Original Article

Usefulness of Serum High Sensitivity-CRP Measurement for Differentiating Nonalcoholic Steatohepatitis from Simple Steatosis in Patients with Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a disorder which ranges over a spectrum, extending from simple steatosis to steatohepatitis (NASH). Significant proportion of NASH could progress to cirrhosis. However, it has remained difficult to differentiate between NASH and simple steatosis by clinical examination. Nowadays serum high sensitivity C-reactive protein (hs-CRP) is an inflammatory marker that predicts severity of metabolic syndrome.

Aim: To study whether serum hs-CRP including other metabolic parameters could differentiate and predict NASH in patients with NAFLD.

Methods: Seventy-six patients with biopsy proven NAFLD (mean age 53 ± 10.5), 15 with simple steatosis (mean age 49.5 ± 11.3) and 61 with NASH (mean age 53.9 ± 10.2) were investigated. Baseline characteristics including BMI and waist-hip ratio of all subjects were evaluated. Serum fasting plasma glucose, lipid, hs-CRP, leptin, adiponectin, TNF-α and Homeostasis Model Assessment Method (HOMA-IR) were measured. Metabolic syndrome was assessed according to National Cholesterol Program's Adult Treatment Panel III. Liver biopsies were graded according to Brunt criteria. Diagnostic accuracy of predictors of NASH and advance fibrosis was examined prospectively.

Results: The median (min-max) of serum hs-CRP was higher in NASH compared with simple steatosis [1.9 (0.2-14.2) vs 0.97 (0.2-12.3) mg/L]. And the optimal cut-off value of serum hs-CRP in differentiating NASH and simple steatosis, assessed by ROC analysis was 0.88 mg/L, with sensitivity of 85.3%, specificity of 46.7%, and accuracy of 88.1%. In univariate analysis, factors associated with NASH were female sex, presence of metabolic syndrome criteria > 2, FPG, HOMA-IR and serum hs-CRP ≥ 0.88 mg/L. In multivariate analysis, serum hs-CRP ≥ 0.88 mg/L (OR 4.9; 95% CI 1.3-18.5 $p = 0.02$) and presence of metabolic syndrome criteria > 2 (OR 5.0; 95% CI 1.3-19.8 $p = 0.02$) were independent predictors of NASH. When both factors were concomitantly positive, the accuracy to detect NASH as determined by ROC analysis was 0.7 (95% CI 0.6-0.9) and had an accuracy of 82.9%.

Conclusions: Serum hs-CRP is a useful non-invasive marker that differentiates between NASH and simple steatosis in NAFLD patients.

Key words : NAFLD, NASH, C-reactive protein, metabolic syndrome

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of fat in the liver in the absence of significant alcohol consumption and other causes of chronic liver disease such as viral hepatitis or drugs[1,2]. The incidence and prevalence of this disease are thought to be rising as the prevalence of obesity and diabetes has dramatically increased in the past 10-20 years. Recently, it has been suggested that nearly 24% of adults may have NAFLD, making it the leading cause of abnormal liver enzymes worldwide[3-6].

NAFLD includes both simple fatty liver or bland steatosis and nonalcoholic steatohepatitis (NASH), defined by the presence of lobular necroinflammatory activity with or without the presence of perisinusoidal fibrosis on liver biopsy. Differentiating between the two is important because up to 28% of patients with steatohepatitis, may progress to cirrhosis[7], which carry a risk of both liver failure requiring liver transplantation and hepatocellular carcinoma, in contrast to simple steatosis which often remains stable for a number of years and will probably never progress in many patients[8,9].

To date, the only reliable method to differentiating simple steatosis from NASH is by liver biopsy, which is an invasive procedure associated with a number of complications. Developing noninvasive biochemical assays and clinical models to accurately distinguish steatosis from NASH would potentially eliminate the risk of liver biopsy in some patients while still providing prognostic information. Noninvasive approaches for assessing the severity of fibrosis in NAFLD have included a combination of clinical features and routine laboratory investigations[10-13].

