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Original Article

Prognostic value of high sensitivity C-reaction protein in non-insulin dependent diabetes mellitus patients with non-alcoholic fatty liver disease

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Abstract: Background and purpose: High sensitivity C-reaction protein (hsCRP) has been used as a significant predictive factor of cardiovascular events in patients with non-insulin dependent diabetes mellitus (NIDDM). However, existing reports in regards to the significance of hsCRP in predicting the progression of hepatic complications in NIDDM patients are too sparse to deliver clear results. This study is aimed at investigating the prognostic value of hsCRP in NIDDM patients with non-alcoholic fatty liver disease (NAFLD). Methods: 1128 NIDDM patients with a definite diagnosis of NAFLD were enrolled and followed for one year. The baseline body mass index (BMI), waist-hip circumference ratio (WHR), serum aspartate aminotransferase (AST), presence of hypertension, alanine aminotransferase (ALT), serum hsCRP, total cholesterol (Tch), fasting blood glucose (FBG), triglycerine (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and hepatitis B surface antigen (HBsAg) were recorded to analyze the significance of hsCRP in predicting the short-term progression from NAFLD to non-alcoholic steatohepatitis (NASH). Results: One year after baseline, 32% of the NAFLD patients suffered progression to NASH and the percentages of NASH were respectively 8.2%, 12.5%, 33.8% and 72.6% in 4 groups with quartered baseline serum level of hsCRP; there was significant difference among the 4 groups in percentage of NASH ($P<0.001$). With sex, age, WHR, BMI, hypertension, TG, TCH, HDL-C, LDL-C, FBG and HBsAg included, the calibrated regression model gave the OR values of 1.000, 1.669, 6.635 and 32.131 in in 4 quartered baseline serum levels of hsCRP. Conclusion: High serum level of hsCRP is an independent risk factor of short-term progression to NASH in patients with NIDDM and NAFLD. Those NIDDM patients with NAFLD that present with high serum level of hsCRP should be subjected to regular monitoring, lifestyle intervention and medication.

Keywords: Non-insulin dependent diabetes mellitus, non-alcoholic fatty liver disease, high sensitivity C-reaction protein, non-alcoholic steatohepatitis

Introduction

C-reactive protein (CRP) is an acute phase protein synthesized and released by hepatocytes under the effect of inflammatory cytokines, playing roles in different types of inflammatory responses. Past reports have showed that serum CRP levels predict the pathogenesis and development of primary and secondary cardiovascular disorders [1, 2]. Especially, it has been suggested that the increased CRP level is a significant predictive factor of cardiovascular events in patients with non-insulin dependent diabetes mellitus (NIDDM) [3, 4]. The concrete mechanisms of CRP in promoting the development of cardiovascular events, especially in patients with NIDDM, have not yet been entirely clarified. However, according to some existing researches, NIDDM is usually characterized by overexpression of inflammatory markers and the serum CRP levels are associated with the degree of insulin resistance in Chinese patients with NIDDM [5, 6].

Although the value of CRP in predicting the development of cardiovascular events in diabetic patients has been primarily confirmed, the reports in regards to the value of CRP in the prognostic evaluation of non-alcoholic fatty liver disease (NAFLD) in diabetic patients are still very sparse. Non-alcoholic steatohepatitis (NASH) is the progressive stage of NAFLD, fol-

Table 1. Baseline data of NIDDM patients with NAFLD at different levels of serum hsCRP

	G1 (n=280)	G2 (279)	G3 (284)	G4 (n=285)	P
Sex					0.053
Male	202	202	179	200	
Female	78	77	105	85	
Age (y)	50.1±13.8	51.2±13.4	49.4±12.6	50.4±12.7	0.427
WHR	0.814±0.005	0.822±0.004	0.838±0.010	0.861±0.012	<0.001
BMI	23.17±1.83	23.28±2.39	23.08±1.58	25.43±2.08	<0.001
TG	1.85±0.69	1.95±1.02	1.83±0.51	3.10±0.32	<0.001
TCH	4.63±0.37	4.66±0.48	4.62±0.32	5.08±0.42	<0.001
HDL-C	1.47±0.32	1.38±0.30	1.40±0.31	1.36±0.29	<0.001
LDL-C	2.31±0.34	2.30±0.37	2.30±0.35	2.52±0.36	<0.001
FBG	4.92±1.81	5.18±2.66	4.86±1.32	8.16±0.85	<0.001
hypertension					0.599
Yes	81	92	96	88	
No	199	187	188	197	
HBsAg					0.081
Yes	22	31	39	40	
No	258	248	245	245	
NASH					<0.001
Yes	23	35	96	207	
No	257	244	188	78	

lowed by serious hepatic dysfunction and even failure. The pathogenesis of NASH is multifactorial with hepatic insulin resistance playing a central role in the disease progression [7, 8]. Thus, serum CRP levels may be in correlation with the progression of NAFLD and indicate the development of NASH. In recent years, the prevalence of NAFLD has increased in China, especially amongst some less physically fit groups, such as obese children and administrative officers [9, 10]. In order to investigate the prognostic significance of CRP in diabetic patients with NAFLD, we analyzed the correlation between the baseline serum high sensitive CRP (hsCRP) levels and the risk of NASH amongst Chinese adults with NIDDM complicated with NAFLD.

