

RAPID COMMUNICATION

Alterations of biliary biochemical constituents and cytokines in infantile hepatitis syndrome

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Abstract

AIM: To investigate the biliary biochemical constituents and cytokines in infantile hepatitis syndrome (IHS).

METHODS: From 42 IHS subjects and 21 controls, serum and biliary biochemical constituents, including total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ -GT), total bile acid (TBA), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) both in bile and serum, were assayed. The subjects with IHS were divided into a cholestasis group ($n = 21$) and a hepatitis group ($n = 21$).

RESULTS: In the cholestasis group, serum TBIL, DBIL, ALT, γ -GT, TBA, IL-6 and TNF- α levels were higher than those in the control ($P < 0.01$); and also the biliary TBIL, DBIL, γ -GT and TBA levels were lower than those in the control, whereas biliary IL-6 and TNF- α levels were higher than those in the control ($P < 0.01$). In the cholestasis group, serum IL-6 and TNF- α levels were lower than those in bile ($P < 0.01$). In the hepatitis group, serum DBIL, ALT, γ -GT, TBA, IL-6 and TNF- α levels were higher than those in the control ($P < 0.01$ or 140.57 ± 70.32 vs 79.06 ± 35.25 , $P < 0.05$), while biliary TBIL, DBIL, γ -GT and TBA levels were lower than those in the control ($P < 0.01$), and biliary IL-6 and TNF- α levels were higher than those in the control ($P < 0.01$). In the hepatitis group, serum IL-6 and TNF- α levels were also lower than those in bile ($P < 0.01$). Serum TBIL, DBIL, γ -GT, IL-6 and TNF- α levels in the cholestasis group were higher than those in the hepatitis group, while biliary IL-6 and TNF- α levels in the cholestasis group were higher than those in the hepatitis

group. Biliary IL-6 and TNF- α were found to be more significantly increased than serum IL-6 and TNF- α in IHS ($P < 0.01$). The biliary IL-6 and TNF- α levels were positively correlated with serum DBIL, TBA and γ -GT levels in IHS subjects.

CONCLUSION: Biliary biochemical constituents alter in coincidence with pathological changes in hepatocellular injury. Cholestasis is more serious in IHS patients of cholestasis subtype. Assay of biliary IL-6 and TNF- α levels can be specific and sensitive to determine the inflammatory status of impaired liver in IHS.

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Key words: Infantile hepatitis syndrome; Biliary biochemical constituents; Biliary cytokines; Interleukin-6; Tumor necrosis factor- α

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INTRODUCTION

Infantile hepatitis syndrome (IHS), with a morbidity of 1/2500 in live-born infants^[1], comprises a series of symptoms, including jaundice, splenohepatomegalia, changes of texture of the liver, hepatic dysfunction in onset mainly in the neonatal period and infancy. It has been reported that the biochemical constituents and cytokines in blood alter when the disorder attacks^[2]. However, what changes of those biochemical constituents and cytokines in bile can be, and what relationships between IHS and physiologic jaundice in those constituents can be, remains a puzzle in pediatric practice. In this study, we focused on the alterations of biochemical constituents and cytokines in serum and bile obtained from subjects attacked by IHS.

MATERIALS AND METHODS

Subjects

According to the diagnostic criteria^[3], IHS was defined as: (1) age < 1 year; (2) jaundice; (3) splenohepatomegalia and changes of texture of the liver; and (4) alteration in hepatic function. Forty-two subjects [29 males and 13

Table 1 Comparison of serum and biliary biochemical constituents among three groups (mean \pm SD, $n = 21$)

