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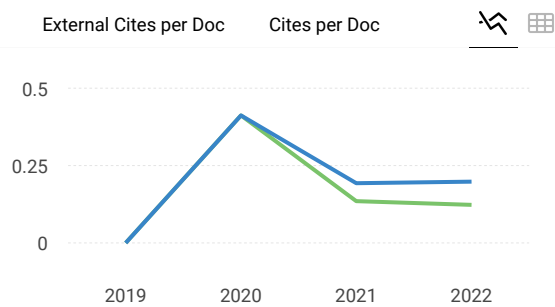
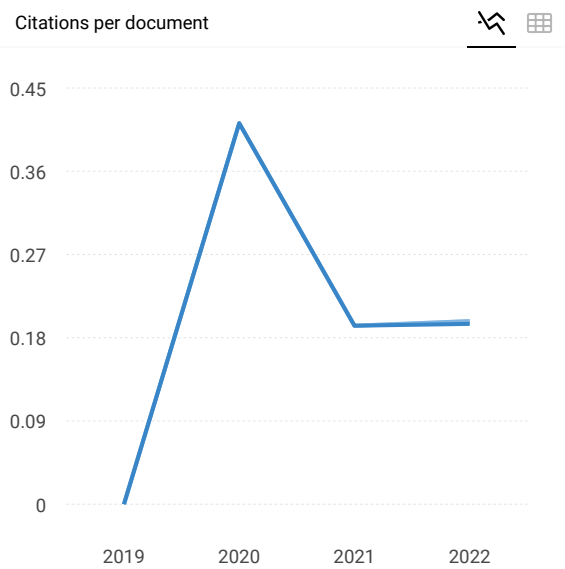
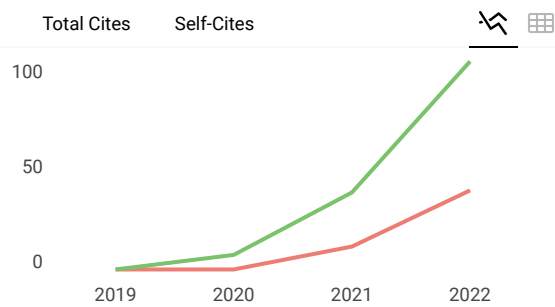
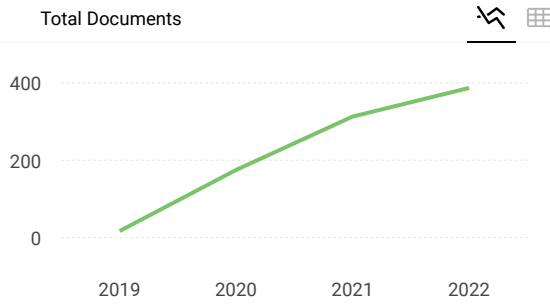
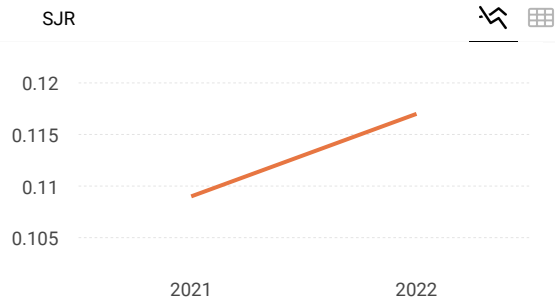
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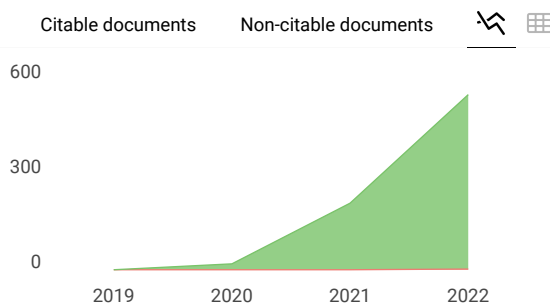
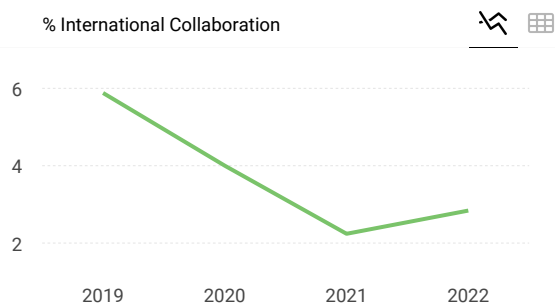
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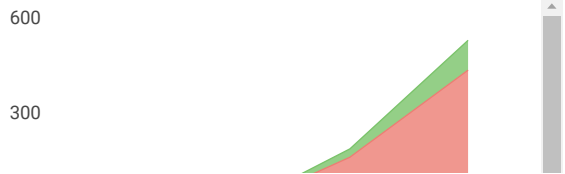
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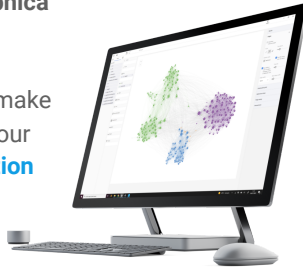
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Accuracy of 2-phase abdominal ultrasound for diagnosing biliary atresia



