

## Serum hs-CRP was correlated with treatment response to pegylated interferon and ribavirin combination therapy in chronic hepatitis C patients

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### Abstract

**Background/aims** Serum high sensitivity C-reactive protein (hs-CRP) is a surrogate marker for cardiovascular disease risks and related mortality. However, the features of hs-CRP in chronic HCV infection (CHC) patients have not been fully addressed. This study aimed to elucidate the characteristics of hs-CRP and its correlation with clinical profiles in CHC patients.

**Methods** Ninety-five CHC patients and 95 age- and sex-matched healthy controls were enrolled for serum hs-CRP level, biochemical, and metabolic profiles examinations. Sequential changes of hs-CRP levels in CHC patients receiving peginterferon/ribavirin combination therapy were also evaluated.

**Results** The mean hs-CRP level of CHC patients was significantly higher than that of healthy controls ( $0.97 \pm 0.11$  vs.  $0.24 \pm 0.07$  mg/L,  $P < 0.001$ ). There was no significant correlation between hs-CRP and both virological and histological factors. CHC patients with a high LDL-C level had significantly higher mean hs-CRP ( $1.38 \pm 0.20$  mg/L) than that of patients without ( $0.59 \pm 0.06$  mg/L) ( $P < 0.001$ ). Hs-CRP level was significantly decreased in 83 patients after peginterferon/ribavirin combination therapy ( $0.24$  vs.  $0.62$  mg/L,  $P < 0.001$ ), particularly in 68 patients achieving a sustained virological response ( $0.25$  vs.  $0.64$  mg/L,  $P < 0.001$ ).

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**Conclusion** CHC patients had a higher hs-CRP level than healthy controls which could be ameliorated after peginterferon/ribavirin combination therapy.

**Keywords** High sensitivity C-reactive protein · Hepatitis C virus · Antiviral therapy

## Introduction

Hepatitis C virus (HCV) infection is one of the most important causes of cirrhosis and hepatocellular carcinoma and has a strong impact on public health worldwide. HCV is lymphotropic as well as hepatotropic in nature [1]. Replication of HCV in diseased extrahepatic organs and tissues may have direct cytopathic effects, thus leading to a wide spectrum of extrahepatic manifestations, such as thyroid dysfunction, glucose abnormalities, lipid disorders, mixed cryoglobulinemia, and autoimmunity [2–8]. The related mechanisms in terms of liver injury and extrahepatic figures were not fully clarified. Systemic inflammatory response triggered by HCV per se and/or its subsequent immune cascades and cytokine storms may play a major role in it [9].

In the context of cytokines, high-sensitivity C-reactive protein (hs-CRP) has been shown to be closely related to the occurrence of systemic inflammatory response [10]. Serum hs-CRP levels were elevated in patients with insulin resistance and were correlated with metabolic syndrome [11, 12]. Moreover, hs-CRP has been shown to predict future risks for cardiovascular disease and related mortality [13, 14]. With respect to hepatological views, hs-CRP is a liver specific acute-phase protein, and its expression in hepatocytes is closely related with proinflammatory cytokines, such as tumor necrosis factor- $\alpha$ , interleukin-1, and interleukin-6 [15]. There were discrepant results of the association of CRP level and anti-HCV seropositivity in different study groups [16–21]. The feature of hs-CRP in chronic HCV infection (CHC) patients has not been fully addressed and remains a puzzle. Furthermore, study regarding the sequential changes of hs-CRP levels in terms of current standard-of-care antiviral therapy has never been evaluated.

The present study aimed to clarify the characteristics and the associated factors of hs-CRP in CHC patients. We also aimed to assess the impact of antiviral therapy on the sequential changes of hs-CRP levels.

## Methods

The ethical committee of Kaohsiung Medical University Hospital approved the study before it began. All subjects gave written informed consent before enrollment.

## CHC patients

Ninety-five treatment-naïve CHC patients were consecutively enrolled. All patients were seropositive for HCV antibodies (anti-HCV) and HCV RNA by polymerase chain reaction (PCR) for more than 6 months. All liver biopsies, which were obtained within 6 months prior to enrollment, showed chronic hepatitis of different severity. Patients with a concurrent hepatitis B (seropositive for hepatitis B surface antigen), human immunodeficiency virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease,  $\alpha_1$ -antitrypsin deficiency, decompensated cirrhosis (Child-Pugh class B or C), overt hepatic failure, a current or past history of alcohol abuse ( $>40$  g ethanol per day), psychiatric condition, or with evidence of hepatocellular carcinoma were excluded.

