Questioning Diagnostic Value of Serum Matrix Metalloproteinase 7 for Biliary Atresia

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Background: **Matrix metalloproteinase 7 (MMP7) has been suggested as a promising biomarker in diagnosing biliary atresia (BA). This study aimed to assess the diagnostic accuracy of serum MMP7 in BA in the Middle Eastern population.** ***Methods and materials:*** **In this cross-sectional study, neonates and infants with direct hyperbilirubinemia admitted to Namazi referral hospital, Shiraz, Iran, were studied. Baseline demographic and clinical characteristics and blood samples were obtained on admission. MMP7 serum concentration was measured using an enzyme-linked immunosorbent assay (ZellBio GmbH, Ulm, Germany).** ***Results:*** **44 infants with a mean age of 65.59 days were studied. Of these patients, 13 cases were diagnosed with BA, and 31 cases' cholestasis related to other etiologies. Serum MMP7 concertation was 2.13 ng/mL in the BA group and 1.85 ng/mL in the non-BA group. MMP7 was significantly higher in those presented with either dark urine or acholic stool. The predictive performance capability of the MMP7 was not significant in the discrimination of BA from the non-BA group based on receiver operating characteristic curve analysis (area under curve: 0.6, 95% confidence interval: 0.45–0.75). In the optimal cut of point 1.9, the sensitivity and specificity were 84.6% and 45.1%, respectively. Further combination of MMP7 with Gamma-glutamyl transferase (GGT), alkaline phosphatase, direct and total bilirubin, and dark urine or acholic stool was not remarkably boosted the diagnostic accuracy of the test. Interestingly, GGT at a cut-off point of 230 U/L was 84.6% sensitive and 90.3% specific for BA.** ***Conclusion:*** **Our results are not consistent with previous studies on this subject. Considering more conventional and available tests like GGT besides conducting future studies with greater samples and different geographical areas is recommended. (J Clin Exp Hepatol 2023;13:265–272)**

Biliary Atresia

Biliary atresia (BA) is characterized by the bile ducts' obstruction of extra- and/or intra-hepatic areas, which can rapidly progress into biliary cirrhosis and hepatic failure. The exact etiology is still unknown, though many environmental factors such as toxic substances, viruses, and genetic susceptibility have been related to this entity. Patients with BA can be asymptomatic at birth, but within a few weeks, they present with cholestatic signs and symptoms; soon after, fibrosis and cirrhosis would be developed, especially in undiagnosed or untreated cases.[1–3] BA is not a prevalent disease; an incidence rate of 1:15000 to 1:20000 live births in European and northern American populations and 1:6000 to 1:9000 live births in the Asian population were reported. Unfortunately, there is not a definite treatment for BA; however, after the recruitment of Kasai portoenterostomy (KPE), which tries to remove this blockage and restore bile flow, and advancement in liver transplantation, ten-year overall survival ranged from 66.7% to 89% based on a systematic review.[4,5]

One of the most important prognostic factors is the early diagnosis and performing KPE, which can

Keywords: biliary atresia, matrix metalloproteinase 7, kasai portoenterostomy, cholestasis
Received: 30.7.2022; *Accepted:* 1.10.2022; *Available online 2 December 2022*
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Abbreviations: ALP: Alkaline phosphatase; BA: Biliary atresia; DB: Direct bilirubin; GGT: Gamma-glutamyl transferase; KPE: Kasai portoenterostomy; LT: Liver transplantation; MMP7: Matrix metalloproteinase 7; MRCP: Magnetic resonance cholangiopancreatography; PFIC: Progressive familial intrahepatic cholestasis; PIBD: Paucity of interlobular bile ducts; TSB: Total serum bilirubin
https://doi.org/10.1016/j.jceh.2022.10.001

dramatically change the outcome. Thus, many efforts have been made to diagnose BA early and efficiently. To approach neonates with cholestasis, clinicians routinely start with requesting an array of laboratory studies to assess the degree of cholestasis, hepatocellular injury, and function, followed by a wide range of screening and confirmatory diagnostic modalities including hepatobiliary ultrasound, hepatobiliary scintigraphy, magnetic resonance cholangiopancreatography, and intraoperative cholangiography and liver biopsy. Although the latter is considered a gold standard diagnosis, it should be done in those with high levels of suspicion due to its aggressive nature.[1,6]