C-reactive protein (CRP) is an acute phase-reactant protein synthesized by the liver that is also elevated in chronic inflammatory states. CRP levels have been shown to be closely related to obesity, in particular central or visceral fat deposition. Traditional assays were not sufficiently sensitive to monitor low levels of CRP elevation and therefore high-sensitivity assays have been developed (hs-CRP)[14]. Recently, elevated serum hs-CRP was reported to be a strong predictor of future cardiovascular events and also related to metabolic syndrome and atherosclerosis[15-20]. Moreover, elevated serum hs-CRP was reported to be a diagnostic tool or predictor of disease progression in patients with of NAFLD. Currently, there is no histological confirmation and grading to distinguish between simple steatosis and steatohepatitis[21-25]. Therefore, we aimed to study whether serum hs-CRP including other metabolic parameters could differentiate and predict NASH in patients with NAFLD.

PATIENTS AND METHODS

From October 2008 to September 2009, we included patients who had chronic elevation of serum alanine aminotransferase (ALT) and/or aspatate aminotransferase (AST) more than 1.5 × upper limit normal on 2 occasions in the past 6 months and fatty liver was diagnosed by abdominal ultrasound. All patients were negative for hepatitis B or C viral markers, and no evidence of other causes of chronic liver disease such as autoimmune liver disease, Wilson's disease and hemochromatosis. Additionally, all patients had no history of herbal or hepatotoxic medication as well as history of alcohol drinking more than 20 gm/week. All patient underwent percutaneous liver biopsy. The patients who had liver histology compatible with nonalcoholic fatty liver disease (NAFLD) either simple steatosis or NASH base on Brunt criteria were included in this study. We also excluded patient who had clinical evidence of active infection and inflammation at the time of serum analysis. This study protocol was approved by the Hospital Ethical Committee and was carried out according Helsinki Declaration guideline. All participants were informed consent prior to the study.

Demographic data

The following data were recorded at the time of liver biopsy: age, sex, body mass index (BMI), history of diabetes mellitus, hypertension and hyperlipidemia. Metabolic syndrome was assessed according to modified Asia-Pacific guidelines of the NCEP III which are (1) fasting plasma glucose ≥ 110 mg/dL, (2) central obesity (waist circumference ≥ 90 cm in men and ≥ 80 cm in women, (3) triglyceride level ≥ 150 mg/dL, (4) blood pressure ≥ 130/85 mmHg or on treatment, (5) HDL ≤ 40 mg/dL or ≤ 50 mg/dl in men and women, respectively.

Biochemical tests

After 10-hours overnight fast, venous blood sample were drawn for liver biochemical test, glucose, total cholesterol, low-density cholesterol (LDL-C),

high-density cholesterol (HDL-C), triglyceride, adiponectin and leptin (Quantikine adiponectin and Quantikine leptin; R&D systems, Oxford, UK). Serum high sensitivity CRP (hs-CRP) was measured on the same day by nephelometry method and determined by using a standard curve then reported in unit of milligram per litre (mg/L). The index of insulin resistance (IR) was calculated on the basis of fasting plasma glucose and insulin, according to the homeostsis model assessment (HOMA-IR), which was equal to fasting insulin (MIU/mL) × fasting plasma glucose (mmol/L)/ 22.5.

Histological assessment

Liver specimens were fixed in 10% buffered formalin, and were analyzed by a single pathologist unawared of clinical and biochemical data. All specimens were stained with hematoxylin and eosin stain, and Masson trichome stain. Histology was scored according to Brunt criteria[26]. Steatosis was graded from 0 to 3, ballooning degeneration was graded as mild, moderate and marked and necroinflammation was graded from 0 to 3. Fibrosis was graded 0 (absent) to 4 (cirrhosis) and significant fibrosis was defined as grade ≥ 2. Steatohepatitis was defined by steatosis plus ballooning degeneration and/or necroinflammation.

