Original Article

Serum Immunoglobulin (Ig) Levels Predict Degree of Hepatic Fibrosis in Patients with Chronic Hepatitis B and Chronic Hepatitis C Infections

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ABSTRACT

Background: A number of non-invasive methods are currently available for use as an alternative to liver biopsy, but their clinical value remains to be determined. The aim of this study was to determine the association between serum immunoglobulin (Ig) and significant hepatic fibrosis (Metavir score ≥F2) in chronic hepatitis B (CHB) and chronic hepatitis C (CHC) infections.

Method: Sixty patients with CHB and CHC who underwent liver biopsy at Ramathibodi Hospital, Bangkok, between December 1, 2006 and November 30, 2007 were recruited. Biochemical, serologic, virologic data, serum globulin, serum Ig (IgA, IgG, IgM and total Ig) determined by nephelometry, and liver biopsy histologic findings were collected. In discriminating patients with significant histological fibrosis, serum globulin and Ig provided the best area under the receiver operating characteristic curves (AUROC).

Results: There were 37 male and 22 female subjects, with a mean age of 45.8 ± 12.78 years. Thirty-five patients (58.33%) had CHB, of whom 36 (60%) had significant liver fibrosis. The respective mean serum IgM, IgG, IgA, total Ig and serum globulin levels were 1.4 ± 0.8,17.1 ± 4.5, 3.4 ± 1.8,21.9 ± 5.6 and 37.6 ± 6.2, and the AUROCs were 0.57, 0.67, 0.72, 0.71 and 0.72 were significant fibrosis, respectively. By multivariate analysis, three important predictors of significant fibrosis were serum globulin level (OR 5.10; 95% CI 1.07-24.22, $p = 0.04$), aspartate aminotransferase to platelet count ratio index (APRI) (OR 9.99; 95% CI 2.45-40.73, $p = 0.001$) and serum IgA (OR 5.35; 95% CI 1.29-22.17, $p = 0.021$).

Conclusion: Serum globulin, APRI and serum IgA can serve as noninvasive markers of significant hepatic fibrosis. This observation, if confirmed, has important implications for the management of patients with CHB and CHC infections.

Key words : hepatic fibrosis, chronic hepatitis, serum immunoglobulin

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INTRODUCTION

Noninvasive diagnosis of liver fibrosis is an emerging issue for chronic liver disease. Several noninvasive methods have been studied for the diagnosis of hepatic fibrosis, including certain clinical, blood marker and signal analyses (ultrasound, magnetic resonance imaging, elastography).[1-3] Several blood tests have been studied with multivariate analysis to identify direct and/or indirect blood markers, and these have been chosen to evaluate clinically significant fibrosis. However blood tests or markers were not accurate and were not predictive of liver histology. An ideal noninvasive diagnostic test for hepatic fibrosis should be simple, accurate, readily available and inexpensive.

Hypergammaglobulinemia is often noted in chronic liver disease. The mechanism of hypergammaglobulinemia in liver disease is complex, and includes increased synthetic rates as well as decreased catabolism.[4] It also involves reduction of Kuffer cell clearance of antigens delivered from the GI tract.

T-cells and B-cells were activated by interaction of antigens.[5,6] Consequently, elevated serum immunoglobulins are considered a consequence rather than a cause of liver cirrhosis.[6] Recent studies have found that immunoglobulins exert a direct effect on hepatic fibrogenesis, and that IgG stimulates the proliferation of hepatic stellate cell and the expression of smooth muscle alpha-actin.[7] Hepatic stellate cells are also important in the mediation of liver fibrosis.[8]

Recent studies have found a strong association between serum immunoglobulin level and the extent of hepatic fibrosis in patients with CHB and CHC infection.[9,10]

This study was aimed at assessing the role of serum immunoglobulins for prediction of significant hepatic fibrosis in CHB and CHC infection.

PATIENTS AND METHOD

Patients

From January 1, 2006 to December 31, 2007, patients with chronic HBV and HCV infection who had undergone percutaneous liver biopsy were prospectively recruited from the GI clinic, Ramathibodi Hospital. The diagnosis of HBV or HCV infection was based on the finding of seropositivety for either hepatitis B surface antigen (HBs Ag) or HCV RNA respectively. Exclusion criteria were coinfection with HIV, presence of other liver diseases, hepatocellular carcinoma, other autoimmune diseases, inflammatory or metabolic disease associated with hypergammaglobulinemia (e.q. infection in the previous month), and history of immunomodulatory and /or immunosuppressive drugs or antiviral therapy within the preceding 6 months.

