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ORIGINAL CONTRIBUTION

Vitamins B status and antioxidative defense in patients with chronic hepatitis B or hepatitis C virus infection

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Abstract
Background & Aims The impact of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection upon B vitamins status and antioxidative defense in infected patients was examined.
Methods Dietary record and blood levels of B vitamins and oxidative stress–associated biomarkers were determined for 195 healthy controls, 132 HBV, and 114 HCV patients.
Results HBV-infected patients had significantly higher levels of total cholesterol, free fatty acids (FFA), and lower ghrelin level ($p < 0.05$); and HCV-infected patients had significantly higher Ishak inflammation score and lactate dehydrogenase activity ($p < 0.05$). HBV patients had significantly lower red blood cell (RBC) vitamins B_2 and B_6 levels, and HCV infection significantly decreased vitamins B_2, B_6 and folate levels in RBC and/or plasma ($p < 0.05$). Correlation coefficients of RBC vitamin B_2 versus serum FFA in HBV patients, RBC vitamins B_2 and B_6 versus HCV RNA and Ishak inflammation score, and plasma vitamin B_6 vs Ishak inflammation score in HCV patients were <-0.5. HBV-infected patients had significantly higher oxidized glutathione level and lower glutathione peroxidase activity ($p < 0.05$), but HCV patients had significantly lower superoxide dismutase and catalase activities ($p < 0.05$).
Conclusion HBV or HCV infection enhanced oxidative stress and lowered B vitamins in circulation. In order to avoid other healthy risk, nutrition status should be monitored and limitation or supplementation of certain nutrients might be helpful for HBV- or HCV-infected patients.

Keywords Hepatitis B virus · Hepatitis C virus · B vitamins · Oxidative stress · Lipid metabolism

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GPX	Glutathione peroxidase
GSH	Glutathione
GSSG	Oxidized glutathione
Hb	Hemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High density lipoprotein
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein

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MDA Malondialdehyde
SOD Superoxide dismutase
XO Xanthine oxidase

Introduction

Hepatitis B, resulted from hepatitis B virus (HBV) infection, and hepatitis C, resulted from hepatitis C virus (HCV) infection, are two major etiologies of liver diseases in many countries including Taiwan [1, 2]. Both HBV and HCV infections are often chronic and able to progress into liver fibrosis, cirrhosis, and hepatocellular carcinoma [2, 3].

B vitamins including vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6 (pyridoxine), vitamin B_{12}, and folic acid are involved in many important physiological functions such as energy metabolism, protein biosynthesis, and cell reproduction. So far, less attention is paid to the influence of HBV or HCV infection upon the status of B vitamins in circulation. On the other hand, ghrelin is an important metabolism-associated hormone and favors a positive energy balance [4]. Our previous study found that liver cancer patients had lower ghrelin and higher cholesterol levels in circulation than healthy controls [5]. However, the variation of ghrelin level and lipid metabolism in HBV- or HCV-infected patients (without cirrhosis or cancer) remains unknown.

It has been proposed that oxidative stress contributes to the progression and deterioration of viral hepatitis [6, 7]. The study of Chrobot et al. [8] reported that HBV or HCV infection increased oxidative stress via decreasing SOD and catalase activities. However, that study included children only and did not examine the impact of HBV or HCV upon the variation of other non-enzymatic antioxidants such as glutathione and vitamin C. Hepatic oxidative DNA damage in liver biopsy samples from HBV- and HCV-infected patients has been investigated [9]. However, liver biopsy examination for patients with viral infection may not be always practical or feasible. In order to enhance understanding regarding the impact of HBV and HCV infection upon oxidative stress and antioxidant defense, both HBV- and HCV-infected patients were included, and oxidative stress–associated biomarkers in circulation from HBV- or HCV-infected patients were examined in this study.

The purpose of this study was to examine the status of B vitamins, lipid profile, and antioxidative defense in HBV- or HCV-infected patients. This study provided several novel clinical findings to elucidate the possibility of nutritional intervention for virus-infected patients in order to prevent the prevalence and complications of advanced liver diseases.