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Background: Biliary atresia is a dangerous neonatal disorder characterized by an inflammatory and fibrotic obliteration of the extrahepatic biliary tree, which results in progressive hepatic failure and, if ignored, death from end-stage liver disease. Due to clinical similarities that distinguish biliary atresia from other causes of neonatal cholestasis, early identification is often challenging. Ultrasound is recommended in screening for infantile cholestasis because it is inexpensive, non-invasive, safe from radiation and does not require anesthesia. It is hoped that abdominal ultrasound can be an alternative for diagnosing biliary atresia. This observational design analyzes abdominal ultrasound diagnostic tests for biliary atresia.

Method: This study uses medical data records of children who were diagnosed with biliary atresia and underwent liver biopsy and 2-phase abdominal ultrasound. Statistical analyses using the Kruskal-Wallis and Mann-Whitney U-test. The chi-square test was employed in qualitative data to establish the significance of differences between groups. The results were considered significant if the p-value was less than 0.05. Diagnostic performance was measured using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results: With a liver biopsy investigation, there were 89 participants with biliary atresia and 71 subjects without. Abdominal ultrasonography had a sensitivity of 77.5%, a specificity of 69%, a positive predictive value of 78.4%, a negative predictive value of 71%, and an accuracy of 73.7%.

Conclusion: Two-phase abdominal ultrasonography can help diagnose biliary atresia early.

Keywords: Cholestasis, ultrasonography, liver biopsy, screening.

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INTRODUCTION

Biliary atresia is a severe birth disorder that leads to progressive hepatic failure and, if left untreated, death from end-stage liver disease. One in every 8,000 to 18,000 live births is affected by biliary atresia, which is more common in Asians and Africans than in Europeans.¹ The major therapy for biliary atresia is portoenterostomy, and preoperative diagnosis of portoenterostomy for biliary atresia is required early and accurately because portoenterostomy 90 days after birth has a better life expectancy.^{2,3} The timing and precision of biliary atresia diagnosis are connected to longer age (> 30 days) and more extensive fibrosis, increasing the requirement for liver transplantation.⁴ Portoenterostomy had a 10-year survival advantage in newborns in Canada and France at 30 days of age (49% and 48%) than those operated on at >90 days (15% and 26%).^{5,6}

The diagnosis of biliary atresia in infants is a global problem. Early clinical indications of biliary atresia are difficult to distinguish from those of infant cholestasis, such as hepatitis, making early detection challenging.⁷ If clinical symptoms of jaundice are present at 2 weeks of age, the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) suggests testing neonates for cholestasis.⁸ Although intraoperative cholangiography is the gold standard for detecting biliary atresia, liver biopsy is the most accurate method, with a 98% sensitivity and 93% specificity.^{9,10} However, a liver biopsy is an intrusive, very expensive, and potentially dangerous procedure.¹¹

Many new diagnostic techniques for biliary atresia have been discovered, including abdominal ultrasound, liver biopsy, scintigraphy, and magnetic resonance cholangiopancreatography.¹¹ Abdominal ultrasonography is advised

as the initial step in screening since it is inexpensive, non-invasive, radiation-free, and does not require anesthesia.³ When performed by a qualified radiologist, ultrasonography alone can reveal biliary atresia.¹² The triangular cord sign is a specific finding on ultrasound that reflects fibrotic tissue in the porta hepatis in biliary atresia, with a sensitivity of 95% and a specificity of 89%, and abnormal gallbladder morphology is the most significant predictor, with a sensitivity of 85% and a specificity of 94%.^{3,11,12}

To identify biliary atresia from neonatal hepatitis, abdominal ultrasonography in biliary atresia is used to focus on measuring gallbladder length, contractility, or both in the fasted infant. Biliary atresia is assumed to be a tiny or undetectable gallbladder, but this can also be found in newborn hepatitis.¹³ This observational study design analyzes abdominal ultrasound diagnostic tests for biliary atresia. It is envisaged that 2-phase abdominal ultrasonography will

be used to diagnose biliary atresia.