Group	TBIL ($\mu\text{mol/L}$)	DBIL ($\mu\text{mol/L}$)	ALT (nkat/L)	γ -GT (nkat/L)	TBA ($\mu\text{mol/L}$)
Serum					
Cholestasis	188.76 \pm 72.88 ^{b,d}	130.76 \pm 48.18 ^{b,d}	126.80 \pm 82.12 ^b	266.20 \pm 92.73 ^{b,d}	118.62 \pm 43.58 ^b
Hepatitis	125.91 \pm 64.28	68.67 \pm 36.75 ^b	96.11 \pm 53.69 ^b	140.57 \pm 70.32 ^a	111.89 \pm 51.54 ^b
Control	124.89 \pm 62.07	6.19 \pm 2.55	24.23 \pm 7.17	79.06 \pm 35.25	14.50 \pm 9.76
Biliary					
Cholestasis	78.13 \pm 58.86 ^b	52.63 \pm 42.43 ^b	8.07 \pm 4.21	167.00 \pm 100.06 ^b	180.05 \pm 216.98 ^b
Hepatitis	89.95 \pm 59.12 ^b	63.56 \pm 53.39 ^b	8.21 \pm 4.15	278.55 \pm 201.10 ^b	266.63 \pm 268.09 ^b
Control	252.36 \pm 108.81	174.08 \pm 92.56	7.22 \pm 3.41	783.50 \pm 363.28	643.63 \pm 80.67

^a $P < 0.05$ vs control; ^b $P < 0.01$ vs control; ^d $P < 0.01$ vs hepatitis group.

Table 2 Comparison of serum and biliary IL-6 and TNF- α among three groups (mean \pm SD, $n = 21$, ng/L)

Group	Serum IL-6	Serum TNF- α	Biliary IL-6	Biliary TNF- α
Cholestasis	63.76 \pm 26.67 ^{b,d}	493.11 \pm 137.26 ^{b,d}	286.92 \pm 102.02 ^{b,d,f}	1625.89 \pm 563.41 ^{b,d,f}
Hepatitis	40.81 \pm 19.32 ^b	305.30 \pm 94.45 ^b	183.64 \pm 76.94 ^{b,f}	1068.55 \pm 383.35 ^{b,f}
Control	11.46 \pm 6.54	214.32 \pm 78.40	40.11 \pm 19.89	535.80 \pm 168.69

^b $P < 0.01$ vs control group; ^d $P < 0.01$ vs hepatitis group; ^f $P < 0.01$ vs serum.

females; average age 56 d (range: 33-120 d)] suffering from IHS, being divided into cholestasis subtype ($n = 21$) and hepatitis subtype ($n = 21$) according to the color of stool^[4], were randomly selected from in-patients in Wuhan Children's Hospital. Another 21 subjects [12 males and 9 females; average age 43 d (range: 23-68 d)], being ruled out from IHS and diagnosed as breast-feeding jaundice, were chosen as controls.

Methods

In fasting condition, next morning after admission to the hospital, non-anticoagulated venous blood was collected from all patients, and the blood specimens were examined instantly. The bile specimens were collected by infant duodenum drainage tube. The procedure of draining was carried out as mentioned below^[5]. Before draining, the subjects were instructed to fast for 4 h and given intravenous transfusion for essential nutrition, and given diazepam intravenously or chloral hydrate orally if restless. Then, the infants were placed at right arm reclining and the head was retained by an assistant; after applying a small amount of liquid paraffin and dispelling the tampon at the end of the tube, the operator inserted the tube through right nasal cavity to the stomach at a depth of 30-35 cm and gastric juice was drained out; then through the pylorus, the tube accessed the duodenum at the depth of 40-45 cm and duodenal juice was drained out. The draining was considered successful if yellow draining juice was obtained, or the head of the draining tube was ascertained in the duodenum by X-ray.

The biliary and serum total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ -GT) and total bile acid (TBA) were assayed by CL-7200 Fully-automated Chemistry Analyzer provided by Shimadzu Co. Ltd. The biliary and serum interleukin-6 (IL-6) and tumor necrosis factor- α

(TNF- α) were assayed by double antibody Sandwich-ELISA with the apparatus from Micro Reader-3 and the kit from R&D Co. Ltd.

Statistical analysis

One-way ANOVA (F -test, SNK-test) was employed to determine the differences of data among the three groups. Student's t test was applied for the differences in same constituent between biliary and serum data. Pearson correlation analysis was adopted to explore the relationships among the data. Statistical analyses were performed using SPSS 12.0 software.

