Original Research Paper

Volume - 13 | Issue - 10 | October - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pediatric Surgery

SERUM MATRIX METALLOPROTEINASE-7 AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER FOR EXTRAHEPATIC BILIARY ATRESIA

Chiranjiv Kumar	M. Ch, Department of Pediatric Surgery, Maulana Azad Medical College, New Delhi-110002, India.
Simmi K Ratan	M. Ch, Department of Pediatric Surgery, Maulana Azad Medical College, New Delhi-110002, India.
Nitin Jain	M.Ch, Department of Pediatric Surgery, Maulana Azad Medical College, New Delhi – 110002, India
Shasanka Shekhar Panda*	M. Ch, Department of Pediatric Surgery, Maulana Azad Medical College, New Delhi-110002, India. *Corresponding Author
Bidhan Chandra Koner	M.D., Department of Biochemistry, Maulana Azad Medical College, New Delhi-110002, India.
Anju Garg	M.D., Department of Radiodiagnosis, Maulana Azad Medical College, New Delhi-110002, India.
Varuna Mallya	M.D., Department of Pathology, Maulana Azad Medical College, New Delhi-110002, India.
Raghvendra Singh	M.D., Department of Pediatrics, Maulana Azad Medical College, New Delhi-110002, India.
Prafful Kumar	M.Ch, Department of Pediatric Surgery, Maulana Azad Medical College, New Delhi – 110002, India
Sujoy Neogi	D.N.B., Department of Pediatric Surgery, Maulana Azad Medical College, New Delhi-110002, India.

ABSTRACT **Aim:** Serum Matrix metalloproteinase-7 as a diagnostic and prognostic biomarker for extrahepatic biliary atresia **Methods:** This was a prospective, single center, case control study where consecutive infants less than 6 months of age with obstructive jaundice (n=20) at MAMC from April 2021 to March 2023 were enrolled. Age and sex-matched controls (n=20) were also taken. Serum MMP-7 were tested in all 40 infants. In Case group, additional tests performed were, CBC, LFT to derive APRI and FIB-4 scores, Ultrasound Abdomen, Hepatobiliary scintigraphy, Per-operative cholangiogram, Pre and/or intraoperative liver biopsy. **Results:** The participants were substratified into EHBA (n = 11) and NH (n = 9) based on post priming hepatobiliary scintigraphy and Per-operative cholangiogram. Median serum MMP-7 level in EHBA was 23 ng/ml that was found to be 6 times higher than Neonatal hepatitis and 13 times higher than in normal infants. Median serum MMP-7 levels in NH group and normal infants were 4ng/ml and 1.8ng/ml respectively. MMP-7 values in males were higher than 4.99 (based on the cut offs from review of literature) that was statistically significant as compared to female counter parts ($p<0.05$). Based on age categorization, almost 50% of children <90 days old and all children >90 days had MMP-7 value >4.99 which had significant statistical values for both case and control groups ($p<0.05$). The Metavir and Ishak fibrosis category were divided into F0 – no fibrosis, Early and Advanced fibrosis stages. From among the Metavir fibrosis group which did not have any fibrosis, 33.3% had MMP-7 >4.99, whereas all patients from early and advanced stages had MMP-7 >4.99. **Conclusion:** The high concentrations of MMP-7 in EHBA may be used to substratify patients of neonatal cholestasis into EHBA and NH. The strong correlation of MMP-7 with histopathological injury has also suggested that MMP-7 may be used as a prognostic biomarker of liver fibrosis in EHBA.

KEYWORDS : Matrix metalloproteinase-7, neonatal hepatitis, extra-hepatic biliary atresia, Metavir scoring, Ishak scoring.

INTRODUCTION

BA is the most frequent surgical cause of cholestatic jaundice in neonates. The common histopathological picture is one of inflammatory damage to the intra- and extrahepatic bile ducts with obliteration of the biliary tree with rapid progression to endstage cirrhosis if not diagnosed early in life. BA is the most common clinical indication for pediatric liver transplantation globally. [1-3]

We need to differentiate neonatal cholestasis into neonatal hepatitis (NH) and extra-hepatic biliary atresia (EHBA) as it is essential for opting medical versus surgical management for the patients. Early intervention is very important for optimal outcome in EHBA. Due to diagnostic dilemma in a patient with NH may underwent unnecessary anesthesia and laparotomy. So there is need for rapid non-invasive diagnostic test which is both, sensitive-and-specific.

