

Original Article

Circulating *P*-Cresylsulphate Level is Associated with Hepatocellular Carcinoma in Chronic Hepatitis B or C Virus Infection

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Objectives: *p*-cresylsulphate (PCS) is metabolized in the liver and is derived from the gastrointestinal tract. Previous studies have also suggested that, in addition to the kidneys, the liver is an essential and independent organ in determining serum PCS levels. However, little is known about the role of total PCS in hepatocellular carcinoma (HCC) with concurrent chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. We aimed to investigate whether total serum PCS levels were altered in patients with HCC. In addition, the association between total serum PCS levels and pretreatment hematological profiles was evaluated.

Methods: Total serum PCS concentrations were measured using the Ultra Performance LC System in 76 HCC patients with either HBV or HCV infection, and 76 healthy control subjects.

Results: HCC patients with chronic HCV infection had significantly higher levels of total PCS compared with those in HCC patients with chronic HBV infection and healthy controls. Multiple logistic regression analysis revealed that total PCS was an independent risk factor for HCC, even after full adjustment for known biomarkers. Pearson's correlation analysis showed that total serum PCS level was positively associated with age, hypertension, HCV, chronic kidney disease stage, and white blood cell count. In addition, platelet levels, hemoglobin, hematocrit, estimated glomerular filtration rate, red blood cell count, and lymphocyte count were negatively associated with total PCS levels.

Conclusion: These results suggest that total serum PCS level is associated with the presence of HCC, and that a higher total serum PCS level may be important for the pathogenesis of HCC.

Key words: chronic hepatitis B, chronic hepatitis C, hepatocellular carcinoma, total *p*-cresylsulphate

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Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infect hundreds of millions of people worldwide, and cause a spectrum of chronic liver diseases.^{1,2} In up to 20% of patients, chronic HBV or HCV infection causes progressive hepatic fibrosis and cirrhosis, and approximately 10 – 20% of cirrhotic patients may develop hepatocellular carcinoma (HCC) within 5 years.^{3,4} Previous studies have demonstrated that chronic HBV and HCV infections are important causes of HCC, as they are associated with > 80% of cases worldwide,⁵ and previous studies have indicated that the mortality rate is rising.⁶ Hence, investigating risk factors for the development of HCC in patients infected with HBV or HCV is of utmost importance.

Previous studies have demonstrated that HCV is associated with an increased risk of albuminuria, progression of diabetic nephropathy, and progression of chronic kidney disease (CKD) to end stage renal disease (ESRD).⁷ As renal function deteriorates, levels of pro-inflammatory cytokines and uremic retention toxins increase.⁸ It has been previously shown that the levels of uremic retention solutes are associated with the elevated production of reactive oxygen species (ROS) and inflammatory mediators in CKD patients.⁹ *p*-Cresol (4-methylphenol, molecular weight 108.1 Da) is a small molecule derived from the ingestion of the amino acids tyrosine and phenylalanine. In humans, total *p*-cresylsulphate (PCS) exists predominantly as conjugated PCS, and a rare, sometimes undetectable form of unconjugated *p*-cresol (free-form).^{10,11} PCS is strongly bound to protein and is poorly cleared by conventional hemodialysis.¹⁰ The concentration of PCS in uremic plasma is approximately 10 times higher than that in normal control subjects.¹² PCS induces endothelial dysfunction by increasing leukocyte transmigration into the en-

dothelium and enhancing baseline leukocyte activity; it can also induce the production of ROS,¹³ which may contribute to the deterioration of renal function,¹⁴ vascular damage,^{15,16} cardiovascular events, and all-cause mortality in CKD patients.^{17,18}

