

## RESEARCH ARTICLE

## Correlation between Length-to-width Ratio of Gallbladder and Gamma-glutamyl Transferase Value in Biliary Atresia

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### Abstract

Biliary atresia represents one of the most prevalent etiologies for neonatal cholestasis. Unmanaged biliary atresia can be fatal. Ultrasonography is the primary diagnostic test because it's accurate, cost-effective, and available. Various ultrasound findings can assist in diagnosing biliary atresia; the length-to-width ratio of the gallbladder is a particularly advantageous method, offering a short examination time, objectivity, and ease of use, with an accuracy rate of 78.9%. In cases with unconventional ultrasound findings, gamma-glutamyl transferase is believed to be able to complete the diagnostic process with an accuracy rate of up to 80%. The optimal cut-off value differs between studies, making it challenging to use as a benchmark for biliary atresia detection. In this study, researchers aim to further investigate the relationship between length-to-width ratio and gamma-glutamyl transferase in cases of biliary atresia, compared to the liver biopsy results in these patients and the optimal cut-off. This study employed an observational analytic approach with a retrospective design. The sample population consisted of all patients with neonatal cholestasis who underwent laboratory and ultrasonographic examinations at Dr. Soetomo Academic General Hospital Surabaya between 2019 and 2023. The study population comprised 82 patients. A significant relationship ( $p$ -value $<0.001$ ) was observed between the length-to-width ratio of the gallbladder and biliary atresia, as well as between gamma-glutamyl transferase and biliary atresia (area under the curve: 0.7–0.8). However, the analysis between the length-to-width ratio of the gallbladder and the value of gamma-glutamyl transferase showed  $p$ -value=0.066, which means no significant relationship was observed between the length-to-width ratio and gamma-glutamyl transferase.

**Keywords:** Biliary atresia, gamma-glutamyl transferase, length-to-width ratio, ultrasound

### Introduction

The reported incidence of biliary atresia worldwide varies from 1 in 8,000 to 19,000 live births, with females outnumbering males (1.4:1), with the highest incidence in East Asia. The reason for this high incidence remains unexplained. While epidemiological data on biliary atresia in Indonesia is not yet widely available, it is the most common cause of obstructive cholestasis identified in the first three months of life. Furthermore, in advanced stages, it can contribute to neonatal mortality. Consequently, biliary atresia is often considered physiological neonatal jaundice in its early phase.<sup>1–3</sup>

The diagnosis of biliary atresia is often based on clinical judgment, as there is no specific examination. Laboratory and imaging findings

frequently overlap with other etiologies of cholestasis. In selecting imaging modalities, some studies state that ultrasonography (USG) is the modality of choice to diagnose biliary atresia and the most common non-invasive examination performed in preparation for further action.<sup>4,5</sup>

A study conducted in China obtained a comparison of triangular cord signs with gallbladder classification in biliary atresia patients; the accuracy of gallbladder classification was 10% higher when compared to triangular cord signs. Meanwhile, using the gallbladder's length-to-width ratio (LTWR), a high diagnostic value was obtained with an area under the curve of 0.844.<sup>6</sup> In contrast to the previous study conducted at Dr. Soetomo Academic General Hospital, there was no significant relationship between gallbladder wall echogenicity or gallbladder contractility on

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Received: 4 September 2024; Revised: 12 December 2024; Accepted: 23 December 2024; Published: 28 December 2024

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liver biopsy results.<sup>7</sup>

Several studies have been conducted to detect biliary atresia early, but many aspects still need to be studied. In practice, ultrasound examination in children requires conducive conditions and a short implementation time. A multidisciplinary approach is expected to make the diagnosis more accurate.<sup>8</sup>

Another indicator often used is the gamma-glutamyl transferase (GGT) value, which is believed to significantly improve the accuracy of biliary atresia diagnosis. In a previous study, a sensitivity of 85% with a specificity of 88% was obtained,<sup>9</sup> and in another study, the sensitivity was 86.7% and specificity 65%.<sup>10</sup> However, the optimal cut-off value differed from each study, making it difficult to adapt as a benchmark for early detection of biliary atresia.

