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Original Article

Study on the clinical expressions of MMP-1, MMP-2 and CD14 protein in liver tissues of infants with biliary atresia

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Abstract: Objective: To observe and explore the clinical expressions of MMP-1, MMP-2 and CD14 protein in liver tissues with different grades of liver fibrosis in infants with biliary atresia. Methods: A total of 20 infants with liver fibrosis and biliary atresia treated in our hospital from January 2015 to June 2017 were selected as the experimental group, while ten infants without hepatobiliary system disease were enrolled as the control group. The expression levels of MMP-1, MMP-2 and CD14 proteins in liver tissues were detected by enzyme-linked immunosorbent assay (ELASA) and immunohistochemistry. Results: The expressions of MMP-2 and CD14 protein in liver tissues of grade I, II and III in experimental group were significantly higher than those in control group ($P<0.05$). There were no significant differences in MMP-1 expression of all grades and MMP-2 and CD14 protein expressions of grade IV between two groups ($P>0.05$). The positive expressions of MMP-1 and MMP-2 protein were positively correlated with the degree of liver fibrosis ($r=0.53$, $P<0.05$; $r=0.57$, $P<0.05$). The positive expression of CD14 protein were negatively correlated with the degree of liver fibrosis ($r=-0.42$, $P<0.05$). Besides, there was a positive correlation between the expression of MMP-1 and MMP-2 protein ($r=0.67$, $P<0.05$). In addition, CD14 was negatively correlated with MMP-1 and MMP-2 protein expressions ($r=-0.38$, $P<0.05$; $r=-0.41$, $P<0.05$). Conclusion: The formation of liver fibrosis in infants with biliary atresia was positively correlated with the expressions of MMP-1 and MMP-2, and negatively related to the expression of CD14 protein.

Keywords: MMP-1, MMP-2, CD14, biliary atresia

Introduction

Biliary atresia (BA) is a pediatric disease with the obstruction of biliary system caused by progressive fibrosis of bile duct, presenting a continual pathological change course in clinic. Infants with BA usually suffer high bilirubin jaundice, white clay-colored feces and hepatomegaly 2 weeks after birth. And other symptoms like slow growth, skin itching, high portal pressure appear from 3 weeks to 2 years old [1]. Kasai procedure is an important treatment which can make bile drainage unobstructed. However, most infants still need liver transplantation in the end because of the occurrence of progressive liver fibrosis, cholestatic cirrhosis and portal vein hypertension. Progressive liver fibrosis is a critical factor that can limit the efficacy of surgery, thereby, preventing the progress of liver fibrosis can significantly improve the efficacy of surgery [2]. In addition, both matrix metalloproteinase (MMP) and cluster of differentiation antigen 14 (CDl4) play key roles in liver fibrosis [3, 4]. However, there are no relevant reports on whether MMP and CDl4 can promote liver fibrosis jointly. Therefore, 20 infants with liver fibrosis and biliary atresia were enrolled in this study and the expression levels of MMP-1, MMP-2 and CD14 protein in their liver tissues were detected. The results were as follows.

Clinical data and methods

General information

A total of 20 infants who were diagnosed with BA and liver fibrosis in our hospital from January 2015 to June 2017 were included as subjects. Inclusion criteria: BA existed in infants was confirmed by surgery and pathology after Kasai; all the infants were treated with Kasai procedure.

Exclusion criteria: Patients with severe complications of liver cirrhosis combined with portal hypertension, alimentary tract bleeding and intrahepatic lithiasis and so on; patients with severe cardiovascular, nephritic, endocrine, hematologic, and neurological diseases; patients with hepatic failure. There were 12 male patients and 8 female patients, aged from 50 to 183 days during the operation. According to Ohkuma's classification, these patients were divided into 4 grades: 6 cases in grade I, 7 cases in grade II, 5 cases in grade III and 2 cases in grade IV [4]. The other ten infants (6 males and 4 females) with hepatic rupture and treated with hepatic repair, aged 48 to 175 days, were selected as the control group. And there were no significant differences in age, age in days and sex between the two groups (P>0.05), and the data were comparable. The study had been approved by the Ethics Committee of the hospital. In addition, the patients' family members were aware of the diagnosis and treatment strategy and the informed consent had been signed.