Matrix metalloproteinase 7 (MMP7), also known as Matrilysin, is a proteinase excreted by bile duct cells, and increased serum levels have been associated with biliary atresia. This potential biomarker can pave the way toward a better understanding of BA and thereby its use as a noninvasive diagnostic tool in neonates and infants with cholestasis.[7,8]

To the best of our knowledge, the diagnostic accuracy of MMP7 has not been studied in the region; thus, considering the remarkable potential diagnostic role of MMP7 in the early diagnosis of BA and probable racial differences, we aimed to assess the diagnostic accuracy of serum MMP7 in BA in neonates and infants with cholestasis in the main referral center of southern Iran.

MATERIAL AND METHODS

Population and Design

A cross-sectional study was conducted on neonates and infants less than three months of age with direct hyperbilirubinemia admitted to Namazi referral hospital under the affiliation of Shiraz University of Medical Sciences, Shiraz, Iran, in the period between March 2020 and April 2021. Direct hyperbilirubinemia is defined if direct bilirubin is greater than 1 mg per deciliter (mg/dL) and more than 20% of total serum bilirubin if total serum bilirubin is higher than 5 mg/dL. Those whose parents did not consent to participate in the study were excluded.

The final diagnosis of BA and inspissated bile syndrome were set by intraoperative cholangiography in those with high suspicion of BA. Definite diagnoses of paucity of interlobular bile ducts, progressive familial intrahepatic cholestasis, and idiopathic neonatal hepatitis were achieved with liver biopsy. Tyrosinemia and galactosemia were also diagnosed with high levels of serum succinylacetone and measurement of Galactose-1-phosphate uridylyl transferase activity, respectively.

Data Gathering

Baseline demographic characteristics, past medical history, family history, and disease course were gathered through history taken from the patient's parents at the time of admission. Anthropometric measures, hepatomegaly, and splenomegaly were assessed by physical examinations. All the patients were examined with abdominal ultrasonography for triangular cord signs or possible choledochal cysts. Blood samples were obtained for the basic metabolic panel, liver function tests, and complete blood count on day first of admission, and the sera were stored at - 20 °C (based on kit protocol) immediately after extraction for MMP7.

MMP7 Measurement

MMP7 serum concentration was measured using an enzyme-linked immunosorbent assay (ZellBio GmbH, Ulm, Germany). Briefly, the diluted serum samples were added in duplicate to 96-well plates coated with MMP7 antibody and then incubated at 37 °C for 60 min. After washing, the chromogen solution was added, and the plate was further incubated for 10 min. Following the termination of the reaction with the stop solution, the optical density was measured at 450 nm using a spectrophotometric microplate reader. The concentration of MMP7 was calculated from a standard curve. To achieve a baseline as a control group, samples from sixteen healthy persons under three months of age were gathered and checked for MMP7 concentrations.

Ethical Consideration

The ethical committee of Shiraz University of Medical Sciences has approved this study (IR.SUMS.MED.REC.1400.531). The committee waived the requirement for informed consent since we used left blood samples from routine investigations along with patient anonymity.

Statistical Analyses

Continuous and categorical variables were presented as mean ± standard deviation or number (%), respectively. Shapiro–Wilk test was used to evaluate the normal distribution. The student's two sample t-test and Pearson correlation were used for analyzing that data. The receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic ability of the MMP7 test to discriminate the patients. In addition, the unconditional logistic regression model was used to assess the predictive power of concurrent tests. The data were analyzed using SPSS software (Version 16.0. Chicago, SPSS Inc., United States). For ROC curve analysis, MedCalc statistical software (Version 20.1.4) was employed. We considered $\alpha = 0.05$ in all tests.

RESULTS

Baseline Characteristics

In this survey, 44 infants aged between 17 and 109 days with a mean age of 65.59 ± 19.10 days were studied. Of

Table 1 Demographic Characteristics and Baseline Data in BA and Non-BA Patients.