PCS emanates from the gastrointestinal tract. The precursors of this solute are generated through the metabolism of dietary protein by the intestinal flora. PCS is subsequently metabolized by cytosolic enzymes in hepatocytes, before finally being released into the systemic circulation. Previous studies have indicated that in addition to the kidneys, the liver is an essential and independent organ for determining serum PCS levels.¹⁹ Interestingly, PCS was shown to play a role in aberrant adipose tissue metabolism, insulin resistance, reallocation of fat in the body,²⁰ hampering of calcium deposition and osteoprotegrin expression in human mesenchymal stem cells. Lipid metabolism plays a role in HCC development²¹ and insulin resistance (measured by HOMAIR), regardless of the presence of diabetes. It has been shown to be significantly associated with HCC development in patients with chronic HCV infection.²² Therefore, PCS might play a role in insulin resistance and lipid metabolism and may contribute to the pathogenesis of HCC. To investigate the role of PCS in HCC, total serum PCS levels were measured in HCC patients with HCV or HBV infection. Total serum PCS levels were further assessed and pretreatment hematological profiles were evaluated in this population.

Materials and Methods

Study participants

From January 2016 to January 2018, 76 consecutive patients were enrolled, 36 had both HCC and chronic HCV infection (14 men and 22 women; age: 69 ± 9 years), and 40 had both HCC and chronic HBV infection (29 men and 11 women; age: 63 ± 10 years). Among the

36 patients with HCV infection, 28 had HCV genotype 1b and 8 had HCV genotype 2a. In addition, 76 healthy adults (58 men and 18 women; age: 59 ± 6 years; body mass index (BMI): 24.0 ± 3.0 kg/m²) were enrolled as the control group; these patients did not have anti-HCV antibodies and were HBsAg and HIV negative, with an alcohol consumption of < 20 g/day and a normal alanine aminotransferase (ALT) level. Chronic HBV was defined as being positive for HBsAg (Abbott Laboratories, North Chicago, IL, USA) for at least 6 months. Chronic HCV infection was defined as the presence of serum HCV-RNA, as assayed using reverse transcription polymerase chain reaction (Amplicor Roche/Promega v.2 Diagnostic Test, Branchburg, NJ, USA). The diagnosis of cirrhosis was based on laboratory data, physical findings combined with imaging studies or liver biopsy. Severity of cirrhosis was assessed according to the Child-Pugh score, and HCC was diagnosed based on histological examinations of resected specimens or biopsies. The exclusion criteria were alcohol or drug abuse, other HCV genotypes, autoimmune, neoplastic, thyroid, and psychiatric diseases, a serum ferritin level > 800 ng/mL, HIV coinfection, and heart or renal failure. The study protocol and procedure were approved by the Ethics Committee of I-Shou University and E-Da Hospital. Written informed consent was obtained from each participant prior to their enrollment.

Clinical and laboratory assessments

According to the World Health Organization criteria,²³ a diagnosis of type 2 diabetes mellitus was made when the fasting blood glucose level was ≥ 126 mg/dL on at least 2 occasions, or the patient was receiving ongoing treatment with hypoglycemic agents. Hypertension was defined as persistent elevation of systolic blood pressure (≥ 140 mmHg) and/or diastolic blood pressure (≥ 90 mmHg). Patients receiving antihypertensive therapy were also

defined as having hypertension. The estimated glomerular filtration rates (eGFRs) were calculated using the CKD-EPI 2-concentration race equation,²⁴ and the status of CKD was confirmed by follow-up eGFR measurements after 3 months. The modified National Kidney Foundation classification of CKD was used.²⁵ Serum biochemical parameters and complete blood cell counts were also measured after overnight fasting, including triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), creatinine, iron, transferrin iron binding capacity (TIBC), alkaline phosphatase (ALKP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), red cell distribution width-coefficient of variation, red cell distribution width-standard deviation (RDW-SD), total bilirubin, hemoglobin, hematocrit, white blood cell count (WBC), platelets, albumin, alpha-fetoprotein (AFP), and prothrombin time, all of which were measured using standard commercial methods on a parallel, multichannel analyzer (Hitachi 7170A, Tokyo, Japan) as described in a previous report.²⁶ The AST to platelet ratio index (APRI) was calculated as $[\text{AST (IU/L)} / \text{platelet count (10}^9\text{/L)}] \times 100$.