Patients and methods

Study cohort

We prospectively followed 1128 diabetic patients that were presented to our department and definitely diagnosed as NAFLD. Most of the patients were participants in annual regular physical examination whereas the others took regular re-examinations for diabetes mellitus. The inclusion criteria were: 1) taking annual physical examinations; 2) definite diagnosis of NIDDM and NAFLD; 3) no manifestations of NASH in first round of physical examination; 4) signing informed consent. The exclusion criteria were: 1) past history of autoimmune hepatitis, hepatic dysfunction, cirrhosis, or drug dependence; 2) alcoholism; 3) medication with herbal medicine or supplements for bodybuilding; 4) incomplete medical records.

Physical examination

The physical examination included measurement of body height, body weight, waist circumference, hip circumference, and calculation of body mass index (BMI) and waist-hip circumference ratio (WHR). The physical examination was performed in our independent examining room and the results were recorded and compared between two clinicians to ensure the accuracy of data.

Laboratory tests

The laboratory tests included routine blood and urine analysis, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum hsCRP, total cholesterol (Tch), fasting blood glucose (FBG), triglycerine (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and hepatitis B surface antigen (HBsAg). The samples were each tested 3 times with a time interval of 4 hours and the average values were recorded.

Ultrasonographic examination

The ultrasonographic examination of liver was performed by experienced ultrasonographers, using 3.5 MHz probe. The patients were placed in the horizontal position with an empty stomach. NASH was defined as ultrasonographic

Table 2. Regression models: the risk of progression from NAFLD to NASH in NIDDM patients at different baseline serum levels of hsCRP

	Monovariate regression		
	OR	95% CI	P
G1	1.000	-	-
G2	1.603	0.921-2.791	0.095
G3	5.706	3.487-9.336	<0.001
G4	29.654	17.990-48.880	<0.001
	Calibrated model 1		
	OR	95% CI	P
G1	1.000	-	-
G2	1.666	0.942-2.947	0.079
G3	6.350	3.377-11.941	<0.001
G4	32.924	13.184-82.221	<0.001
	Calibrated model 2		
	OR	95% CI	P
G1	1.000	-	-
G2	1.669	0.940-2.962	0.080
G3	6.635	3.487-12.624	<0.001
G4	32.131	12.668-81.501	<0.001

manifestations of fatty liver and either AST or ALT more than 1.5 times the upper limit.

Statistical methods

All statistical analyses were performed using SPSS 13.0. The measurement data was compared among different groups using one-way ANOVA analysis and LSD pairwise comparison was also performed. Since the serum hsCRP levels were unevenly distributed, the raw data was subjected to logarithmic transformation with all zero-values included into the analysis as 0.02 mg/L. The enumeration data was compared using chi-square test. The risk factors of NASH amongst diabetic patients with NADLH were analyzed using logistic regression. P<0.05 was considered statistically significant.

Results

Baseline data

A total of 1128 cases of NIDDM complicated with NAFLD (783 males and 345 females) were included into the study cohort, with an average age of 50.3 years (22-72 years). The cases were divided into 4 groups according to the quartiles of baseline hsCRP; the quartiles were respectively 0.35 mg/L, 0.67 mg/L and 1.83 mg/L. The groups were significantly different from each other (P<0.05) in WHR, BMI, TG, TCH, HDL-C, LDL-C and FBG. Specifically, the cases with baseline hsCRP<1.83 mg presented with similar serum levels of WHR, BMI, TG, TCH, HDL-C, LDL-C and FBG; however, the cases with baseline hsCRP≥1.83 mg presented with significantly higher serum levels of the indexes above (except HDL-C) than the other cases. Of the 1128 cases, 132 were hypertensive, 357 were HBsAg positive and 361 developed NASH within one year after baseline. The percentages were not significantly different among the 4 groups in hypertension (P=0.599) and HBsAg (P=0.081). One year after baseline, the percentages of NASH were respectively 8.2%, 12.5%, 33.8% and 72.6%; there was significant difference among the 4 groups in percentage of NASH (P<0.001). See **Table 1** for details.

Logistic regression

As the results of logistic regression showed, the model including baseline hsCRP only indicated that the risk of progression to NASH increased along the serum level of hsCRP; the OR values were respectively 1.000, 1.603, 5.706 and 29.654 in the 4 groups. With sex, age, WHR, BMI and hypertension included, the calibrated model 1 gave the OR values of 1.000, 1.666, 6.350 and 32.924 in the 4 groups. With TG, TCH, HDL-C, LDL-C, FBG and HBsAg included, the calibrated model 2 gave the OR values of 1.000, 1.669, 6.635 and 32.131. See **Table 2** for details.