Materials and methods

Patients and healthy subjects

This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was proved by Ethical Committee of the Medicine Faculty at Chung Shan Medical University. From January 2008 to February 2009, 132 patients with chronic hepatitis B and 114 patients with chronic hepatitis C who had not previously been treated were eligible to enter this study. Patients co-infected with both HBV and HCV and patients with habitual alcohol intake, and any other liver diseases (alcohol-, drug-, or obesity-induced liver disease, autoimmune hepatitis, hemochromatosis, alfa-1 antitrypsin deficiency, Wilson disease or cirrhosis) were excluded. Patients with serum creatinine >1.5 mg/dL, absolute neutrophil count <1,000/μL, platelet count <50,000/μL, or hemoglobin <10 g/dL were also excluded. One hundred and ninety-five healthy subjects were recruited in Chung Shan Medical University Hospital between January and September 2008. These subjects, confirmed by blood and ultrasound examination, did not suffer from HBV, HCV, or other liver diseases and were included as control group for comparison. The baseline characteristics of healthy controls, HBV and HCV patients are presented in Table 1. Informed consent for study participation was obtained from 132 HBV, 114 HCV patients, and 195 healthy control subjects.

Clinical evaluation

Chronic HBV infection was confirmed by the presence of serum hepatitis B virus surface antigen (HBsAg), HBeAg, and HBV DNA. HBsAg and HBeAg were measured by radioimmunoassay (Abbott Laboratories, Chicago, IL, USA) and electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA), respectively. After DNA extraction, plasma HBV DNA level was analyzed by the real-time TaqMan polymerase chain reaction with an ABI Prism 7,900 HT sequence detection system (Applied Biosystems, Foster City, CA, USA). Quantitative HCV RNA was measured by Cobas Monitor HCV v 2.0 (Roche Diagnostics, Branchburg, NJ, USA) and qualitative HCV RNA by Amplicor HCV v 2.0 (Roche Diagnostics, Branchburg, NJ, USA). HCV genotypes were determined by a linear probe assay (Inno-Lipa HCV, Innogenetics, Belgium). Percutaneous liver biopsy was performed for patients, and liver necro-inflammation and fibrosis were assessed according to the Ishak scoring system [10].

Table 1 Baseline characteristics in healthy subjects (control), HBV, and HCV patients

Parameters	Control $n = 195$	HBV $n = 132$	HCV $n = 114$
Gender, M/F	100/95	72/60	59/55
Age	51.4 ± 8.5	48.7 ± 7.3	45.9 ± 9.0
Body mass index (kg/m^2)	25.3 ± 3.0[a]	24.5 ± 2.8[a]	23.9 ± 3.4[a]
Serum ALT (U/L)	33 ± 7[a]	311 ± 38[b]	304 ± 47[b]
Serum AST (U/L)	28 ± 6[a]	241 ± 30[b]	260 ± 36[b]
HBeAg	Negative	Positive	Negative
HBsAg	Negative	Positive	Negative
HBV DNA, × 10^5	–[c]	5.7 ± 2.2	–
HCV RNA, × 10^5	–	–	6.3 ± 1.9
HCV genotype 1a/1b/2a/2b	NA	NA	15/42/37/20
Ishak fibrosis score	NA	2.17 ± 1.12[a]	2.58 ± 1.34[a]
Ishak inflammation score	NA	4.28 ± 1.61[a]	7.05 ± 2.04[b]
Bilirubin (mg/dL)	0.45 ± 0.18[a]	0.50 ± 0.17[a]	0.39 ± 0.22[a]
Alpha fetal protein (ng/L)	17 ± 3[a]	34 ± 15[a]	29 ± 10[a]
Albumin (g/dL)	3.35 ± 0.49[a]	3.58 ± 0.53[a]	3.77 ± 0.41[a]
Other diseases			
COPD	1	4	1
Gout	0	3	4
Diabetes	2	4	3
Renal insufficiency	0	0	1
Hypertension	5	7	3

Values are means ± SD

NA not available

[ab] In a row without a common letter differ, $p < 0.05$

[c] Too low to be detected

Dietary record and nutrients analyses

A 3-day dietary record including meal, snack, and drink was obtained from control subjects, HBV, and HCV patients. Nutrient composition in diet was calculated based on Taiwan Nutrient Databases [11]. All subjects did not take vitamins and minerals supplement.