Variables (Units)		Total	BA (n = 13,29.5%)	Non-BA (n = 31,70.5%)	*P* value
Age, day		65.59 ± 19.10	69.3 ± 12.79	65.45 ± 21.28	0.548
Gender	Female	21 (47.7)	8 (61.5)	13 (41.9)	0.235
	Male	23 (52.3)	5 (38.5)	18 (58.1)	
Birth weight (grams)		3091 ± 501	3084 ± 549	3094 ± 489	0.950
Congenital malformation		9 (20.5%)	2 (15.4)	7 (22.6)	0.589
Dark urine		34 (77.3%)	13 (100)	21 (67.7)	**0.020**
Pale stool		19 (43.2%)	12 (92.3)	7 (22.6)	**<0.001**
Hepatomegaly		26 (59.1%)	11 (84.6)	15 (48.4)	**0.026**
Splenomegaly		6 (13.6%)	2 (15.4)	4 (12.9)	0.827
Gallbladder	Semi-distended	15 (34.1)	0 (0)	15 (48.4)	<0.001
	well distended	9 (20.5)	0 (0)	9 (29.0)	
	Contracted	14 (31.8)	8 (61.5)	6 (19.4)	
	Distended	1 (2.3)	0 (0)	1 (3.2)	
Absent		5 (11.4)	5 (38.5)	0 (0)	
Triangular cord sign		4 (9.1)	4 (30.8)	0 (0)	0.005
MMP7 concentration (ng/mL)		1.93 ± 0.64	2.13 ± 0.44	1.85 ± 0.69	0.184

Data were presented as Mean ± SD/number (%) for continuous/categorical data.
BA, Biliary atresia, MMP7, Matrix metalloproteinase 7.

these, 52.3% (n = 23) were boys. Birth weight varied from 1900 to 4600 g and averaged 3092 ± 501 g. Of these patients, 13 cases were labeled as biliary atresia, and 31 cases' cholestasis related to other causes (Paucity of interlobular bile ducts,[7] Progressive familial intrahepatic cholestasis,[7] Inspissated bile syndrome,[4] idiopathic neonatal hepatitis,[4] tyrosinemia,[4] Galactosemia,[3] choledochal cyst,[1] hemophagocytic lymphohistiocytosis[1]). There was not any significant difference between patients having BA and Non-BA in terms of age (P = 0.548), gender (P = 0.235), and birth weight (P = 0.950), while there were significant differences between groups in the presence of dark urine, acholic stool, hepatomegaly, and gallbladder condition (P-value <0.05).

Table 2 Mean (SD) of MMP7 and its Relationship with Clinical and Paraclinical Findings.

Variables		N	Mean ± SD	*P*-value
Sex	Male	23	2.05 ± 0.51	0.223
	Female	21	1.81 ± 0.75	
Associated congenital malformation	No	35	1.91 ± 0.68	0.585
	Yes	9	2.04 ± 0.46	
Dark urine	No	10	1.45 ± 0.79	***0.037***
	Yes	34	2.08 ± 0.51	
Acholic stool	No	25	1.78 ± 0.74	***0.043***
	Yes	19	2.14 ± 0.40	
Hepatomegaly	No	18	1.97 ± 0.69	0.776
	Yes	26	1.91 ± 0.12	
Splenomegaly	No	38	1.89 ± 0.67	0.288
	Yes	6	2.20 ± 0.25	
Triangular cord sign	No	40	1.94 ± 0.64	0.776
	Yes	4	1.85 ± 0.70	
Group	BA	13	2.13 ± 0.44	***0.011***
	Control	16	1.63 ± 0.52	

BA, Biliary atresia

The baseline serum MMP7 concertation varied from 0.3 Nanograms per milliliter (ng/mL) to 2.9 ng/mL in the studied population with a mean of 1.93 ± 0.64 ng/mL (2.13 ± 0.44 in BA group and 1.85 ± 0.69 in non-BA group) (see Table 1). There was no significant relationship between MMP7 and age (r = 0.014, *P* = 0.928). An inverse but insignificant relationship was observed between MMP7 and their weight at admission (r = −0.187, *P* = 0.225). MMP7 was significantly higher in those presented with either dark urine (2.08 versus 1.45, *P* = 0.037) or acholic stool (2.14 versus 1.78, *P* = 0.043). There was not any statistically significant difference between the mean of MMP7 in terms of gender, concurrent congenital malformation, hepatomegaly, splenomegaly, and the presence of triangular cord sign (*P* > 0.05 in all cases). (Table 2).