To determine total PCS serum levels, samples were deproteinized by the addition of 3 parts methanol to 1 part serum. Total PCS was measured in serum ultrafiltrates obtained using a UPLC assay. A UPLC assay, using a photodiode array (PDA) detector set at 280 nm, was performed at room temperature on an ACQUILITY UPLC[®] BEH phenyl column of 2.1 mm \times 100 mm. The buffer flow was 0.4 mL/min using 10 mM NH₄H₂PO₄ (pH = 4.0) (A) and 100% acetonitrile (B) with a gradient from 82.5% A/17.5% B to 55% A/45% B, over 9 min. Under these conditions, p-cresol sulphate was detected at 260 nm and appeared at 1.7 min. There were standard curves from total PCS at 0.5, 1, 2.5, 5, and 10 mg/L. Processed like-serum samples had average r^2

Table 1. Clinical characteristics of the study subjects.

Variable	HCC with HCV	HCC with HBV	Controls	p-value
N	36	40	76	
Age (years)	69.2 ± 9.1	62.6 ± 10.2	59.0 ± 6.1	< 0.0001
Gender, male (n, %)	14 (38.9)	29 (72.5)	58 (76.3)	0.0003
Current smoking (n, %)	10 (27.8)	12 (30.0)	19 (25.0)	0.840
Antiviral therapy (n, %)	10 (27.8)	18 (45.0)	-	0.120
Body mass index (kg/m ²)	23.6 ± 4.1	23.5 ± 4.1	24.0 ± 3.0	0.790
Total-cholesterol (mg/dL)	150.8 ± 46.2	176.0 ± 51.0	198.6 ± 32.3	0.0003
Triglyceride (mg/dL)	87.0 (52.0 – 97.3)	66.0 (58.5 – 115.8)	101.0 (74.5 – 132.5)	0.381
HDL-cholesterol (mg/dL)	50.9 ± 7.2	46.9 ± 19.6	53.7 ± 13.6	0.425
LDL-cholesterol (mg/dL)	79.0 ± 24.9	89.1 ± 20.8	110.4 ± 26.3	0.009
Serum Fe (µg/dL)	115.4 ± 54.1	104.8 ± 43.8	-	0.497
TIBC (µg/dL)	292.0 ± 79.4	280.9 ± 50.9	-	0.589
Aspartate aminotransferase (U/L)	71.0 (48.0 – 118.3)	49.5 (36.5 – 80.0)	28.0 (23.3 – 33.0)	< 0.0001
Alanine aminotransferase (U/L)	63.0 (38.8 – 111.0)	34.0 (22.5 – 53.0)	29.0 (22.3 – 39.8)	< 0.0001
Alkaline phosphatase (U/L)	421.5 (314.3 – 886.0)	329.5 (238.0 – 525.3)	227.0 (186.0 – 265.0)	< 0.0001
Prothrombin time (seconds)	11.3 ± 0.9	12.9 ± 10.9	10.1 ± 0.5	0.477
APRI	1.1 ± 0.8	0.6 ± 0.5	0.1 ± 0.1	< 0.0001
Child-Pugh class (A/B,C)	34 / 2	38 / 2	-	0.914
Total bilirubin (mg/dL)	1.1 (0.9 – 1.5)	1.0 (0.7 – 1.5)	1.1 (0.9 – 1.5)	0.675
Creatinine (mg/dL)	1.0 (0.8 – 1.2)	1.1 (1.0 – 1.2)	1.1 (1.0 – 1.2)	0.173
eGFR (mL/min/1.73 m ²)	75.3 ± 25.6	69.8 ± 23.8	75.2 ± 13.3	0.366
Hemoglobin (g/dL)	11.9 ± 1.8	13.0 ± 2.2	14.6 ± 1.4	< 0.0001
Hematocrit (%)	35.1 ± 5.0	38.1 ± 5.9	43.1 ± 3.5	< 0.0001
White blood cell count (10 ⁹ /L)	6.215 ± 3.270	6.169 ± 4.115	5.190 ± 1.178	0.048
Platelet (10 ³ /µL)	106.8 ± 46.4	146.5 ± 70.4	231.4 ± 52.0	< 0.0001
RDW-SD (fL)	48.9 ± 5.5	48.8 ± 6.2	41.2 ± 3.8	< 0.0001
RDW-CV (%)	15.0 ± 1.9	15.9 ± 5.2	13.3 ± 1.4	0.029
Albumin (g/dL)	3.7 ± 0.4	3.8 ± 0.5	4.4 ± 0.2	< 0.0001
Alpha-fetoprotein (ng/mL)	90.3 (32.0 – 203.4)	182.0 (31.0 – 2726.0)	-	0.252
Prothrombin time (sec)	11.2 (10.9 – 11.7)	11.0 (10.5 – 11.7)	10.0 (9.8 – 10.4)	0.477
Total p-cresylsulphate (mg/L)	5.7 (2.1 – 10.7)	2.6 (0.9 – 7.0)	1.6 (0.5 – 3.9)	< 0.0001