Limited literature explicitly mentions the relationship between gallbladder LTWR and GGT values in patients with biliary atresia. The researcher is interested in continuing research on this relationship, which will be compared with the liver biopsy results in these patients.

This analysis is expected to provide new insights into the correlation of LTWR and GGT values in patients with biliary atresia. Additionally, it furnishes data regarding the newly established cut-off value of length and the length-to-width ratio for biliary atresia. With a better understanding of this relationship, early detection can be done appropriately so that the treatment and management of patients with biliary atresia can be further improved and more effective radiology services can be provided. This study aims to investigate further the relationship between length-to-width ratio and gamma-glutamyl transferase in cases of biliary atresia, comparing the liver biopsy results in these patients and the optimal cut-off.

## Methods

This study was an observational analytic study with a retrospective approach. The sample population consisted of patients with neonatal cholestasis who underwent laboratory and ultrasound examinations at Dr. Soetomo Academic General Hospital Surabaya. The Health Research Ethics Committee of Dr. Soetomo Academic General Hospital has approved the procedure with a letter of exemption registration number 1670/

LOE/301.4.2/V/2024. Data were collected from all patients diagnosed with cholestasis based on clinical patients who had undergone laboratory examination, ultrasonography, and liver biopsy from January 2019 to December 2023 with accessible medical record data. Laboratory results must have a difference of less than 30 days from the ultrasound examination to reduce the bias factor in this study. The final diagnosis was obtained from histopathological results or final diagnosis data based on clinician agreement seen from medical record data.

Researchers identified patients with neonatal cholestasis who had undergone ultrasound examination and then re-evaluated the ultrasound findings by researchers under the supervision and guidance of 2 pediatric radiology consultants and compared them with laboratory and histopathological results of liver biopsy from medical record data. The inter-observer agreement was evaluated with Cohen's kappa coefficient test, with a significance level of  $p < 0.05$ . An agreement value of 0.8–0.9 was obtained. Subsequently, the between variables were compared to identify the significance value. The results were considered significant if the  $p$ -value was  $< 0.05$  with a 95% confidence interval (CI). The data were analyzed using SPSS 23 statistical software.

The data obtained were then analyzed and presented as research results. The cut-off used in this study, LTWR  $> 5.2$  and GGT value  $> 188$ , follows the prior study conducted by Wang et al.<sup>6</sup> and Zhou and Zhou<sup>11</sup> in China. Relevant guidelines and regulations are carried out in all methods.

## Results

The sample was 82 patients, with 49 males (59.8%) and 33 females (38.4%). The final diagnosis of biliary atresia was more prevalent in males, with 23 males (56.1%) and 18 females (43.9%). The age group of  $\leq 30$  days represented the smallest sample size, comprising only two individuals (2.4%), whereas the age group of  $\geq 121$  days had the largest sample size, with 28 individuals (34.2%). The age group of  $\geq 121$  days also had the highest prevalence of biliary atresia, accounting for 39% of the cases. The distribution of samples according to age group is presented in Table 1.

**Table 1 Age Distribution**

Range of Age (Days)	Diagnosis		Total n=82 (%)
	Biliary Atresia n=41 (%)	Non-biliary Atresia n=41 (%)	
≤30	0 (0)	2 (4.9)	2 (2.4)
31–60	8 (19.5)	10 (24.4)	18 (22.0)
61–90	11 (26.9)	12 (29.2)	23 (28.0)
91–120	6 (14.6)	5 (12.2)	11 (13.4)
≥121	16 (39.0)	12 (29.3)	28 (34.2)

