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Diagnostic value of MMP-1 in chronic liver diseases

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Abstract

Background: Chronic liver disorders in children constitute an increasing concern with major public health impacts, the MMP is degrading diverse extracellular matrix macromolecules and involves tissue reshaping and repair in physiological processes. The purpose of this research was to assess the diagnostic usefulness of MMP-1 in children with chronic liver disorders. Methods: 60 children undertook this research; Group I (hepatic group): 60 children with chronic liver disease, Group II (control group): 20 seemingly healthy youngsters, The entire history of taking, comprehensive clinical examination, abdominal US and laboratory testing like a complete blood count and liver function tests have been performed for all children (Alt, AST, Albumin, PT, PTT, INR, Total and direct bilirubin), Liver biopsy was conducted in all patients, with human enzyme-connected immunosorbent testing measuring Serum MMP-1 levels (ELISA). Results: the mean MMP-1 in hepatic group was 11.2±2.1 ng/ml and in control group 32.7±17.4 ng/ml. In the hepatic group controlled, $p<0.001$, MMP-1 was statistically lower in order to measure the performance of MMP-1 in liver fibrosis (>F1), AUC was 0.872 (95 percent confidence interval: 0.709-0.487), $p<0.001$. Sensitivity was 95% at a cutoff threshold < 11.3 ng/ml and specificity was 95%. ROC testing was conducted to evaluate MMP-1 performance in detecting severe liver fibrosis (>F3); AUC $p<0.001$. The sensitivity was 90% at cutoff point < 4.5 ng/ml and the specificity was 80%. Conclusion: Fibrosis identification in children with chronic liver disorders might benefit from MMP-1.

Keywords: Matrix metalloproteinase, MMP-1, Liver, Fibrosis.

1. Introduction

In many of the body's metabolic processes, liver plays an important function including the control of protein, fat and carbohydrates metabolism, storage of vitamins, activation, detox and excretion of waste products. Chronic liver disorders are an increasing concern in children with major public health repercussions. Many infant liver illnesses are in reality a forerunner of chronic adult hepatopathy, cirrhosis and hepatocellular carcinoma [1].

Different extracellular matrix macromolecules are decomposed by matrix metalloproteinases (MMP) and participate in physiological processes of tissue reshaping and repair. Theoretically, disordered MMP activity might lead to such clinical conditions as gradual fibrosis, cancer invasion and connective tissue degeneration [2].

Most kinds of chronic liver illness are linked with hepatic fibrosis, in which the breakdown rate of extracellular matrix proteins is low, compared to the increasing production rate [3].

According to their substrate specificity, matrix metalloproteinases are classed as three main types: collagenases, gelatinases and stromelysins. Interstitial collagenase (EC 3.4.24.7, MMP-1), among others, cleaves into one and three-quarters products the native helix of fibrillary collagenase I, II and III, particularly at the same place. These products are vulnerable to further degradation by other proteinases. Due to collagen type I and III preponderance in the fibrotic liver, the production of MMP-1 would be essential to start collagen breakdown [4].

The purpose of this research was to assess the diagnostic usefulness of MMP-1 in children with chronic liver disorders.

2. Patients and methods

This case-control study was carried out on 60 children who attended the outpatient hepatology clinic at Benha university hospitals and National liver institute, Menoufia University after informed written consent was obtained from the parents or caregivers of enrolled children after explanation of the study, the laboratory work was done in clinical pathology department, Benha University. 20 apparently healthy children matched age and sex was taken as a control group. All subjects were classified into:

- **Group I (hepatic group**): included 60 children with chronic liver disease, their mean age was 21.1±5.3 years, , they were 58 (58.3%) males and 23 (41.7%) females.
- **Group II (control group**): included 20 apparently healthy children, their mean age was 14.3±4.4 years, they were 39 (48.3%) males and 32 (51.7%) females.

2.1. Inclusion criteria

Children with chronic liver diseases as chronic viral hepatitis (B and C), autoimmune hepatitis, metabolic liver disease, liver tumors, and liver cysts less than 18 years old. their diagnosis was based on 2019 Practice Guidance and Guidelines From the American Association for the Study of Liver Diseases [5].

2.2. Exclusion criteria

Children with chronic liver diseases with congenital diseases and associated with other comorbid disease (ex: renal, cardiovascular, ….).

The study was approved by Ethical committee of Benha University. And an informed written consent

was taken from parents or caregivers of enrolled children, after full explanation of the aim of the study.

