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Differential Elevation of Serum Matrix Metalloproteinases 1 and 2 in Pediatrics Chronic Liver Diseases

ABSTRACT

Background/Aim: Non invasive diagnosis of hepatic fibrosis has become the focus because of the limited biopsy, especially in the screening of hepatic fibrosis. Matrix metalloproteinases (MMPs) are thought to play an essential role in liver injury associated with tissue remodeling. The present study aims to investigate levels of two major collagen-degrading enzymes, matrix metalloproteinase (MMP-1& MMP- 2) in children with viral and parasitic liver diseases.

Methods: Serum samples from 75 children with chronic hepatitis B (n = 12), hepatitis C (n = 22), Bilharziasis (n = 34), and healthy children (n = 30) were collected for measuring the levels of MMP-1 and MMP-2 using ELISA technique. Liver function tests were measured using auto-analyzer. Abdominal ulltrasonography was also done.

Results: The results of this study showed a significant increase in MMP-1 level in children with Bilharziasis, whereas serum MMP-2 level was significantly increased in children with hepatitis B and C. Serum MMP-1 and MMP-2 levels were correlated with liver function indices and liver biopsy scores. MMP-1 level showed negative correlation with ALT (r= -0.274, P< 0.05), AST (r= -0.343, P< 0.01) ALP (r = -0.322,P<0.05), total bilirubin (r= - 0.357, P< 0.01), GGT(r= -0.244, P<0.05). Significant positive correlation was obtained with ALT/AST ratio (r=0.250, P≤ 0.05), serum albumin (r=0.249, P< 0.05), and prothrombin concentration (r= 0.236, P< 0.05). Level of MMP-2 showed positive correlation with ALP(r= 0.249, P<0.05). According to the severity of liver disease, levels of MMP-1 showed significant decrease whereas MMP-2 showed significant increase. The sensitivity level of MMP-1 was 0, 0 & 76.5 % and of MMP-2 was 50, 48.3 & 61.8 % for HBV, HCV and Bilharzial respectively at specificity 100% for both markers.

Conclusion: Since serum MMP_S activities were significantly varied according to the cause as well as the stage of liver fibrosis, an individual profile of these parameters might serve as an easy accessing serum marker to monitor the progression of liver disease.

Keywords: Hepatic fibrosis, non invasive diagnosis, matrix metalloproteinases

INTRODUCTION

Hepatic fibrosis is the result of an excessive accumulation of extracellular matrix components, in part due to increased synthesis and deposition of various collagens (Type 1 collagen in particular) and noncollagenous glycoproteins {1}. Indirect evidence indicates insufficient extracellular matrix degradation as a factor contributing to the pathological accumulation of hepatic extracellular matrix {2}, but the role of specific matrix- degrading enzymes in this process is still controversial.

Matrix metalloproteineases (MMPs) are important group of enzymes that degrade the extracellular matrix and other extracellular proteins {3}. MMPs are synthesized by hepatic stellate cells that are involved in degradation of these extracellular matrix proteins (fibrolysis). The family of MMPs consists of collagenases, glatenases and recently described membrane type MMPs (MT-MMPs). MMPs are secreted in latent form and their activation is regulated by a family of tissue inhibitors of metaloproteinases (TIMMs) {4}. Importantly the physiological balance between degradation and deposition of extra-cellular matrix (ECM) is maintained by the tissue inhibitors of MMPs (TIMPs). All TIMPs are secreted proteins that have the potential to reversibly inhibit active forms of MMPs {5}.

Matrix metalloproteinases (MMPs) are central to tissue remodeling {6}, however little is known about the temporal pattern and differential regulation of hepatic MMP expression in the course of chronic human liver disease {7}. Therefore, the present work was planned to evaluate the expression of two major collagen–degrading enzymes, matrix metalloproteinases 1 (MMP-1) and 2 (MMP-2) activities in hepatocellular damage in children with viral and bilharziasis infections.