METHODS

Study population

This retrospective analysis included infants with cholestasis who were treated at the Department of Child Health at Dr. Soetomo General Academic Hospital in Surabaya, Indonesia, from January 2011 to January 2021. Only 160 of 993 children with cholestasis met inclusion (underwent liver biopsy and abdominal ultrasonography) and exclusion criteria (incomplete medical record). The newborns were separated into two groups based on their final diagnosis: 89 with biliary atresia and 71 without.

Liver biopsy and histopathological evaluation

Liver biopsies were performed on all patients in both the BA and non-BA groups. For liver biopsies, true-cut needles were employed. Biopsy specimens were formalin-fixed and embedded in paraffin before being stained with hematoxylin-eosin, Masson's trichrome, reticulin, and Perl's stains. Bile duct proliferation,

bile obstruction, multinuclear giant cells, inflammatory cell infiltration, liver parenchymal necrosis, portal edema, and the absence of sinusoid fibrosis were all seen in a liver biopsy for biliary atresia.

Abdominal ultrasound

Ultrasound Philips 5-12MHz was used for 2-phase abdominal ultrasound. All patients were fasted for at least 4 hours prior to assessment, and their GB contractility was assessed 30 minutes after feeding. The parameters evaluated included gallbladder abnormalities (irregular wall, wall thickness, and GB length), TC sign (thickness greater than 4 mm), and hepatic subcapsular flow.

Statistical analysis

The descriptive data were presented as a median or as a number (%). The Kruskal-Wallis non-parametric test was employed for quantitative data to establish statistical significance among all groups. The Mann-Whitney U-test was employed to establish significance between individual groups when the Kruskal-Wallis test showed

significance. The chi-square test was employed in qualitative data to establish the significance of differences between groups. The results were considered significant if the p-value was less than 0.05. Diagnostic performance was measured using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The Statistical Package for the Social Sciences application (SPSS 19.0) was utilized for statistical analysis.

RESULTS

The number of infants with cholestasis from January 2011 to January 2021 was 939 infants. Two hundred forty-two infants were included (had liver biopsy and abdominal ultrasound) and 82 infants were excluded because of incomplete medical records. One hundred sixty infants were divided into the biliary atresia group (89 infants) and the non-biliary group (71 infants). Clinical, laboratory, abdominal ultrasound, and histopathological data of the study groups are shown in [Table 1](#).

With a median of 119 days, the

Table 1. Clinical, laboratory, abdominal ultrasound and histopathological data of the studied groups

Characteristic	Biliary atresia		Total (n=160)	p-value*
	Yes (n=89)	No (n=71)		
Age (%)				0.133
≤ 60 days	8	12	20	
>60 days	81	59	140	
Median (days)			119 (37-943)	
Gender (%)				0.039
Boys	38(43.2)	43(59.7)	81(50.7)	
Girls	51(56.8)	28(40.3)	79(49.3)	
Clinical				
Yellowish skin	89(100)	71(100)	160(100)	-
Clay stool	85(95.5)	22(31)	107(66.9)	0.001
Dark urine	47(52.8)	16(22.5)	63(39.4)	0.001
Hepatomegaly	69(77.5)	26(36.6)	95(59.4)	0.001
Laboratory				
Conjugated bilirubin (mg/dL)	9.6 (0.17- 26.05)	7.15 (1.36-22.5)	8.85 (0.17-26.05)	0.103
Total bilirubin (mg/dL)	12.5 (1.8-55)	8.8 (2.3-32.38)	11.1 (2.3-55)	0.872
AST (μ/L)	210 (10-589)	174 (25-768)	197 (10-768)	0.05
ALT (μ/L)	161 (9-1042)	132 (11-741)	143.5 (9-1042)	0.212
Abdominal ultrasound				
Triangular cord sign	34 (38.2)	10(14.1)	44(27.5)	0.001
Abnormal gall bladder	61 (68.5)	29(40.8)	90(56.3)	0.001
Hepatic subscapular flow	32 (36)	11(15.5)	43(26.9)	0.004
Histopathological				
Ductal proliferation	82(92.1)	9(12.7)	91(56.9)	0.001
Fibrosis	83(93.3)	18(25.4)	101(63.1)	0.001