All children incorporated in this study were subjected to full history taking, complete clinical examination, abdominal US, and laboratory investigations as complete blood count (CBC), and liver function tests (Alt, AST, Albumin, PT, PTT, INR, Total and direct bilirubin)

PELD score (pediatric end stage liver disease score) was used to assess disease severity scoring system for children under 12 year of age [7], MELD score: (Model for end stage liver disease score) was used for patients above 12 year of age [8]. Chronic liver disease is classified into Child - PUGH class A to C employing the added score from above [9].

2.3. Statistical analysis

The data were coded, entered and processed on computer using SPSS (version 24). The results were represented in tabular and diagrammatic forms then interpreted. Mean, standard deviation, range, frequency, and percentage were use as descriptive statistics. The following tests were done: Chi-Square test X^2 was used to test the association variables for categorical data. Student's t-test was used to assess the statistical significance of the difference between two population means in a study involving independent samples, with normal distribution. ROC curve was used to determine cut off values of TNF alpha, BCL2 and lymphocytic counts with optimum sensitivity and specificity in prediction of HAI and MODS in critically ill children.

3. Results

There was no statistical difference between hepatic and control groups regarding age, or gender, table (1).

Most patients (104 patients, 56.7%) had score A, 11 patients (9.2%) had score B, and 5 patients (4.2%) had score C. The mean PELD Score was 13.9±9.3, and the mean MELD Score was 12±3.1. AST to Platelet Ratio Index (APR) score, and Fibrosis-4 (FIB4 score), where used as a non-invasive biomarkers for assessment of liver fibrosis in hepatic group the mean APR score was 1.1±0.85, and the mean FIB4 score was 0.53±0.54in hepatic group.

The mean MMP-1 was 14.2±5.1 ng/ml in hepatic group, and 61.7±20.4 ng/ml in control group. MMP-1 was statistically lower in the hepatic group that controls, $p<0.001$, Figure 1.

There was a significant positive correlation between MMP-1 and (hemoglobin, WBCs, and platelets), while there was a significant negative correlation between MMP-1 and (ALT, AST, PT, PTT, INR, and albumin). And there was a significant negative correlation between MMP-1 and (PELD score, MELD score, APR score, FIB-4 score, and CHILD-PUGH score). While there was no significant correlation between MMP-1 and age, anthropometric measures, or US parameters, table 3.

Table (1) Sociodemographic data of the studied groups.

		Hepatic group		Control group		Test	P value
		N=120	%	N=60	%		
Age/years	**Mean ±SD**	13.1±5.3		14.3±4.4		t=0.961	0.320
Gender	**Male**	70	58.3%	29	48.3%	X^2= 1.6	0.212
FH of liver	**Negative**	115	95.8%	59	98.3%	X^2= 0.4	0.827
disease	**Positive**	5	4.2%	1	1.7%		

t: Student t-test; X^2: Chi square test; FH: family history.

Figure 1: Diagnosis of the hepatic group

Table (2) Liver biopsy findings in hepatic group.

		Hepatic group	
	Biopsy	**N=120**	**%**
Liver biopsy	**Apoptotic cells**	19	15.9 %
	Hydropic swelling no steatosis	19	15.9 %
	Mosaic like appearance	30	25%
	Interface hepatitis	29	24.1%
	Regular hepatocytes	6	5%
	Distended mononuclear phagocytic cells	6	5%
	Hepatocytes showed diffuse hydropic and balloning degeneration.	6	5%
	Mononuclear inflammatory cells	5	4.1%
	A3	27	22.5%

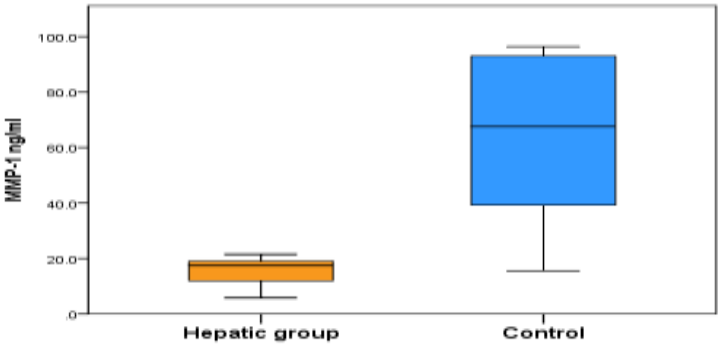

Fig. (1) MMP-1 in the studied groups.

Table (3) Correlation between MMP-1 and patients data.