Discussion

NAFLD is a pathological condition associated with obesity, NIDDM and metabolic syndrome. The natural history of NAFLD can be divided into three stages: 1) fatty liver stage, which is characterized by fat accumulation in hepatocytes without inflammatory infiltration or fibrosis; 2) NASH stage, which is characterized by fat accumulation, inflammatory infiltration, necrosis of hepatocytes and mild to moderate hepatic dysfunction; 3) cirrhosis stage, which is characterized by serious fibrosis and hepatic dysfunction [11-13]. In Western countries, NAFLD has been considered to be the commonest cause of chronic hepatic damage and about 20 to 30% of the NAFLD patients present with NASH, which leads to a series of serious

hepatic disorders such as cirrhosis, hepatic failure and malignant tumors [14]. Overall, NASH has an incidence of no less than 2% in the US and the EU [13]. A prospective study using paired biopsies showed that 23% of the NAFLD patients developed borderline NASH within 3 years after baseline [15]. Our data showed that 32% of the NAFLD patients suffered development of NASH within one year after baseline. This rapid progression might be partly attributed to the diabetic condition of these patients, which suggested that the hepatic function among the patients with diabetes mellitus should be regularly monitored to achieve early diagnosis and intervention.

Since NIDDM is associated with insulin resistance, an important mechanism in different types of chronic organic injuries, regular examinations for NIDDM patients should not only be comprehensive, but also judiciously focused. Although cardiovascular events have always been considered to be the major causes for excess mortality among the patients with NIDDM, the threat of hepatic failure and encephalopathy should not be underestimated. CRP is a sensitive marker of inflammation used to predict the prognosis of a series of cardio-, cerebro- and reno-vascular disorders in patients with diabetes mellitus [16-19]. However, the existing literature has not investigated the value of CRP in predicting the prognosis of NAFLD in patients with diabetes mellitus. According to our study, the risk of short-term progression to NASH increased along the serum levels of hsCRP, respectively 8.2% (35/280), 12.5% (35/279), 33.8% (96/284) and 72.6% (207/285) in 4 groups with different serum levels of hsCRP from low to high. In addition, our results also showed that those patients with higher serum levels of hsCRP did present with higher serum levels of TG, TCH, LDL-C and FPG, which might suggest that elevated baseline hsCRP indicated the presence of a number of peroxidative and vasoactive substances, which caused persistent damage to secretory and epithelial cells.

In our study, the regression model calibrated with different indexes in physical and laboratory examinations showed that high serum level of hsCRP was an independent risk factor of short-term progression to NASH in patients with NIDDM and NAFLD; the risk in group 4 (hsCRP>1.83 mg/L) was 32.1 times higher than it was in group 1 (hsCRP<0.35 mg/L). The interrelationship between hsCRP and the development of NASH is not entirely clear. However, past researches have showed that CRP is one of the markers of local inflammation, endothelial dysfunction and tumorigenesis [20, 21]. The liver injury in NIDDM patients with NAFLD might be caused by microthrombosis and accumulation of collagenous fibrils. In addition, as a pro-inflammatory cytokine, high level of CRP might also have direct and persistent damage to the hepatocytes. There are also other possible mechanisms of CRP in the progression of NAFLD. The excessive CRP might be originated from some fat tissues, rather than the fat accumulated in hepatocytes, in those obese patients [21, 22]; under such condition, the increased serum level of hsCRP reflected the insufficient hepatic functions. The correlation between serum level of CRP and progression of NAFLD might be reciprocal causation with multiple factors and mechanisms involved. For the perspective of long-term prognosis, the serum level of CRP is also a potential predictive factor of cirrhosis, hepatic failure and malignant tumors. Thus, for those NIDDM patients with NAFLD, who present with high serum levels of CRP, regular monitoring, lifestyle intervention and medication should be recommended to slow down the progression of NAFLD.

Our study does still have some limitations. First, it was a short-term observational study, so the values of serum hsCRP in predicting some terminal events, such as hepatic failure, cirrhosis and malignant tumors, were not evaluated. Second, the diagnosis of NAFLD and NASH was made using ultrosonography rather than biopsy, which had higher sensitivity and specificity. Last but not least, it was a single-center study and a larger sample size was difficult to achieve. However, our study has primarily laid the foundation for further investigation into the value of serum hsCRP as a predictive factor in the prognostic evaluation of the patients with NIDDM and NAFLD. Our study may also have some implications in the value of serum hsCRP in predicting some other serious events amongst the patients with diabetes mellitus or metabolic syndrome.

As stated above, the patients with NIDDM and NAFLD suffered even more rapid progression to

NASH than their non-NIDDM counterparts and high serum level of hsCRP was an independent risk factor of short-term progression to NASH in patients with NIDDM and NAFLD. Those patients with NIDDM and NAFLD that present with high serum level of hsCRP are more prone to the development of NASH and they should be subjected to regular monitoring, lifestyle intervention and medication in order to slow down the progression of NAFLD.

Disclosure of conflict of interest

None.

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