Gamma glutamyl transferase (GGT), is one of the factors measured in biochemical liver function tests and is widely used to differentiate BA from non-BA, with an accuracy of 76% to 88% at a cutoff value of ~300 IU/L.[4,5] Various other non-invasive biomarkers like Nitric oxide [6] and Interleukin-33/ST2 Receptor [7] were also reported to be helpful in differentiating EHBA from neonatal hepatitis and correlation with liver fibrosis progression in EHBA patients respectively.

Although radiologic investigations (such as ultrasound, MRI, and hepatobiliary scintigraphy) were reported to be helpful in differentiating BA from other obstructive cholestasis, they are time consuming and costly.[8-11] Liver biopsy has the best positive predictive value of 90.7% reported by Russo et al.[12], but is associated with considerable morbidity. Therefore, an effective biomarker would be extremely valuable for the preoperative diagnosis of BA.

Matrix metalloproteinase-7 (MMP-7), a protease responsible for tissue remodeling, was discovered to be associated with liver fibrosis in patients with EHBA. [13,14] MMP-7 demonstrated good accuracy in diagnosing EHBA and holds promise for future clinical application. Furthermore, its correlation with liver fibrosis indicated its potential use as a therapeutic target or prognostic biomarker. The strong correlation of MMP-7 with histopathological injury has also suggested that MMP-7 may be used as a prognostic biomarker of liver fibrosis

and a follow-up marker in EHBA.

The current study was designed to study the role of MMP-7 as a serum biomarker to differentiate between EHBA and NH. The study had also been configured to evaluate the functional and prognostic significance of serum MMP-7 levels in EHBA by looking into its correlation with liver inflammation and fibrosis. The relevance of APRI and FIB-4 scores in quantifying liver injury vis-à-vis serum MMP-7 has also been studied in patients with EHBA.

MATERIAL AND METHODS

This prospective, single center, case control study was conducted in the Department of Paediatric surgery, Maulana Azad Medical College & Associated Lok Nayak Hospital, New-Delhi after seeking approval of the Institutional Ethics Committee (F.1/IEC/MAMC/83/01/2021/ No.343, Date-23.02.2021) from April 2021 to March 2023. Consecutive infants less than 6 months of age of obstructive jaundice (defined as a serum direct bilirubin>1 mg/dl and 20% of total bilirubin) presenting with acholic stools (n=20) were enrolled in the study. An equal number (n = 20) of age-and sex-matched infants presenting for unrelated noninfectious conditions not involving the liver and not known to affect the serum MMP-7 levels were included as controls.

Predesigned templates were used to record demographic parameters, clinical findings, complete blood count, liver function tests, and findings upon ultrasound evaluation and post priming hepatobiliary scintigraphy.

At the time of routine blood sampling, an additional 2.5-mL sample was collected in a plain vial for serum MMP-7 estimation. The sample was transported to the laboratory in ice, centrifuged at 2500 rpm for 3 min, and stored in aliquots at − 80°C to avoid loss of bioactive MMP-7. Commercially available human MMP-7 enzyme-linked immunosorbent assay (ELISA) kit (Bioassay technology, Shanghai, China), was used to quantify serum MMP-7 and the values were recorded in ng/ml.

Participants who demonstrated gut activity on post priming hepatobiliary scintigraphy were labeled as NH, whereas others were labeled as suspect EHBA and further evaluated with a POC.[15]

A wedge liver biopsy was taken at the time of surgery for EHBA and processed for standard histopathology. Staging of fibrosis and activity assessment were done using the METAVIR scoring system[6] and the Ishak's modification of Knodell's Histological Activity Index score.[6] The METAVIR scores were as follows: Inflammatory activity (A0 = none; A1 = mild; A2 = moderate; and A3 = severe inflammation) and fibrosis stage (F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with few septa; F3 = portal fibrosis with many septa; and F4 = cirrhosis). The Ishak scores for necrosis and inflammatory activity were as follows: histological grading scores ranging 0–18; re-stratified as mild 0–6, moderate 7–12 and marked 13–18 and fibrosis stage were: F0 = no fibrosis; F1 = fibrous expansion of some portal areas, F2 = fibrous expansion of most portal areas; F3 = fibrous expansion of most portal areas with occasional bridging, F4 = fibrous expansion of most portal areas with marked bridging; F5 = marked bridging with occasional nodules/ incomplete cirrhosis; F6 = probable or definite cirrhosis. Patients were re-grouped into early (METAVIR stage 1, 2; Ishak's Stage 1–3) and advanced fibrosis (METAVIR stage 3, 4; Ishak's stage 4–6).

