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FULL PAPER

Serum matrix metalloproteinase 7 (MMP-7) and liver function in biliary atresia

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Biliary atresia is one of the main factors leading to the need for liver transplantation in children. Currently, there is no non-invasive approach to diagnosing biliary atresia and differentiating it from other causes of cholestasis. This study aimed to evaluate matrix metalloproteinase 7 (MMP-7) levels as a diagnostic biliary atresia biomarker and its correlation with laboratory parameters. This cross-sectional study included infants with biliary atresia admitted to Dr. Soetomo General Academic Hospital, Surabaya. Blood samples, as well as baseline clinical and demographic data, were collected when admitted. MMP-7 levels were evaluated by enzyme-linked immunosorbent assay. There were 85 infants with biliary atresia, mean age 11 (7-35) weeks, 46 (54.1%) boys, with the onset of jaundice at 2 (1-20) weeks. The MMP-7 levels were 1.91 (0.39 - 9.95) ng/ml, Albumin 4.06 (1.41 - 4.79) g/dl, Aspartate aminotransferase (AST) 235.37 ± 130.48 U/L, , Alanine aminotransferase (ALT) 143.2 (30 - 641) U/L, Gamma-glutamyl transpeptidase (GGT) 361 (23.9-3746) U/L, direct bilirubin 8.75 ± 4.28 mg/dl, and total bilirubin 12.34 ± 6.36 mg/dl. MMP-7 showed a positive correlation with albumin levels (r=0.232; p=0.033), but correlated negatively with AST (r=-0.252; p=0.020) and ALT (r=-0.275; p=0.011) levels. Direct or total bilirubin, hemoglobin, leukocyte, platelet, and coagulation function levels were not associated with MMP-7 (p>0.05). There is a positive correlation between MMP-7 and albumin, but the relationship between MMP-7 and serum transaminases is reversed. MMP-7 may be used as a non-invasive diagnostic marker for biliary atresia.

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KEYWORDS

MMP-7; biliary atresia; albumin; transaminases enzyme.

Introduction

Biliary atresia is a progressive fibroinflammatory disease of bile ducts. It is a major cause of liver transplantation in children [1]. Biliary atresia presents with jaundice, hepatomegaly, and acholic stools [2]. The biliary atresia diagnosis can be made through supporting examinations of abdominal ultrasonography (USG), hepatobiliary scintigraphy, endoscopic retrograde cholangiopancreatography (ERCP), and liver

biopsy. Prompt diagnosis in biliary atresia is crucial, as a timely intervention with the Kasai operation yields the most favorable results in terms of averting the need for liver transplantation. Studies have found various probable causes of biliary atresia, such as genetic vulnerability, immune system involvement, and viral infections. These factors can lead to the blockage of the bile ducts outside the liver and the development of liver fibrosis [3].

Prolonged inflammation, with an immunological response, transformation of biliary epithelial cells into mesenchymal cells, deposition of the matrix, and uncontrolled formation of new blood vessels have been proposed as factors contributing to the development of liver fibrosis [4]. Routine biochemical tests are performed to evaluate the degree of cholestasis, the degree of hepatocellular injury, and the hepatocyte functions. However, there is no established noninvasive test to predict the diagnosis of biliary atresia.

Matrix metalloproteinases (MMPs) are a group of enzymes that are responsible for the degradation of components of the extracellular matrix (ECM) through the use of zinc-dependent catalysis. This enables cellular migration and tissue reorganization. MMPs are synthesized as zymogens, which are dormant forms that become activated upon being released from cells [2]. Previous studies have indicated that the serum MMP-7 assay is very sensitive and specific in differentiating biliary atresia from other forms of neonatal cholestasis. Several prior studies have explored the diagnostic potential of serum biomarkers in differentiating biliary atresia from other forms of neonatal cholestasis, with a particular focus on the role of matrix metalloproteinases, including MMP-7. For instance, research published in leading pediatric and gastroenterology journals has demonstrated the sensitivity and specificity of serum MMP-7 levels in identifying biliary atresia, suggesting that MMP-7 could serve as a promising non-invasive biomarker for this condition. The diagnostic sensitivity and specificity were determined to be 98.67% and 95.00%, respectively, with the negative predictive value found to be 98.28% [5]. Serum levels of MMP-7 have shown promise in indicating the seriousness of liver fibrosis and might be helpful in the identification of biliary atresia in infants suffering from cholestasis [6]. We hypothesize that serum MMP-7 levels can serve as a highly sensitive and specific non-invasive biomarker for distinguishing biliary atresia from other forms of neonatal cholestasis. In addition, we posit that variations in serum MMP-7 levels correlate with the severity of liver fibrosis in biliary atresia, thus offering a potential tool for assessing disease progression. The variability in MMP-7 serum levels across different populations and age groups necessitates the establishment of standardized reference ranges for accurately interpreting results. This study aimed to evaluate serum matrix metalloproteinase-7 levels as a diagnostic biomarker of biliary atresia and their association with laboratory parameters.