Data are expressed as mean ± SD, number (percentage), or median (interquartile range). HBV: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TIBC: transferrin iron binding capacity; APRI: aspartate aminotransferase to platelet ratio index; RDW-SD: red cell distribution width-standard deviation; RDW-CV: red cell distribution width-coefficient of variation. HDL: high-density lipoprotein; LDL: low-density lipoprotein; eGFR: estimated glomerular filtration rate.

values of 0.999 ± 0.001 . Quantitative results were obtained and calculated as concentrations (mg/L). The method detection limit of the assay was 1 mg/L.²⁷

Statistical analysis

Continuous, normally distributed variables are expressed as mean ± standard deviation, and non-normally distributed variables are presented as median (interquartile range). The Kolmogorov-Smirnov test was used to

evaluate the normality of distribution. Differences in variables between groups were tested using one-way analysis of variance (ANOVA) for normally distributed variables, followed by Tukey's pairwise comparison. Categorical data are expressed as a number (percentage), and inter-group comparisons were performed using the χ^2 test or Fisher's exact test. Logarithmic transformations were analyzed to correct for the skewed distribution of serum triglycerides, AST, ALT, alkaline phosphatase (ALKP), total

bilirubin, creatinine, alpha-fetoprotein, prothrombin time, and total PCS.

In multivariate logistic regression analysis, model 1 evaluated the relationship of each parameter with the presence of HCC, and model 2 used multivariate forward stepwise logistic regression analysis that included variables with a p -value < 0.1 in model 1. We further divided the distribution of total PCS in pooled data into tertiles using 1.25 mg/L and 4.4 mg/L as cutoff values to define three patient groups (lowest tertile: < 1.25 mg/L, $n = 50$; middle tertile: 1.25 to 4.4 mg/L, $n = 51$; and highest tertile: > 4.4 mg/L, $n = 51$). General linear and logistic regression models were used to estimate the significant trends across increasing tertiles and to estimate the odds ratios (ORs) of HCC in each tertile, using the lowest tertile as the reference category. Multivariate adjusted ORs are presented with 95% confidence intervals (CI). Pearson's correlation coefficient analysis was used to examine the correlations between serum total PCS and the other parameters. All analyses were carried out using SAS statistical software, version 8.2 (SAS Institute Inc.; Cary, NC, USA). All tests were two-sided, and a p -value < 0.05 was considered to indicate a statistically significant difference.