Aspartate-to-Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) scores were calculated from the recorded information.[7]

Statistical analysis was carried out using SPSS 23.0 (IBM). Serum MMP-7 levels, APRI, and FIB-4 were assessed for normal distribution using Shapiro–Wilk test. MMP-7 levels were then compared among the three groups using Kruskal–Wallis test, followed by pair-wise comparison using Wilcoxon rank-sum test. The P value was adjusted using Bonferroni correction. Receiver-operating characteristic (ROC) curves were drawn to assess the diagnostic performance of MMP-7 in EHBA versus NH and NH versus controls groups. The results were presented as cutoff, sensitivity, specificity, and likelihood ratio. Area under ROC was calculated. The association of MMP-7 and APRI and FIB-4 scores with METAVIR and Ishak activity grades and fibrosis staging was assessed using Jonkheere–Terpstra test and Wilcoxon rank sum test, respectively. P <0.05 was considered significant.

RESULTS

The participants were substratified into EHBA (n = 11) and NH (n = 9) based on post priming hepatobiliary scintigraphy and Per-operative cholangiogram.

The median serum MMP-7 level in normal infants (n = 20) was 1.8 ng/mL (IQR: 0.43–4.76 ng/mL). The median serum MMP-7 level was 23 ng/mL in EHBA (n = 11; IQR: 19.94–27.85) which was approximately 6-fold higher as compared to the values observed in NH (n = 9; median: 4 ng/mL, IQR: 1.77–7.25) and approximately 13-fold higher than values observed in normal infants. The levels in NH were 2.2-fold higher than those in normal infants.

The demographic variables such as age, weight and gender were categorized conveniently based on the cut offs from review of literature. (Table.1) Gender details show that in males, the MMP-7 values were higher than 4.99 as compared to female counter parts and this was statistically significant in both cases and control group ($p<0.05$). Based on age categorization, almost 50% of children <90 days old and all children >90 days had MMP-7 value >4.99 and this was statistically significant for both case and control groups ($p<0.05$).

The Box and whisker plot shows the distribution of MMP-7 among patients with EHBA and NH. (Fig.1) Middle horizontal depicts the median value, whereas, the upper and lower lines of the box represent the 75th and 25th centile respectively. The reference horizontal line is the mean value of MMP which is around 15 ng/dl. Median Values for EHBA and NH are around 23 and 4 respectively. In case of EHBA the median is more towards the lower centile which in case of NH is almost towards the center.

The correlation matrix table above shows the values of MMP-7 plotted against the various parameters of inflammation. (Table.2) It shows a positive correlation for the parameters AST, Total Bilirubin, ALP and Serum GGT, whereas it shows a negative correlation between platelet count and ALT. Serum GGT has a correlation coefficient of 0.9 which shows a very strong positive correlation and is highly statistically significant. (Fig.2)

The area the ROC curve for MMP-7 (ng/ml) predicting EHBA vis-à-vis NH was 0.83 at 95% confidence interval and CI: 0.63 – 1:00. This signifies a good diagnostic performance with Area under ROC > 0.5 which was significant at a value of 0.012. (Fig.3) At a serum cut-off off of 4.99 ng/ml, nearly 100 % of patients could be correctly classified as EHBA, with a sensitivity of cent percent and a specificity of 67% with standard error of 0.1.