SUBJECTS AND METHODS

Subjects for this study were obtained from patients with liver fibrosis diagnosed by histopathological examination. One hundred and five children were enrolled in this study from Al-Hussein hospital and Menofyia Liver Institute inpatient and outpatient clinic; they were divided as follows:

1. HBV group: 12 patients
2. HCV group: 29 patients
3. Bilharzial group: 34 patients
4. Control group: 30 apparently healthy children with no history or clinical evidence of liver disease or any other diseases

All individuals were subjected to full history and clinical examination.

Specimen collection

Under complete aseptic technique, 5 ml of blood was taken from the patients and controls. Blood samples were divided into two portions, the first one (1.8 ml) was added to 0.2 ml Na-citrate (3.8 m%) in test tube to obtain plasma, while the remaining portion was allowed to clot naturally in another test tube. Serum was separated and stored at -80 °C, until tested, whereas plasma was used immediately to measure prothrombin time and prothrombin concentration.

Analytical determination

Using ELISA techniques of kits purchased from Dia-Sorin Biomedical Company, Hepatitis B surface antigen (HBsAg), Hepatitis B Core Antibody IgM (HBcAb-IgM), and Hepatitis B Core Antibody IgG (HBcAb-IgG), were measured according to the methods of Boniolo et al {8}, Tedder and Wilson {9} and Hoofangle et al {10} respectively. Anti-HCV was detected by third generation ELISA kit from the Biochem. Immunosystem Company. {11}. HCV-RNA extraction was carried out by reverse transcription polymerase chain reaction (RT-PCR) according to the method described by Ravaggi et al {12}. Anti-bilharzial antibodies were detected by indirect haemagglutination test (IHA) according to the method of Hoshino et al {13}, using kits of Fumoze-France. Serum levels of AST, ALT, alkaline phosphatase (ALP), total protein, albumin, total bilirubin and GGT were measured using EKTOCHEM 750XRC Analyzer. Prothrombin time and prothrombin concentration were also estimated in plasma, according to method of Poller {14}.

Total MMP-1 concentration was measured by ELISA technique using kit of Oncogene Research Products Company-Sandiago, USA, according to the method of Zhang et al. {15}**,** while total MMP-2 concentration was measured by immunoassay technique according to the method of Murphy, {16}**,** using kit of R&D Systems Company- USA.

Abdominal Ultrasonography

To assess the size and echopattern of the liver, the size of the spleen and the presence of ascitis or any other abnormalities in the abdomen, abdominal ultrasonography was done using RT-X200 Prob 3.5 MHZ convex of general Electric Company – USA.

Statistical analysis

The data obtained were presented in tables as mean ± standard error. The difference between two groups was calculated using unpaired t-test, while the differences among more than two groups were calculated using F-test (One way analysis of variance, ANOVA). Correlation of variable was tested by Pearson's test. The analysis was run on IBM compatible computer using the SPSS/PC+ statistical package (SPSS Inc. Chicago, IL). Sensitivity, specificity and cut-off values were calculated according to Sox et al., {16}.

RESULTS

Serum levels of MMP-1 & MMP-2 in all patients (n=75) were significantly increased as compared with control. The recorded increases were 45.2% and 18.12% respectively (Table-I). The data revealed significant increases in MMP-1 level only in Bilharzial children as compared to control group ($P \leq 0.05$). However, a significant increase was recorded in levels of MMP-2 in patients with viral hepatitis B&C as compared with the healthy children ($P \leq 0.05$).

The correlation between the biochemical markers (MMP-1 & MMP-2) and liver function indices in patients with liver diseases (n=75) are illustrated in Table (II). Serum level of MMP-1 showed a significant negative correlation with ALT ($r=0.274$, $P \leq 0.05$). AST ($r=0.343$, $P \leq 0.01$), ALP ($r=0.322$, $P \leq 0.05$), total bilirubin ($r=0.357$, $P \leq 0.01$) and GGT ($r=0.244$, $P \leq 0.05$) while positive correlation was recorded with ALT/AST ratio ($r=0.250$, $P \leq 0.05$), serum albumin ($r=0.249$, $P \leq 0.05$) and prothrombin concentration ($r=0.236$, $P \leq 0.05$). To sum - up, the level of MMP-1 was related to good liver function. Level of MMP-2 showed positive correlation only with ALP ($r=0.249$, $P \leq 0.05$).