Blood sampling

A peripheral blood sample, 15 mL, from each subject was drawn after an overnight fasting. One ml whole blood was used for vitamin B_1 analysis. The other 14 mL blood samples was treated to separate plasma or serum from erythrocyte.

Biochemical measurements

Blood level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, albumin, red blood cell (RBC) count, hemoglobin (Hb), total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride, creatinine, and uric acid was determined by an autoanalyzer (Dr. Lange LP 420, Berlin, German). Serum free fatty acid (FFA) content was determined by using a commercial kit (Wako Chemicals, Richmond, VA, USA). Lactate dehydrogenase (LDH) activity in serum was determined using photometric method by an automated instrument (Shimadzu CL-7300, Tokyo, Japan). Plasma immunoreactive ghrelin concentration was measured using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA). Plasma level of Se, Cu, and Zn was determined by flame atomic absorption spectrometry (Perkin-Elmer Model 5000; Norwark, CT, USA). The plasma level of α-tocopherol and β-carotene was quantified by a reverse-phase HPLC method [12]. Plasma level of total vitamin C (ascorbic acid and dehydroascorbic acid) was determined by an HPLC method, in which monolithic column and UV detector were equipped [13]. Glutathione (GSH) and oxidized glutathione (GSSG) concentrations in plasma were determined by commercial colorimetric GSH and GSSG assay kits (OxisResearch, Portland, OR, USA). The activity of catalase, Cu–Zn superoxide dismutase (SOD) and glutathione peroxidase (GPX) in plasma was determined by catalase, SOD, and GPX assay kits (Calbiochem, EMD Biosciences, Inc. San Diego, CA, USA), respectively. Xanthine oxidase (XO) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm [14].

B vitamins analyses

The level of vitamins B_1, B_2, and B_6 in whole blood, plasma, or RBC was determined by HPLC methods [15–17]. The status of vitamins B_1, B_2, and B_6 was determined as thiamine diphosphate, flavin adenine dinucleotide, and pyridoxal-5′-phosphate, respectively. Folate and vitamin B_{12} (cobalamin) were analyzed by radioprotein-binding assays (Bio-Rad Laboratories, Richmond, CA, USA). For folate determination, folic acid as pteroylglutamic acid was used for calibration, and its ^{125}I-labeled analog was used as the tracer. For cobalamin determination, cyanocobalamin was used for calibration, and its ^{57}Co-labeled analog was the tracer for cobalamin assay.

Lipid oxidation determination

Malondialdehyde (MDA) level in plasma was measured via an HPLC method [18]. Briefly, 0.1 mL plasma was mixed with thiobarbituric acid and phosphoric acid. After heating to 95 °C for 1 h, sample was cooled down and mixed with 0.1 mL methanol. HPLC equipped with a Nova-Pak C18

column (Waters, Millipore, Milford, MA, USA) was used to quantify MDA level. Plasma level of 8-isoprostane was determined via a method described in Konishi et al. [19]. Briefly, 0.5 mL plasma was diluted with a phosphate buffer and applied to an 8-isoprostane affinity column (Cayman Chemical, Ann Arbor, MI, USA). The 8-isoprostane was eluted from this column by adding 1.5 mL of 95% ethanol and further evaporated under nitrogen. The level of 8-isoprostane was measured by an EIA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol.