The various parameters of Metavir & Ishak Activity, Metavir fibrosis and Ishak fibrosis stage have been categorized and frequency distribution denotes that 20% cases showed no activity, and 45% cases do not show any signs of fibrosis. Almost 55 % have EHBA and 45% show neonatal hepatitis. (Table.3)

The MMP-7 groups based on the cut of value of 4.99 was compared with the Metavir fibrosis category and the Ishak fibrosis category along with the General Diagnosis category into Neonatal hepatitis and Extra hepatic biliary atresia. The Metavir and Ishak fibrosis category were divided into F0 – no fibrosis, Early and Advanced fibrosis stages. From among the Metavir fibrosis group which did not have any fibrosis, 33.3% had MMP-7 >4.99, whereas, cent percent from among early and advanced stages had MMP-7 >4.99. The same held true for Ishak fibrosis stage and both were highly significant at statistical P value 0.000. Moreover, cent percent of EHBA had high MMP-7 values and 33.3% of NH had MMP-7 values >4.99 respectively. This was also significant statistically. (Table.4)

The mean square plot between APRI scores and serum MMP-7 values has been depicted in the figure below. (Fig.4) The middle line is the reference line between 2 and 3 around which the various categories are distributed and most of them are towards the reference line with few extreme values. On the other hand, the distribution of FIB-4 mean score against MMP-7 scores show a perfect distribution around the middle reference line.

DISCUSSION

EHBA is a rare, progressive cholangiopathy with multifactorial etiology in which fibrosing injury to extrahepatic bile ducts (EHBDs) in response to an unknown insult leads to cholestasis and jaundice [6] The pathophysiology involves obstructive cholangiopathy of the extrahepatic bile duct, which culminates into liver fibrosis, cirrhosis,

and eventual liver failure. The outcomes are suboptimal despite the best possible treatment; age at surgery is one of the crucial factors contributing to the final outcomes.[15]

MMP-7 is a protease enzyme which is a member of the MMP family consisting of structural related zinc-dependent endopeptidases.[16,17] MMP-7 has been implicated in tissue remodeling during biliary atresia associated liver fibrosis.[18] The expression of MMP-7 is weak in normal liver and is highly upgraded in response to progressive biliary atresia.[19,20]

The mean age of presentation of neonatal cholestasis cases is 88.25±43.4 days whereas median age of presentation of EHBA in the current study is 125 days (IQR 55-168 days). Singh et al. [18] had reported median age of presentation of EHBA in his study as 87 days (IQR 74–121 days; n=25). Yang et al. [1] have reported a mean of 54 days (0–6 months; n = 75), Wu et al. [2] have reported a mean age of 42.4 ± 3.6 days (n = 36), while Jiang et al. [3] reported a median age of 59 days (IQR 48–69 days; n = 187). Because the levels of MMP-7 are dependent on the extent of liver fibrosis, the levels are likely to rise with age and progression of disease pathology.

The study has established the serum levels of MMP-7 (median value 1.8 ng/mL, IQR 0.43–4.77 ng/mL; n = 20) in normal infants across the age range of 33 days to 165 days, and there were no age-related differences in this bracket. These data may serve as a baseline reference for further studies. Singh et al. [18] in his study had reported serum levels of MMP-7 as median value 1.2 ng/mL, (IQR 1.08–1.32 ng/mL; n = 45) across the age range of 59 days to 103 days in normal infants. The reported value of MMP-7 in normal infants from China [3] is 2.4-fold higher (2.86 ng/mL, IQR: 1.32–5.32 ng/mL, n = 54).

The study has witnessed that the serum levels of MMP-7 levels are 13-fold higher in EHBA (median MMP-7: 23 ng/mL, IQR: 19.94–27.85) as compared to normal babies and 6-fold higher as compared to those with NH. Singh et al. [18] in his study had reported serum levels of MMP-7 levels are 23-fold higher in EHBA (median MMP-7: 28.1 ng/mL, IQR: 19.1–30.7) as compared to normal babies and 15-fold higher as compared to those with NH. The results are in concurrence with the previous reports. [1-3] However, there is a wide variation in median values between different studies, with the highest values being reported by Yang et al. [1] from China with a rise of 42-fold when compared to age-matched controls. This could be ascribable to various factors such as the age of the patient, ethnicity, differences in sample collection, technique of measurement (including dilution technique and kit used), sample storage, and severity of liver injury. However, two studies from China itself have reported a difference in MMP-7 values [1,3] although the age group of the patients under study is not grossly different (54 days in Yang et al. [1] and 59 days in Jiang et al. [3] for EHBA). Wu et al. [2] have reported upon the youngest age group and the serum MMP-7 levels are the lowest of all.