Statistical analysis

The demographic and laboratory data were first compared between the simple steatosis and NASH group using the Student's *t*-test for continuous variables and χ^2 or Fisher exact test for categorical variables. Continuous data were summarized as mean ± SD or median (minimum - maximum) as appropriate and categorical variables as frequencies and percentages. Serum hs-CRP was then compared between the two groups. In order to evaluate the ability of the independent variables to discriminate with respect to diagnosis and explore the appropriate cut-off, we then performed a receiver operating characteristic (ROC) curve analysis. The cutoff value that associated with the optimal combination of sensitivity and specificity was determined. Variable with p-value < 0.05 were selected to enter stepwise multivariate logistic regression analysis to determine factors associated with NASH. Data analysis was performed with STATA version 9.0. A p-value < 0.05 was taken as statistically significant.

Table 1. Demographic data of all NAFLD patients (n = 76)

Parameters	Mean ± SD or number (%)
Age (years)	53 ± 10
Female	40 (52.6%)
History of diabetes	32 (42.1%)
History of hypertension	44 (57.9%)
History of dyslipidemia	61 (80.3%)
Body weight (kg)	82.7 ± 8.8
BMI (kg/m^2)	27.8 ± 3.5
Waist-hip ratio (WHR)	0.9 ± 0.06
FBS (mg/dL)	120.8 ± 37.8
AST (U/L)	62.5 ± 33.3
ALT (U/L)	119.4 ± 43.4
Cholesterol (mg/dL)	212.3 ± 50.5
Triglyceride (mg/dL)	158.6 ± 57.4
LDL (mg/dL)	136.8 ± 43.7
HDL (mg/dL)	44.8 ± 9.1

RESULTS

During the study period, 81 patients who had chronic elevation of serum aminotransferase with negative results of other specific causes and were clinically suspected NAFLD were included and underwent percutaneous liver biopsy. Five patients were excluded because of liver biopsy results did not compatible with NASH. Two patients were drug induced liver injury and 3 patients were non-specific liver change. A total of 76 NAFLD patients were included for analysis, 15 with simple steatosis and 61 with NASH. Demographic data of all patients are shown in Table 1.

Comparison between simple steatosis and NASH patients

Of the demographic and laboratory data, there were significant differences between two groups including sex (p = 0.03), number of patients with presence of metabolic syndrome criteria > 2 (p = 0.02), FBS (p = 0.03), HOMA-IR (p = 0.04) as shown in Table 2. The median of serum ALT, AST, BMI, WHR as well as serum adiponectin and leptin were not significantly difference between two groups.

The median (min-max) of serum hs-CRP was higher in patients with NASH compared to those with simple steatosis (0.97; 0.2-12.3 compared with 1.9; 0.2-14.2, respectively) but the difference was not statistically different (p = 0.05).

The receiver operating characteristic (ROC) analysis was applied for serum hs-CRP to differentiate

Table 2. Comparison between simple steatosis and NASH

Parameters	Simple steatosis (N = 15)	NASH (N = 61)	*p*-value
Age (years) (mean ± SD)	49.5 ± 11.3	53.89 ± 10.2	0.14
Female	4 (26.7%)	36 (59.0%)	0.03
History of diabetes	5 (33.3%)	27 (44.3%)	0.44
History of hypertension	6 (40%)	38 (62.3%)	0.12
History of dyslipidemia	14 (93.3%)	47 (77.1%)	0.16
BMI (kg/m^2)	28.2 ± 0.8	27.7 ± 0.5	0.66
WHR	0.96 ± 0.2	0.9 ± 0.08	0.21
Metabolic syndrome criteria > 2	9 (60%)	54 (88.5%)	0.02
ALT (U/L)115.7 ± 9.1	120.3 ± 5.8	0.72	
FBS (mg/dL)107.3 ± 5.5	124.1 ± 5.2	0.03	
TG (mg/dL)138.7 ± 5.5	163.5 ± 5.2	0.13	
Insulin (μIU/mL)	13.6 (4.3-23)	16 (5.2-54.5)	0.1
HOMA-IR	1.8 (0.6-3)	2.3 (0.6-7.7)	0.04
HOMA-IR ≥ 2.5	2 (13.3%)	28 (45.9%)	0.02
hs-CRP (mg/L)	0.97 (0.2-12.3)	1.9 (0.2-14.2)	0.05
Adiponectin (ng/mL)	3064.2 (1197.8-4797.7)	3267.8 (986-11711)	0.21
Leptin (pg/mL)	11226.8 (2001.1-21017.7)	14553.7 (2445-60759)	0.07
Adiponectin / Leptin	0.3 (0.08-0.7)	0.3 (0.05-1.3)	0.23