Statistical analysis

Each measurement was analyzed from 132 HBV, 114 HCV patients, and 195 healthy subjects. Data were assessed for normality using the Shapiro–Wilk test. Skewed data were logarithmically transformed before statistical analysis. Data were subjected to analysis of variance (ANOVA), and difference with $p < 0.05$ was considered to be significant. All data presented in this study were mean ± SD. Correlation between two variables was calculated by simple regression analysis (Minitab Inc., State College, Philadelphia, PA, USA).

Results

HBV or HCV infection significantly increased ALT and AST levels, and Ishak fibrosis and inflammation scores (Table 1, $p < 0.05$). HCV-infected patients had significantly higher Ishak inflammation score than HBV-infected patients ($p < 0.05$). HBV patients had significantly lower dietary fiber and calcium intake than other two groups (Table 2, $p < 0.05$). The dietary intake of vitamin B_6, folic acid, and Se in HCV groups was similar as healthy controls ($p > 0.05$), but with greater SD values. As shown in Table 3, when compared with healthy controls, HBV patients had significantly higher levels of total cholesterol, LDL cholesterol, FFAs, uric acid, and LDH activity as well as lower plasma ghrelin level ($p < 0.05$), and HCV patients had significantly higher levels of FFAs, uric acid, and LDH activity ($p < 0.05$).

Blood levels of B vitamins are shown in Table 4. HBV patients had significantly lower RBC vitamins B_2 and B_6 levels than healthy controls ($p < 0.05$). HCV infection significantly decreased vitamin B_2, vitamin B_6, and folate levels in plasma and RBC ($p < 0.05$). As shown in Table 5, the correlation coefficients of RBC vitamin B_2 vs serum FFA in HBV patients, RBC vitamins B_2 and B_6 vs HCV RNA and Ishak inflammation score in HCV patients, and plasma vitamin B_6 vs Ishak inflammation score were <-0.5.

Table 2 Daily dietary intake in healthy subjects (control), HBV, and HCV patients

Parameters	Control $n = 195$	HBV $n = 132$	HCV $n = 114$
Energy, kcal	1,732 ± 231[a]	1,808 ± 187[a]	1,590 ± 284[a]
Protein, g	73 ± 19[a]	70 ± 26[a]	68 ± 16[a]
Carbohydrate, g	214 ± 48[a]	237 ± 36[a]	220 ± 51[a]
Fat, g	68 ± 20[a]	75 ± 32[a]	61 ± 25[a]
Cholesterol, mg	190 ± 42[a]	214 ± 29[a]	208 ± 30[a]
Dietary fiber, g	15.1 ± 3.7[b]	12.7 ± 2.3[a]	16.0 ± 3.1[b]
Vit A, μg RE[c]	774 ± 72[a]	801 ± 64[a]	735 ± 80[a]
Vit D, μg	7.08 ± 1.42[a]	8.13 ± 0.75[a]	6.71 ± 1.68[a]
Vit E, mg	11.3 ± 2.8[a]	12.2 ± 2.3[a]	11.0 ± 3.1[a]
Vit C, mg	62 ± 11[a]	71 ± 14[a]	66 ± 9[a]
Vit B_1, mg	1.16 ± 0.24[a]	1.10 ± 0.19[a]	1.32 ± 0.25[a]
Vit B_2, mg	1.21 ± 0.17[a]	1.03 ± 0.31[a]	1.17 ± 0.20[a]
Vit B_6, mg	1.79 ± 0.31[a]	1.66 ± 0.28[a]	1.59 ± 0.75[a]
Vit B_{12}, μg	3.57 ± 0.66[a]	3.61 ± 0.58[a]	3.48 ± 0.70[a]
Folic acid, μg	342 ± 36[a]	367 ± 24[a]	354 ± 72[a]
Ca, mg	730 ± 154[b]	514 ± 126[a]	697 ± 181[b]
Fe, mg	11.2 ± 3.3[a]	12.1 ± 1.8[a]	10.9 ± 3.1[a]
Zn, mg	9.3 ± 2.1[a]	10.8 ± 3.0[a]	10.2 ± 2.5[a]
Cu, mg	3.8 ± 1.0[a]	3.6 ± 1.2[a]	4.1 ± 1.5[a]
Se, μg	109 ± 17[a]	121 ± 14[a]	115 ± 43[a]