The serum MMP-7 levels were higher in samples obtained from infants with EHBA at >90 (n = 8) days of age compared with <90 (n = 3) days of age. The arbitrary value of 90 days was chosen based on the prognostic cutoff for surgery.[34] Singh et al.[18] in his study had reported serum MMP-7 levels were comparable in samples obtained from infants with EHBA at >90 (n = 11) days of age compared with <90 (n = 14) days of age. Subgroup stratification at cutoff of 30 days and 60 days was not possible as the study cohort had 1 patient and 4 patients in the respective subgroups. Wu et al. [2] have, however, shown that the serum MMP-7 levels were significantly higher in EHBA patients beyond 30 days of age. The difference may be explained by the difference in the progression of the disease pathology with age and establishment of liver fibrosis.

In current study, based on age categorization, almost 50% of children <90 days old and cent percent of children >90 days had MMP-7 value >4.99 which had significant statistical values for both case and control groups.

Singh et al. [18] in his study had reported diagnostic role of serum MMP-7 for biliary atresia and established a serum cutoff level of 4.99 ng/mL to stratify neonatal cholangiopathy into EHBA and NH with sensitivity, specificity, and negative predictive values of 96%, 90.4%, and 95%, respectively. This compares favorably with the data reported in literature. [1-3]

The METAVIR scoring system and the Ishak's modification of Knodell's Histological Activity Index were used individually to quantify the processes of "inflammatory activity" and "fibrosis." Both these parameters were found to correlate strongly with the serum MMP-7 levels. These observations have further suggested the potential role of MMP-7 as a noninvasive prognostic biomarker or a surrogate indicator of the severity of liver injury. These observations also suggest indirectly that follow-up MMP-7 levels after Kasai's portoenterostomy may be used to monitor the progress of the disease pathology. Wu et al. [2] reported a positive correlation between serum MMP-7 levels and collagen content in the liver. Jiang et al. [3] had documented an association with liver fibrosis but not with inflammatory activity over the METAVIR scale, which was contrary to our observations. The discrepancy in observations may partly be explained by the age difference of the patients in the two studies.

The utility of APRI in EHBA as a predictor of liver fibrosis and cirrhosis, native liver survival, and prognosis has been disputed between studies.[21-24] Reports pertaining to the utility of FIB-4 in biliary atresia as a serological indicator of liver fibrosis are also conflicting. [25,26] However, Hwang et al. documented a positive correlation of both APRI and FIB-4 with progressing clinical categories in EHBA.[27] In current study, distribution of FIB-4 mean score against MMP-7 scores show a perfect distribution around the middle reference line, but not the same with APRI. These findings may be explained by the observations from the meta-analysis by Lin et al. [28] wherein a pooled analysis of the data from forty different studies suggested that the score is more valid for cases with advanced pathology.[28]

The limitations of this study was small sample size and conducted at a single center. The study design is suited to determine the diagnostic value of MMP-7 levels for EHBA and differentiating it from NH. However, it would deserve further study to establish the prognostic value of MMP-7 as a biomarker of liver injury.

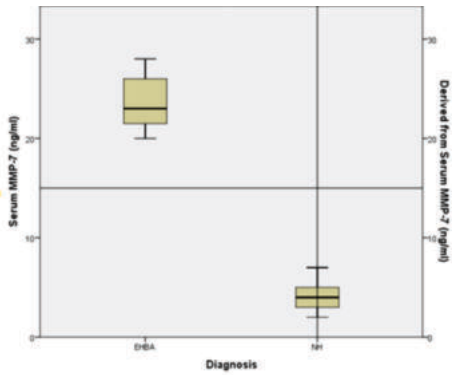

Fig.1 Box And Whisker Plot Showing Distribution Of Serum MMP-7 Among Patients With EHBA And NH.

