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# Elevated Serum Macrophage Migration Inhibitory Factor Levels in Post-operative Biliary Atresia

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**OBJECTIVE:** Biliary atresia (BA) is one of the most common causes of neonatal cholestasis. Macrophage migration inhibitory factor (MIF) is an important mediator of inflammation and immune response in various diseases. The objective of this study was to examine the possible roles of MIF in BA.

**METHODS:** Forty-eight BA paediatric patients who had undergone a Kasai operation and 22 healthy children were recruited. The mean ages of the patients and controls were $8.47 \pm 0.74$ and $7.64 \pm 0.41$ years, respectively. The patients were categorised into two groups according to their serum levels of total bilirubin (TB) (TB < 2 mg/dL; no jaundice, and TB ≥ 2 mg/dL; persistent jaundice). The serum MIF levels were determined using commercially available enzyme-linked immunosorbent assay.

**RESULTS:** The mean serum MIF level of the BA children was higher than that of healthy controls ($0.43 \pm 0.04$ pg/mL *vs.* $0.27 \pm 0.02$ pg/mL; $p < 0.001$). However, there was no difference in serum MIF levels between BA patients with jaundice and those without jaundice. Further analysis revealed that there was no difference in serum MIF levels of BA patients without portal hypertension compared to that of BA patients with portal hypertension.

**CONCLUSION:** MIF production was elevated in BA patients compared to normal controls. It is likely that MIF plays a role in the pathophysiology of post-operative BA patients. However, the elevated MIF levels are not associated with either jaundice status or portal hypertension. [*Asian J Surg* 2009;32(2):109–13]

**Key Words:** biliary atresia, jaundice, macrophage migration inhibitory factor, portal hypertension

## Introduction

Biliary atresia (BA) is a progressive obliterative process involving the extrahepatic and intrahepatic bile ducts in the newborn. It is characterised by worsening cholestasis, hepatic fibrosis, and cirrhosis, which lead to portal hypertension and a decline in hepatic synthetic function.[1] BA affects approximately 1 in 8,000 to 1 in 15,000 live births. It is the most common cause of neonatal jaundice and the prime indication for paediatric liver transplantation worldwide.[2] Approximately 70% to 80% of children with BA finally require liver transplantation, which is indicated when Kasai portoenterostomy fails and symptoms of end-stage liver disease appear.[3,4] Aetiology and pathogenesis of BA have remained elusive. No agent has been confirmed as the cause of this disorder. The variability in patient presentation and outcome suggests that multiple factors may play a potential role in BA. A number of possible aetiologies of BA include defects due to viral infection or toxin exposure, defects in morphogenesis, genetic predisposition, defects in prenatal circulation, and immune or autoimmune dysregulation.[2]

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Macrophage migration inhibitory factor (MIF), identified nearly four decades ago, is considered a pleiotrophic lymphocyte and macrophage cytokine, but in a number of reports[5] has also been suggested to be an endocrine factor. Initially, MIF was reported as an immune response regulator extracted from the supernatants of T lymphocytes and was found to inhibit the random migration of macrophages. Its activity is associated with macrophage phagocytosis and delayed-type hypersensitivity.[6] Nowadays, MIF is being cloned[7] and its structure has been well characterised by crystallisation, nuclear magnetic resonance spectroscopy and various biochemical methods. MIF can be distinguished from other cytokines by a number of properties. It is expressed in large varieties of tissues including both immune and non-immune cells. The most critical functions of MIF are the regulation of macrophage functions, lymphocyte immunity, and endocrine functions. It plays an important role in the pathogenesis of several inflammatory diseases, including septic shock, rheumatoid arthritis, delayed-type hypersensitivity, inflammatory lung disease and cancer.[8]

In addition, MIF also promotes angiogenesis, and correlates with cell differentiation. This constitutes a link to BA based on experimental evidence of a vascular/ischaemia aetiology for BA as bile ducts receive their blood supply exclusively from the hepatic arterial circulation; thus, hepatic arterial ischaemia results in bile duct strictures as observed after liver transplantation.[9] Nevertheless, the pathologic role of MIF in BA has not yet been extensively studied. Recently, associations between MIF and various liver diseases have been explored in alcoholic liver disease and chronic hepatitis.[10,11] It has been demonstrated that serum and tissue expression of MIF were increased in alcoholic liver disease.[10] Moreover, serum MIF levels were elevated and correlated with alanine aminotransferase in chronic hepatitis. Therefore, serum MIF levels appear to reflect the severity of tissue injury in hepatitis B.[11]

MIF also plays a role in promoting the Th1 immune response in the development of acute hepatitis and hepatic injury as demonstrated in hepatitis models in mice.[12] Arikan et al suggested that the −173C allele of the MIF gene might be associated with susceptibility to BA.[13] The present study has been aimed at investigating the serum MIF levels in BA and to evaluate whether there is any association between serum MIF and outcome parameters in BA patients after Kasai operation.