## Community-based controls

A total of 95 age-, sex-matched healthy adults aged 30 years or more who were seronegative for antibody to hepatitis C virus (anti-HCV) were invited for hs-CRP examination during their multi-purpose health surveillance in The Clinic of Department of Preventive Medicine, Kaohsiung Medical University Hospital. The controls were recruited with the same exclusion criteria of CHC patients.

## Laboratory measurements

Anti-HCV was detected by using a third-generation commercially available enzyme-linked immunosorbent assay kit (AxSYM 3.0, Abbott Laboratories, Chicago, IL, USA). HCV RNA levels were detected by a qualitative polymerase chain reaction (PCR) assay (Cobas Amplicor Hepatitis C Virus Test, version 2.0; Roche Diagnostics, Branchburg, NJ, USA; detection limit: 50 IU/ml). HCV genotypes were determined by the method described by Okamoto et al. [22].

Serum levels of aspartate and alanine aminotransferase (AST and ALT), gamma-glutamyl transpeptidase (rGT), fasting plasma glucose (FPG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were determined using a multichannel autoanalyzer (Hitachi Inc., Tokyo, Japan). Hs-CRP levels were analyzed before and 6 months after antiviral therapy with a latex turbidimetric immunoassay (Wako Chemicals, Neuss, Germany). The analytical detection limit for this method is 0.06 mg/L. When samples containing a known level of CRP were tested ten times, the coefficient of variation of the test was under 10%. Individuals with serum hs-CRP  $>10$  mg/L were excluded in the current study because of the possibility of concurrent inflammatory or infectious diseases [23].

## Histological analyses

For each CHC patient, a liver biopsy specimen of at least 2 cm in length was taken and fixed in 10% formalin buffer. Biopsy samples were stained with hematoxylin-eosin and the results were then reported by one pathologist who was blinded to the treatment of each patient. Histological grading of chronic hepatitis was made based on histological activity index (HAI) of Knodell et al. Liver fibrosis was staged into F0-4 according to the Metavir scoring systems.

## Regimens and treatment responses

CHC patients were treated with peginterferon- $\alpha$ -2a (PEGASYS, Roche, Basel, Switzerland), 180  $\mu$ g/week subcutaneously, plus oral ribavirin at a dose of 1000–1200 mg/day for 48 weeks if they were infected with HCV genotype 1 (HCV-1) or peginterferon- $\alpha$ -2a, 180  $\mu$ g/week, plus oral ribavirin at a dose of 800 mg/day for 24 weeks if they were infected with HCV genotype 2 (HCV-2), with a 24-week follow-up period.

Evaluation of efficacy of antiviral treatment was based on sustained virological response (SVR), defined as PCR-negativity by the end-of-treatment and throughout the follow-up period. Non-responders were defined as persistence of HCV RNA levels throughout the treatment course.

## Statistical analysis

Hs-CRP levels were expressed as mean (SD) or median (interquartile range, IQR). Comparisons between groups were

made using the Student's test or the Mann–Whitney  $U$  test for continuous variables and the  $\chi^2$  or Fisher exact probability test for categorical data if suitable. HCV RNA levels were analyzed with values  $\log_{10}$  transformed before  $t$  test analysis. Statistical correlations between serum hs-CRP and other parameters were determined by the non-parametric Spearman test. Paired  $t$  test or Wilcoxon signed rank test was used to compare the changes of serum hs-CRP levels before and after antiviral therapy in CHC patients. A multivariate analysis based on a logistic regression study is used to establish associations as appropriate. The procedures were performed using the SPSS 12.0 statistical package (SPSS, Chicago, IL, USA). All statistical analyses were based on two-sided hypothesis tests with a significance level of  $P < 0.05$ .