Values are means ± SD

[ab] In a row without a common letter differ, $p < 0.05$

[c] Retinol equivalent

Compared with healthy controls, plasma levels of MDA and 8-isoprostane in HBV or HCV patients were significantly increased ($p < 0.05$, Fig. 1), in which HCV infection led to more production of 8-isoprostane than HBV infection ($p < 0.05$). As shown in Table 6, HBV or HCV infection resulted in significantly lower levels of vitamin C and GSH and higher XO activity ($p < 0.05$). Furthermore, HBV patients had significantly higher GSSG level and lower GPX activity than healthy controls and HCV patients ($p < 0.05$), but HCV patients had significantly lower SOD and catalase activities than other two groups ($p < 0.05$). Plasma level of Se, Cu, and Zn was not significantly different among controls, HBV, and HCV patients ($p > 0.05$).

Discussion

Elevated serum uric acid in HCV-infected patients has been reported [20]. Our present study found that increased circulating uric acid also occurred in HBV-infected patients, which could be explained by enhanced XO activity in those infected patients. In addition, we found that LDH activity was markedly elevated in HBV- or HCV-infected patients.

Table 3 Metabolic profile in healthy subjects (control), HBV, and HCV patients

Parameters	Control $n = 195$	HBV $n = 132$	HCV $n = 114$
Plasma glucose (mg/dL)	103 ± 14^{a}	99 ± 18^{a}	92 ± 5^{a}
HOMA-IR	1.98 ± 0.81^{a}	2.27 ± 1.03^{a}	2.05 ± 0.74^{a}
Serum total cholesterol (mg/dL)	128 ± 21^{a}	178 ± 30^{b}	133 ± 23^{a}
HDL cholesterol (mg/dL)	47 ± 7^{a}	44 ± 9^{a}	50 ± 6^{a}
LDL cholesterol (mg/dL)	61 ± 11^{a}	99 ± 21^{b}	67 ± 16^{a}
Serum triglyceride (mg/dL)	101 ± 17^{a}	121 ± 21^{a}	109 ± 14^{a}
Serum FFA (μmol/L)	267 ± 19^{a}	360 ± 31^{c}	306 ± 22^{b}
Serum creatinine (mg/dL)	0.53 ± 0.14^{a}	0.59 ± 0.17^{a}	0.60 ± 0.24^{a}
Serum uric acid (μmol/L)	178 ± 23^{a}	237 ± 19^{b}	251 ± 30^{b}
LDH activity (U/L)	155 ± 13^{a}	266 ± 25^{b}	304 ± 19^{c}
Plasma ghrelin (pmol/L)	141 ± 20^{b}	102 ± 18^{a}	132 ± 14^{b}

Values are means ± SD

[abc] In a row without a common letter differ, $p < 0.05$

Table 4 B vitamins in whole blood, plasma or red blood cell (RBC) from healthy subjects (control), HBV, and HCV patients

Parameters	Control $n = 195$	HBV $n = 132$	HCV $n = 114$
Whole blood vitamin B_1 (nmol/L)	107 ± 12^{a}	98 ± 15^{a}	91 ± 19^{a}
RBC vitamin B_1 (ng/g Hb)	425 ± 21^{a}	413 ± 25^{a}	408 ± 19^{a}
Plasma vitamin B_2 (nmol/L)	66.3 ± 2.2^{b}	67.1 ± 1.8^{b}	57.0 ± 3.1^{a}
RBC vitamin B_2 (nmoL/g Hb)	2.82 ± 0.30^{c}	2.41 ± 0.26^{b}	1.97 ± 0.33^{a}
Plasma vitamin B_6 (nmol/L)	20.8 ± 1.4^{b}	21.5 ± 1.0^{b}	16.6 ± 2.0^{a}
RBC vitamin B_6 (pmoL/g Hb)	332 ± 20^{c}	298 ± 24^{b}	260 ± 18^{a}
Plasma vitamin B_{12} (pmol/L)	328 ± 22^{a}	323 ± 19^{a}	311 ± 27^{a}
Plasma folate (nmol/L)	32.4 ± 2.6^{b}	30.7 ± 3.1^{b}	27.2 ± 2.3^{a}