Methods

Immunohistochemistry detection of the expression of MMP-1, MMP-2 and CD14 protein: Immunohistochemical staining streptavidin-perosidase (SP) method was applied. MMP-1, MMP-2 and CD14 monoclonal antibodies were all mouse anti-human monoclonal antibodies, which were purchased from Beijing Zhongshan Bio-Tech Co., Ltd. Each group was given negative control and DAB was applied for development. The expressions of MMP-1, MMP-2 and CD14 were regarded as positive when their membrane appeared yellow or brown yellow particles. Meanwhile, 10 fields of vision (400 ×) from each slice were chosen randomly; and the degree of positive expression of these proteins was judged by the staining intensity and the percentage of positive cells [5]. Staining intensity scoring: 0 point for non-staining, 1 for yellow, 2 for brown yellow, and 3 for tawny. Scoring of positive cell percentage: 0 point for below 5%; 1 point for 6%~25%; 2 for 26%~50%; 3 for above 50% [6]. The synthetic score was calculated by multiplying staining intensity score by the score of positive cells percentage (0-1 point was regarded as negative (-); 2-5 points as weakly positive (+); 6-8 points as moderately positive (++); 9 points as strongly positive (+++)).

ELISA detection of the expression of MMP-1, MMP-2 and CD14 proteins: The blood plasma samples were collected in a sterile vessel, then the plasma and liver samples were diluted at 1:10 by dilution and tested with MMP-1, MMP-2 and CD14 kits, which was purchased from Wuhan Boster Biological Technology Co., Ltd. ELISA meter was zeroed at the wavelength of 450 nm by blank control hole to determined OD value of each well. If the value was 2.1 times greater than the negative control, it could be regarded as positive.

Statistical analyses

Data were analyzed by the SPSS 23.0 statistical software. The measurement data were expressed as mean ± standard deviation, and the comparison among three groups was examined by one-factor analysis of variance and Bonferroni test. The enumeration data were expressed as case and percentage, and χ^2 test was used for comparison among three groups and χ^2 partition test was used for pair-comparison. Pearson correlation analysis was used for correlation analysis of MMP-1, MMP-2 and CD14 protein expression in liver tissue. P<0.05 was considered that differences were statistically significant.

Results

Expression of MMP-1 and MMP-2 in liver tissues of biliary atresia

There were no significant differences in MMP-1 protein in grade I, II and III between experimental group and the control group (2,612.42± 681.61) ng/ml (P=0.69, P=0.38, P=0.47), while there were differences in MMP-2 protein in grade I, II and III between experimental group and control group (2,423.51±643.71) ng/ml (all P<0.01), while the expression level of MMP-1 and MMP-2 protein in experimental group in grade IV did not obviously differ from control group (P=0.81, P=0.37). See **Table 1**.

Expression of CD14 protein in liver tissues of biliary atresia

There were differences in CD14 protein in grade I, II and III between experimental group and control group (2,657.28±592.61) ng/ml (all P<0.01), while the expression level of CD14 protein in grade IV in experimental group did

Table 1. The expression of MMP-1 and MMP-2 in liver tissues of biliary atresia

Group	Liver fibrosis grade	Case	MMP-1 protein (ng/ml)	MMP-2 protein (ng/ml)
Control group	Grade 0	10	2,612.42±681.61	2,423.51±643.71
Experimental group	Grade I	6	2,672.39±654.24	3,625.72±523.27**
	Grade II	7	2,698.33±625.37	3,316.45±872.12**
	Grade III	5	2,747.58±631.67	2,797.63±846.21**
	Grade IV	2	2,621.92±652.18	2,455.58±622.52

Note: Compared with the control group, **P<0.01.

Table 2. The expression of CD14 protein in liver tissues of biliary atresia

Group	Liver fibrosis grade	Case	CD14 protein (ng/ml)
Control group	Grade 0	10	2,657.28±592.61
Experimental group	Grade I	6	4,564.84±712.25**
	Grade II	7	4,329.73±751.42**
	Grade III	5	3,924.54±762.18**
	Grade IV	2	2,708.63±694.55

Note: Compared with the control group, **P<0.01.

not significantly differ from control group (P=0.37). See **Table 2**.

Immunohistochemistry detection of expression of MMP-1, MMP-2 and CD14 protein in liver tissues of biliary atresia

By immunohistochemistry detection, MMP-1 and MMP-2 were expressed in portal tract area fibrosis while CD14 protein was expressed in pseudolobuli, which could promote liver fibrosis. See **Figures 1** and **2**.