Results

Comparisons between the baseline

features and total PCS levels among HCC patients with HCV, HCC patients with HBV, and controls are shown in Table 1. The control subjects were predominantly male, and the median age of patients with HCC and HCV (69 years) was significantly higher than that of patients with HCC and HBV (63 years), and the control subjects (59 years) ($p < 0.0001$). The patients with HCC and HCV had significantly lower levels of hemoglobin, hematocrit, and platelets than those in patients with HCC and HBV, and the control subjects ($p < 0.0001$), as well as significantly lower total cholesterol, LDL-C, and albumin levels compared with those in the control subjects (all $p < 0.01$). Furthermore, the patients with HCC and HCV had significantly higher levels of ALT, ALKP, APRI, and total PCS than those in patients with HCC and HBV and in control subjects ($p < 0.0001$), as well as a significantly higher AST, WBC count, and RDW-SD levels compared with those in the control subjects ($p < 0.05$). There were no significant differences in the levels of current smoking, BMI, triglycerides, HDL-C, total bilirubin, creatinine, eGFR, and prothrombin time among the three groups. There were also no significant differences in the levels of antiviral therapy, Fe, TIBC, Child-Pugh class, and AFP between patients with HCC and HCV and those with HCC and HBV. Furthermore, among the 36 patients with HCV and HCC, 10 had received antiviral therapy.

Table 2. Logistic regression analysis with the presence of hepatocellular carcinoma as the dependent variable.

Variable	Multivariate Model 1		Multivariate Model 2	
	Odds ratio (95% CI)	p -value	Odds ratio (95% CI)	p -value
AST	1.15 (1.09 - 1.21)	< 0.0001	1.34 (1.12 - 1.59)	0.001
ALT	1.04 (1.02 - 1.07)	< 0.0001		
ALKP	1.02 (1.01 - 1.03)	< 0.0001		
Total bilirubin	0.98 (0.94 - 1.03)	0.441		
Hemoglobin	0.48 (0.36 - 0.64)	< 0.0001		
WBC count	1.25 (1.04 - 1.51)	0.017		
Albumin	0.01 (0.00 - 0.03)	< 0.0001	0.06 (0.00 - 0.75)	0.030
Total p -cresylsulphate	1.29 (1.15 - 1.46)	< 0.0001	1.43 (1.06 - 1.94)	0.020

CI: confidence interval; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALKP: alkaline phosphatase; WBC: white blood cell.

Model 1: multivariate logistic regression analysis of each variable after adjusting for age, gender.

Model 2: multivariate stepwise regression analysis including all the variables that demonstrated a p -value < 0.1 in model 1 listed in the table after adjusting for age, gender.

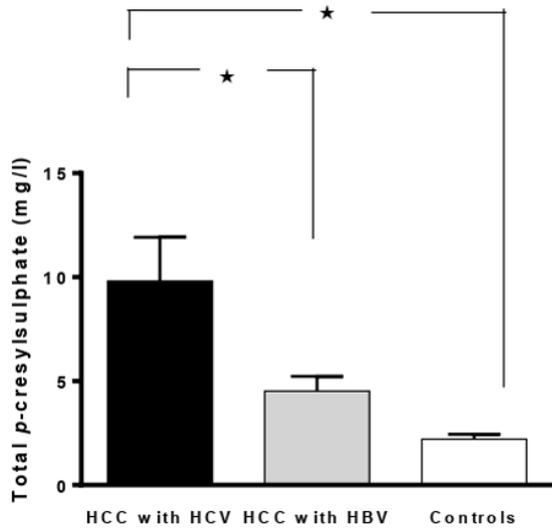


Fig. 1 Patients with hepatocellular carcinoma (HCC) and chronic hepatitis B or C virus infection had significantly higher levels of total p-cresylsulphate (PCS) compared with the controls. HCC patients with chronic hepatitis C virus infection patients had significantly higher levels of total PCS compared with HCC patients with chronic hepatitis B virus infection. The bars represent the mean \pm SD. Differences between groups were analyzed by one-way analysis of variance and t-test.

However, no patients reached sustained virologic response. Moreover, among the 40 patients with HBV and HCC, 18 had received antiviral therapy. The total PCS levels were not significantly different between HBV patients with antiviral therapy and those without (3.4 ± 4.3 mg/L vs. 5.5 ± 4.5 mg/L, respectively $p = 0.145$). In addition, patients with HCC and

chronic HCV infection had significantly higher levels of total PCS compared with those in patients with HCC and chronic HBV and in controls ($p < 0.0001$) (Fig. 1).