Values are means ± SD

[abc] In a row without a common letter differ, $p < 0.05$

Table 5 Correlation coefficients of B vitamins versus clinical and metabolic features in HBV- and HCV-infected patients

	RBC vitamin B_1	Plasma vitamin B_2	RBC vitamin B_2	Plasma vitamin B_6	RBC vitamin B_6	Plasma folate
HBV infected						
HBV DNA load	−0.055	−0.037	−0.405	0.016	−0.318	−0.102
ALT	−0.094	−0.058	−0.319	−0.063	−0.285	−0.205
AST	−0.103	−0.091	−0.241	−0.025	−0.206	−0.153
Fibrosis score	−0.021	−0.082	−0.258	0.022	−0.224	−0.092
Inflammation score	−0.100	−0.095	−0.306	−0.066	−0.351	−0.140
Serum FFA	−0.118	−0.084	−0.529*	−0.050	−0.451	−0.075
Serum LDL cholesterol	−0.070	−0.067	−0.463	−0.018	−0.389	−0.039
Serum uric acid	−0.062	−0.071	−0.212	−0.087	−0.194	−0.116
Plasma ghrelin	−0.078	0.049	0.389	−0.029	0.268	0.119
HCV infected						
HCV RNA	−0.073	−0.361	−0.646*	−0.436	−0.624*	−0.314
ALT	−0.086	−0.290	−0.378	−0.378	−0.462	−0.280
AST	−0.109	−0.214	−0.267	−0.304	−0.321	−0.205
Fibrosis score	−0.064	−0.138	−0.274	−0.186	−0.210	−0.146
Inflammation score	−0.142	−0.395	−0.605*	−0.504*	−0.537*	−0.259
Serum FFA	−0.077	−0.205	−0.323	−0.189	−0.280	−0.182
Serum LDL cholesterol	−0.045	−0.096	−0.089	−0.104	−0.106	−0.041
Serum uric acid	−0.095	−0.173	−0.286	−0.202	−0.303	−0.142
Plasma ghrelin	0.060	0.033	−0.077	−0.047	−0.106	−0.108