Table.1 Sociodemographic Association Among Study Participants

Parameters	Variables	MMP <4.99	MMP >4.99	Total	CASES	CONTROLS
Gender	FEMALE	1	3	4	Pearson Chi square = 23.08 Fisher exact test = 12.7, df = 4, P value = 0.004	Pearson Chi square = 21.0 Fisher exact test = 7.4, df = 2, P value = 0.05
	MALE	5	11	16		
	Total	6	14	20		
Age	< 90 days	6	6	12	Pearson Chi square = 29.57 Fisher exact test = 17.7, df = 4, P value = 0.014	Pearson Chi square = 21.0 Fisher exact test = 7.08, df = 2, P value = 0.04
	>90 days	0	8	8		
	Total	6	14	20		
Weight	<2.8 kg	2	2	4	Pearson Chi square = 24.095 Fisher exact test = 13.6, df = 4, P value = 0.41	Pearson Chi square = 21.0 Fisher exact test = 8.7, df = 2, P value = 0.09
	>2.8 kg	4	12	16		
	Total	6	14	20		

Table.2 Correlation Of Matrix Metalloproteinase-7 Levels With Other Facets Of Extrahepatic Biliary Atresia

		Serum MMP-7 (ng/ml)
Serum MMP-7 (ng/ml)	Pearson Correlation	1
	Sig. (2-tailed)	
	N	20
AST (SGOT)-U/L	Pearson Correlation	.190
	Sig. (2-tailed)	.422
	N	20
Serum Bilirubin (Total)-mg/dl	Pearson Correlation	.126
	Sig. (2-tailed)	.597
	N	20
ALT (SGPT)-U/L	Pearson Correlation	-.038
	Sig. (2-tailed)	.873
	N	20
ALP-(U/L)	Pearson Correlation	.411
	Sig. (2-tailed)	.072
	N	20
Serum G.G.T.-(IU/L)	Pearson Correlation	.900**
	Sig. (2-tailed)	.000
	N	20
Platelet count (10*9/L)	Pearson Correlation	-.463*
	Sig. (2-tailed)	.040
	N	20

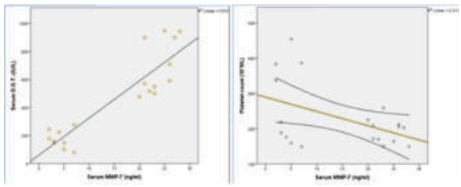

Fig. 2 Correlation Of Serum Matrix Metalloproteinase-7 Levels With Serum G.G.T. And Platelet Count

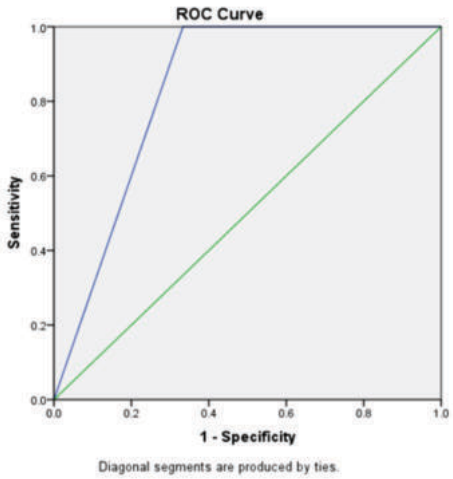

Fig. 3 Receiver-operator Characteristic Curve Analysis Showing Serum Matrix Metalloproteinase-7 Levels As A Diagnostic Biomarker For Extrahepatic Biliary Atresia.

Table. 3 Quantification Of Fibrosis And Inflammation In Liver Biopsy Specimen In Neonatal Cholestasis Cases With METAVIR & Ishak's (modified Knodell's Histological Activity Index) Scoring Systems

Categories	Grades	N = 20	Percentage
METAVIR & Ishak Activity Grade	None	4	20 %
	Mild	7	35 %
	Moderate	6	30 %
	Severe	3	15 %
METAVIR Fibrosis Stage & Category	F0	9	45 %
	Early (F1-F2)	3	15 %
	Advanced (F3-F4)	8	40 %
Ishak Fibrosis Stage & Category	F0	9	45 %
	Early(F1-F3)	3	15 %
	Advanced(F4-F6)	8	40 %
Diagnosis	Neonatal Hepatitis	9	45 %
	EHBA	11	55 %

Table.4 Correlation Between Inflammation And Fibrosis Upon Liver Histopathology (METAVIR/ishak Scoring) And Serum Matrix Metalloproteinase-7

Parameters	Variables	MMP <4.99	MMP >4.99	Total	CASES
Metavir Fibrosis Category	F 0	6	3	9	Pearson Chi square = 35.04 Fisher exact test = 21.15, df = 6, P value = 0.000
	Early	0	3	3	
	Advanced	0	8	8	
	Total	6	14	20	
Ishak Fibrosis Category	F 0	6	3	9	Pearson Chi square = 35.04 Fisher exact test = 21.2, df = 6, P value = 0.000
	Early	0	3	3	
	Advanced	0	8	8	
	Total	6	14	20	
Diagnosis	NH	6	3	9	Pearson Chi square = 35.05 Fisher exact test = 22.5, df = 4, P value = 0.000
	EHBA	0	11	11	
	Total	6	14	20	