The positive expression of MMP-1 and MMP-2 protein was positively correlated with the degree of liver fibrosis (r=0.53, P<0.05; r=0.57, P<0.05). And the positive expression of CD14 protein was negatively correlated with liver fibrosis (r=-0.42, P<0.05). See **Tables 3** and **4**.

The positive expression of MMP-1 and MMP-2 protein was positively correlated (r=0.67, P<0.05); the expression of CD14 was negatively correlated with the expression of MMP-1 and MMP-2 protein (r=-0.38, P<0.05; r=-0.41, P<0.05). See **Tables 5** and **6**.

Discussion

The prognosis of infants with biliary atresia depends on age, type of atresia, severity of liver fibrosis, postoperative complications and many other factors [6]. Progressive liver fibrosis is an important factor that affects the efficacy of surgery. Therefore, prevention of the development of liver fibrosis after kasai procedure plays an important role in improving the efficacy of surgery.

Liver fibrosis is the result of the synthesis of extracellular matrix (mainly for type I and type II collagen) exceeding its degradation. In the course of biliary atresia, a large number of cytokines are released due to virus infection or local immunoreactive inflammatory abnormalities, which cause apoptosis and necrosis of the bile duct epithelial cells [7]. Among the numerous cytokines, CD14 is a promoting factor of liver fibrosis, and the matrix metalloproteinases (MMPs) also play key roles in the conversion of extracellular matrix.

MMPs are a kind of proteinase depending on zinc ion, with cell-extracellular matrix elements as hydrolysis substrates, and more than ten kinds of MMPs have been found [8]. MMP-1 mainly degrades type I and III collagen, prevents collagen deposition in liver and plays an active role in the prevention of hepatic fibrosis [9]. Research has found that MMP-1 plays a more important part in the occurrence of hepatic fibrosis, in which it presents higher expression than MMP-2 [10]. MMP-2 is an important gelatinase, which mainly degrades denatured interstitial collagens, intact type IV collagen (the main component of basement membrane), type V collagen and non-collagen protein such as elastin and fibronectin [11, 12]. MMP-2, an important pathological factor in inducing and promoting hepatic fibrosis, can degrade normal basement membrane in liver sinusoid, destroy the internal environment of liver survival and trigger the activation of hepat-