	MMP-1	
Variables	**r**	**P value**
Age/ year	-0.129	0.089
Weight/ cm	0.138	0.170
Height/cm	0.088	0.334
BMI	0.109	0.112
Liver span/cm	0.107	0.146
Spleen span/cm	0.029	0.785
Hemoglobin(gm/dl)	0.327*	<0.001
WBCs(l)	0.457*	<0.001
Platelets (10^3/l)	0.446*	<0.001
ALT(IU/L)	-0.389*	0.008
AST(IU/L)	-0.448*	<0.001
PT(Seconds)	-0.361*	<0.001
PTT(Seconds)	-0.582*	<0.001
INR	-0.404*	<0.001
Albumin (g/L)	-0.312*	<0.001
PELD score	-0.688	<0.001
MELD score	-0.617*	<0.001
APR score	-0.653*	<0.001
FIB-4 score	-0.557*	<0.001
CHILD-PUGH score	-0.522*	<0.001

r = Correlation coefficient; *Significant; ALT: alanine aminotransferase, AST: aspartate aminotransferase, PT: prothrombin time, PTT: Partial thromboplastin time, INR: international normalized ratio. MMP: Matrix Metalloproteinases

MMP-1 level was significantly lower in patients who had hepatomegaly, splenomegaly, or ascites than who didn't (p=0.031, p=0.018, amd p<0.001, respectively). According to degree of fibrosis; MMP-1 level decreased significantly with increasing the degree of fibrosis, the mean MMP-1 in F1 was 19.2±1.5, in F2= 13.1±0.7, in F3= 8.8±0.4, and in F4=6.2±0.5 ng/ml, p<0.001. According to Histological activity index (HAI); MMP-1 level decreased significantly with increasing the degree of fibrosis, the mean MMP-1 in A1 was 18.3±2, in A2= 14.4±5.3, and in A3=9±3.5 ng/ml, p<0.001. According to CHILD-PUGH Score; MMP-1 level decreased significantly with increasing the degree of severity of liver disease, the mean MMP-1 in score A was 16.3±4.4, in score B= 8.8±3.5, and in score C=7±0.8 ng/ml, p<0.001.

ROC analysis was done to assess the performance of MMP-1 to detect liver fibrosis (>F1); AUC was 0.972 (95% confidence interval: 0.909-0.987), p<0.001. At a cutoff point < 43.3 ng/ml, the sensitivity was 95% and specificity was 95%. ROC analysis was done to assess the performance of MMP-1 to detect sever liver fibrosis (>F3); AUC was 1, p<0.001. At a cutoff point < 235 ng/ml, the sensitivity was 100% and specificity was 100%, Fig. (3).

4. Discussion

Both liver damage and regeneration are associated with complicated extracellular matrix (ECM) mechanisms. While normal ECM breakdown is an essential characteristic of tissue healing and remodelling, abnormal ECM turnover leads to a range of disorders of the liver. The key enzymes involved in ECM breakdown are matrix metalloproteinases (MMPs). In addition to reshaping the ECM, MMPs govern immunological responses. Autoimmune hepatitis metabolism of the liver was the most common

diagnosis in this investigation. 23 patients (19.1%) had viral hepatitis {18%) had HCV and HBV}, and 17 (9.2%) had cholestasis for differential diagnosis, with a chronic hepatitis.

Our results were comparable to Behairy et al, [10], they reported that 25% were diagnosed with chronic hepatitis (3,3% Dubin Johnson Syndrome, 15% with glycogen storage and 6,7% with Wilson disease), 43,4% with chronic hepatitis [Autoimmune hepatitis 20% with chronic hepatitis, of unknown aetiology 11,7% with steatohidone] (5 percent Alagille syndrome and 5 percent progressive familial intrahepatic cholestasis).

A decreased incidence of autoimmune illness was documented in Hanif et al. [11] and Dhole et al. [12] autoimmune hepatitis was found in 10.9 percent of patients with CLD and in 13 (8.6 percent) of the cases. Hepatitis C Infection was the major cause of the CLD in 4 (2.7%) individuals in a research conducted by Abou-Taleb et al., (13), neomatal cholestasis disorders (41.05%) and metabolic liver diseases (35.1%).

In two studies from Pakistan metabolic liver disease represented 36 percent and 36.5 percent of CLD (14,15). Sathe [16] also showed that metabolic liver disease accounted for 41.2% of CLD patients in a Western India research.

In the research Thajeel et al. [17] analysed trace elements for a hundred individuals with chronic hepatitis and discovered that the largest proportion was of chronic liver disease, while the lower proportion was of autoimmune hepatitis, for bile abnormalities and undiagnosed chronical hepatitis.