Multivariate logistic regression analysis (Table 2) demonstrated that the biochemical variables associated with the presence of HCC were AST, ALT, ALKP, hemoglobin, WBC count, albumin, and total PCS (model 1). However, only AST, albumin, and total PCS levels remained significant in model 2, with ORs of 1.34 (95% CI: 1.12 – 1.59), 0.06 (95% CI: 0.00 – 0.75), and 1.43 (95% CI: 1.06 – 1.94), respectively.

The increases in serum total PCS levels showed a significant linear trend with the incidence of HCC (Table 3). Using total PCS < 1.25 mg/L as the reference, the ORs for HCC increased across the groups. Even after adjustments for the confounding variables, this trend remained statistically significant (Model 2). In a fully adjusted model (Model 3), the ORs (95% CI) for HCC in the second and third tertile were 1.81 (0.44-8.38) and 6.16 (1.38-16.79), respectively compared to those in the first tertile (p for trend = 0.022).

Pearson's correlation analyses revealed that total PCS serum level was positively correlated with age, hypertension, HCV, CKD stage, and WBC count. In addition, platelet, hemoglobin, hematocrit, eGFR, red blood cell (RBC)

Table 3. Odds ratios (95% Confidence interval, CI) for impact of total p-cresylsulphate serum levels on hepatocellular carcinoma according to the tertiles of total p-cresylsulphate levels.

Factor	Tertiles of total p-cresylsulphate			p for trend
	Q1 (95% CI)	Q2 (95% CI)	Q3 (95% CI)	
All subjects				
No. of cases/reference	17 / 33	22 / 29	37 / 14	0.0003
Cut off serum total PCS (mg/l)	< 1.25	1.25 - 4.4	> 4.4	
Crude odds ratios	1.00	1.47 (0.66- 3.33)	5.13 (2.24 - 12.32)	0.0002
Model 1	1.00	1.12 (0.46 - 2.68)	3.88 (1.61 - 9.73)	0.003
Model 2	1.00	1.35 (0.36 - 5.30)	5.25 (1.33 - 13.45)	0.021
Model 3	1.00	1.81 (0.44 - 8.38)	6.16 (1.38 - 16.79)	0.022

Values shown are cut-offs of serum total p-cresylsulphate levels of all subjects, and ORs with 95% CIs. Model 1: Adjusted for age and sex. Model 2: Model 1 with further adjustment of alanine aminotransferase, hemoglobin, and albumin. Model 3: Model 2 with further adjustment of creatinine and white blood cell count. PCS: p-cresylsulphate.

Table 4. Pearson's correlation analysis of total p-cresylsulphate with clinical laboratory data.

Factor	r	p-value
Age	0.324	< 0.0001
Gender	0.001	0.991
Diabetes mellitus	0.086	0.176
Hypertension	0.243	0.0001
Hepatitis B virus	0.046	0.490
Hepatitis C virus	0.179	0.007
CKD stages	0.271	0.0004
Systolic blood pressure	0.117	0.077
Diastolic blood pressure	0.010	0.879
Body mass index	- 0.141	0.086
AST	0.095	0.243
ALT	0.080	0.325
ALKP	0.161	0.066
APRI	0.120	0.143
Platelet	- 0.231	0.004
Total bilirubin	- 0.001	0.991
Hemoglobin	- 0.238	0.003
Hematocrit	- 0.224	0.006
RDW-SD	0.064	0.562
RDW-CV	- 0.035	0.753
Albumin	- 0.051	0.532
Creatinine	0.062	0.467
eGFR	- 0.197	0.020
White blood cell count	0.248	0.039
Red blood cell count	- 0.204	0.012
Monocyte count	0.172	0.077
Neutrophil count	0.097	0.322
Lymphocyte count	- 0.350	0.0002

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALKP: alkaline phosphatase; APRI: aspartate aminotransferase to platelet ratio index; RDW-SD: red cell distribution width-standard deviation; RDW-CV: red cell distribution width-coefficient of variation; eGFR: estimated glomerular filtration rate.

count, and lymphocyte count were negatively correlated with total PCS (Table 4).