Data in Table (III) shows increasing tendency in MMP-1 levels with a decrease in severity of liver disease from Child's A to C while a significant increase was observed in the level of MMP-2, with severity of liver affection (ANOVA) test.

According to ultrasonographic finding (Table IV), MMP-1 level showed a significant increase in 57 patients of enlarged spleen, using t-test and 25 patients with PPF, using ANOVA test. However, MMP-2 showed a significant decrease only in 4 patients with diffuse and periportal fibrosis by ANOVA test.

Sensitivity results of MMP-1 was 0% for viral patients and 73.5 % for patients with bilharziasis at specificity level of 100% (Fig. 1) and the sensitivity level for MMP-2 was 50,41.4, and 35.3 % for patients with HBV, HCV and bilharziasis respectively at specificity level of 100% (Fig. 2).

DISCUSSION

Liver fibrosis is characterized by imbalanced deposition and degrading of extra-cellular matrix (ECM). Many factors are involved in this process. Therefore, it is difficult to evaluate the fibro-proliferative activity {18}.

In our study, we compared serum level of MMP1 & MMP2 to observe whether a difference between patients with viral or parasitic liver damage is demonstrated or not. Our results indicate that there is a significant increase in level of MMP-1 in patients with bilharzial liver affection; whereas increased level of MMP-2 is related to viral liver damage whether due to HBV or HCV. Thus, expression of the two Metalloproteinases differs according to the cause of liver damage.

Consistent with our findings, Gomez and co-author {19} agreed with the concept that matrix MMPs differ in their expression in Schistosomal portal fibrosis; they also reported that MMP-2 expression was always less intense than MMP-1. Moreover, Bin-Bin et al., {18} and Kasahara et al., {20} found that in patients with chronic hepatitis C, serum MMP-2 levels were significantly higher especially in patients with no response to interferon than in those with sustained and transient response. They also found that serum MMP-1 levels did not differ hence; they concluded that serum MMP-2 level might be useful for estimating the degree of fibrosis in patients with HCV.

This study also showed that expression of these two enzymes was different according to the extent of liver damage, while serum MMP-1 decreased , serum MMP-2 increased with the progress of liver affection from Child's A to C as well as with the deterioration of liver function.

Our finding that serum level of MMP-1 was related to good liver function (as evidenced by its negative correlation with ALT, AST, ALP, GGT & total bilirubin and its positive correlation with ALT/AST ratio, serum albumin, and prothrombin concentration) is supported by the finding that its level was also decreased with the progression of liver damage from Child's A to C. In contrast, MMP-2 was found to be increased with the progression of liver cell damage (from Child's A to C). A previous study reported that reversal of aminotransferase ratio (ALT/AST) with prolonged prothrombin time is positive predictor markers in hepatic cirrhosis {21}.

The normal liver contains the sub endothelial space which separates the epithelium (hepatocytes) from the sinusoidal endothelium. This space contains a basement membrane – like matrix composed of non- fibril – forming collagens including type IV {22}. A hall mark of early liver injury is the replacement of this normal sub endothelial matrix, which contains laminin, type IV collagen, and fibronectin, with one enriched in inter-stitial collagens types I of III {23}. This replacement may lead to deterioration of hepatocellular function {24}.

MMP-1 shows a substrate specificity for the native forms of interstitial collagens type I and III {25}. Current evidence indicates that except for the granulocyte MMP-8, MMP-1 is the only collagen with specificity for native interstitial type 1 of III collagens produced in the liver. Absent or reduced MMP-1 activity may therefore shift the balance between fibrogenesis and fibrolysis, which are delicately maintained in normal tissues, towards accumulation of native interstitial collagens. Moreover, it is likely that the enhanced expression of MMP-2 may contribute to the alteration of normal liver matrix in the perisinusodial space, thus affecting liver cell function and favouring the progression of the fibrotic process {1}.