All patients had a detailed demographic and medical history taken, and underwent a through physical examination. Routine blood chemistry and complete blood count were performed. An informed consent was obtained in all cases.

Measurements

Serum biochemical marker and virological assay

Liver function tests, alfa-fetoprotein and coagulogram were measured. Serum aminotranferase platelet ratio index was calculated from the formula: serum aspartate aminotransferase/ULN × 100/platelet (10^9/L).

Hepatitis B surface antigen (HBs Ag), Hepatitis Be antigen (HBe Ag), Anti HBe, and HCV genotype were determined. HBV DNA and HCV RNA were assayed by Amplicor method.

Immunological test

Serum immunoglobulin levels were determined by nephelometry, using standard laboratory techniques (Beckman Coulter Instruments, Brea, CA, USA). Serum globulin levels were measured using routine standard laboratory techniques.

Histological analysis

Liver biopsy specimens were fixed, paraffin embedded and routinely stained with hematoxylin-eosin and trichrome stains. All biopsy specimens were interpreted by two pathologists, and were reported along the METAVIR group scoring system. Pathologic findings were staged on a scale of F0 to F4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3= numerous septa without cirrhosis, F4 = cirrhosis). Histological activity, a measure of intensity of necroinflammatory lesions was graded as follow: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

Statistical analysis

Patients baseline characteristics were descriptively summarized and reported as mean ± SD, median or proportions. The diagnostic value of the marker was assessed by receiver operating characteric (ROC)

curve analysis. The cutoff for optimal measure such as sensitivity and specificity were determined from ROC curves. Demographic characteristics and serum biochemical values between significant and non-significant fibrosis were compared by Student *t*-test or Mann-Whitney test in abnormal distribution for continuous variables and x^2 test or Fisher exact test for categorical variable. Univariate analysis was performed on variables between patients with and without fibrosiss. Significant variables from the univariate analysis (p <0.05) were subjected to multivariate analysis to identify independent factor. All statistical analyses were done with stata, version 10.

RESULTS

Patients characteristics

From January 1, 2007 to December 31, 2007, 67 patients with CHB and CHC were recruited. Seven patients were excluded, 5 with steatohepatitis, and 2 with inadequate liver biopsy tissue. Of the remaining 60 patients, 35 (58.33%) had CHB and 25 (42.37%) had CHC with a mean age of 45.8 ± 12.78. Patients characteristics and biochemical markers were shown in Table 1.

Table 1. Baseline characteristics of the study population (n = 60).

Characteristic	Mean ± SD
Age (years)	45.8 ± 12.78
Male : female	37 (61.67%) : 23 (38.33%)
Drinking	7 (11.67)
CHB : CHC	35 (58.33%) : 25 (42.37%)
Hct (%)	41.22 ± 4.17
White cell count	6622 ± 2103.26
Platelet count (103/L)	214 ± 57.5
PT (sec)	12.09 ± 0.85
INR	1.02 ± 0.07
AP (U/L)	88 ± 30
GGT (U/L) median	76.5 (23-430)
AST (U/L) median	60 (13-407)
ALT (U/L) median	111 (31-488)
TP (g/dL)	78.78 ± 5
Albumin (g/dL)	41.15_4.59
Globulin (g/dL)	37.5 ± 6.16
TB (mg/dL)	0.74 ± 0.32
DB (mg/dL)	0.27 ± 0.10
AFP (ng/dL) median	3.98 (0.61-154.4)
Serum immunoglobulin	
Ig M median	1.18
Ig G	17.09 ± 4.45
Ig A	3.37 ± 1.76
Total Ig	21.93 ± 5.6
0-1	24 (40%)
2-4	36 (60%)

Thirty-seven (61.67%) patients were male. Nineteen (54.29%) CHB patients were HBe Ag positive. Median HBV DNA in 35 CHB patients were 3.23×10^6 cp/ml. Of 25 HCV patients, 11 were of (44%) genotype 1 and 14(56%) genotype 2 & 3. Median HCV RNA in 25 CHC patients were 8.52×10^6. Mean serum globulin level was 37.5 ± 6.16 g/dL. Mean serum IgG, IgA, IgM, total immuno-globulin levels were 17.09 ± 4.45, 3.37 ± 1.76, 1.42 ± 0.82 and 21.93 ± 5.6 mg/dL respectively. Thirty-six (60%) patients had significant fibrosis.