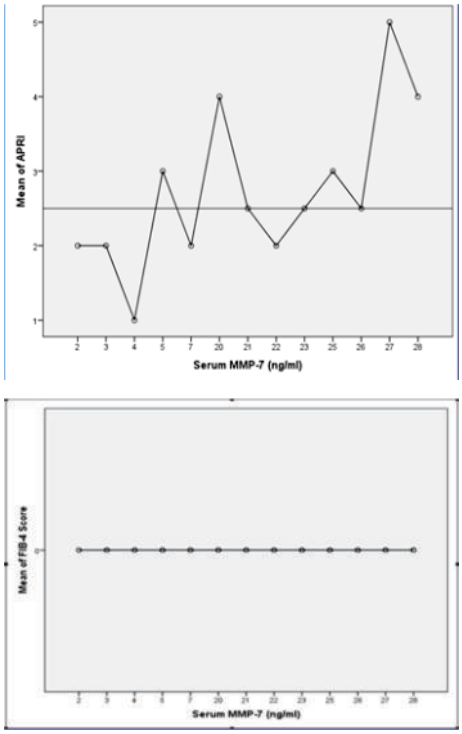

Fig.4 Predictive Accuracy Of Aspartate-to-platelet Ratio Index And Fibrosis-4 Markers In Quantifying Liver Injury Vis-à-vis Serum Matrix Metalloproteinase-7

CONCLUSIONS

The diagnostic and prognostic value of MMP-7 and its role as noninvasive biomarker in the diagnosis and management of EHBA has been strongly suggested by the study. The high concentrations of MMP-7 in EHBA may be used to sub-stratify the patients of neonatal cholangiopathy into EHBA and NH. The strong correlation of MMP-7 with histopathological injury has also suggested that MMP-7 may be used as a prognostic biomarker of liver fibrosis and a follow-up marker in EHBA. The role of APRI and FIB-4 as markers of liver fibrosis in the management of EHBA could not be verified.

REFERENCES

1. Yang L, Zhou Y, Xu PP, et al. Diagnostic accuracy of serum matrix metalloproteinase-7 for biliary atresia. Hepatology. 2018;68(6):2069–77
2. Wu JF, Jeng YM, Chen HL, et al. Quantification of serum matrix metallopeptide 7 levels may assist in the diagnosis and predict the outcome for patients with biliary atresia. J Pediatr. 2019;208:30–7.
3. Jiang J, Wang J, Shen Z, et al. Serum MMP-7 in the diagnosis of Biliary atresia. Pediatrics. 2019;144(5):e20190902.
4. Chen X, Dong R, Shen Z, Yan W, Zheng S. Value of gamma-glutamyl transpeptidase for diagnosis of biliary atresia by correlation with age. J Pediatr Gastroenterol Nutr. 2016; 63(3):370–3.
5. Tang KS, Huang LT, Huang YH, et al. Gamma-glutamyl transferase in the diagnosis of biliary atresia. Acta Paediatr Taiwan. 2007;48(4):196–200.
6. Goel P, Bhatnagar V, Das N, Kalaivani M. Evaluation of blood levels of nitric oxide as a means of differentiation between neonatal hepatitis and extrahepatic biliary atresia: A pilot study. J Indian Assoc Pediatr Surg. 2015;20(3):139-42.
7. Liu J, Yang Y, Zheng C, et al. Correlation of Interleukin-33/ST2 Receptor and Liver