## Results

### Comparison of baseline characteristics between CHC patients and controls

The basic demographical and clinical features of the 95 CHC patients and the 95 healthy controls were shown in Table 1. The mean viral load of the CHC patients was  $5.50 \pm 0.96$  log IU/mL. Forty (42.1%) patients were of HCV-1 infection. The CHC patients had a significantly higher level of AST, ALT, and rGT and a significantly lower level of TG, total cholesterol, HDL-C and LDL-C than healthy controls (all  $P < 0.001$ ). The mean and median hs-CRP of CHC patients were  $0.97 \pm 0.11$  mg/L, and 0.62 (IQR = 0.30, 1.09) mg/L,

**Table 1** Basic demographical and clinical features of CHC patients and controls

|  | CHC patients ( $N = 95$ ) | Healthy controls ( $N = 95$ ) | $P$ value |
|--|---------------------------|-------------------------------|-----------|
| Age (years) <sup>a</sup>                                 | $52.8 \pm 10.3$           | $53.1 \pm 10.3$               | 0.87      |
| Sex (male), $n$ (%)                                      | 45 (47.4)                 | 45 (47.4)                     | 1         |
| BMI ( $\text{kg}/\text{m}^2$ )                           | $24.5 \pm 3.4$            | $24.2 \pm 3.3$                | 0.59      |
| Blood sugar ( $\text{mg}/\text{dL}$ ) <sup>a</sup>       | $98.6 \pm 23.6$           | $103.0 \pm 31.6$              | 0.28      |
| Triglycerides ( $\text{mg}/\text{dL}$ ) <sup>a</sup>     | $94.5 \pm 46.1$           | $125.1 \pm 63.5$              | <0.001    |
| Total cholesterol ( $\text{mg}/\text{dL}$ ) <sup>a</sup> | $168.0 \pm 34.3$          | $206.6 \pm 42.3$              | <0.001    |
| HDL-C ( $\text{mg}/\text{dL}$ ) <sup>a</sup>             | $42.6 \pm 14.6$           | $56.3 \pm 14.6$               | <0.001    |
| LDL-C ( $\text{mg}/\text{dL}$ ) <sup>a</sup>             | $102.5 \pm 30.0$          | $123.3 \pm 36.7$              | <0.001    |
| AST (IU/L) <sup>a</sup>                                  | $123.2 \pm 5.15$          | $23.6 \pm 8.6$                | <0.001    |
| ALT (IU/L) <sup>a</sup>                                  | $157.7 \pm 82.1$          | $25.1 \pm 13.1$               | <0.001    |
| r-GT (U/L) <sup>a</sup>                                  | $70.6 \pm 67.2$           | $29.5 \pm 19.5$               | <0.001    |
| hs-CRP, median (IQR)                                     | 0.62 (0.30, 1.09)         | 0.10 (0.05, 0.20)             | <0.001    |
| < 1 mg/L, $n$ (%)  | 65 (68.4)                 | 92 (96.8)                     | <0.001    |
| 1–3 mg/L, $n$ (%)  | 25 (26.3)                 | 2 (2.1)                       |           |
| >3 mg/L, $n$ (%)   | 5 (5.3)                   | 1 (1.1)                       |           |

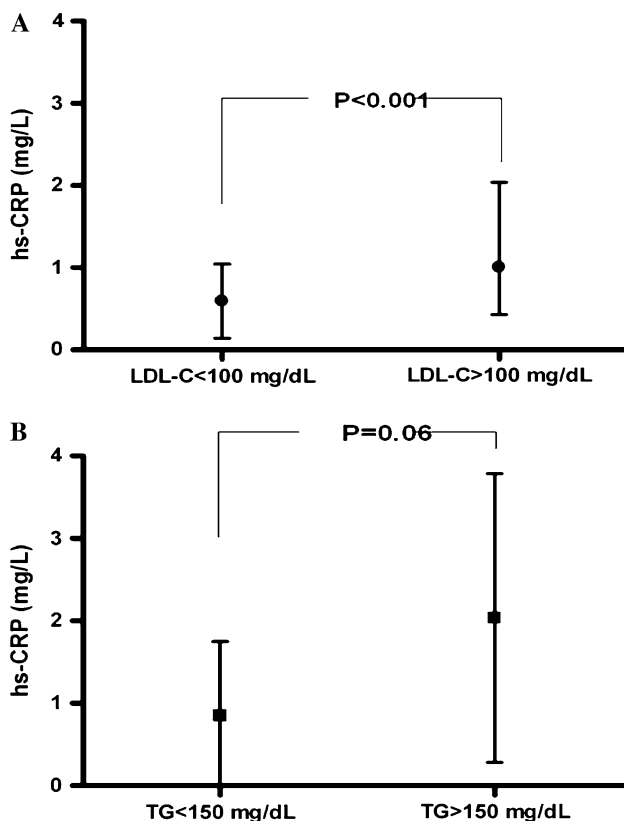
CHC chronic hepatitis C, BMI body mass index, AST alanine aminotransferase, ALT aspartate aminotransferase, rGT r-Glutamyl transferase, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, hs-CRP high sensitivity C-reactive protein, IQR interquartile range