* Correlation coefficient was higher than 0.5 or lower than −0.5

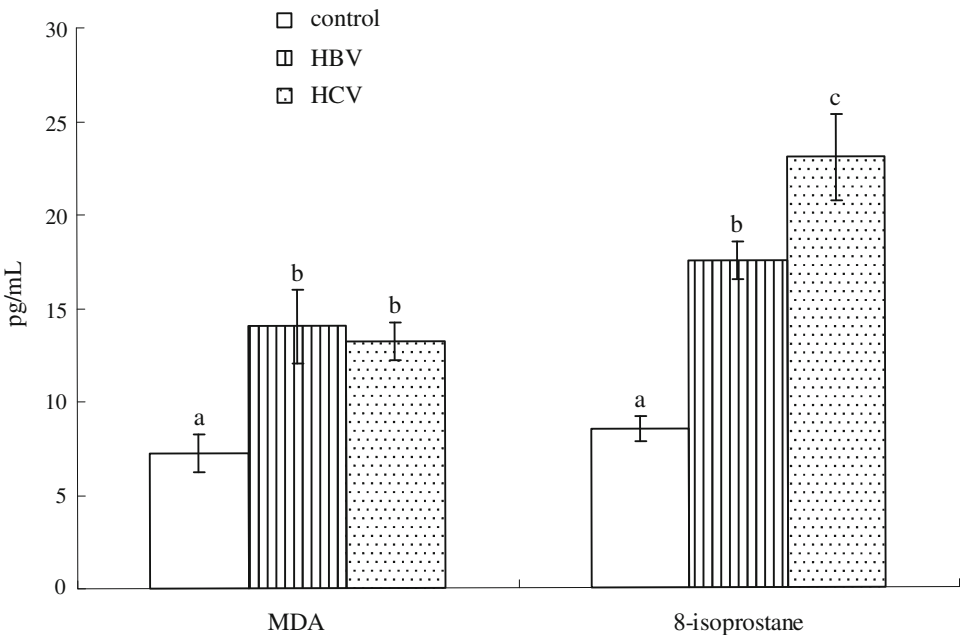

Fig. 1 Plasma level of malondialdehyde (*MDA*) and 8-isoprostane in healthy subjects (*control*), HBV and HCV patients. Data are mean ± SD. [abc] Among bars without a common letter differ, $p < 0.05$

Table 6 Plasma level of α-tocopherol, β-carotene, vitamin C, glutathione (GSH), oxidized glutathione (GSSG), selenium, copper, zinc, activity of glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), and xanthine oxidase (XO) in healthy subjects (control), HBV and HCV patients

Parameters	Control $n = 195$	HBV $n = 132$	HCV $n = 114$
α-tocopherol (μmol/L)	17.4 ± 2.3^{a}	16.8 ± 3.0^{a}	17.1 ± 2.5^{a}
β-carotene (μmol/L)	0.59 ± 0.17^{a}	0.76 ± 0.29^{a}	0.63 ± 0.25^{a}
Vitamin C (μmol/L)	31.5 ± 3.7^{b}	26.2 ± 2.4^{a}	25.9 ± 3.1^{a}
GSH (μmol/L)	15.6 ± 1.0^{c}	11.5 ± 1.8^{a}	13.4 ± 1.6^{b}
GSSG (μmol/L)	0.25 ± 0.07^{a}	0.79 ± 0.16^{b}	0.33 ± 0.10^{a}
Se (μg/L)	217 ± 9^{a}	214 ± 11^{a}	202 ± 13^{a}
Cu (μg/L)	570 ± 38^{a}	596 ± 45^{a}	608 ± 40^{a}
Zn (μg/L)	603 ± 56^{a}	611 ± 42^{a}	608 ± 51^{a}
GPX (U/L)	267 ± 32^{b}	209 ± 17^{a}	251 ± 24^{b}
SOD (U/mL)	20.5 ± 0.28^{b}	19.4 ± 0.17^{b}	15.3 ± 0.23^{a}
CAT (U/mL)	14.0 ± 0.38^{b}	13.1 ± 0.45^{b}	10.2 ± 0.26^{a}
XO (U/L)	1.65 ± 0.45^{a}	2.53 ± 0.78^{b}	2.66 ± 0.61^{b}

Values are means ± SD

[abc] In a row without a common letter differ, $p < 0.05$

Because increased uric acid level and LDH activity may impair cardiac or hepatic functions, monitoring the variation of uric acid and LDH for patients with viral hepatitis could benefit understanding their hepatitis progression.

Viral infection is a cause of lipid metabolism disturbance [21, 22]. Kim et al. [22] further indicated that HBV expression promoted lipid accumulation in hepatic cells by mediating sterol regulatory element–binding protein 1. In our present study, HBV patients had similar dietary lipid intake as healthy controls, but higher circulating levels of total cholesterol, LDL cholesterol, and free fatty acids than healthy controls. These findings agreed that HBV infection was a contributor toward abnormal lipid metabolism. Furthermore, we found that RBC vitamin B_2 level was negatively correlated with serum free fatty acids in HBV patients. It is reported that the administration of vitamin B_2, via acting as precursors of flavin adenine dinucleotide and flavin mononucleotide, could enhance activity of flavin-dependent acyl-CoA dehydrogenases and ameliorate lipid storage myopathies [23, 24]. Thus, it is possible that vitamin B_2 was used to modify HBV-induced lipid metabolism disorder in these patients, which caused vitamin B_2 depletion. Therefore, limiting dietary lipid intake or supplying vitamin B_2 might be helpful for these patients to minimize the disturbance of lipid metabolism. In addition, we notified that HBV patients had lower plasma ghrelin level. Ghrelin is an appetite stimulant, and its function is to cause a positive energy balance by decreasing fat utilization through growth hormone independent mechanisms [25]. Decreased plasma ghrelin level has been observed in patients with advanced cancer, cachexia, and weight loss [26]. The lower ghrelin level as we observed in HBV patients might favor the development of catabolic status (or so called negative energy balance) in these patients. Thus, in order to avoid the occurrence of negative energy balance, appropriate nutritional intervention should be considered for these HBV patients, especially when lipid intake is restricted.