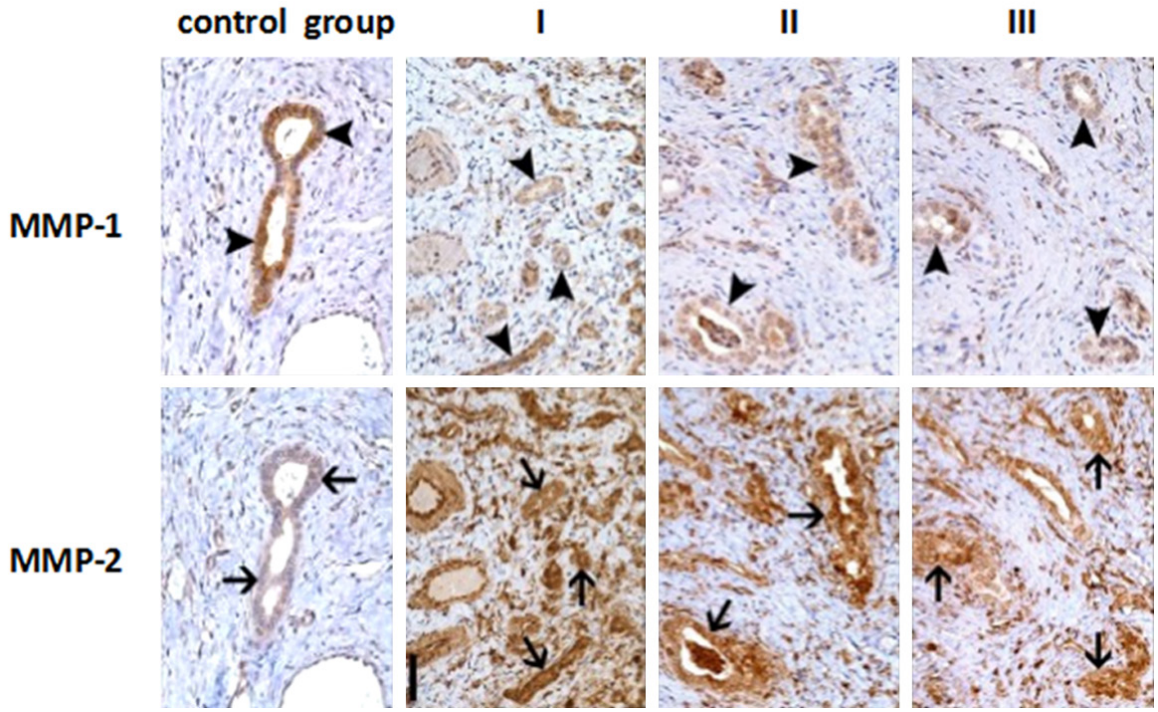

Figure 1. The expression of MMP-1 and MMP-2 in normal liver tissues and liver tissues of biliary atresia (400 ×).

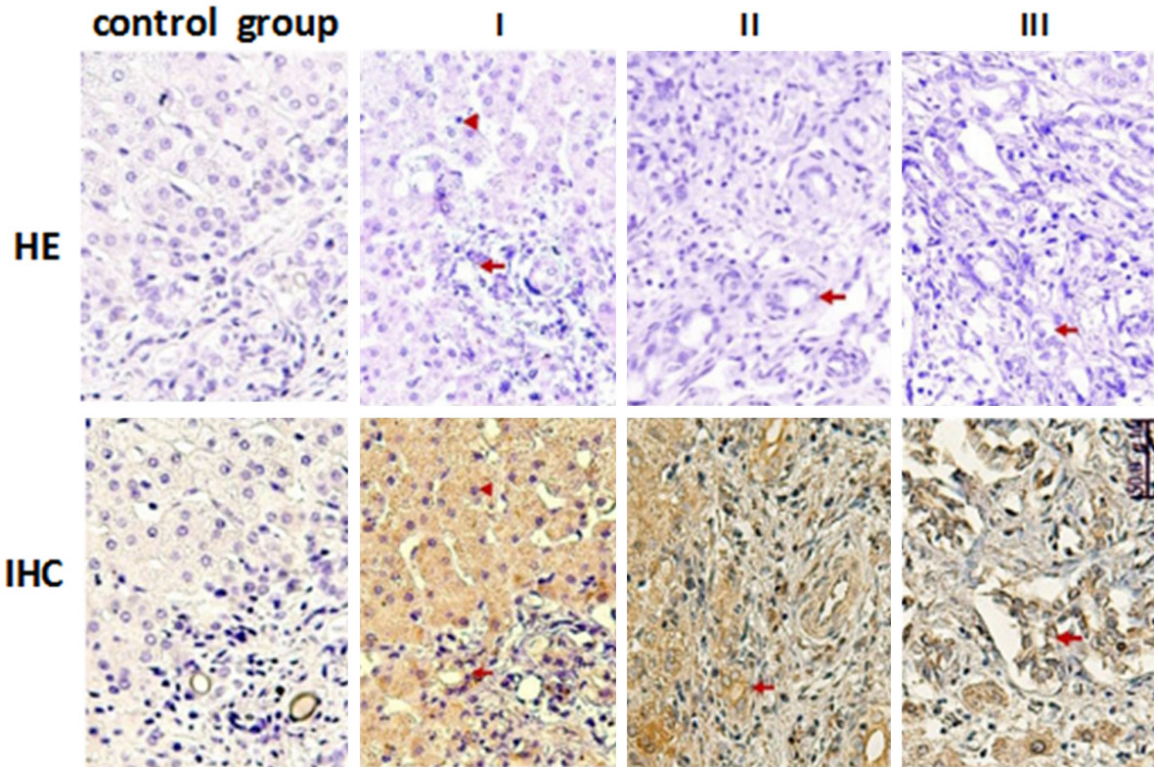

Figure 2. The expression of CD14 in normal liver tissues and liver tissues of biliary atresia (400 ×).

ic stellate cell (HSC) [13-15]. The results of this study indicated that the expressions of MMP-1 and MMP-2 protein of liver tissue in infants with biliary atresia were obviously higher than that in normal ones. Moreover, proteins' expressions increased as the hepatic fibrosis in biliary atre-

Table 3. The expression of MMP-1 and MMP-2 protein in patients with different liver fibrosis