Diagnostic Ability of MMP7

To evaluate the diagnostic performance of MMP7, it was initially compared with the control group (Healthy population). MMP7 levels were significantly higher in the BA group (2.13 ± 0.44) than the control (1.63 ± 0.52) groups (*P* = 0.010) (Figure 1-A).

Moreover, to assess MMP7 discriminating power in differentiating between patients and healthy individuals, the ROC curve analysis was performed. According to ROC analysis results that indicated in Figure 2 and table 4, in the optimal cut of point 1.8 ng/mL with 0.81 (95% CI: 0.62–0.93) area under curve (AUC), 92.3% sensitivity, and 68.7% specificity, the MMP7 test had a powerful performance to diagnose BA.

Predictive Performance for Etiology of Cholestasis (BA Versus Non-BA Cholestasis)

After the test capability was confirmed in the diagnosis of patients, we divided the patients into two groups (BA and non-BA cholestasis). A comparison of means in Figure 1-B shows that there is no statistically significant difference between the two groups. In order to evaluate the predictive power of the test, ROC curve analysis was again recruited. The ROC curve analysis demonstrated that the predictive performance capability of the test is not significant regarding this issue (AUC: 0.6, 95%CI: 0.45–0.75). In this case, in the optimal cut of point 1.9, the sensitivity and specificity were 84.6% and 45.1%, respectively. The detailed outcomes of the analyses are demonstrated in Figure 3 and Table 4.

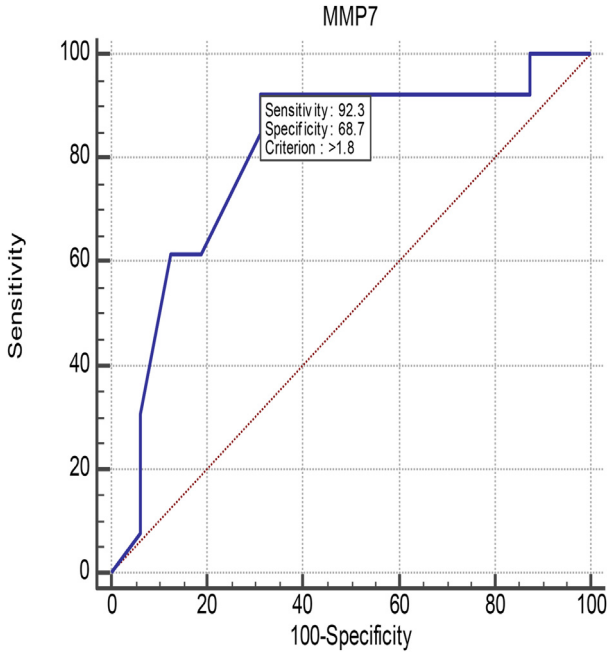

Figure 2 ROC curve analysis of MMP7 for discrimination of BA patients from control group. BA, biliary atresia; MMP7, matrix metalloproteinase 7; ROC, receiver operating characteristic.

Predictive Performance of Combined MMP7 and Other Clinical and Laboratory Data

To enhance the diagnostic performance of MMP7 at a more advanced level, it was analyzed combined with total bilirubin, direct bilirubin, alkaline phosphatase (ALP),