Kamal, [18], stated that 3 percent of youngsters under the age of 19 had chronic hepatitis C infections in Upper Egypt and 9 percent in Lower Egypt. No instances of hepatitis C infection were reported in Hanif et al., [11] during their research. However, hepatitis C infection was the cause of CLD in 31.66% of children in the Tahir et al. (19) investigation. And in a research from Nigeria by Ahmed et al. [20], 57.1 percent of the children tested with viral aetiology were CLDs. There have been 21.4 percent non-infectious instances of bile atresia, cholestatic hepatitis, hepatoblastoma and galactosaemia. Of the 24 positive patients with serological viral antigen markers (57.1 percent), 18 were positive for hepatitis B surface antigen (HbsAg), 10 were positive for HbeAg and six had a high viral load of HBV DNA. Five (11.9%), some with co-infections, children have been positive for hepatitis C and anti-HCV antibodies.

The etiological spectrum of CLDs vary depending on age, research site, illness frequency, availability of diagnostic tools, physician experience and referral pattern [21].

The incidence of idiopathic CLD ranges from 5 to 10% in various regions of the globe, such as the UK [22]. Unlike Dar et al., [23], from India, 50% of CLD patients remained idiopathic. As follows, additional investigations, Thajeel et al. [17], from Iraq, have not diagnosed 25% of cases. This high prevalence of idiopathic CLD in these investigations reflects budget constraints and the lack of modern and specialised diagnostic instruments in order to determine the underlying aetiology of CLD. The proportion of idiopathic CLD lowers when specific diagnostics tools are found, such as the introduction of HbsAg and anti-HCV, which have transferred many previously designated idiopathic CLDs. There is still a considerable proportion of patients among these patients waiting to develop appropriate diagnostic tools for idiopathic and etiological diagnosis [22].

In the current investigation, the mean MMP-1 in the hepatic group was 34.2±5.1 ng/mL while the control group was 45.7±27.4 ng/mL. In the checking hepatic group, MMP-1 was significantly lower, $p<0.001$. Similarly, Murawaki et al. [24] found that levels of blood MMP-1 were considerably lower compared with controls of minimum chronic hepatitis (CH) patients ($P<0.001$), mild CH ($P<0.0001$), moderate CH ($P<0.0001$) and CH-C patients ($P<0.0001$).

Our findings were agreed with [25] because MMP-1 was statistically lower (3.6±0.8µg/mL) than F1&F2 (5.6±0.6µg/mL) patients (<0.001).

In Ando et al. [1], serum MMP levels were compared to those in healthy controls in patients with early NASH (n=24) and advanced NASH (n=9). In early NASH patients, serum levels of MMP-1 were more likely than in late NASH patients.

In the recovery phase of experimental liver fibrosis, MMP-1 has a key function to play in the degradation of the extracellular matrix in rats yet MMP-1 appears to trigger the differentiation of MMP-1 expressing cells. MMP-1 is hypothesised to be involved in NASH pathological progression. MMP-1 may serve a kick-starting function as a quick response to oxidative stress, that means increased MMP-1 levels help the NASH develop [1].

In the research, Attallah et al., [25] agree: "Fibro-check: combination of direct and indirect indicators for liver fibrosis in patients with chronic hepatitis C," and showed that F2-F4 patients were related with lower MMP-1 levels than patients with F1, $p<0.001$. While in Ando et al., [1], serum fibrosis MMP-1 levels (F1 group) are greater than those in healthy statistically significant controls ($P = 0.019$). There was, however, no link between MMP-1 and the fibrosis stage.

Our results were in accord with the study by Murawaki et al. [24], the serum MMP-1 level was closely linked with the degree of periportal necrosis (Kruskal–Wallis test $P<0.0001$), the level of intralobular necrosis and the degree of portal ($P<0.005$) and the degree of liver fibrosis, while the relationship between serum MMP-1 levels and liver histological findings was examined. The association between serum levels MMP-1 and HAI was shown by Spearman (ć= −0,641; P<0,0001). In Ando et al.[1], in steatosis grade 2 and inflammation grades 1 and 2 serum MMP-1 levels were greater than in healthy controls, but no link was identified between histological serum levels and degree. (AUC) MMP-1 was 0.98 to discriminate

cirrhotic liver patients from healthy people and 0.78 to discrimination cirrhotic liver patients from non-cirrhotic patients. With 98% sensitivity and 97% efficiency, the diagnostic potential for MMP-1 for differentiating between healthy people was quite high. Cirrhosis in CHC was identified by MMP-1 with 71% sensitivity and 73% efficiency.

5. Conclusion

A validated non-invasive fibrosis marker in paediatric patients would be highly valuable. This signal might assist doctors in therapy and act as a longitudinal measure of therapy success without recurring liver biopsies. Our research results show that MMP-1 is effective for fibrosis diagnosis in children with chronic liver disease.

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Author contribution

Authors contributed equally in the study**.**

Conflicts of interest

No conflicts of interest

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