Table 3. Diagnostic efficacy using serum hs-CRP to differentiate between simple steatosis and NASH

Serum hs-CRP (mg/L)	NASH (N = 61)	Simple steatosis (N = 15)
≥0.88	52 (85%)	8 (53%)
<0.88	9 (15%)	7 (47%)

between patients with simple steatosis and NASH and showed that the AUC was 0.7 (95%CI = 0.5-0.8). We also found that cut-off value of serum hs-CRP (0.88 mg/L to differentiate between simple steatosis and NASH yielded a sensitivity of 85.3%, specificity of 46.7%, positive predictive value of 86.7%, negative predictive value of 43.8% and accuracy of 77.6% (Table 3).

Predictive factors of non-alcoholic steatohepatitis and non-alcoholic fatty liver disease patients

In univariate analysis, four parameters were significantly associated with NASH including female sex, presence of metabolic syndrome criteria > 2, HOMA-IR ≥ 2.5 and serum hs-CRP ≥ 0.88 mg/L (Table 4). In multivariate logistic regression analysis, serum hs-CRP ≥ 0.88 mg/L and metabolic syndrome criteria > 2 were independently associated with NASH with OR of 4.9 (95% CI = 1.3-18.2; $p = 0.017$) and 5.0 (95% CI = 1.3-19.8; $p = 0.023$), respectively (Table 5).

The ROC analysis was applied when using model that comprised of serum hs-CRP ≥ 0.88 mg/L and presence of metabolic syndrome criteria >2 to differentiating between NASH and simple steatosis, yielded (AUC) of 0.73 (95%CI = 0.6-0.9). Comparison of the efficacy between the model that included both parameters with each parameter to differentiate NASH, we found that AUC of model that included both serum hs-CRP (0.88 mg/L and presence of metabolic syndrome > 2 were significant higher compared with each parameter ($p = 0.04$) (Figure 1 and Table 6).

Finally, using this model as a diagnostic tool for differentiating between NASH and simple steatosis yielded a sensitivity of 98.4%, specificity of 20%, positive predictive value of 83.3%, negative predictive value of 75% and accuracy of 82.9% (Table 7).

DISCUSSION

Our study demonstrates that NASH patients tend

Table 4. Univariate analysis of predictive factor of NASH.

	OR	95%CI	*p*-value
Female	4.0	1.1-13.9	0.03
Presence of metabolic syndrome criteria > 2	5.1	1.4 -18.9	0.01
HOMA-IR ≥ 2.5	5.5	1.2-26.6	0.03
Serum hs-CRP ≥ 0.88 mg/L	5.1	1.5-17.4	0.01

Table 5. Multivariate logistic regression analysis of predictive factor of NASH.

	Coefficient (95%CI)	OR (95%CI)	*p*-value
Serum hs-CRP ≥ 0.88 mg/L	1.6 (0.3-2.9)	4.9 (1.3-18.2)	0.017
Presence of metabolic syndrome criteria > 2	1.6 (0.2-3)	5 (1.3-19.8)	0.023