RESULTS

Comparison of serum biochemical constituents and cytokines among three groups

Serum TBIL, DBIL, ALT, γ -GT, TBA, IL-6 and TNF- α levels were significantly higher in the cholestasis group than those in controls ($P < 0.01$). Moreover, serum DBIL, ALT, γ -GT, TBA, IL-6 and TNF- α levels were markedly higher in the hepatitis group compared to the controls ($P < 0.01$ or $P < 0.05$); while there was no significant difference in serum TBIL level between the hepatitis and control group. In addition, serum TBIL, DBIL, γ -GT, IL-6 and TNF- α levels in the cholestasis group were markedly higher than those in the hepatitis group ($P < 0.01$), but ALT and TBA levels were not obviously different between these two groups (Tables 1 and 2).

Comparison of biliary biochemical constituents and cytokines among three groups

Biliary TBIL, DBIL, γ -GT and TBA levels both in cholestasis and hepatitis groups were significantly lower than those in controls ($P < 0.01$), while biliary IL-6 and TNF- α levels in those two groups were notably higher

than those in controls ($P < 0.05$). In contrast to that in the hepatitis group, the value of biliary IL-6 and TNF- α in cholestasis group markedly increased ($P < 0.01$), but TBIL, DBIL, γ -GT and TBA levels in both groups were not significantly different. However, the value of biliary ALT was not obviously different among the three groups (Tables 1 and 2).

Comparison between biliary and serum cytokines

Both in cholestasis and hepatitis groups, the levels of IL-6 and TNF- α in serum were notably lower than those in bile ($P < 0.01$) (Table 2).

Relationships between biochemical constituents and cytokines

Biliary IL-6 was obviously correlated with serum DBIL, ALT and γ -GT with the coefficient of correlation of 0.4621, 0.4152 and 0.5376, respectively ($P < 0.05$). Moreover, the biliary TNF- α was significantly correlated with serum DBIL, ALT and γ -GT with the coefficient of correlation of 0.3972, 0.4309, 0.4713, respectively ($P < 0.05$). However, the serum IL-6 and TNF- α were not correlated with serum TBIL, DBIL, ALT, γ -GT, and TBA.

DISCUSSION

Serum biochemical constituents, such as TBIL, DBIL, ALT, γ -GT and TBA, are used to monitor liver function in medical practice. Among those constituents, bilirubin is a breakdown product of hemoglobin, and total and direct bilirubin (TBIL and DBIL) are usually measured to screen for or to monitor jaundice caused by liver or gall bladder dysfunction. Alanine aminotransferase (ALT), an enzyme found mainly in the liver, is released into the bloodstream when the liver is damaged or diseased. Gamma-glutamyl transpeptidase (γ -GT), existing in the endochylema of the hepatocyte and epithelium of the intrahepatic bile duct and being mainly synthesized by mitochondria in hepatocytes, is discharged to the duodenum through bile duct. Therefore, rise of γ -GT indicates hepatocytic dysfunction or obstruction of extra-hepatic bile duct. Especially, detection of biliary γ -GT can differentiate extra-hepatic biliary atresia and IHS, for bile not consisting of γ -GT when biliary atresia occurs^[5]. Total bile acid (TBA) is an exclusive index reflecting hepatic synthesis, secretion, metabolism and hepatocellular dysfunction, and it has been shown that its specificity, sensitivity and stability tendency in numerical hepatobiliary disorder in liver are all superior to the conventional liver function examinations^[6].

Cytokines are small proteins released by cells that have a specific effect on interactions and communications between cells or on behavior of cells, and participate in many pathophysiological progresses in hepatobiliary disorders. Biliary cytokines are produced by hepatocytes, macrophages and epithelium of bile duct. It has been reported that biliary IL-6 was exclusive for diagnosis of angiocholitis^[7] and TNF- α could reflect the extent of angiocholitis^[8]. We previously confirmed that under cholestatic condition, biliary IL-6 and TNF- α levels increased and were correlated with hepatocellular impairment and cholestasis in rabbit, thereby indicating

that IL-6 and TNF- α could reflect the extent of hepatocellular necrosis and angiocholitis^[9].