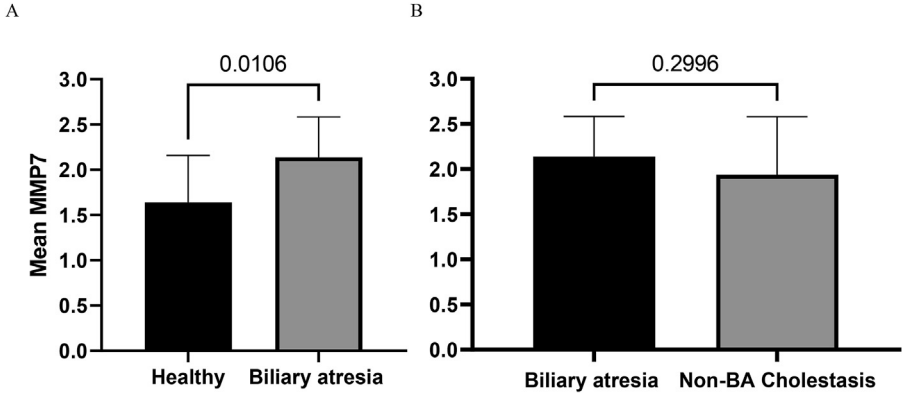

Figure 1 Mean (SD) of MMP7 in nanograms per milliliter (ng/mL) is compared between BA and control (A) and non-BA cholestatic group (B). BA, biliary atresia; MMP7, matrix metalloproteinase 7.

Table 3 Indices of ROC Curve Analysis for Evaluating the Diagnostic Ability of MMP7 and Predictive Ability of MMP7, Total Bilirubin, Direct Bilirubin, ALP, GGT and Dark Urine, Acholic Stool.

Index	Value (95% CI)						
	MMP7	Total bilirubin	Direct bilirubin	ALP	GGT	Dark urine	Acholic stool
Cut off point	>1.9	>6.9	>3.5	>1850	**>230**	–	–
AUC	0.6 (0.45–0.75)	0.51 (0.36–0.66)	0.56 (0.4–0.71)	0.65 (0.49–0.79)	**0.94** (0.83–0.99)	0.66 (0.51–0.79)	0.84 (0.70–0.93)
Sensitivity	84.6 (54.5–98)	100 (75.2–100)	92.3 (63.9–99.8)	61.5 (31.5–86.1)	**84.6** (54.5–97.6)	100 (75.2–100)	92.3 (63.9–98.7)
Specificity	45.1 (27.3–63.9)	25.8 (11.8–44.6)	35.4 (19.2–54.6)	80.6 (62.5–92.5)	**90.3** (74.2–97.6)	32.2 (16.6–51.3)	77.4 (58.9–90.4)
PPV	39.2 (30.3–48.9)	36.1 (31.4–41.0)	37.5 (30.6–44.8)	57.1 (36.6–75.4)	**78.5** (54.9–91.6)	38.2 (32.6–44.1)	63.1 (46.7–77.0)
NPV	87.5 (64.8–96.3)	100 (100–100)	91.6 (61.2–98.7)	83.3 (71.1–91.0)	**93.3** (79.5–98.0)	100 (100–100)	96.0 (78.3–99.3)
Accuracy	56.8 (41.0–71.6)	47.7 (32.4–63.3)	52.2 (36.6–67.5)	75.0 (59.6–86.8)	**88.6** (75.4–96.2	52.2 (36.9–67.5)	81.8 (67.2–91.8)

Note: It is more appropriate to use predictive value in diagnostic/predictive tests while sensitivity-specificity in screening tests.
AUC: Area Under Curve; CI: Confidence Interval; PPV: Positive Predictive Value; NPV: Negative Predictive Value, MMP7: Matrix metalloproteinase 7, APL: Alkaline Phosphatase, GGT: Gamma-glutamyl transferase. Numbers are percentages except for AUC.

Table 4 Indices of ROC curve analysis for evaluating the diagnostic ability of MMP7 and predictive ability of MMP7, total bilirubin, direct bilirubin, ALP, GGT and dark urine, acholic stool.

Index	Value (95% CI)						
	MMP7	Total bilirubin	Direct bilirubin	ALP	GGT	Dark urine	Acholic stool
Cut of point	>1.9	>6.9	>3.5	>1850	>230	–	–
AUC	0.6(0.45–0.75)	0.51(0.36–0.66)	0.56(0.4–0.71)	0.65(0.49–0.79)	0.94(0.83–0.99)	0.66(0.51–0.79)	0.84 (0.70–0.93)
Sensitivity	84.6(54.5–98)	100(75.2–100)	92.3(63.9–99.8)	61.5(31.5–86.1)	84.6(54.5–97.6)	100(75.2–100)	92.3 (63.9–98.7)
Specificity	45.1(27.3–63.9)	25.8(11.8–44.6)	35.4(19.2–54.6)	80.6(62.5–92.5)	90.3(74.2–97.6)	32.2(16.6–51.3)	77.4 (58.9–90.4)
PPV	39.2(30.3–48.9)	36.1(31.4–41.0)	37.5(30.6–44.8)	57.1(36.6–75.4)	78.5(54.9–91.6)	38.2(32.6–44.1)	63.1 (46.7–77.0)
NPV	87.5(64.8–96.3)	100(100–100)	91.6(61.2–98.7)	83.3(71.1–91.0)	93.3(79.5–98.0)	100(100–100)	96.0(78.3–99.3)
Accuracy	56.8(41.0–71.6)	47.7(32.4–63.3)	52.2(36.6–67.5)	75.0(59.6–86.8)	88.6(75.4–96.2	52.2(36.9–67.5)	81.8 (67.2–91.8)