Predictor of significant fibrosis

Variables associated with the presence of significant fibrosis were first assessed by univariate analysis (Table 2) AST, ALT, DB, TP, serum globulin, PT, APRI, total immunoglobulin, serum IgG and IgA were associated with significant fibrosis. Subsequent multivariate analysis (Table 3) showed that only APRI (OR 9.9; 95% CI 2.45-40.73, p = 0.001), serum IgA (OR 5.35; 95% CI 1.29-22.17, p = 0.02) and serum globulin (OR 5.1; 95% CI 1.07-24.22, p = 0.04) were independent predictors of significant fibrosis.

Serum globulin and serum immunoglobulin in predicting significant liver fibrosis

The ROC curves of serum globulin, total immunoglobulin, serum IgG, IgA IgM and APRI for predicting significant fibrosis were plotted as shown in Figure 1. The AUC of serum globulin, total immunoglobulin, serum IgG, IgA IgM and APRI were 0.72, 0.71, 0.68, 0.72, 0.57 and 0.80 respectively.

Based on the ROC, serum globulin cutoff point of 34.5 g/dL was shown to predict significant fibrosis, with sensitivity of 75% and specificity 50%, PPV60% and NPV 66.67%. Total immunoglobulin cutoff point of 18.6 g/dL was shown to predict significant fibrosis, with sensitivity of 74.29%, specificity 52.17% PPV 60.83% and NPV 66.98%. IgG cutoff point of 15 g/dL was shown to predict significant fibrosis, with sensitivity of 77.78%, specificity 50% PPV 60.87 and NPV 69.23%. IgM cutoff point of 1.15 g/dL was shown to predict significant fibrosis, with sensitivity of 65.71%,

specificity 52.17%, PPV 57.87% and NPV 60.34%. IgA cutoff point of 2.5 g/dL was shown to predict significant fibrosis, with sensitivity of 77.14%, specificity 43.48%, PPV57.71% and NPV 65.54%. APRI cutoff of 0.4 was shown to predict significant fibrosis, with sensitivity of 80.56%, specificity 58.33% PPV 70.04% and NPV 75%.

DISCUSSION

Several studies on prediction of significant fibrosis in chronic liver disease have been published in the past few years. Forns *et al*. proposed the routinely measured variables of GGT, cholesterol, platelet count and prothrombin time in combination with age.This model could predict significant fibrosis in 51% of patients with an AUC of 0.94.[11] Wai *et al*. proposed a simple and elegant model of AST-to-platelet ratio index (APRI), which predicted significant fibrosis as determined by the Ishak scoring system with an AUC of 0.80-0.88. APRI had a lower accuracy for determining more subtle grades of fibrosis.[12] Fibrotest, pre-

Table 2. Univariate Analysis of variables associated with the presence of significant fibrosis.

Variable	No significant fibrosis	Significant fibrosis	*p*-value
Age;mean(SD)	42.41(13.62)	48.05(11.85	0.094
Gender male; n (%) female (%)	 20 (55.56) 16 (44.44)	 17 (70.83) 24 (29.17)	 0.233
Hct; mean (SD)	42.22 (5.06)	7 (27.17)	0.165
WBC; mean (SD)	6423.33 (1836.88)	6754.44 (2279.19)	0.554
Plt; mean (SD)	235,875 (54,782)	199,416 (55,433)	0.015
PT; mean (SD)	11.84 (0.78)	12.25 (0.86)	0.066
PTT; mean (SD)	28.06 (2.45)	29.50 (2.36)	0.028
AP; mean (SD)	80.87 (25.9)	93.55 (32.20)	0.11
AST; median (range)	49 (13,93)	77 (30,407)	0.002
ALT; median (range)	97 (31,322)	120 (46,488)	0.048
GGT; median (range)	57 (23,180)	83 (26,430)	0.179
TP; mean (SD)	76.47 (5.02)	80.32 (4.44)	0.002
Alb; mean (SD)	41.84 (5.31)	40.68 (4.04)	0.342
Globulin; mean (SD)	34.62 (4.63)	39.54 (6.31)	0.001
TB;median (range)	0.6 (0.3, 1.7)	0.7 (0.3, 1.7)	0.637
DB; mean (SD)	0.24 (0.06)	0.29 (0.12)	0.031
Total Ig; mean (SD)	19.52 (3.93)	23.51 (6.01)	0.003
IgG; mean (SD)	15.53 (3.43)	18.11 (4.79)	0.026
IgA; mean (SD)	2.64 (1.28)	3.86 (1.88)	0.008
IgM; median (range)	1.14 (0.59,3.46)	1.32 (0.49,5.04)	0.31
APRI: mean (SD)	0.31 (0.12,0.89)	0.71 (0.26,2.83)	0.0001