<sup>a</sup> Expressed as mean  $\pm$  SD

respectively, which was significantly higher than that (mean =  $0.24 \pm 0.07$  mg/L; median = 0.10, IQR = 0.05, 0.20 mg/L) of healthy controls ( $P < 0.001$ ). When subjects were stratified into three groups dependent of severity of cardiovascular risks [14], 65 (68.4%) CHC patients had hs-CRP levels less than 1 mg/L, 25 (26.3%) patients of 1–3 mg/L, and 5 (5.3%) patients of  $>3$  mg/L. CHC patients had a significantly higher proportion of the groups carrying hs-CRP  $> 1$  mg/L than healthy controls ( $P < 0.001$ ).

Clinical profiles correlated with hs-CRP level in CHC patients

Lipid profiles including TG ( $r^2 = 0.055$ ,  $P = 0.023$ ), total cholesterol ( $r^2 = 0.072$ ,  $P = 0.009$ ) and LDL-C levels ( $r^2 = 0.048$ ,  $P = 0.033$ ), were positively correlated with hs-CRP level in CHC patients. Further analysis demonstrated that 46 patients with LDL-C level  $> 100$  mg/dL had significantly higher mean hs-CRP ( $1.38 \pm 0.20$  mg/L) than that of other 49 patients with LDL-C level  $< 100$  mg/dL ( $0.59 \pm 0.06$  mg/L) ( $P < 0.001$ ) (Fig. 1a). For those patients with high TG level ( $>150$  mg/dL), the mean hs-CRP level ( $2.01 \pm 0.55$  mg/L) was substantially higher than that of patients without high TG level ( $0.85 \pm 0.10$  mg/L) ( $P = 0.06$ ) (Fig. 1b).



**Fig. 1** a The mean hs-CRP level between CHC patients with LDL-C level  $< 100$  mg/dL and  $>100$  mg/dL. b The mean hs-CRP level between CHC patients with TG level  $< 150$  mg/dL and  $>150$  mg/dL

Eighty-four (88.4%) CHC patients were regarded as high hs-CRP group, defined as 75 percentile of the healthy controls (0.197 mg/L). As shown in Table 2, univariate regression analysis demonstrated that the factors associated with high hs-CRP included fibrosis score, genotype non-1, ALT and total cholesterol levels.

The independent predictive value of age, sex, BMI, AST, ALT, baseline HCV RNA levels, grade and stage of liver histopathology, blood sugar, total cholesterol, TG, HDL-C and LDL-C was determined by using stepwise logistic regression analysis.

Total cholesterol level was the only independent factor associated with high hs-CRP in CHC patients (odds ratio = 1.04, 95% CI = 1.01–1.08,  $P = 0.004$ ).

Sequential changes of hs-CRP levels related to treatment response

A total 85 of the 95 CHC patients received peginterferon plus ribavirin combination therapy, including 36 HCV-1 and 49 HCV-non 1 patients, respectively. Two patients were excluded for comparison because of serum hs-CRP

