation of hepatic interferon-gamma-producing T cells in response to CMV, compared to minimal BA response to other viruses or the response seen in control groups [35].

Recent meta-analysis data has revealed that BA patients with ongoing CMV infection experience notably worse outcomes than CMV-negative patients, particularly in terms of jaundice clearance. The possible negative impact of CMV infection could stem from infection occurring before KPE, resulting from direct viral involvement or activation of secondary immune responses. This avenue allows for clinical treatment observations, although further validation is warranted through placebo-controlled randomized studies, multicenter trials, and collaboration with disease-specific organizations [36, 37].

Despite the relatively high rate of CMV infection in children with BA, the direct correlation between positive CMV expression and damage to the biliary system remains poorly elucidated. A study suggests that CMV mRNA expression is primarily observed in vascular endothelial cells, biliary epithelium, the basal membrane of bile ducts, connective tissue in the portal area, matrix cells in the gallbladder capsule, and infiltrating mononuclear lymphocytes. During infection, the virus can replicate in both hepatocytes and cholangiocytes, leading to direct damage to the liver and biliary duct system. Additionally, it induces immune-related damage in infected cells, potentially through a direct cytopathic effect or by eliciting an immune response. The pres-

ence of inclusion bodies is notably seen in hepatocytes and vascular epithelial cells, particularly in the epithelial cells of the biliary ducts [38].

In the context of this study, the serology test results did not show significant associations with the incidence of BA. This may be attributed to the low sensitivity and specificity of serology tests in detecting CMV infection, and these examinations cannot replace CMV PCR tests conducted on liver tissue. Moreover, discrepancies in the availability of data between the two groups or incomplete medical record information can also influence statistical outcomes. Nonetheless, CMV infection is considered significant in the pathogenesis of BA due to both direct viral assault and the induction of immune-mediated pro-inflammatory responses that lead to cellular damage. To comprehensively explore the relationship between CMV infection and BA, prospective multicenter studies incorporating appropriate controls are essential.

The findings of this study indicate that there was no significant relationship between anti-toxoplasma serology test results and the incidence of BA ($p = 0.159$). Neonatal cholestasis can manifest as a result of various underlying conditions. Establishing an early etiological diagnosis is crucial due to the potential impact on prognosis when treatment is initiated within the first 60 days of life for children with BA. Congenital toxoplasmosis can present either asymptomatically in newborns or with neurological, ophthalmological, or gastrointestinal symptoms. The transmission of the infection occurs through exposure to contaminated cat feces, consumption of contaminated vegetables, fruits, and milk [39].

However, neonatal cholestasis as a consequence of congenital toxoplasmosis is not frequently reported. T. gondii, the causative agent of toxoplasmosis, poses significant risks to pregnant women when acquired during the first or second trimester, potentially leading to severe consequences for the fetus. Furthermore, this study demonstrates no significant association between anti-rubella serology test results and the incidence of BA ($p = 0.440$). Currently, there is limited research data directly linking rubella infection to BA. Suspected perinatal rubella infection has been linked to congenital anomalies that could give rise to BA-like presentations. However, substantial empirical data supporting this connection are lacking. Conjugated hyperbilirubinemia can be attributed to diverse factors, including TORCH infections (toxoplasmosis, rubella virus, CMV, and herpes simplex), genetic disorders (such as Alagille syndrome, α1-antitrypsin deficiency, and cystic fibrosis), metabolic disorders (like tyrosinemia, galactosemia, hypothyroidism, and inborn errors of bile acid metabolism), choleduct cyst obstruction, and spontaneous bile duct perforation.

CONCLUSION

In conclusion, this study reveals that age at first time visiting specialist, birth weight, gestational age, and domicile are all significantly linked to the incidence of biliary atresia. However, no substantial relationship was found between sex and the serology test results for anti-CMV, anti-rubella, and anti-toxoplasma antibodies and the occurrence of biliary atresia in children with cholestasis. Future research endeavors on a larger scale, incorporating the analysis of additional risk factors, are necessary to enhance the data validation and comprehensiveness of the findings in this study.

What is already known on this topic. BA is associated with pre- and post-natal as well as the environmental factor risks. Limited researches are about BA and BA in cholestasis children in Indonesia.

What this study adds. BA case in cholestasis infants is lower than non-BA one. This study confirms that age at first time visiting specialist, gestational age, birth weight, and domicile are strongly linked to BA. Normal birth weight compromises 7.4 times folds developing non-BA cholestasis.

Acknowledgements: we are grateful to the "Dr. Soetomo" General Academic Hospital, Airlangga University for supporting research. Their generous funding allowed us to conduct our study and complete our work.

Authors' contributions: all authors listed have made a substantial, direct, and intellectual contribution to the work and approved the final manuscript for publication.

Conflict of interest: the authors declare no conflict of interests

Financial support: none declared

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