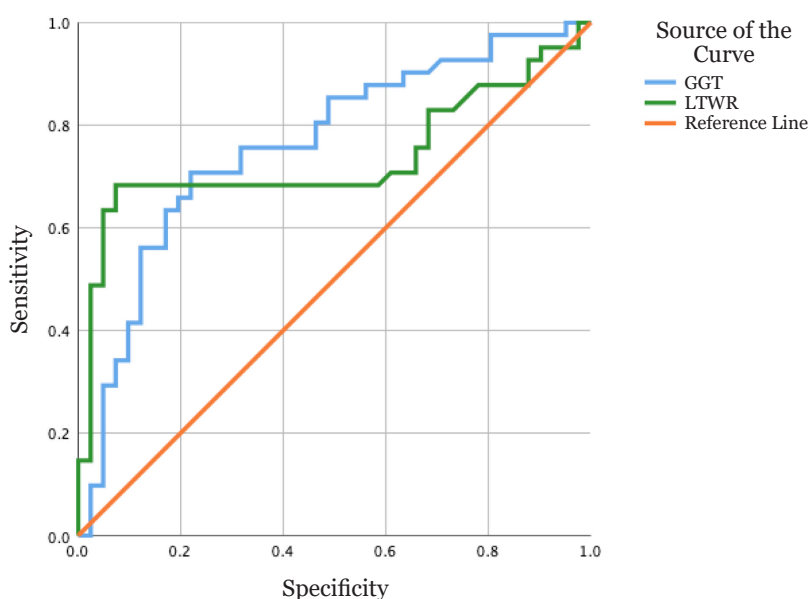
**Table 2 LTWR and GGT Data**

	Abnormality	Median	Minimum	Maximum
LTWR	<0.05	4.345	1.17	21.31
GGT	<0.05	369.550	12.80	3,964.20

Length-to-width ratio (LTWR) data were obtained from the results of reviewing the abdominal ultrasonography results performed by researchers under the supervision of 2 pediatric consultant radiology specialists with the results of the agreement test (kappa) on significant gallbladder length-to-width ratio data with a strength >0.9 and abnormal data distribution so that the data characteristics were grouped by median (4.34), minimum (1.17) and maximum (21.31). Gamma-glutamyl transferase (GGT) data was obtained from medical records with

abnormal data distribution results, so the data characteristics are grouped by median (369.55), minimum (12.8), and maximum (3,964.20). Data on LTWR and GGT is presented in Table 2.

Data analysis was performed by examining the relationship of each variable to biliary atresia, calculations using 95% confidence intervals and p-values were considered significant if  $p < 0.05$ , LTWR and GGT have a  $p\text{-value} < 0.001$  which means there is a very substantial relationship with biliary atresia. Then, the ROC curve was made, the area under the curve was measured, and the



**Figure 1 ROC Curve**

study cut off with a 95% confidence interval, as presented in Figure 1. The cut-off value of length to width ratio of gallbladder was >4.75, and the cut-off value of GGT was >390.

Table 3 illustrates that diagnostic tests conducted for the two variables, compared with histopathological results, demonstrated that LTWR of the gallbladder exhibited the highest accuracy among the variables, at 78.04%. Upon applying the research cut-off (>4.75), this accuracy value increased to 80.48%.

The results of the analysis of the relationship between the gallbladder's LTWR and the value of GGT in biliary atresia showed a value of  $p=0.066$  ( $p>0.05$ ) with a correlation coefficient of 0.204, which means there is no significant relationship between the LTWR and the value of GGT (Table 4).

**Discussion**

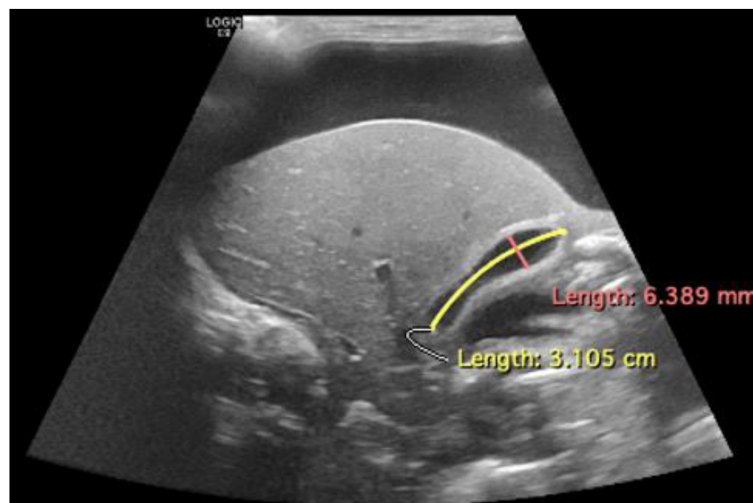
Females are more common in cases of biliary atresia, and in this study, biliary atresia data were more dominant in men (56.1%) than in women (43.9%). This aligns with previous research conducted at Dr. Soetomo Academic General Hospital, where there was more male sample than female.<sup>7</sup> However, the previous literature did not explain the relationship between gender and biliary atresia. Epidemiologically, biliary atresia can be found in both males and females, with a probability percentage that is not very different. Still, sometimes, there is a gap in the prevalence of men and women; in general, of all congenital abnormalities that have been reported, men dominate the majority. In biliary atresia,