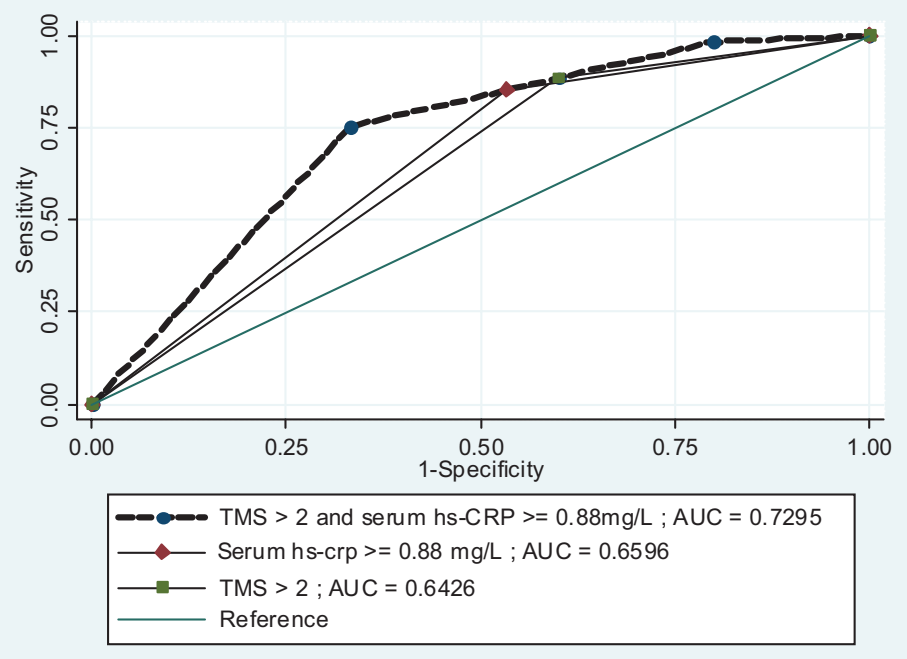

Figure 1. ROC analysis comparison between model that include serum hs-CRP ≥ 0.88 mg/L and metabolic syndrome > 2 with each parameter alone for differentiating NASH.

Table 6. Comparison between model that include serum hs-CRP ≥ 0.88 mg/L and metabolic syndrome > 2 with each parameter alone for differentiating NASH.

	AUC	95%CI
Serum hs-CRP ≥ 0.88 mg/L and metabolic syndrome >2	0.7	0.6-0.9
Metabolic syndrome criteria > 2	0.6	0.5-0.8
Serum hs-CRP ≥ 0.88 mg/L	0.7	0.5-0.8

to be more likely to be female, more insulin resistant, have more metabolic syndrome than patients with simple steatosis. Mean serum AST, ALT as well as BMI are not significant different between two groups in this study. Focusing on serum hs-CRP, we find that median of serum hs-CRP is higher in NASH compare with simple steatosis group but this difference does not reach statistical significant. Nevertheless, when we use ROC analysis to calculate the optimal cut-off value of serum hs-CRP to differentiating NASH, serum hs-

CRP ≥ 0.88 mg/L can discriminate these two entity with statistical significant and also yield high sensitivity and accuracy (85.3% and 77.6%, respectively). Moreover, in multivariate analysis, serum hs-CRP ≥ 0.88 mg/L and metabolic syndrome > 2 are independent predictor of NASH. We then use both two factors as a predictive model to differentiate between NASH and simple steatosis, ROC analysis shows that this model can be use as a good predictive tool which yields AUC of 0.73. Furthermore, when both factors are concomitantly positive, which mean serum hs-CRP ≥ 0.88 mg/L combine with metabolic syndrome > 2, the sensitivity for diagnosis of NASH is nearly 100%.

Recently, NAFLD was considered as a systemic low-grade inflammation and could contribute to accelerated atherosclerosis. Additionally, there is now a growing body of evidence, suggesting that NAFLD is likely to be associated with an early mediator of atherosclerosis and increased cardiovascular (CVD) risk[27]. Our study supports previous studies that demonstrated relationship between serum hs-CRP and severity of liver histology in NAFLD patients[24,25]. And we propose a optimal cut-off level of serum hs-CRP for differentiating simple steatosis and NASH, so clinicians can easily use this non-invasive serum testing in clinical practice. The combination of the number of metabolic syndrome and serum hs-CRP can be used as a screening and diagnostic tool to differentiate between these two conditions. Identifying patients at risk for developing NASH will assist the clinician in determining the prognosis and treatment plans.

In conclusion, our data supports that serum hs-CRP is associated with NASH in patients with NAFLD. And also can be use as non-invasive diagnostic and screening test of NASH especially when combined with metabolic syndrome parameters. Nevertheless, further studies with larger population are needed to validation of this model and to determine long term prognostic value of serum hs-CRP in patients with NAFLD.