Unlike proteinases that degrade normal basement membrane constituents (i.e., type IV collagenases, such as MMP-2, which degrades type IV collagen) and thereby indirectly promote net matrix accumulation, interstitial collagenase MMP-1 degrades type I and III collagen that could potentially be beneficial {23}. Milani and coworkers agreed with this concept and they concluded that expression of MMP-2 in the absence of MMP-1 expression may be responsible for the quantitative and qualitative changes of extracellular matrix observed in chronic liver disease {1}.This study suggests that the differential expression of matrix metalloproteinases (MMP-1 and MMP-2) in patients with bilharzial and viral liver damage explains in part the rapid progression of liver cirrhosis in patients with viral liver disease than in those with bilharziasis.

In conclusion, expression of MMP-1 and MMP-2 differs according to the cause of the disease. They also differ according to the severity of liver damage. MMP-1 expression is related to good liver function and to less severe hepatic damage.

Table I: Serum levels of MMP-1 and MMP-2 in total and different studied groups (mean ± SE)

	Control (n=30)	HBV (n=12)	HCV (n=29)	Bilharzial (n=34)	Total (n=75)
MMP-1(ng/ml)	4.2 ± 0.3	4.1 ± 0.4	4.1 ± 0.2	8.5 ± 0.3 *	6.1±0.3*
% Change		2.4 %	4.8%	102 %	45.2 %
MMP-2(ng/ml)	653.37 ±18.4	849.33±31.3*	792.61±34.4*	726.65 ± 37.6	771.8±22.6*
% Change		30 %	*21.3 %*	11.2 %	18.1 %

Table II: Pearson correlation between MMP-1, MMP-2 with liver function parameters in all patients with liver diseases (n=75)

	MMP - 1	MMP - 2
ALT	-0.274*	0.037
AST	-0.343*	- 0.050
ALT/AST	0.250*	0.100
Alk. Phosphtase	-0.322*	0.293*
T.Protein	0.164	0.009
Albumin	0.249*	- 0.122
T.Bilirubin	-0.357*	0.211
GGT	-0.244*	0.190
Prothrombin %	0.236*	0.006

*significant at level < 0.05

Table IV: Serum levels of MMP-1 and MMP-2 according to ultrasonographic finding (mean ± SE)

	MMP – 1 (ng/ml)	**MMP – 2** (ng/ml)
Spleen		
Enlarged(n=57)	6.5 ± 0.4*	758.1±25.4
Not (n=18)	4.8 ± 0.4	815 ± 48.9
Ascitis		
Present (n=15)	5.8 ± 0.6	847 ± 38.6
Not (n=60)	6.2 ± 0.4	752.8± 26.1
Liver:		
Hepatomegaly (n=13)	5.0 ± 0.7	791.6 ± 47.7
Shrunken (n=2)	4.4 ± 0.1	654 ± 29.0
PPF (n=25)	8.5 ± 0.5*	731.1 ± 41.5
Diffusepathology (n=12)	3.9 ± 0.3	802.4± 41.7
diffuse+PPF (n=4)	7.4 ± 1.1	508.5 ± 80.5*
coarse texture (n=14)	4.5 ± 0.3	836.0 ± 43.5
coarse texture+PPF (n=5)	5.8 ± 1.1	928.0 ± 64.2

*significant difference at level < 0.05 using t-test for spleen and ascites or F- test for liver ultrasonographic finding

Fig 1: Scatter diagram of MMP- 1(ng/ml) level. The Horizontal line represents the cut-off value of 7.68 (mean +2SD of control). The sensitivity is 0% for HBV and HCV and 76.5% for Patients with Bilharziasis at a specificity level of 100%