**Table 3 AUC between Literature and This Study Cut-off**

	AUC	Significance	Literature Cut-off	Study Cut-off
LTWR	0.735	<0.001	≥5.2	>4.75
GGT	0.761	<0.001	>188	>390

**Table 4 Diagnostic Performance of LTWR and GGT in the Determination of Biliary Atresia**

	Sensitivity	Specificity	PPV	NPV	Accuracy	OR
LTWR ≥5.2	60.97%	95.12%	92.59%	70.90%	78.04%	30.469
LTWR >4.75	68.29%	92.68%	90.32%	74.51%	80.48%	27.282
GGT >188	85.36%	51.22%	63.63%	77.77%	68.29%	6.125
GGT >390	70.73%	78.04%	76.31%	72.72%	74.39%	8.593



**Figure 2 Gallbladder LTWR Measurement Example**

the opposite is true. There is a theory that biliary atresia is an immune-mediated disease, as the frequency of human leukocyte antigen (HLA) B12 in atresia patients increases compared to cases. Epidemiologically, women are more likely to develop immune-mediated conditions compared to men.<sup>12,13</sup>

This study divides age into five age range groups, namely  $\leq 30$  days, 31–60 days, 61–90 days, 91–120 days, and  $\geq 120$  days. Most samples were in the age range  $\geq 120$  days, with 28 patients divided into 16 biliary atresia diagnoses and 12 non-biliary atresia diagnoses. This could be due to low awareness of biliary atresia and early diagnosis that cannot be done in primary health facilities. Therefore, referred patients to Dr. Soetomo Academic General Hospital are advanced patients. It is recommended that patients with suspicion of biliary atresia should be diagnosed at the age of  $< 60$  days because the age of recommendation for determining operative action in patients with biliary atresia is optimal in the age range of  $< 60$ –90 days. This limitation is related to the success rate of post-treatment in biliary atresia cases. If there are findings that lead to cirrhosis, then operative action should ideally be carried out in the age range of 30–60 days; supporting examinations have a role in helping to establish a definitive diagnosis so that the principle of early diagnosis enforcement is prioritized.<sup>3,5,14</sup>

Abdominal ultrasound examinations routinely performed on neonatal cholestasis patients at Dr. Soetomo Academic General Hospital include triangular cord sign, subcapsular hepatic flow, and the ratio of the diameter of the hepatic artery to the portal vein, but in this study, the researchers added the variable length to width ratio of the gallbladder. The data processing results are presented alongside the results of the LTWR of the gallbladder, which was found to have a highly significant relationship ( $p < 0.001$ ) with biliary atresia. The above findings are similar to research conducted by Choochuen et al.,<sup>15</sup> showing the highest sensitivity in the length-to-width ratio of the gallbladder (71.7%) and the highest specificity in the triangular cord sign (95.9%). Other studies conducted by Zhang et al.<sup>8</sup> showed the superiority of the length-to-width ratio of the gallbladder. Although there is a slight difference in the results of data analysis, this can be caused by differences in the age distribution of the sample and the

length-to-width ratio measurement technique. This study measured the outer to outer wall, as shown in Figure 2. In contrast, in Choochuen et al.,<sup>15</sup> measurements were made on the inner to inner wall.