AUC: Area Under Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value, MMP7: Matrix metalloproteinase 7, APL: Alkaline Phosphatase, GGT: Gamma-glutamyl transferase. Numbers are percentages except for AUC.
Note: It is more appropriate to use predictive value in diagnostic/predictive tests while sensitivity - specificity in screening tests.

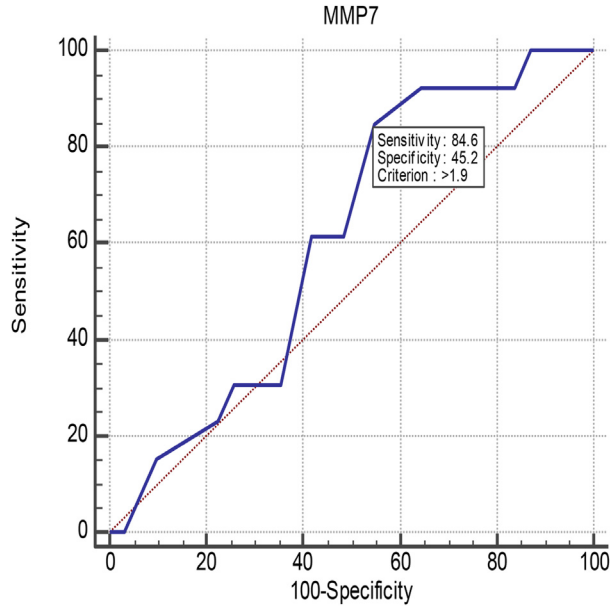

Figure 3 ROC curve analysis of MMP7 for distinguishing BA patients from non-BA cholestatic patients. BA, biliary atresia; MMP7, matrix metalloproteinase 7; ROC, receiver operating characteristic.

Gamma-glutamyl transferase (GGT), dark urine, and acholic stool. These variables first were analyzed separately for BA versus non-BA cholestasis population via ROC curve analysis. These clinical and common laboratory characteristics have almost similar to results to MMP7 test, except for GGT, which was found to be the most accurate test with 94.6% sensitivity and 90.3% specificity. The in-detail results of the ROC curve analysis are demonstrated in Table 3.

To further analyze and determine the simultaneous predictive power of other tests with MMP7, a logistic regression model was initially used to predict the probability of a pair of tests, then using rock curve analysis based on the best cut-off point of predictive power simultaneously. The results were summarized in the form of substandard level and 95% confidence interval in Table 5. Indicators based on cutting points, including sensitivity, specificity, positive and negative predictive value, and accuracy of concurrent use of tests, are also summarized in Table 4. Compared to the use of single tests, it seems that their simultaneous use increases the accuracy.

DISCUSSION

KPE is considered vital for BA patients considering the progressive nature of the disease, autotoxication of biliary contents to the liver, and inability to get liver transplantation—the ultimate treatment for BA, due to potential immediate and longer-term sequelae. Age plays a crucial role in achieving the best possible outcome after KPE. Previous studies have suggested that the age between 60 and 100

Table 5 Indices of ROC Curve Analysis for Assessing Predictive Ability of MMP7 and Simultaneously with Total Bilirubin, Direct Bilirubin, Direct to Total Bilirubin Ratio, ALP, GGT and Dark Urine, Acholic Stool.