Experimental

Study design

This cross-sectional study included 85 infants with biliary atresia at Dr. Soetomo General Academic Hospital, Surabaya, from January 2022 to April 2023. This study included infants with biliary atresia aged one month to 12 months. Congenital abnormalities, infection, sepsis, and hemodynamic disorders were all the exclusion criteria.

All subjects underwent laboratory examination, abdominal ultrasonography, and liver biopsy. The diagnosis of biliary atresia is based on a combination of clinical signs (prolonged jaundice and acholic stools), physical examination (jaundice, hepatomegaly, and splenomegaly), laboratory tests (cholestasis), 2-phase abdominal

ultrasonography, and confirmed by the finding of luminal EHBD obstruction following liver biopsy.

Serum levels of routine blood count, bilirubin, albumin coagulation parameters, liver biomarkers such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Gamma-glutamyl transpeptidase (GGT) were analyzed. Serum MMP-7 concentration was measured by enzyme-linked immunosorbent assay according to the manufacturer's protocol. Serum MMP-7 concentration was measured by enzyme-linked immunosorbent assay according to the manufacturer's protocol. Blood samples are collected via venipuncture. The serum is separated from blood cells by centrifugation at approximately 2000-3000 g for 10 minutes at room temperature. The serum is aliquoted and stored at -80 °C until analysis to prevent degradation of proteins.

Ethical clearance

The study has obtained written ethical approval from the Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya. Before conducting the study, the parents were provided with all the necessary information, and their consent was obtained.

Statistical analysis

The data's normality was evaluated using the Kolmogorov-Smirnov tests. Statistical analysis used Chi-square or Fisher exact tests to evaluate qualitative variables. Spearman's rank correlation test was performed to analyze the associations among variables. The statistical analyses using the SPSS 25.0 software, with a p-value of less than 0.05 interpreted as statistically significant.

Results and discussion

A total of 85 infants with biliary atresia, 46 (54.1%) boys and 39 (49.5%) girls mean age 11 (7-35) weeks were included in this study. The onset of jaundice was 7 (1-33) weeks (Table 1).

In this study, the mean albumin levels in biliary atresia were 4.06 (1.41-4.79) g/dl, direct bilirubin 8.75 ± 4.28 mg/dl, and total bilirubin 12.34 ± 6.36 mg/dl. The level of GGT, AST, and ALT were 361 (23.9-3746) U/L, 235.37 ± 130.48 U/L, and 143.2 (30-641) U/L (Table 2).

TABLE 1 Basic characteristics of the subjects

Variable	n (%)
Sex	
Boys	46 (54.1 %)
Girls	39 (49.5 %)
	Median (Min-Max)
Age (weeks)	11 (7-35)
Duration of illness (week)	7 (4-33)
Onset of jaundice (weeks)	2 (1-20)

TABLE 2 Laboratory measurement of the subjects at the first presentation

Variable	Mean ± SD
WBC (10^3)	13.16 ± 5.35
Platelet (10^3)	410.78 ± 208.26
AST (U/L)	235.37 ± 130.48
	Median (Min-Max)
Hb (g/dl)	10.60 (3.40-16.80)
Albumin (g/dl)	4.06 (1.41-4.79)
Direct Bilirubin (mg/dl)	8.75 ± 4.28
Total Bilirubin (mg/dl)	12.34 ± 6.36
APTT	33.2 (11.4-70.6)

PPT	11.9 (9 – 121,4)
GGT (U/L)	361 (23.9-3746)
ALT (U/L)	143.2 (30-641)
MMP-7 (ng/mL)	1.91 (0.39-9.95)

WBC: White Blood Cell; AST: Aspartate aminotransferase; APTT: Activated partial thromboplastin time; PPT: Prothrombin time; GGT: Gamma-glutamyl transpeptidase; ALT: Alanine aminotransferase: MMP-7: Matrix metalloproteinase 7.

Table 3 indicates a positive correlation between MMP-7 levels and albumin levels (r=-.033; p=0.232). MMP-7 levels correlated negatively with AST (r = -0.252; p = 0.020) and ALT (r = -0.275; p = 0.011) levels.

TABLE 3 Correlation between MMP-7 and laboratory parameters

MMP-7	**Hb**	**WBC**	**Platelet**	**Albumin**	**Direct bilirubin**	**Total bilirubin**
r	0.198	0.086	0.776	0.232	-0.211	-0.196
p	0.069	0.435	0.776	0.033*	0.052	0.073
MMP-7	**APTT**	**PPT**	**GGT**	**AST**	**ALT**	
r	-0.140	-0.042	-0.166	-0.252	-0.275	
p	0.201	0.701	0.128	0.020*	0.011*	

Hb: Hemoglobin; WBC: White Blood Cell; MMP-7: Matrix metalloproteinase 7; APTT: Activated partial thromboplastin time; PPT: Prothrombin time; GGT: Gamma-glutamyl transpeptidase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

The exclusion of biliary atresia must be made early, as the consequences of failure to make this diagnosis are devastating. Cholestasis due to biliary atresia is clinically difficult to differentiate. It can be quite challenging to differentiate biliary atresia's clinical and biochemical characteristics from other etiologies of cholestasis [1]. However, clinical signs such as acholic stools may help to identify biliary atresia rapidly. Immediate referral for liver biopsy or intraoperative cholangiogram should be made [7]. Studies suggest that direct bilirubin levels of less than 2.5 mg/dL, a GGT of less than 150 U/L, and normal percutaneous cholangiogram results have been shown to have 100% sensitivity in excluding biliary atresia. In general, compared to clinical findings and abdominal ultrasound, liver biopsy has a sensitivity of 98% and specificity of 84% in diagnosing biliary atresia [8]. While early diagnosis and Kasai surgery are considered the most reliable indicators of outcome, it's important to note that these diagnostic methods can be invasive, may not be accessible in all medical facilities, and can be time-consuming. Therefore, markers are needed for early diagnosis and treatment [1].