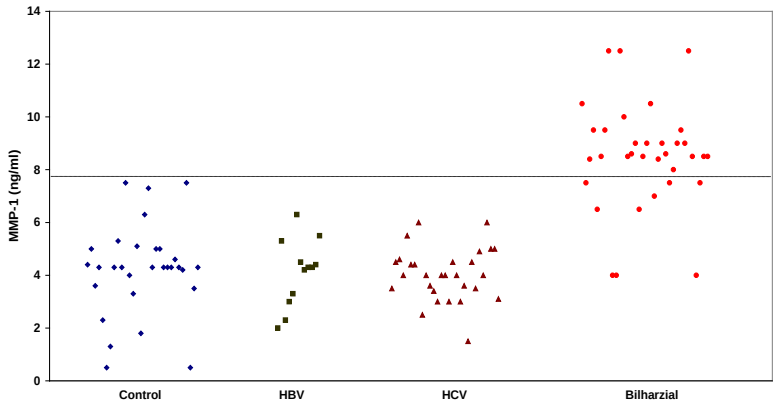
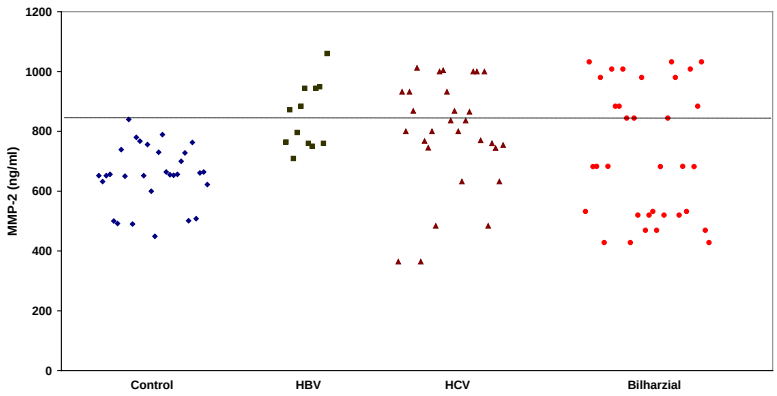


Fig 1: Scatter diagram of MMP- 2(ng/ml) level. The Horizontal line represents the cut-off value of 854.29 (mean +2SD of control). The sensitivity is 50%, 48.3%, 61.8% for Patients with HBV, HCV and Bilharziasis respectively at a specificity level of 100%