Index	Value (95% CI)							
	MMP7	MMP7+Total bilirubin	MMP7+Direct bilirubin	MMP7+ALP	MMP7+GGT	MMP7+Dark urine	MMP7+ Acholic stool
AUC	0.6 (0.45–0.75)	0.62 (0.46–0.76)	0.60 (0.44–0.74)	0.66 (0.51–0.80)	0.94 (0.83–0.99)	0.68 (0.53–0.81)	0.86 (0.72–0.94)
Sensitivity	84.6 (54.5–98)	84.6 (54.5–98.0)	92.3 (63.9–99.8)	76.9 (46.1–94.9)	92.3 (63.9–99.8)	84.6 (54.5–98.0)	92.3 (63.9–98.7)
Specificity	45.1 (27.3–63.9)	48.3 (30.1–66.9)	45.1 (27.3–63.9)	64.5 (45.3–80.7)	87.1 (70.1–96.3)	58.0 (39.0–75.4)	77.4 (58.9–90.4)
PPV	39.2 (30.3–48.9)	40.7 (31.2–50.9)	41.3 (33.0–50.1)	47.6 (34.1–61.4)	75.0 (54.2–88.3)	45.8 (34.4–57.6)	63.1 (46.7–77.0)
NPV	87.5 (64.8–96.3)	88.2 (66.5–96.5)	93.3 (67.1–98.9)	86.9 (70.4–94.9)	96.4 (80.3–99.4)	90.0 (70.8–97.0)	96.0 (78.3–99.3)
Accuracy	56.8 (41.0–71.6)	59.0 (43.2–73.6)	59.0 (43.2–73.6)	68.1 (52.4–81.3)	88.6 (75.4–96.2)	65.9 (50.0–79.5)	81.8 (67.2–91.8)

Note: It is more appropriate to use predictive value in diagnostic/predictive tests while sensitivity - specificity in screening tests.
AUC: Area Under Curve; CI: Confidence Interval; PPV: Positive Predictive Value; NPV: Negative Predictive Value, MMP7: Matrix metalloproteinase 7, APL: Alkaline Phosphatase, GGT: Gamma-glutamyl transferase. Numbers are percentages except for AUC.

days as a golden time for KPE procedure. Hence, post-KPE survival deeply depends on early diagnosis.[9–11]

On the other hand, BA and other conditions causing infantile cholestatic jaundice mostly present with similar clinical manifestations and laboratory findings. For better distinguishing BA from non-BA cholestatic diseases, many modalities have been proposed, and none of the invasive tests have an acceptable accuracy within the first two months. Therefore, the existing diagnostic methods of BA diagnosis mostly rely on invasive procedures such as surgical exploration and intraoperative cholangiography and liver biopsy, and histopathological examinations. Based on a recent meta-analysis, respective sensitivity and specificity are as follows: 77% and 93% for biliary sonography, 96% and 58% for magnetic resonance cholangiopancreatography; 87% and 78% for acholic stool; 84% and 97% for serum liver function tests; 96% and 73% for hepatobiliary scintigraphy; 98% and 93% percutaneous liver biopsy. Taking into consideration of the beneficial and disadvantageous aspects of each method, the availability, cost, and invasiveness of the method, besides its diagnostic accuracy, is a key element in decision-making.[12,13]

Serum biomarkers as a screening or prognostic tool have been widely used for many clinical entities, predominantly in oncology. Several diagnostic biomarkers have been introduced for BA diagnosis, though due to lack of high-level, none of them has found a way into clinical practice.[14] MMP7 has been reported to be a promising biomarker in diagnosing BA. Previous studies have shown that MMP7 may have a role in the pathogenesis of BA by various mechanisms that enhance inflammation and fibrosis. Hepatic MMP7 expression was found to be correlated with the extent of liver fibrosis, even with minimal cholestasis after the performance of a KPE, and its colocalization with CK-7 is a reliable marker for cholangiocyte proliferation which highlights the potential diagnostic and prognostic biomarker or therapeutic target in conditions involving biliary system including BA.[8,15,16]

In our study, 44 patients with infantile cholestasis were surveyed to assess the diagnostic accuracy of serum MMP7 for BA. MMP7 levels were significantly higher in the BA group (2.13 ng/mL) than the control group of healthy individuals (1.63 ng/mL). This was predictable based on MMP7 synthesis mechanisms and expression in conditions with biliary tree inflammation.[17]