*mann whitney u test

majority of babies come at more than 60 days of age, and girls outweigh boys in the BA group. The BA group had significantly higher amounts of clay stool, dark urine, hepatomegaly, the TC sign, an abnormal gall bladder, hepatic subscapular flow, ductular proliferation, and fibrosis in liver biopsy, and aspartate aminotransferase (AST). The non-hepatic control group, on the other hand, had all yellowish skin. Conjugated bilirubin, total bilirubin, and alanine aminotransferase (ALT) levels were not substantially different (Table 1). According to the study's findings, when compared to the gold standard of liver biopsy testing, abdomen ultrasonography had a sensitivity of 77.5%, a specificity of 69%, a positive predictive value of 78.4%, a negative predictive value of 71%, and an accuracy of 73.7%.

DISCUSSION

Age is important for the prognosis of biliary atresia. Early detection and referral are very influential on the outcome. The age (> 30 days) and extent of fibrosis are associated with the timing and accuracy of biliary atresia diagnosis, increasing the need for liver transplantation.¹ A portoenterostomy performed 90 days after birth prolongs life.^{2,3} A portoenterostomy done within the first 60 days of life resulted in a 70% increase in bile flow, whereas a portoenterostomy performed beyond 90 days resulted in a 30% bile flow, bile flow is only < 25%.¹⁴ In this study, 51 infants (31.8%) came to the hospital for the first time at the age of ≤ 60 days and 109 infants (68.2%) were aged > 60 days with a median of 155 days. In developing countries, 55% of patients come at more than 120 days old.¹⁵ There were more infants with biliary atresia in girls than boys. This is in accordance with research in the United States, which states that baby girls are at risk of experiencing biliary atresia by 1.43 times compared to baby boys.¹⁶

Symptoms found in infants with biliary atresia are related to bile flow disturbances such as yellowish skin, pale stools, dark urine and enlarged liver.^{17,18} Clay stool color was found in 95.5% of infants with biliary atresia in this study ($p=0.001$). Clay stool color had a sensitivity of 92.5% and a specificity of 55.6%, while non-biliary atresia had a sensitivity of 44.4%.¹⁹ Dark

urine was reported by 52.8% of infants with biliary atresia and 22.5% of infants without ($p=0.001$). Liver enlargement occurred in 69 children (77.5%) with biliary atresia ($p=0.001$). On palpation of the abdomen, biliary atresia is suspected when hard hepatomegaly is felt, generally in the left or middle lobe.¹⁴

Bilirubin in the urine is a key feature in biliary atresia, but it is not specific.¹⁷ Conjugated bilirubin in biliary atresia of 9.6 mg/dL was higher than non-biliary atresia of 7.15 mg/dL. According to prior investigations, conjugated bilirubin has a 100% sensitivity, a 99.9% specificity, a 5.9% positive predictive value, and a 100% negative predictive value.²⁰ The level of AST is higher in biliary atresia than in non-biliary atresia ($p=0.05$). Sensitivity was 84%, specificity was 97%, positive predictive value was 62.5%, and negative predictive value was 79.3%.¹⁰

There is no initial diagnostic test for biliary atresia that is 100% accurate, making it difficult to diagnose biliary atresia within the first two months of life. A liver biopsy is a diagnostic test that is used to diagnose neonates with cholestasis and can be used to make a prelaparotomy diagnosis of biliary blockage. A liver biopsy had a sensitivity of 98%, a specificity of 93%, a positive predictive value of 93%, and a negative predictive value of 98%.¹⁰ This study discovered ductal proliferation of 92.1% and fibrosis of 93.3% in liver samples from babies with biliary atresia. Histological features of biliary atresia include ductal proliferation (96.7% sensitivity, 86.7% specificity, and 91.7% accuracy), bile plug (96.7% sensitivity, 63.3% specificity, and 80% accuracy), portal fibrosis, giant cell transformation (sensitivity 73.3%, specificity 86.7% and accuracy 80%).²¹

