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High levels of soluble Major Histocompatibility Complex class I related chain A (MICA) are associated with biliary cast syndrome after liver transplantation

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ABSTRACT

Background: The biliary cast syndrome (BCS) is a frequent problem following liver transplantation. The pathogenesis of this complication is not well understood. Previous research has demonstrated that the soluble form of MICA (sMICA) is significantly higher in patients with chronic liver disease and hepatocellular carcinoma (HCC) than in healthy volunteers. The aim of this study is to investigate the possible involvement of sMICA in the formation of BCS after liver transplantation.
Methods: Serum soluble MICA was retrospectively evaluated in pre- and post-transplant sera from 133 consecutive primary liver transplant patients and in sera from 88 healthy volunteers using sandwich ELISA. Normal distribution of serum sMICA was described by the data obtained from healthy population and sMICA concentration that was greater than the upper bound 95% normal range was considered as high levels of sMICA. Patient records were reviewed to identify patients who developed BCS.
Results: The results demonstrated that 37.6% of patients with end-stage liver diseases had significantly higher pre-transplant serum sMICA than in healthy population. 34.4% of recipients with post-transplant high levels of sMICA developed BCS. In contrast, 17.3% of patients with post-transplant normal levels of sMICA developed BCS. The risk of BCS development is significantly associated with the presence of post-transplant high levels of sMICA ($P=0.0365$). Further analysis disclosed that patients with decreased post-transplant sMICA following liver transplantation had a lower incidence rate of BCS than those with remained high levels of sMICA after transplantation (10.5% vs. 38.7%, $P=0.0302$). Furthermore, log-rank test showed that BCS occurrence was significantly associated with dynamic changes of sMICA among different groups ($P=0.0188$).
Conclusions: Biliary cast syndrome is more likely to develop in recipients who have post-transplant high levels of sMICA. The data suggested that sMICA might have some immunologic effect on BCS development following liver transplant. Monitoring of serum sMICA could have a prognostic value in assessment of patients with liver transplantation.

1. Introduction

Liver transplantation is now the accepted treatment for end-stage liver disease and major improvements of outcome have been seen over the last 20 years. However, despite improvements in 1-year survival rates, biliary/vascular complications are common causes of morbidity in liver transplant recipients. It has been reported that the biliary cast syndrome (BCS), characterized by the presence of biliary casts and debris causing biliary obstruction, occurs in 2.5%–18% of orthotopic liver transplant (OLT) patients [1–5]. This condition has been found to be associated with increased morbidity, mortality, and graft rejection [6,7].

The diagnosis of BCS is typically confirmed by cholangiography [8,9]. Chemical analysis has shown that the casts are composed of bilirubin and collagen, bile acid, and/or cholesterol [2,6,10]. The initial literature on the subject discussed the possibility of biliary obstruction, acute cellular rejection, biliary infection, and placement of biliary drainage tubes as triggers for the development of BCS [11–13]. Recent literature has shown that ischemic factors and biliary strictures may be important in the development of BCS [10]. However, the true pathogenesis of BCS is not clearly understood and patients who cannot be successfully treated by endoscopic or percutaneous means require surgery for removal of the casts or possibly even retransplantation.

MHC class I related chain A (MICA) molecules are glycoproteins expressed on the cellular membrane. Unlike the classical MHC class I molecules, MICA expression is restricted to human fibroblasts, endothelial cells, and many tumor cells. MICA expression is induced by

Abbreviations: MICA, Major Histocompatibility Complex class I related chain A; sMICA, soluble MICA; $sMICA_{high}$, high levels of serum sMICA; BCS, biliary cast syndrome; ELISA, Enzyme-linked immunosorbent assay; HCC, hepatocellular carcinoma.

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stress and upregulated in infection [14], tumor transformation [15] and organ transplantation [16]. The engagement of MICA and NKG2D, a common activating natural killer cell receptor [17], strongly activates NK cells and CD8+ T cells, enhancing their cytolytic activity and cytokine production [18]. This pathway is important for the host innate immunity. In addition to the membrane-bound forms, MICA proteins are also cleaved from the cell surface and appear as soluble forms in sera of patients with malignancy [15,19]. Recently, Kohga et al. examined soluble MICA in liver disease and found that the levels of soluble MICA/B were significantly higher in chronic liver disease and hepatocellular carcinoma (HCC) patients than in healthy volunteers [20]. Sera with high levels of soluble MICA (sMICA) can downregulate NKG2D expression in vitro, suggesting that sMICA in the circulation may suppress NKG2D-mediated host innate immunity. Shedding of sMICA in end-stage liver diseases may modulate NKG2D-mediated immune surveillance, supporting the possible involvement of sMICA in regulatory mechanisms after liver allotransplantation.

To date, there is little published work on the relationship between serum sMICA and the outcome of liver transplantation, particularly with the transplant-associated BCS. In this retrospective study, we investigated the presence of sMICA in recipients before and after liver transplantation and analyzed the correlation between serum levels of sMICA and occurrence of BCS.

2. Methods

2.1. Subjects and specimen collection

88 serum samples obtained from healthy volunteers were used to determine the cutoff between normal and high levels of serum sMICA. 133 consecutive primary liver transplant patients operated between 2005 and 2007 were included in this study. The indications for liver transplantation were mainly end-stage chronic liver disease. 16.6% of patients had chronic severe hepatitis, 33.1% liver cirrhosis and 50.4% HCC. All of the donated livers were obtained from non-related deceased donors, and recipient/donor selection was based on ABO blood group compatibility. No attempts were made for HLA matching. The immunosuppressive protocol started at the time of transplantation with basic immunosuppression such as tacrolimus/FK506 with or without mycophenolate mofetil for three months. 37% of patients received two doses of Simulect or Zenapax at 0 and 4–7 days after transplantation. For each patient, one serum was taken immediately prior to liver transplantation, two post-liver transplant sera were taken 3 months and 6 months after transplantation. Sera were stored at −20 °C. The University human experimentation study committee approved the study protocol. For the protection of human subjects, all research data were coded without linking to their identifiers.