## Materials and methods

This study was approved by the Ethics Committee on Human Research of the Faculty of Medicine, Chulalongkorn University. All parents of children with BA and of the healthy controls were informed of the study's objective. Written informed consent was obtained from the parents prior to the children's enrolment into the study.

*Patients*

Forty-eight paediatric patients (23 boys and 25 girls; mean age, 8.47 ± 0.74 years) with BA undergoing Roux-en-Y hepatic portojejunostomy (original Kasai operation) who attended the paediatric liver clinic, and 22 healthy children (nine boys and 13 girls; mean age, 7.64 ± 0.41 years) from Anuban-Ratchaburi primary school were enrolled in the study after acquiring their parents' written informed consent. Children in the healthy control group had normal physical examination results and no underlying disease. Among the 48 patients with BA, none displayed any signs or symptoms of fever, ascending cholangitis or clotting abnormalities at the time of blood sampling. None had received liver transplantation. To compare the various outcomes among BA patients, according to their levels of total serum bilirubin (TB) they were categorised into two groups: patients without jaundice (TB < 2 mg/dL, $n = 25$) and patients with persistent jaundice (TB ≥ 2 mg/dL, $n = 23$). Based on presence or absence of portal hypertension (PH), the sample group was categorised into two groups: patients with PH ($n = 26$), and patients without PH ($n = 22$). PH was validated by the presence of ascities and/or oesophageal varices on endoscopy.

*Laboratory methods*

The serum samples were collected and stored at −70 °C until tested. Serum MIF levels were determined by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D System, Minneapolis, MN, USA) according to the manufacturer's instructions. Recombinant human MIF was used to generate the standard curve. The liver function tests including serum albumin, total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) were performed using an automated Hitachi 912 analyser.

**Table.** Demographic data, liver function test, and serum MIF levels of the entire BA group, BA patients with jaundice, and patients without jaundice

| BA patients (normal) | Total | Jaundice | Jaundice-free | *p* |
|---|---|---|---|---|
| No. of patients | 48 | 23 | 25 | |
| Gender (M/F) | 23/25 | 8/15 | 15/10 | NS |
| Age (yr) | 8.47 ± 0.74 | 6.73 ± 1.07 | 10.07 ± 0.94 | NS |
| Albumin (3.4–5.5 g/dL) | 3.92 ± 0.12 | 3.46 ± 0.18 | 4.35 ± 0.09 | < 0.001 |
| Total bilirubin (0–1 mg/dL) | 5.64 ± 1.07 | 10.91 ± 1.65 | 0.80 ± 0.09 | < 0.0005 |
| Direct bilirubin (0–0.25 mg/dL) | 3.43 ± 0.75 | 6.90 ± 1.22 | 0.25 ± 0.05 | < 0.0005 |
| AST (0–38 U/L) | 144.58 ± 19.80 | 223.43 ± 32.68 | 71.84 ± 12.00 | < 0.001 |
| ALT (0–38 U/L) | 124.19 ± 14.33 | 173.83 ± 21.28 | 78.52 ± 15.02 | 0.001 |
| ALP (39–117 U/L) | 491.48 ± 31.07 | 599.48 ± 36.27 | 392.12 ± 40.19 | < 0.001 |
| GGT (7–50 U/L) | 177.67 ± 24.33 | 252.27 ± 36.82 | 109.04 ± 26.82 | 0.003 |
| MIF (pg/mL) | 0.43 ± 0.04 | 0.44 ± 0.06 | 0.42 ± 0.04 | NS |

Data are expressed as mean ± SEM. Values of *p* indicate differences between the jaundice and jaundice-free patients. AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatise; GGT = gamma-glutamyl transpeptidase; MIF = migration inhibitory factor; NS = not significant.

*Statistical analysis*

Comparisons of demographic and clinical parameters between groups were performed using unpaired *t*-tests. A value of $p < 0.05$ was considered statistically significant. Data are expressed as mean ± SEM.

## Results

MIF concentrations were determined in 48 serum samples from BA patients and 22 serum samples from healthy children. No significant difference of age (healthy controls *vs.* BA patients, 8.47 ± 0.74 years *vs.* 7.64 ± 0.41 years, $p = 0.16$) and gender (male: female, 9:13 *vs.* 23:25) between healthy controls and the BA patients was observed. Comparison between demographic data, liver function test results, and serum MIF levels between BA patients without jaundice and BA patients with persistent jaundice is shown in Table.