REFERENCES

1. Teil MR, James OF, Burt AD, *et al*. The natural history of nonalcoholic fatty liver: a follow up study. Hepatology 1995; 22:1714-9.
2. Powell EE, Cooksley WG, Hanson R, *et al*. The natural history of nonalcoholic steatohepatitis: a follow up study of fourty-two patients for up to 21 years. Hepatology 1990;11:74-80.
3. Browning JD, Szczepaniak LS, Dobbins R, *et al*. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387-95.
4. Bedogni G, Miglioli L, Masutti F, *et al*. Prevalence of and risk factors for nonalcoholic fatty liver disease: The Dionysos nutrition and liver study. Hepatology 2005;42:44-52.
5. Fan JG, Zhu J, Li XJ, *et al*. Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. J Hepatol 2005;43:508-14.
6. Nomura H, Kashiwagi S, Hayashi J, *et al*. Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med 1988;27:142-9.
7. Harrison SA, Neuschwander-Tetri BA. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Clin Liv Dis 2004;8:861-79.
8. Teli MR, James OF, Burt AD, *et al*. The natural history of nonalcoholic fatty liver: a follow-up study. Hepatology 1995; 22:1714-9.
9. Dam-Larsen S, Franzmann M, Andersen IB, *et al*. Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut 2004;53:750-5.
10. Angulo P, Keach JC, Batts KP, *et al*. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356-62.
11. Ratziu V, Giral P, Charlotte F, *et al*. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117-23.
12. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001;121:91-100.
13. Shimada M, Hashimoto E, Kaneda H, *et al*. Nonalcoholic steatohepatitis: risk factors for liver fibrosis. J Hepatology 2002;24:429- 38.
14. Anna W, Arthur JM, Ariel EF. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. Hepatology 2007;46:582-9.
15. Ridker PM, Hennekens CH, Buring JE, *et al*. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342: 836-43.
16. Koenig W, Sund M, Frohlich M, *et al*. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984-92. Circulation 1999;99:237-42.
17. Danesh J, Whincup P, Walker M, *et al*. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Br Med J 2000;321:199-204.
18. Ridker PM, Rifai N, Rose L, *et al*. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of fi rst cardiovascular events. N Engl J Med 2002; 347:1557-65.
19. Ridker PM, Buring JE, Cook NR, *et al*. C-reactive protein, the metabolic syndrome, and the risk of incident cardiovascular events: an 8-year folloe up of 14419 initially healthly American woman. Circulation 2003;107:391-7.

20. Duncan BB, Schmidt MI, Pankow JS, *et al*. Low grade systemic inflammation and the development of type 2 dibetes: the atherosclerosis risk in communities study. Diabetes 2003; 52:1799-805.
21. Park SH, Kim BI, Yun JW, *et al*. Insulin resistance and C-reactive protein as independent risk factors for non-alcoholic fatty liver disease in non-obese Asian men. J Gastroenterol Hepatol 2004;19:694-8.
22. Kurioka S, Murakami Y, Nishiki M, *et al*. Relationship between visceral fat accumulation and antilipolytic action of insulin in patients with type 2 diabetes mellitus. Endocr J 2002;49:459-64.
23. Hanley AJ, Williams K, Festa A, *et al*. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. Diabetes 2005;54:3140-7.
24. Riquelme A, Arrese M, Soza A, *et al*. Non-alcoholic fatty liver disease and its association with obesity, insulin resistance and increased serum level of C-reactive protein in Hispanics. Liver Inter 2009;29:82-8.
25. Cervera JL, Chavez-Tapia NC, Pérez-Bautista O, *et al*. Association among C-reactive protein, fatty liver disease, and cardiovascular risk. Dig Dis Sci 2007;52:2375-9.
26. Brunt EM, Janney CG, Di Bisceglie AM, *et al*. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74.
27. Giovanni T. Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with nonalcoholic fatty liver disease. J Hepatology 2006;45:879-82.