Liver fibrosis grade	Case	MMP-1 protein expression (n)			MMP-2 protein expression (n)		
		+	++	+++	+	++	+++
Grade I	6	3	2	1	3	2	1
Grade II	7	2	4	1	3	3	1
Grade III	5	1	1	3	1	1	3
Grade IV	2	0	0	2	0	0	2
r value			0.53			0.57	
P value			0.032			0.037	

Table 4. The expression of CD14 protein in patients with different liver fibrosis

Liver fibrosis grade	Case	CD14 protein expression (n)		
		+	++	+++
Grade I	6	1	1	4
Grade II	7	2	4	1
Grade III	5	2	2	1
Grade IV	2	2	0	0
r value			-0.42	
P value			0.029	

Table 5. The correlation of CD14, MMP-1 and MMP-2 protein expression in liver tissues of biliary atresia

CD14	MMP-1		r	P	MMP-2		r	P
	+~++	+++			+~++	+++		
+~++	0	7	-0.38	0.011	1	7	-0.41	0.009
+++	6	0			6	0		

Table 6. The correlation of MMP-1 and MMP-2 protein expression in liver tissues of biliary atresia

MMP-1	MMP-2		r	P
	+~++	+++		
+~++	6	0	0.67	0.000
+++	1	7		

sia aggravated. Statistical process also showed that there was a significant correlation between MMP-1 and MMP-2, which was indicative of the close connection between the abnormal expressions of MMP-1 and MMP-2 protein of liver tissue in infants with biliary atresia protein and hepatic fibrosis. The mechanism of MMP-2 protein expression increase in liver tissue in BA patients may be as follows: Hepatic Kupffer cell, bile duct epithelial cell, hepatic stellate cell and hepatocyte can secrete transforming growth factorβ1 (TGF-β1), which is a potent factor in promoting hepatic fibrosis and promoting the synthesis of MMP-2; the expression of MMP-2 can be promoted via balance mechanism, which means in the process of hepatic fibrosis, excessive deposition of type IV collagen can increase the expression of MMP-2 [16].

CD14 is the LPS acceptor anchored by glycosylation. It is expressed on the surface of macrophage, neutrophils and other myeloid cells, which can be used as differentiation marker for identification [17]. In the research of human hepatocytes, it can be found that the product of CD14 is similar to a kind of acute phase reactants (APR) [18]. Although CD14 presents an excessive expression in hepatic fibrosis in biliary atresia, its exact mechanism remains unclear. The intrahepatic cholestasis can cause the increase of endotoxin, the activation of hepatocyte, the damage of bile duct epithelial cells, the phosphorylation of related target proteins in CDl4 signaling pathway by protein tyrosine kinase (PTK), the activation of mitogen activated protein kinase pathway. It also can catalyze the phosphorylation of transcription factors, regulate the expression of related gene proteins, then lead to the upregulation of the expression of CD14 [19, 20].

After the detection of CD14 protein expression in this study, the results showed that there were differences in MMP-1, MMP-2 and CD14 protein in grade I, II, III between the experimental group and control group ($P<0.05$). The BA hepatic fibrosis grading showed that from grade I to grade IV, the expression of MMP-1 and MMP-2 protein presented a gradual upward trend while the expression of CD14 protein presented a gradual downward trend. And the immunohistochemistry detection in the experimental group of this study showed that MMP-1 and MMP-2 protein expressed in portal tract fibrosis, while CD14 protein expressed in pseudolobule, which could promote hepatic fibrosis.

From the above data, a preliminary deduction can be made that the expressions of MMP-2 and CD14 protein present an opposite trend when liver lesion occurs and causes hepatic fibrosis of BA. And there is an inhibitory function between MMP-2 and CD14 protein. MMP-1 and MMP-2 belong to the same type of protein, but there is no direct correlation between them.

In summary, MMP-1, MMP-2 and CD14 protein play important roles in the occurrence and development of hepatic fibrosis of biliary atresia. But the sample size was comparatively small in this research, so the experimental results and specific mechanism still need to be further explored. Since MMP-1, MMP-2 and CD14 protein have close relation with hepatic fibrosis of BA, the further study can focus on the promotion mechanism of hepatic fibrosis for preventing the progress of hepatic fibrosis after BA and prolonging the survival time of patients after BA, and providing bases for improving the curative effect of biliary atresia surgery.

Disclosure of conflict of interest

None.

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