Discussion

The present study demonstrated that serum PCS levels were significantly elevated in patients with HCC and chronic HCV or HBV infection. In addition, multivariate analysis revealed that AST, albumin, and the level of total serum PCS were independently associated with the development of HCC. Moreover, Pearson's correlation analyses showed that the level of total serum PCS was positively corre-

lated with age, hypertension, HCV, CKD stage, and WBC count, and was negatively correlated with platelet count, hemoglobin, hematocrit, eGFR, RBC count, and lymphocyte count. To the best of our knowledge, the current study is the first to report an association between an elevated total serum PCS level and HCC with chronic HCV or HBV infection.

Kidney disease is a common extrahepatic manifestation of HCV infection. Chronic HCV infection not only is related to CKD but also accelerates renal deterioration, leading to ESRD.^{28,29} However, the biological mechanisms by which total PCS is involved in the pathogenesis of HCC have yet to be fully elucidated. PCS is of particular interest among the various uremic toxins because of its high protein binding properties. PCS is about 94% bound to plasma protein, and causes a predictable decrease in the measured dialytic clearance.³⁰ In the present study, total PCS levels were associated with the incidence of HCC, which may provide a clue to the pathogenesis of liver diseases in HCV- and HBV- infected patients. Previous research has demonstrated that accumulated PCS plays a role in disturbing the renin-angiotensin-aldosterone system, activating leukocyte radical production, promoting renal tubular cell damage, and interfering with insulin signaling pathways.³¹⁻³⁵ Importantly, the local (i.e., hepatic) renin angiotensin system (RAS) is thought to contribute to the pathophysiology of liver diseases.³⁶ ROS are involved in the necrosis and apoptosis of hepatocytes, as well as hepatic stellate cell activation.^{37,38} Insulin resistance in metabolically active hepatocytes is thus expected to have important systemic consequences. In addition, insulin resistance, now recognized as a pathological factor in the development of non-alcoholic fatty liver disease,³⁹ is also a determinant of disease progression in chronic HCV and alcohol-induced liver disease.⁴⁰ The results of the present study support the idea³⁶⁻³⁹ that total PCS may act through RAS, ROS,

and insulin resistance to play an important role in the pathophysiology of HCC in patients with chronic HCV or HBV infections.

PCS has been previously shown to inhibit the activity of the human conditionally immortalized Breast Cancer Resistance Protein (BCRP) and PTEC efflux transporters Multidrug Resistance Protein 4 (MRP4) by 25% and 40%, respectively. Because these two efflux transporters are responsible for pumping solutes out of the tubular cell, their inhibition may lead to intracellular substrate accumulation and toxicity. These include various organic acids, of which PCS is one, that may contribute to the progression of CKD and HCC.^{41,42} In addition, PCS was shown to play an important role in aberrant adipose tissue metabolism and the reallocation of fat in the body.²⁰ Metabolic alterations constitute a selective advantage for tumor growth, proliferation, and survival, as they provide the crucial needs of cancer cells, such as increased energy production, macromolecular biosynthesis, and maintenance of redox balance.⁴³ In addition, PCS has a pro-inflammatory effect and produces free radicals, as evaluated by the increased oxidative burst activity of leukocytes at baseline.¹³ Furthermore, the present study demonstrated that patients with HCC had higher WBC counts and total PCS compared with those reported by other study groups (Table 1). Besides, the total PCS levels were significantly positively associated with WBC count (Table 4). The development of HCC is a multi-step process, which may develop from chronic liver injury and inflammation to subsequent fibrosis and or cirrhosis and finally to HCC. It is therefore reasonable to propose that total PCS may act as a pro-inflammatory cytokine and play a role in chronic inflammation, thereby contributing to the pathogenesis of HCC in patients with chronic HCV or HBV infections.^{44,45}