**Table 2** Factors associated with high hs-CRP level in CHC patients

|   | High hs-CRP level |                 | P value |
|---|-------------------|-----------------|---------|
|   | Yes, n = 84       | No, n = 11      |         |
| Sex (male), n (%)                           | 38 (45.2)         | 7 (63.6)        | 0.25    |
| Age (years) <sup>a</sup>                    | $52.4 \pm 10.4$   | $56.0 \pm 8.6$  | 0.28    |
| Metabolic syndrome, n (%)                   | 23 (27.4)         | 4 (36.4)        | 0.72    |
| Hypertension, n (%)                         | 22 (26.2)         | 3 (27.3)        | 1       |
| BMI ( $\text{kg}/\text{m}^2$ ) <sup>a</sup> | $24.6 \pm 3.2$    | $23.0 \pm 4.3$  | 0.13    |
| HCV RNA $> 400$ kIU/mL, n (%)               | 34 (40.5)         | 4 (36.4)        | 1       |
| HCV genotype 1, n (%)                       | 32 (38.1)         | 8 (72.7)        | 0.05    |
| FPG (mg/dL) <sup>a</sup>                    | $99 \pm 25$       | $96 \pm 12$     | 0.66    |
| Triglycerides (mg/dL) <sup>a</sup>          | $96 \pm 48$       | $81 \pm 26$     | 0.29    |
| Cholesterol (mg/dL) <sup>a</sup>            | $172 \pm 34$      | $141 \pm 26$    | 0.01    |
| HDL-C (mg/dL) <sup>a</sup>                  | $43 \pm 15$       | $38 \pm 10$     | 0.29    |
| LDL-C (mg/dL) <sup>a</sup>                  | $105 \pm 29$      | $87 \pm 33$     | 0.07    |
| AST (IU/L) <sup>a</sup>                     | $105 \pm 53$      | $93 \pm 33$     | 0.49    |
| ALT (IU/L) <sup>a</sup>                     | $161 \pm 86$      | $130 \pm 40$    | 0.05    |
| Fibrosis score <sup>a</sup>                 | $1.82 \pm 1.17$   | $2.64 \pm 0.92$ | 0.03    |
| HAI score <sup>a</sup>                      | $5.54 \pm 2.77$   | $6.45 \pm 1.81$ | 0.16    |

High hs-CRP level was defined as 75 percentile level of the healthy control group, 0.197 mg/L

BMI body mass index, CHC chronic hepatitis C, HCV hepatitis C virus, FPG fasting plasma glucose, AST alanine aminotransferase, ALT aspartate aminotransferase, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, hs-CRP high sensitivity C-reactive protein

<sup>a</sup> Expressed as mean  $\pm$  SD

**Table 3** Sequential changes of hs-CRP levels according to treatment response in 83 CHC patients

|  | Before treatment<br>[median (IQR)], mg/L | After treatment<br>[median (IQR)], mg/L | <i>P</i> value    |        |
|--|--|---|-------------------|--------|
| All patients ( <i>n</i> = 83)  | 0.62 (0.33, 1.08)                        | 0.24 (0.10, 0.50)                       | <0.001            |        |
| <i>hs-CRP</i> high sensitivity<br>C-reactive protein,<br><i>IQR</i> interquartile range,<br><i>SVR</i> sustained virological<br>response | SVR patients ( <i>n</i> = 68)            | 0.64 (0.30, 1.08)                       | 0.25 (0.10, 0.49) | <0.001 |
|  | Non-SVR patients ( <i>n</i> = 15)        | 0.61 (0.41, 1.57)                       | 0.18 (0.10, 0.52) | 0.02   |
|  | Relapsers ( <i>n</i> = 7)                | 0.85 (0.41, 2.16)                       | 0.13 (0.10, 0.24) | 0.03   |
|  | Non-responders ( <i>n</i> = 8)           | 0.54 (0.36, 0.93)                       | 0.31 (0.03, 0.88) | 0.33   |

levels >10 mg/L after treatment. Sixty-eight (81.9%) patients had a SVR and 15 (18.1%) were non-SVR patients.

Sequential changes of serum hs-CRP levels related to treatment response were shown in Table 3. There was statistically significant decrease of mean hs-CRP levels among those 83 CHC patients after treatment compared with their pretreatment levels (0.24 vs. 0.62 mg/L,  $P < 0.001$ ). The changes were observed either in SVR group (0.25 vs. 0.64 mg/L,  $P < 0.001$ ) or in non-SVR group (0.18 vs. 0.61 mg/L,  $P = 0.02$ ). Nonetheless, the significant decrease of hs-CRP levels after treatment existed only in seven relapsers (0.13 vs. 0.85 mg/L,  $P = 0.03$ ), but not in eight non-responders (0.18 vs. 0.54 mg/L,  $P = 0.33$ ).

## Discussion

Hs-CRP has been shown to be a competent predictor for future risks of cardiovascular diseases and metabolic abnormalities in apparently healthy persons independently of established major risk factors [24, 25]. It plays a major role in the scenario of an activated systemic inflammatory response, and is also a common feature of various chronic liver diseases, such as nonalcoholic fatty liver disease and nonalcoholic steatohepatitis [26, 27]. The current study demonstrated that the hs-CRP level of CHC patients was significantly higher than that of healthy controls. In addition, hs-CRP level was significantly ameliorated after antiviral therapy. It, therefore, implied that a more profound systemic inflammatory response existed in patients with HCV infection. It also provided evidence that systemic inflammatory response could effectively be relieved after antiviral therapy, particularly in patients achieving a SVR.