LTWR of the gallbladder also offers a higher specificity value when compared to the triangular cord sign examination, so the researchers feel that at least LTWR of the gallbladder can complement the shortcomings of the triangular cord sign in the early detection of patients with suspected biliary atresia.

The literature agrees with a cut-off value for gallbladder LTWR of  $> 5.2.6$ ,<sup>11</sup> However, the researchers redetermined with a new cut-off result of  $> 4.75$ . As shown in Table 4, there is an increase in sensitivity (68.29%) and negative predictive value (74.51%), which causes an increase in accuracy by 2%. Similar research by Zhang et al.<sup>8</sup> also redetermined the cut-off value with a sensitivity value (78.9%) and specificity (66.7%). The appearance of different cut-off values in this study can be caused by the process of ultrasound examination at Dr. Soetomo Academic General Hospital running simultaneously with other non-biliary atresia patients, so there is likely an additional fasting period of patients in this study with patients in other studies.<sup>8,15</sup>

This study analyzed the relationship between GGT in biliary atresia using a cut-off of  $> 188$  with sensitivity (85.36%), specificity (51.22%), PPV (63.63%), NPV (77.77%) and accuracy of 68.29% then recalculated the GGT cut-off with results  $> 390$ , this recalculation caused an increase in specificity (78.04%) and positive predictive value (76.31%) which resulted in an increase in accuracy by 6% (74.39%).

The discrepancy in these findings may be attributed to variations in the demographic composition of the study sample relative to other studies. The current study's sample population was predominantly comprised of individuals within the age range of  $\geq 121$  days. In contrast, other studies conducted by Wang et al.<sup>6</sup> utilized a sample population within the age range of  $< 30$  days. In a survey conducted by Sun et al.,<sup>9</sup> 1,273 children were included in the analysis. The researchers utilized a cut-off value of  $> 300$  for biliary atresia patients. Despite discrepancies in the selected cut-off values, the diagnostic efficacy of GGT on the receiver operating characteristic (ROC) curve exhibited a comparable AUC value

range of 0.7-0.8, consistent with this study's findings.

The results of the data analysis indicate that there is no statistically significant correlation between the gallbladder's LTWR and GGT value. This finding suggests that an increase in LTWR does not necessarily correspond with a proportional increase or decrease in GGT.

GGT is agreed to be one of the potential diagnostic parameters for biliary atresia and suspicion of hepatotoxic conditions associated with liver injury and oxidative stress.<sup>16</sup> However, like other biomarkers, the value of GGT can be influenced by various circumstances, for example, the age range,<sup>17,18</sup> because in this study, the age of the sample was dominated in the age range >121 days, while in the studies conducted by Zhang et al.<sup>8</sup> the age of the sample was in the age range that had a significant relationship was the age group 31–90 days. As for the older age group in this study, it could be accompanied by the possibility of using ursodeoxycholic acid (UDCA), commonly applied to neonatal cholestasis patients.<sup>17</sup> Meanwhile, the researchers found that ultrasonographic findings were relatively more consistent when administering oral therapy. The diagnostic performance of LTWR and GGT value as single variables in assessing biliary atresia was statistically significant.<sup>19,20</sup> The combined use of biomarkers and ultrasound findings, especially LTWR and triangular cord signs, will undoubtedly increase the success rate in early diagnosis of biliary atresia.<sup>21</sup>

Additional research should be conducted using a prospective approach and a larger number of patients to obtain a more representative correlation. Further research could be carried out using age range group variables to ascertain the nature of the relationship between variables in greater detail. An additional avenue for research would be to employ a mixed methods approach, with agreed protocols and a multidisciplinary team, to obtain more representative samples and more homogeneous data.

## Conclusions

In this study, the gallbladder's LTWR has no significant relationship with the value of GGT, and the cut-off value has been recalculated to enhance the precision of each variable.

## Conflict of Interest

There is no ethical/legal conflict involved in the article. All authors have no relevant financial interest related to the material.

## Acknowledgment

Our gratitude goes to all Department of Radiology, Universitas Airlangga staff, and the Research and Development of Dr. Soetomo Academic General Hospital.

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