The results of the present study showed that total serum PCS levels were correlated with HCV infection. HCV is a major cause of

chronic liver disease that frequently progresses to cirrhosis and leads to an increased risk of HCC.⁴⁶ As renal function deteriorates, levels of uremia toxins and proinflammatory cytokines were increased.⁸ Previous studies reported a 7.6% prevalence of HCV infection in 4,185 predialysis patients with CKD⁴⁷ and the prevalence of HCV increased with CKD stage; the five-year cumulative incidence of ESRD was 52.6% and 38.4% in patients with CKD with and without HCV infection, respectively.⁴⁸ Chen et al.⁴⁹ suggested a correlation between HCV and renal function, indicating that HCV infection can lead to CKD and promote progression from CKD to ESRD. Various hepatic and/or extrahepatic manifestations of HCV infection can contribute to renal deterioration and further increase uremia toxin or PCS levels. In addition, the current results showed that total serum PCS levels were positively correlated with CKD stage and negatively associated with eGFR. This indicates that total PCS may be a true uremic retention toxin and that its serum concentration may increase with the progression of renal failure. Researchers have shown that renal dysfunction is related to the risk of clear cell renal cell carcinoma.⁵⁰ PCS can induce proliferation and migration of rat aortic vascular smooth muscle cells⁵¹ and epithelial-mesenchymal transition (EMT) in kidney fibrosis.⁵² Based on these studies, the authors believe that total PCS influences proliferation, EMT,⁵³ and migration in HCC. However, further studies are required to ascertain the role of total PCS in patients presenting with HCC.

Previous studies have indicated that, in addition to the kidneys, the liver was an essential and independent organ for determining serum PCS levels. The production rate of PCS was increased in patients with CKD and early liver cirrhosis and lower in patients with advanced liver cirrhosis.¹⁹ In the present study, of the patients with HCC and HCV or HBV, only four had advanced liver cirrhosis. A total of 72 patients were at an early liver cirrhosis

stage. There were five main limitations to the current study. First, the cross-sectional design and relatively small number of HCC patients limited the ability to infer a causal relationship between the increased total serum PCS level and HCC. Second, the analyses depended on single measurements of total PCS levels in the blood, which may not represent the relationship between total PCS level and HCC over time. It would be interesting to analyze serial changes in total serum PCS levels during the different stages of HCC, to further elucidate the role of circulating total serum PCS in HCC. Third, data on the histological status of the patients with chronic hepatitis were not available due to patient refusal and histological examinations not being included in the guidelines of the Bureau of National Health Insurance in Taiwan. Fourth, the range of prothrombin time in the HCC group was quite wide, because the detection method for prothrombin time may not be reliable due to the sampling technique. Fifth, because it is difficult to enroll the healthy controls whose gender and age exactly matched those of the HCC with HBV or HCV group patients, we adjusted the age and sex in the multivariate model of analysis (Tables 2 and 3). Furthermore, in Table 2, these variables had a mild collinearity problem (condition index = 44.4). However, we used multivariate forward stepwise logistic regression analysis to solve this problem in multivariate model 2. In addition, the existence of unrecognized confounding variables is still possible, although we controlled for many other major risk factors for cancer.

In conclusion, the present study showed that an elevated total serum PCS level was independently associated with an increased risk of HCC in ethnically Chinese HBV or HCV patients, and that there was a potentially close relationship among total PCS, chronic inflammation, and the development of HCC. Total PCS may be involved in complex interactions involving RAS, ROS, or insulin resistance. Our

findings suggest that total PCS may be a hepatic toxin in patients with HCC, especially those with HCV. However, large-scale longitudinal follow-up studies, are necessary to clarify the potential causal relationship between total PCS and HCC as well as the potential mechanism underlying the increase in total PCS in patients with HCC.

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