In the second step, ROC curve analysis was recruited to test the distinguishing performance capability of MMP7 between BA and other non-BA etiologies of cholestasis which was not significant. In this case, in the optimal cut off point 1.9 ng/mL, the sensitivity and specificity were 84.6% and 45.1%, respectively. This is not concordant with previous studies. In a study by Jiang *et al.*' in China, 288 patients with neonatal obstructive jaundice were studied. MMP7 at a cut-off value of 10.37 ng/mL was 95.19% sensitive and 93.07% specific for BA. In this study, the median serum MMP7 levels were 38.89 ng/mL for BA patients compared to 4.4 ng/mL for the non-BA group.[8] In another Chinese study by Wang *et al.*, the diagnostic sensitivity of 98.67% and specificity of 95% of MMP7 for BA were estimated by the study of 135 cholestatic infants. They detected a median MMP7 concentration of 2.86 ng/mL in normal controls, 11.47 ng/mL for non-BA, and 121.1 ng/mL for BA.[1] It is considerable that the measure of MMP7 in the normal population in this study is higher than our numbers in the BA group. In a study from Tehran in northern Iran, 22 patients with BA and 32 patients with non-BA cholestasis were studied. They suggested 7.8 ng/mL for MMP7 as a cut-off point for BA and showed 95.5% of sensitivity and 94.5% specificity. This may be due to ethnical differences and the enzyme-linked immunosorbent assay kit used in different studies.

To reach higher accuracy, we used combinations of MMP7 with some other clinical and laboratory. These variables were initially analyzed via ROC curve analysis. Total bilirubin and the presence of dark urine were 100% sensitive, which makes the ideal screening as they are. An interesting finding was about GGT, which was detected as the most accurate test with 94.6% sensitivity and 90.3% specificity at the cut-off point of 230 units per liter. This is consistent with a previous study which showed 82.8% sensitivity and 81.6% specificity of GGT for the discrimination of BA.[18]

A combination of tests like ALP, GGT, bilirubin, and clinical symptoms increased the diagnostic value of MMP7. For instance, considering ALP and GGT besides MMP7 increases the specificity to 64.5% and 87.1%, respectively. The diagnostic value of MMP7 did not change significantly when compared combination of GGT and MMP7 in study by Jiang.[8] In another study, AUC for MMP7 and GGT (0.98) was remarkably greater than GGT alone (0.9) but relatively similar forMMP7 alone (0.97).[19]

In this study, there was no significant relationship between MMP7 and age, while MMP7 was found to be significantly higher in those presented with dark urine or acholic stool. Wu *et al.* reported that serum MMP7 levels were lower in infants with biliary atresia who underwent a cholestatic workup at a younger age,[20] though our result is consistent with Rohani's study.[21] To the best of our knowledge, no study assessed the relation between MMP7 concentration and clinical findings like presence of dark urine.

We faced some limitations while conducting this study; considering the low prevalence of BA in the region, we could not get enough specimens despite enrolling all patients fulfilling the eligibility criteria in a tertiary referral hospital. We collect the specimens and information on the first day of admission and follow the kit's protocol precisely to enhance the accuracy and reliability of the study.

Based on our findings, MMP7 lacks enough specificity and lower diagnostic accuracy compared to GGT in

diagnosing BA from other causes of neonatal cholestasis. This is not concordant with previous studies and highlights the importance of future multi-centric studies with higher participants in different geographical regions as well as secondary research in this field.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

MHA, SMD, MA, NH, MH, MHI, AT, IS: Conceptualization, Methodology, and revising the manuscript.

FK, AM, AA, AH, SMMM, MS, BD, AA, BM: Data gathering, analysis and drafting the manuscript.

CONFLICTS OF INTEREST

All authors have none to declare.

ACKNOWLEDGMENT

The authors thank personnel of Nemazee hospitals in laboratory and pediatric surgery and pediatric gastroenterology wards for their cooperation and our colleagues in Pediatric Gastroenterology, Hepatology and Nutrition Research Center Shahid Beheshti University of Medical Sciences for their valuable guidance.

FUNDING

None.

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