Patients were identified as having BCS by characteristic appearance on endoscopic or radiographic cholangiography. Patients with pre-OLT diagnosis of sclerosing cholangitis, other underlying extrahepatic biliary conditions or combined liver–kidney transplantations were excluded from this study.

2.2. Quantification of serum sMICA

Detection of sMICA was performed using a sandwich ELISA with two anti-MICA monoclonal antibodies that bind to the different domains of MICA as described by Salih HR et al. [21] and modified in our laboratory. In brief, plates were coated with the capture anti-MICA mAb AMO-1 (IgG1, BAMOMAB GmbH, Habsburgerstrasse, Germany) by incubating with 5 μg/ml in PBS (pH 7.4), then blocked with 200 μl of 2.0% BSA-PBS overnight and washed. Serum samples were diluted at 1:3 in 2% BSA-PBS prior to testing. In addition, sMICA (MICA*008 recombinants provided by Y Zou and P Stastny [22]) at different concentrations was added to the remaining wells in each plate as a standard product. Then plates were incubated for 2 h at 37 °C, and washed. The detection anti-MICA mAb 6B3 (IgG_{2a}) [23] at 1.0 μg/ml in 1.0% BSA-PBS was added into each well and incubated for 2 h at 37 °C. Plates were then washed and anti-mouse IgG2a-HRP (BD Pharmingen, San Jose, CA) at 1:5000 in 1.0% BSA-PBS was added to incubate for 1 h at 37 °C. After being completely washed, plates were developed using the TMB Peroxidase Substrate System (KPL, Gaithersburg, MD). Absorbance was measured at 450 nm. All standard samples were tested in triplicate. Each serum sample was tested in duplicate and the amount of serum sMICA was calculated using the standard curve which was generated from standard concentrations in the same plate.

2.3. Statistical analysis

The records of BCS in patients were obtained within 6 months after liver transplantation; no patients were excluded for any reason. We examined the following covariables: the age and gender of recipients, original liver disease, type of immunosuppression, and blood transfusions during transplantation, immunomodulatory treatment, induction treatment and hospital times (Table 1). Data of sMICA concentration in groups were presented as geometric mean (*G*-mean) and 95% confidence interval (CI = 95%). High level of serum sMICA ($sMICA_{high}$) was defined by a threshold value greater than the upper bound 95% normal range of sMICA. Significance of differences between frequencies was determined by Fisher's exact test. The descriptive data are presented as mean ± SD and their differences were analyzed by Student *T* test. Actuarial BCS rates were computed according to the Kaplan–Meier method [24] and expressed as mean (± SE) percentages. Analysis of log-rank test was used to compare the incidences of BCS between subgroups of patients, which were categorized according their sMICA level changes in three tests. All statistical analysis was done with GraphPad software. Differences were considered significant if $P<0.05$.

3. Results

3.1. Baseline of serum sMICA

133 consecutive primary liver transplant patients and 88 normal control patients were included in the analysis. The baseline characteristics of the transplant patients are presented in Table 1. The patients are separated by their serum sMICA levels three months after transplant. Serum sMICA levels in all samples were accessed using sandwich ELISA method. The standard protein used for quantitative analysis in this assay was the purified soluble recombinant of MICA*008, which only contained α1, α2, and α3 extracellular domains [22]. A standard curve was established in each plate and the amount of sMICA between 100 pg/ml to 2000 pg/μl was shown to have a good linear relationship with the optical density (OD) values ($r = 0.9922$, Fig. 1A). To determine the baseline of serum sMICA, 88 sera samples from local healthy volunteers were tested by sandwich ELISA and the concentration of serum sMICA in each sample was calculated using the standard curve established. The distribution of serum sMICA

Table 1
Characteristics of patients with and without soluble MICA in post-transplant sera.

Variable	$sMICA_{high}$ ($N=52$)	$sMICA_{norm}$ ($N=81$)	*P* value
Gender (male)	44 (84.6%)	75 (92.6%)	0.1435
Age (years)	48.0 ± 10.9	47.7 ± 9.1	0.864
Following up (days)	781 ± 363	891.7 ± 348	0.0811
Number of death	6 (11.5%)	6 (7.5%)	0.6171
Previous liver diseases			
Chronic severe hepatitis	9 (17.3%)	13 (16.0%)	0.8875
Liver cirrhosis	18 (34.6%)	26 (32.1%)	0.1261
HCC	25 (48.1%)	42 (51.9%)	0.2921
Transfusion (package)			
Pre-transplant	1.1 ± 1.0	1.1 ± 1.6	1
During transplant	3.9 ± 3.8	2.4 ± 2.6	0.0078
Post-transplant	1.4 ± 1.4	1.0 ± 3.0	0.3699
Immunomodulatory treatment			
FK506 + MMF[a]	98%	99%	NS
Induction treatment			
Simulect or Zenapax	22 (42.3%)	27 (33.3%)	0.3578
Hospital times (weeks)	4.4 ± 4.7	3.7 ± 3.8	0.347

[a] MMF: Mycophenolate mofetil.