Bilirubin levels should be checked in all infants with jaundice persisting for more than two weeks. In the case of biliary atresia, Kasai surgery is conducted within 45 days following birth to normalize the bile flow. However, the outcome of Kasai surgery is variable, and up to 80% of children still require liver transplantation after Kasai surgery [9]. Indications for liver transplantation in biliary atresia include failure of the Kasai operation, recurrent cholangitis, and complications such as portal hypertension, hepatopulmonary syndrome, and portopulmonary hypertension [10]. Increasing evidence suggests that viral infection triggers a proinflammatory response that injures biliary epithelium, leading to rapidly progressive cholangiopathy in biliary atresia [11].

By activating and inhibiting cytokines and chemokines, Matrix metalloproteinases (MMPs) have a crucial function in immunological responses, cellular regeneration, apoptosis, and the formation of new blood vessels (angiogenesis) [12,13]. Moreover, MMPs are also related to normal physiological processes such as trophoblast invasion and embryonic development [12].

MMP-7, or matrilysin-7, is secreted by biliary epithelial cells, Kupffer cells, and periportal hepatocytes [12]. The MMP-7 expression was found in hepatocytes located near the portal area, as well as in the proliferation of ducts and the epithelium of the bile ducts [14]. It is mainly expressed by cholangiocytes and in response to epithelial damage [15]. MMP-7 is most capable of degrading elastin in elastic tissues. MMP-7 can potentially promote hematogenous metastases in the portal veins that terminate in the liver [12]. MMP-7 plays a part in the Wnt/β-catenin signaling cascade, decreases cell-to-cell interaction by releasing E-cadherin, and promotes inflammation as well as fibrosis using osteopontin (OPN) and TNF-α [16].

Serum MMP-7 levels were 6-fold higher ($p < 0.001$) and positively correlated with the hepatic MMP-7 gene ($r = 0.548$, $p = 0.007$) [14]. Serum MMP-7 is a useful marker for diagnosing biliary atresia but failed to predict liver transplantation within one year in Japan [17]. Systematic review and meta-analysis studies have indicated that the MMP-7 marker is a valuable diagnostic tool for identifying biliary atresia in infants with cholestasis, however, it should not be used as a single test for diagnosing biliary atresia [18]. In this study, serum MMP-7 levels were 1.91 (0.39-9.95) ng/mL. The findings of this study are consistent with prior studies on MMP-7 in biliary atresia. A serum MMP-7 level higher than 1.43 ng/ml indicated the possibility of biliary atresia [6]. The sensitivity and specificity of MMP-7 for predicting biliary atresia were 96% (95% CI: 94-98%) and 91% (95% CI: 87-94%), respectively. The area under the curve of the MMP-7 level for the diagnosis of biliary atresia was 0.9847 [19].

MMP-7 may be a sensitive biomarker for predicting biliary atresia and liver fibrosis [5,20]. A previous study showed that GGT in biliary atresia <30 days was 834.2±475.3 IU/L, with the highest diagnostic value at 61-90 days and the lowest diagnostic value at ≥121 days [21]. MMP-7 could indicate an initial phase of damage to the bile ducts before the development of more serious blockages in the bile flow [22]. MMP-7 levels correlated with the stage of fibrosis in liver biopsies. However, combining serum MMP-7 with gamma-glutamyl transferase improves diagnostic accuracy compared to MMP-7 alone [23].

Conclusion

Serum MMP-7 showed a positive correlation with albumin but a negative correlation with transaminases. MMP-7 can be used to simplify the diagnostic algorithm to predict biliary atresia in infants with cholestasis. To advance the understanding and clinical utility of MMP-7 in diagnosing biliary atresia, future research should focus on several key areas. Firstly, large-scale, multicenter studies are needed to validate the findings on MMP-7's diagnostic accuracy across diverse populations and to establish universal reference ranges. Secondly, longitudinal studies should investigate the prognostic value of MMP-7 levels following therapeutic interventions such as the Kasai procedure or liver transplantation, assessing their correlation with long-term outcomes and disease progression. Prospective studies with larger sample sizes are needed to evaluate the accuracy of MMP-7 in predicting biliary atresia and prognostic markers for liver damage.

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Authors' Contributions

The author contributed to all research processes, including preparation, data

collection, data analysis, and approval for publication of this manuscript.

Conflict of Interest

The authors declare no competing interests related to this study.

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