Abdominal ultrasound has long been regarded as an important diagnostic technique for biliary atresia. On abdominal ultrasound, the triangle cord sign, gallbladder length, and gallbladder contractility can contribute to diagnosing biliary atresia.¹⁹ The abdominal ultrasonography exhibited a sensitivity of 77.5%, specificity of 69%, positive predictive value of 78.4%, negative predictive value of 71%, and accuracy of 73.7% in this study. There was a triangular

cord sign in 38.2% of patients ($p=0.001$), an abnormal gall bladder in 68.5% ($p=0.001$), and hepatic subscapular flow in 36% ($p=0.004$). When a triangular cord sign (sensitivity 73%, specificity 100%), wall (sensitivity 91%, specificity 95%) and shape (sensitivity 70%, specificity 100%), abnormal gallbladder, no gallbladder found (sensitivity 93%, specificity 92%) were found, abdominal ultrasound accuracy was 98.3% in children with biliary atresia.²² Abdominal ultrasonography in conjunction with other tests such as pale stool color/scintigraphy/MRCP may be the best and primary choice of examination.¹⁰ The triangular cord sign in conjunction with gallbladder anomalies has a sensitivity of 95% and a specificity of 89%, whereas no gallbladder has a specificity of 99%.¹¹

The triangular cord sign is a particularly characteristic finding in biliary atresia. In Japan, abdominal ultrasonography was used to diagnose biliary atresia in 85 neonates with cholestasis if a triangle cord sign of 3 mm, gallbladder length of 15 mm, and gallbladder contractility of 68% (12 weeks) or 25% (12 weeks) was noted. The triangle cord sign is 95% specific, and gallbladder contractility is 87% sensitive. The positive predictive value of the triangular cord sign when combined with gallbladder problems (low gallbladder contractility or gallbladder length 15 mm or both) is 98%. When paired with a normal gallbladder (normal gallbladder length and normal gallbladder contractility), the triangular cord sign has a 100% negative predictive value.¹²

The gallbladder is not seen on abdominal ultrasonography or there is an irregularity in the gallbladder wall or the shape of the gallbladder wall, with a sensitivity of 95.7%, specificity of 89.3%, and accuracy of 92.2%.²³ The sensitivity and specificity of hepatic subscapular flow on abdominal ultrasonography are 100% and 86%, respectively.²³ An abdomen ultrasound examination exhibited a sensitivity of 100%, a specificity of 94.4%, and an accuracy of 97%, according to Lee et al. When a triangle cord sign (cut off 3.4mm) and gallbladder anomalies are discovered, abdominal ultrasonography is utilized to identify biliary atresia.³ A study of 188 children with cholestasis who had

a 2-phase abdominal ultrasonography while fasting (for 4 hours) and not fasting (for 4 hours) found that when fasting, the accuracy of the diagnosis of biliary atresia was 86.3% and 93.5% when not fasting. Abdominal ultrasonography has an accuracy of 88.7% when conducted by a junior radiologist and 90.4% when performed by a consultant radiologist to diagnose biliary atresia.²⁴⁻²⁷

The weakness of this study is that the data is retrospective, prone to bias and subjectivity due to abdominal ultrasound operators and not all infants with cholestasis in the first 2 months are examined by abdominal ultrasound. Although this study has many weaknesses, it provides important information regarding the performance of abdominal ultrasound which can be used as an alternative examination for diagnosing biliary atresia. Furthermore, the researchers offered data on the characteristics of abdominal ultrasound in newborns with biliary atresia, which might be used as a starting point for future research.

CONCLUSION

Abdominal ultrasound is a non-invasive examination and the results obtained are faster than liver biopsies, so it is expected to be an alternative examination for the early diagnosis of biliary atresia.

AUTHOR CONTRIBUTION

All authors have contributed to this research process, including conception and design, analysis and interpretation of the data, article drafting, critical revision of the article for important intellectual content, and final approval.

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CONFLICT OF INTEREST

The author declared that there is no conflict of interest in the research and writing of the publication. The main researcher will use this publication as a graduation requirement in the Specialist Medical Education Program as a pediatrician

at the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

ETHICAL CLEARANCE

Research ethics were obtained from the Health Research Ethics Committee of the Clinical Research Unit (CRU) at Dr. Soetomo General Hospital, Surabaya, and a letter of exemption Ref. No.: 0330/LOE/301.4.2/ II/2021.

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