REFERENCES

1. *Milani S, Herbst H, Schuppan D, Grappone C,Pellegrini G, et al. Differential expression of matrix- metalloproteinase -1 and -2 genes in normal and fibrotic human liver. Am. J. Pathol. 1994; 144:pp.528-537.*
2. *Feng X, Yau D, Holbrook C, Reder AT. Type I interferons inhibit interleukin-10 production in activated human monocytes and stimulate IL-10 in T cells: implications for Th1-mediated diseases. J. Interferon. Cytokine. Res. 2002; 22:pp.311-9.*
3. *Armin B, Katrin P, Byron AK, Didier M, Jeans S, Johannes N, Gary AB. Matrix metalloproteinases and their inhibitors in human traumatic spinal cord injury. BMC-Neurol, 2007; 7:17.*
4. *Walsh KM, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase – 2 (MMP-2) and tissue inhibitors of metalloproteinases- 1 and -2 (TIMP-1 and TIMP- 2) as noninvasive markers of liver disease in chronic hepatitis C. Dig.Dis.Sci.1999; 44:pp.624-630.*
5. *Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J. Cell. Sci. 2002; 115:pp.3719-3727.*
6. *Roy R, Yang J, Mosses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J. Clin. Oncol. 2009; 27: pp.5287-5297.*
7. *Lichtinghagen R, Bahr MJ, Wehmeier M, et al. Expression and coordinated regulation of matrix metalloproteinases in chronic hepatitis C. and hepatitis C virus – induced liver cirrhosis. Clin. Sci. 2003; 105: pp.373-382.*
8. *Boniolo A, Dovis M, Matteja R. The use of enzyme-linked immunosorbent assay for screening hybridema antibodies against hepatitis B surface antigen. J. Immunology Meth. 1982; 49: pp.1.*
9. *Tedder RS, Wilson CR. IgM antibody response to hepatitis B core antigen in actute and chronic hepatitis B. J. Hyg. Canb. 1981; 86: pp.163.*
10. *Hoofnagle JH, Gerety RJ, Barker LF. Antibody to hepatitis B virus core in man. Lancet. 1973; 2: pp.869-873.*
11. *Alter HJ. Transfusion associated non-A, non-B hepatitis: The first decade. In: Viral Hepatitis and Liver Disease. Zuckermann AJ (ed.). Alan R, Liss, Inc., New York: 1988; 537- 42.*
12. *Ravaggi A, Primi D, Caraini E. Direct RT-PCR amplification of HCV RNA from human serum. RT-PCR methods. Appl. 1992; 1: pp.291-292.*
13. *Hoshino S, Camargo ME, Da Silva LC. Standardization of a haemagglutination test for schistosomiasis with formalin-treated erythrocytes. Am. Trop. Med. Hyg. 1980; 19: pp.463-470.*
14. *Poller L. Laboratory control of anticoagulant therapy. Sem Thromb Hemostasis, 1986; 12: pp.13- 19.*
15. *Zhang J, Fujimoto N, Iwata K, et al. A one- step sandwich enzyme immunoassay for human matrix metalloproteinase – 1 (interstitial collagenase) using monoclonal antibodies. Clin. Chim. Acta. 1993; 219:pp.1-14.*
16. *Murphy G. In Handbook of Proteolytic Enzymes, Barrett AJ et al. eds., Academic Press, San Diego; 1998; 1199-1205.*
17. *Sox HC, Blatt MA, Higgins MC, Marton KI. Medical decision making. Butterworth-Heinemann, USA 1989; pp. 67-146.*
18. *Bin-Bin Z, Wei C, Hong L, et al. Diagnostic value of platelet derived growth factor– β, matrix metalloproteinase-1 and tissue inhibitor of matrix metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. World J. Gastroenterol. 2003; 9: pp.2490-2496.*
19. *Gomez DE, Lorenzo MD, Alonso DF, Andrade ZA. Expression of metalloproteinases (MMP-1, MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) in Schistosomal portal fibrosis. Am. J. Hyg. 1999; 61:pp. 9-13.*
20. *Kasahara A, Hayashi N, Mochizuki K, et al. Circulating matrix metalloproteinase- 2 and tissue inhibitor of metalloproteinase-1 as serum markers of fibrosis in patients with chronic hepatitis C. Hepatol. 1997; 26: pp.574-583.*
21. *Ahmed IS, Musfireh S, Asif M, Arif MS. Alanine aminotransferase/Aspartate aminotransferase ratio reversal and prolonged prothrombin time: A specific indicator of hepatic cirrhosis. J Ayub. Med. Coll. Abbottabad. 2007; 19:pp. 22-24.*
22. *Roskams T, Rosenbaum J, De Vos R, et al. Heparan sulfate proteoglycan expression in chronic cholestatic human liver diseases. Hepatology. 1996; 24:pp.524-532.*
23. *Rockey DC. Pthophysiology of Liver Disease; The cell and molecular biology of hepatic fibrogenesis. Clin. Liver Dis. 2000; 4; pp.319-55.*
24. *Jarnagin WR, Rockey DC, Koteliansky VE, et al. Expression of variant fibronectins in wound healing: Cellular source and biological activity of the EIIIA segment in rat hepatic fibrogenesis. J. Cell. Biol. 1994; 127:pp.2037-2048.*
25. *Albanis E, Friedman SL. Hepatic Fibrosis: Pathogenesis and Principles of Therapy. Clin. Liver Dis. 2001; 2:pp.315-334.*