Table 3. Factor associated with significant fibrosis: multiple logistic regression.

Factor	Coeffictent	SE	OR (95%CI)	*p*-valve
Serum globulin	5.10	4.05	5.1 (1.07-24.22)	0.04
Serum IgA	3.88	2.31	5.35 (1.29-22.17)	0.021
APRI	7.16	3.21	9.99 (2.45-40.73)	0.0001

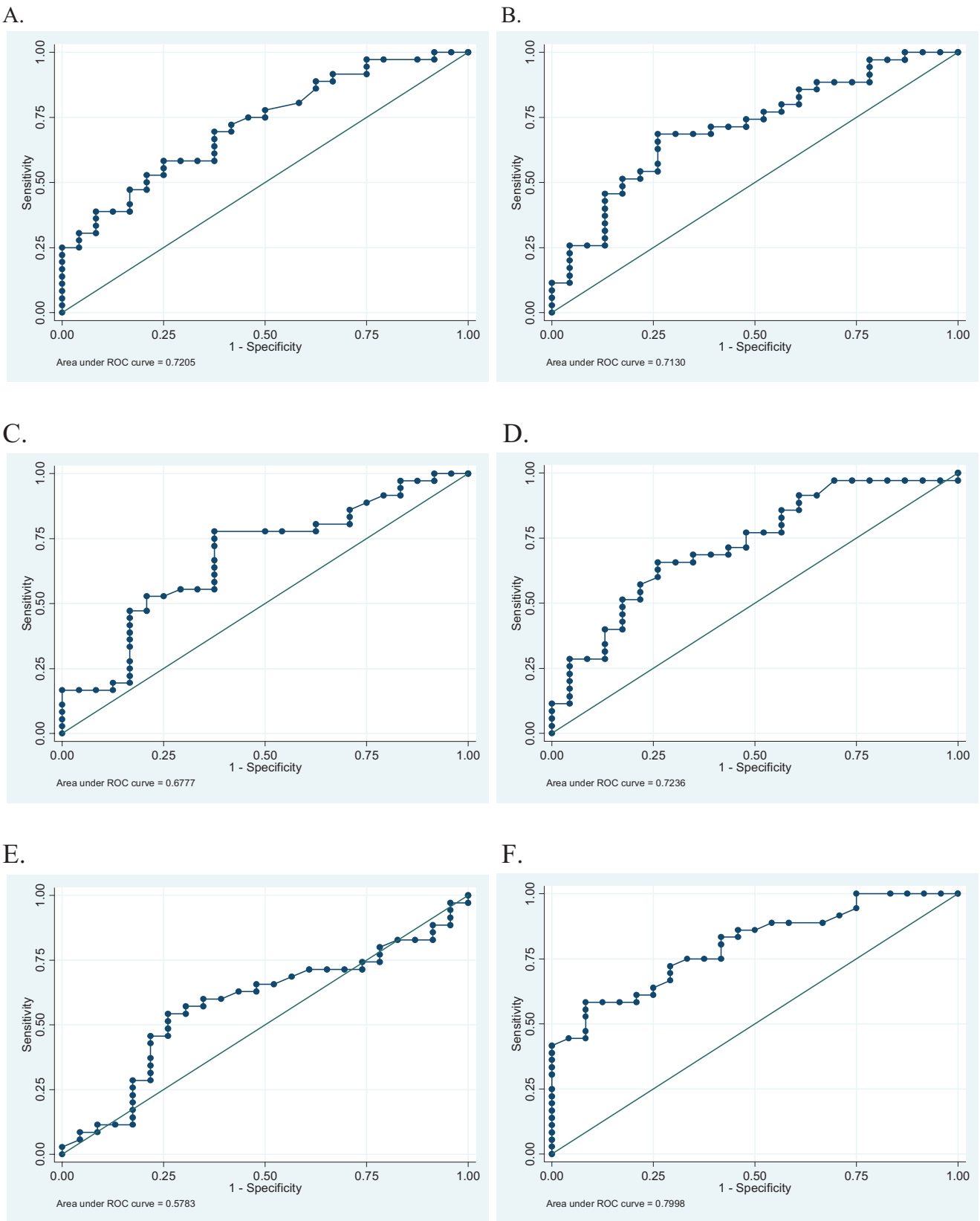

Figure 1. ROC curve of serum globulin = 0.72 (A), totalimmunoglogulin = 0.71(B), IgG = 0.68 (C), IgA = 0.72 (D), IgM = 0.57 (E), APRI = 0.8 (F) in prediction of significant fibrosis.

dictive model for 5 markers index (alpha2 macroglobulin, haptoglobulin, GGT, bilirubin and apolipoprotein A1), had a reported AUC of 0.83-0.85.[13,14] This study was not available.

In the present study, serum immunoglobulins (total, IgA, IgG and IgM) and serum globulin were used to identify serum markers for significant liver fibrosis in HBV and HCV infections. Recent studies suggest that immunoglobulins may play an important role in the pathogenesis of hepatic fibrosis. In a study by Shen *et al*., Fc receptor for IgG was expressed on the cell surface of hepatic stellate cell, and IgG was formed to regulate stellate cell differentiation and proliferation. Clinical observation has noted an increased level of immunoglobulin in patients with liver fibrosis and cirrhosis.

In our study, serum AST, ALT, TP, serum globulin, direct bilirubin, age, PT, APRI, serum IgA & IgG and total immunoglobulin were shown to be significantly correlated to liver fibrosis on univariate analysis. However, on multivariate analysis, APRI, serum globulin and serum IgA were the only indepent predictors of significant fibrosis.

In CHB and CHC infections serum globulin, total immunoglobulin, serum IgA and APRI were good predictors of significant liver fibrosis with areas under ROC of 0.71,0.71, 0.72 and 0.79, respectively. The sensitivity and specificity of erum globulin level >34.5 g/dL were 75% and 50%. The sensitivity and