In this study, we observed that, compared to the controls, almost all biochemical constituents in serum were remarkably increased both in cholestasis and hepatitis groups, while those constituents in bile were mostly decreased, which indicated discharging of those from liver to blood and reduction of those in bile when hepatocyte inflamed and cholangiole was embarrassed. When compared between cholestasis and hepatitis groups, the cholestasis-related indexes TBIL, DBIL and γ -GT in serum increased more obviously in the cholestasis group than those in the hepatitis group, while no significant differences in those biochemical constituents in bile between these two groups were observed. In addition, the ALT and TBA, the indexes related to hepatocellular impairment, were not markedly different between the two groups both in serum and bile, manifesting the feature of cholestasis in IHS.

Following the previous animal experiment, we examined the two related cytokines to explore the role of IL-6 and TNF- α in IHS. We found that the two cytokines notably rose in blood and bile of IHS subjects compared to the controls, thereby indicating the inflammatory status in IHS. Furthermore, IL-6 and TNF- α in the cholestasis group increased more significantly than that in the hepatitis group, elucidating the greater severity in cholestasis. On the other hand, in both cholestasis and hepatitis groups, biliary cytokines were more elevated than serum cytokines, which implied biliary cytokines could be a more sensitive clue for diagnosing hepatic impairment.

From the statistical analysis, it was confirmed that biliary IL-6 and TNF- α had a positive correlation with serum DBIL, ALT and γ -GT, but the serum cytokines had not any correlations with serum biochemical constituents. This result illuminated that as cytokines in blood could be influenced by the state of whole body, assaying biliary inflammatory cytokines might be a specific and sensitive test for monitoring the development of IHS. Thus, the results clearly revealed that biliary biochemical constituents altered in coincidence with pathological changes in hepatocellular injury, which can demonstrate the severity of IHS, especially for cholestasis, and the differences between cholestasis and hepatitis subtype in this disorder. Furthermore, it can be concluded that the test of biliary IL-6 and TNF- α might be a specific and sensitive reference to determine the inflammation status of the impaired liver in IHS.

REFERENCES

- 1 McKiernan PJ. Neonatal cholestasis. *Semin Neonatol* 2002; 7: 153-165
- 2 Mei H, Huang JN, Peng HM. Changes of serum IL-8 and IL-10 in infantile hepatitis syndrome. *J Applied Clin Pediatr* 2003; 18: 370-371
- 3 Yan YG, Duan XC, Xu DL. Summary of national symposium for prevention and treatment of children's viral hepatitis. *Zhonghua Erke Zazhi* 1982; 20: 120
- 4 Dong YS. State and Advance of infantile hepatobiliary disorder. *J Chinese Physician* 1999; 27: 7-9
- 5 Huang ZH, Dong YS. [Dynamic examination of duodenal fluid in the differential diagnosis of infantile cholestatic jaun-

- dice]. *Zhonghua Erke Zazhi* 2004; **42**: 54-56
- 6 **Tomer G**, Ananthanarayanan M, Weymann A, Balasubramanian N, Suchy FJ. Differential developmental regulation of rat liver canalicular membrane transporters Bsep and Mrp2. *Pediatr Res* 2003; **53**: 288-294
- 7 **Rosen HR**, Winkle PJ, Kendall BJ, Diehl DL. Biliary interleukin-6 and tumor necrosis factor-alpha in patients undergoing endoscopic retrograde cholangiopancreatography. *Dig Dis Sci* 1997; **42**: 1290-1294
- 8 **Mano Y**, Ishii M, Okamoto H, Igarashi T, Kobayashi K, Toyota T. Effect of tumor necrosis factor alpha on intrahepatic bile duct epithelial cell of rat liver. *Hepatology* 1996; **23**: 1602-1607
- 9 **Huang ZH**, Huang X, Li Y. [Changes and significance of tumor necrosis factor-alpha and interleukin-6 level in plasma and bile during the formation of acute intrahepatic cholestasis in New Zealand white rabbits]. *Zhonghua Ganzangbing Zazhi* 2003; **11**: 313

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