Serum MIF levels in the entire BA group, jaundice group, jaundice-free group, and healthy control group were 0.43 ± 0.04, 0.44 ± 0.06, 0.42 ± 0.04, and 0.27 ± 0.02 pg/mL, respectively. Serum MIF levels were significantly higher in BA patients than in the control group ($p < 0.001$) as shown in Figure.

BA patients were subsequently re-evaluated according to total serum bilirubin levels. We found that the difference in serum levels of MIF was not statistically significant between BA patients without jaundice and BA patients with jaundice ($p = 0.8$). In addition, serum levels of MIF were not significantly elevated in BA patients with PH compared to those without PH (0.43 ± 0.03 pg/mL *vs.* 0.43 ± 0.04 pg/mL; $p = 0.47$).

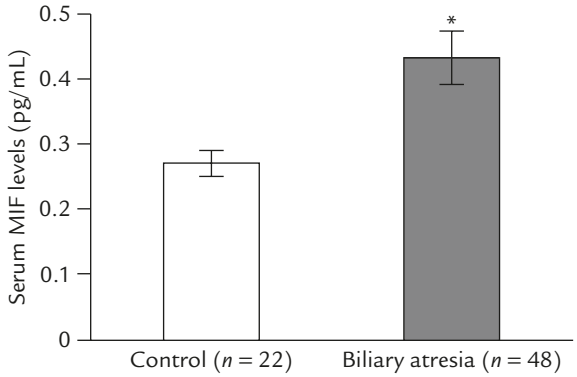


**Figure.** Comparison of serum MIF levels between biliary atresia patients and healthy controls. Data are expressed as mean and SEM. *$p < 0.001$ compared with controls.

## Discussion

Inflammatory cytokines mediate a variety of biological responses that commonly help augment host immunity, repair tissue damage and restore cellular and systemic homeostasis. Although a regulated cytokine response to pathogens or toxins is essential to host defence and tissue repair, cytokine excess and imbalance can result in tissue damage and fibrosis. Macrophage migration inhibitory factor (MIF) was originally described as the protein secreted by activated T lymphocytes capable of inhibiting random

migration of macrophages, concentrating macrophages at inflammation loci, and enhancing their ability to kill intracellular parasites and tumour cells.[14–16] Recent studies have indicated that other cell types such as macrophages, endothelial cells, and fibroblasts, can produce MIF,[17–19] and various other functions have been attributed to this molecule, such as the regulation of cell growth, including tumourigenesis, T cell activation, and angiogenesis.[20,21]

Recent reports have suggested that MIF plays a critical role in inflammatory and immune responses. In particular, MIF has been shown to induce the synthesis of proinflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and IL-8 in immunocompetent cells, and to exert the unique ability of counteracting the inhibition of cytokine production by glucocorticoids.[22] Moreover, it has recently been verified that MIF acts as a powerful stimulator for nitric oxide production.[23] Increased serum MIF levels have been recently documented in several inflammatory diseases such as rheumatoid arthritis,[24] atopic dermatitis,[25] glomerulonephritis,[26] hepatic cirrhosis,[11] and alcoholic liver diseases.[10] However, the role of MIF in the pathogenesis of BA patients has not been fully understood.

Although BA is considered a multi-factorial disorder, activation of the immune system plays a critical part in its development. Recently, a number of inflammatory cytokines and growth factors have been shown to be elevated in sera of patients with BA including IL-8,[27] IL-18,[28] hepatocyte growth factor,[29] endothelin-1[30] and connective tissue growth factor.[31] To the best of our knowledge, no published data are available on serum MIF levels in BA patients. In the present study, we have investigated for the first time the circulating levels of MIF in the serum of BA patients. Significantly higher serum levels of MIF were observed in BA patients than healthy controls. High serum MIF levels in BA children suggest that the pathogenesis of BA might be associated with an inflammatory process. This evidence is supported by the recent study of Arikan et al on the positive association of MIF gene-173G/C polymorphism at the promoter region with BA.[13] In the present study hepatic MIF expression has not been determined. However, it has been shown that the serum levels of MIF were associated with the intensity of MIF expression by hepatocytes in patients with hepatocellular carcinoma and liver cirrhosis.[32] Although the liver may be a potential source of MIF, other extrahepatic tissues may be responsible for the increased MIF production in sera of patients with BA. Other tissues or cells, such as inflammatory cells and fibroblasts in the liver might also contribute to MIF production. Another possible explanation may be that higher MIF levels are secondary to decreased clearance. It might be plausible that increased MIF levels reflect the hepatocellular damage in BA patients or that the elevated MIF levels result from an imbalance between MIF production and MIF clearance.

In conclusion, serum MIF levels were elevated in patients with BA. It is therefore conceivable that MIF is involved in the pathophysiology of BA. However, the elevated serum MIF levels are not associated with either jaundice status or portal hypertension. Further studies are therefore required in order to fully characterise the significance of MIF in BA patients.

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