specificity of serum total immuno-globulin >18.6 g/dL were 74.29% and 52.17%. The sensitivity and specificity of serum IgA >2.5 g/dL were 77.14% and 43.48%. The sensitivity and specificity of APRI >0.4 were 80.56% and 58.33%. However, serum IgG was a fairly good predictor of significant liver fibrosis (AUC = 0.677), while the sensitivity and specificity of IgG level >15 g/dL were 77.78% and 50%, which were than those of serum globulin and serum IgA. Based on the above results, APRI appeared to be the best predictor of significant liver fibrosis. The optimal cutoff of APRI >0.4 was fairly good regarding sensitivity and specificity. This cutoff point of APRI was the differentiated cutoff in Wai *et al*.'s study.[12,15] To date, however, the exact cutoff point for APRI remains uncertain.

In one study, serum IgG and IgA were increased in alcoholic cirrhosis patients, while serum IgM elevation was seen in PBC patients and serum IgG in viral cirrhosis.[16] In our study, serum IgA and IgG levels were found to correlate with significant liver fibrosis, while serum globulin, a simple and convenient marker, was also a good predictor for significant liver fibrosis. In another recent study, a defect in Fc alphaR mediated IgA clearance resulting from down regulation of Fc alphaR on monocytes was proposed in several inflammatory and infectious diseases associated with increased serum IgA levels, including CHC.[17] Lastly, APRI, a simple model proposed by Wai et al, appeared to be an important predictor of significant liver fibrosis in the present study. However, our study had limitations, notably the small sample size.

CONCLUSION

Serum globulin, APRI and serum IgA can serve as noninvasive markers of significant hepatic fibrosis. These observations, if confirmed, have important implications for the management of patients with CHB and CHC.

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