Fibrosis Progression in Biliary Atresia Patients. Front Pediatr. 2019;7:403.
8. He JP, Hao Y, Wang XL, et al. Comparison of different noninvasive diagnostic methods for biliary atresia: a meta-analysis. World J Pediatr. 2016;12(1): 35–43.
9. Kim YH, Kim MJ, Shin HJ, et al. MRI based decision tree model for diagnosis of biliary atresia. Eur Radiol. 2018;28(8):3422–31.
10. Mandelia A, Lal R, Mutt N. Role of hepatobiliary scintigraphy and preoperative liver biopsy for exclusion of biliary atresia in neonatal cholestasis syndrome. Indian J Pediatr. 2017;84(9):685–90.
11. Yoon HM, Suh CH, Kim JR, et al. Diagnostic performance of sonographic features in patients with biliary atresia: a systematic review and meta-analysis. J Ultrasound Med. 2017;36(10): 2027–38.
12. Russo P, Magee JC, Boitnott J, Bove KE, Raghunathan T, Finegold M, Haas J, et al. Design and validation of the biliary atresia research consortium histologic assessment system for cholestasis in infancy. ClinGastroenterolHepatol 2011;9:357-62.
13. Hsieh CS, Chuang JH, Huang CC, et al. Evaluation of matrix metalloproteinases and their endogenous tissue inhibitors in biliary atresia-associated liver fibrosis. J Pediatr Surg. 2005;40(10): 1568–73.
14. Huang CC, Chuang JH, Chou MH, et al. Matrilysin (MMP-7) is a major matrix metalloproteinase upregulated in biliary atresia-associated liver fibrosis. Mod Pathol. 2005;18(7):941–950.
15. Serinet MO, Wildhaber BE, Broue P, Lachaux A, Sarles J, Jacquemin E, Gauthier F, et al. Impact of Age at Kasai Operation on Its Results in Late Childhood and Adolescence: A Rational Basis for Biliary Atresia Screening. Pediatrics 2009;123:1280-6.
16. Woessner JF Jr., Taplin CJ. Purification and properties of a small latent matrix metalloproteinase of the rat uterus. J Biol Chem 1988;263:16918-25.
17. Parks WC, Mecham RP. Matrix Metalloproteinases. Vol. 263. San Diego: Academic; 1988.
18. Singh TR, Goel P, Bajpai M, et al. Serum matrix metalloproteinase-7 a s a dignostic and prognostic biomarker for extrahepatic biliary atresia. J Indian Assoc Pediatr Surg. 2022;27:227-35.
19. Huang CC, Chuang JH, Chou MH, Wu CL, Chen CM, Wang CC, Chen YS, et al. Matrilysin (MMP-7) is a major matrix metalloproteinase upregulated in biliary atresia-associated liver fibrosis. Mod Pathol 2005;18:941-50.
20. Rohani P, Mirrahimi SB, Bashirirad H, et al. Serum matrix metalloproteinase-7 levels in infants with cholestasis and biliary atresia. BMC Pediatr. 2022;22:351.
21. Grieve A, Makin E, Davenport M. Aspartate Aminotransferase-to-Platelet ratio index (APRI) in infants with biliary atresia: Prognostic value at presentation. J Pediatr Surg 2013;48:789-95.
22. Kim SY, Seok JY, Han SJ, Koh H. Assessment of liver fibrosis and cirrhosis by aspartate aminotransferase-to-platelet ratio index in children with biliary atresia. J Pediatr Gastroenterol Nutr 2010;51:198-202.
23. Lampela H, Kosola S, Heikkilä P, Lohi J, Jalanko H, Pakarinen MP. Native liver histology after successful portoenterostomy in biliary atresia. J Clin Gastroenterol 2014;48:721-8.
24. Lind RC, Verkade HJ, Porte RJ, Hulscher JB. Aspartate transaminase-to platelet ratio index is not correlated with severity of fibrosis or survival in children with biliary atresia. J Pediatr Gastroenterol Nutr 2012;54:698-9.
25. Leung DH, Khan M, Minard CG, Guffey D, Ramm LE, Clouston AD, et al. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. Hepatology 2015;62:1576-83.
26. Mansoor S, Yerian L, Kohli R, Xanthakos S, Angulo P, Ling S, et al. The evaluation of hepatic fibrosis scores in children with nonalcoholic fatty liver disease. Dig Dis Sci 2015;60:1440-7.
27. Hwang J, Yoon HM, Kim KM, Oh SH, Namgoong JM, Kim DY, et al. Assessment of native liver fibrosis using ultrasound elastography and serological fibrosis indices in children with biliary atresia after the Kasai procedure. Acta Radiol. 2021;62(8):1088-96.
28. Lin ZH, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: An updated meta-analysis. Hepatology 2011;53:726-36.