Elevated serum hs-CRP level has been consistently demonstrated in the emergence of atherosclerosis [11, 28]. The proinflammatory, proatherogenic effects of hs-CRP that have been documented in endothelial cells include the following features: atherosclerotic streaks and plaques, the emergence of inflammatory processes over intima layer, rupture of plaques and platelet aggregation, activation of glycoprotein IIb/IIIa receptor, cascading coagulating system, and thus lead to thrombus and acute coronary syndrome. HCV infection may lead to insulin resistance and/or

metabolic syndrome, and which were clinical manifestations of systemic inflammatory response [4, 29–31]. Therefore, the association between HCV infection and elevated serum hs-CRP level is plausible. The current proof-of-concept study demonstrated that CHC patients had a higher hs-CRP level than healthy controls. This finding echoed our previous study showing that HCV infection carried a higher prevalence of metabolic syndrome and insulin resistance [4, 32]. In addition, the proportion of CHC patients with hs-CRP level > 3 mg/L, a condition indicative of a high cardiovascular risk, was also higher than healthy controls [14]. The relationships between HCV infection and vascular atherosclerosis remain an argument of debate [33–35]. The current study might provide insight for further studies to clarify subsequent cardiovascular risks in patient with HCV infection.

An association between HCV infection and lipid metabolism has been consistently reported [8, 36]. It has been reported that the LDL-receptor is one of the HCV receptors, and experiments in vitro showed competitive inhibition of binding between HCV and LDL-receptor by LDL-C [36]. In addition, lipoproteins play an important role in the process of HCV infection since complexing of the virus to VLDL-C or LDL-C could promote endocytosis of HCV via the LDL receptor [37]. Taken together, HCV infection is associated with clinically significant lower cholesterol (total cholesterol, HDL-C and LDL-C) and TG levels than those of normal subjects. The current study demonstrated that hs-CRP level was correlated with TG, total cholesterol, and LDL-C levels in CHC patients. For patients with high LDL-C or TG levels, their mean hs-CRP level was significantly or substantially higher than those without. However, advanced liver diseases may be associated with decreasing cholesterol level and the extent of liver injury should be taken into account when addressing this issue. Moreover, the lipid profiles are reflected by multiple factors, such as race, age, gender, life style and meal habits. Therefore, it might be too conclusive to imply that lipid profiles were the major factors correlated with hs-CRP level in CHC patients. Nevertheless, our data may suggest that hs-CRP may be used as a complementary surrogate marker for cardiovascular risks in this special population. Further study addressing the sequential changes and the correlation between lipid profiles and

hs-CRP in a long-term follow-up basis will be needed to elucidate this intriguing observation.

Acute phase reactions with elicited acute phase proteins directly or indirectly from liver are common features in patients with different extent of liver insults ranging from acute liver injury to advanced liver cirrhosis [38]. The features of acute phase proteins include increased C1-inhibitor, C9, C4, and orosomucoid, while decreased transferrin and fetuin/a2HS-glycoprotein in CHC patients who responded to interferon-based antiviral therapy [39]. CRP is the most widely used acute phase protein in clinical setting. However, to our knowledge, the change of hs-CRP subsequent to peginterferon/ribavirin combination therapy has never been studied. Our data demonstrated that serum hs-CRP significantly decreased after pegylated interferon and ribavirin combination therapy. It may suggest that systemic inflammatory response in HCV infection could effectively be relieved after antiviral therapy, particularly in patients achieving a SVR. Intriguingly, among those non-SVR patients, decreases of serum hs-CRP levels were significantly observed only in relapsers but not in non-responders. It has been shown that those relapsers have more favorable outcomes after re-treatment programs compared with non-responders [40]. The decreased systemic inflammatory response in relapsers, which was reflected by significantly decreased hs-CRP level, may in a part contribute to the favorable outcomes. It is noteworthy whether the non-responders carry a higher risk of developing cardiovascular events, awaits further intervention.

In conclusion, the current study demonstrated that CHC patients had a higher serum hs-CRP level than healthy controls. CHC patients with elevated hs-CRP had substantially higher levels of lipid profiles. Furthermore, hs-CRP level was significantly decreased after pegylated interferon and ribavirin combination therapy, particularly for those achieving a SVR. Further studies are warranted to elucidate the causal relationship between HCV infection and cardiovascular risk in terms of hs-CRP level.

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**Conflicts of interest** None.

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