In our present study, HBV patients had lower RBC vitamins B_2 and B_6 levels, but HCV patients had lower plasma and RBC levels of vitamins B_2, B_6, and folate. The

greater SD values for dietary B_6 and folic acid levels in HCV patients implied that some of these HCV patients had insufficient B_6 and folic acid intake, which partially explained the lower B_6 and folic acid levels in circulation of HCV patients. However, the observed folate, vitamins B_2, and B_6 reduction in circulation of these infected patients should be mainly ascribed to viral infection. Especially, HCV infection caused more severe impairment upon host's B vitamins status. The greater correlation coefficients between HCV RNA load vs vitamins B_2 and B_6 also indicated that HCV played a crucial role in decreasing these two vitamins in circulation. In addition, we found that RBC vitamins B_2 and B_6 levels in HCV patients were negatively correlated with inflammation score in HCV patients. Gori et al. [27] reported that patients with low concentration of vitamin B_6 and high concentration of homocysteine had greater inflammatory injury and a high risk of developing cardiovascular diseases. The study of Ullegaddi et al. [28] found that B vitamins supplementation provided both antioxidant and antiinflammatory effects for patients with stroke disease. Thus, the observed lower levels of vitamins B_2 and B_6 in HCV patients might be due to host use these vitamins to attenuate hepatic inflammatory stress.

It is known that vitamin B_2 affects epithelial integrity and rate of prostaglandin biosynthesis [29], and vitamin B_6 is a cofactor for many enzymes involved in metabolism [30]. The insufficiency of these vitamins in circulation of HBV- or HCV-infected patients might further impact many physiological functions and induce other complications. Hence, it is important for these patients, especially HCV patients, to obtain sufficient dietary B vitamins intake in order to maintain normal B vitamins associated physiological functions. B vitamins supplement based on their water-soluble property should be safe and could benefit infected patients to counteract the impairment caused by viral infection and delay the development of advanced liver diseases such as cirrhosis and cancer. However, it remains unknown that vitamin B_6 and other B vitamins could facilitate the replication of HBV or HCV or not. Therefore, the extra supplementation of B vitamins for these patients should be carefully considered.

It has been documented that HBV or HCV infection induced oxidative stress in host [31, 32]. The increase in MDA, 8-isoprostane, and GSSG, and decrease in GSH and vitamin C in circulation as we observed agreed that HBV or HCV infection markedly enhanced oxidative injury in these patients. Particularly, the circulating 8-isoprostane was a more sensitive indicator for HCV-induced oxidative stress. Furthermore, we found that blood GPX, SOD, and catalase activities in these infected patients were markedly reduced, which revealed that HBV or HCV diminished antioxidative defense in these infected patients. On the other hand, we notified that HBV patients had lower circulating GSH level and GPX activity; however, HCV patients had lower SOD and catalase activities. These findings suggested that HBV and HCV infection caused different oxidative features. Although HBV and HCV patients involved in our present study already had sufficient dietary intake of Se and vitamin C, these patients still suffered from severe oxidative injury. Thus, the supplement of nutrients with antioxidant property or foods rich in antioxidant compounds may benefit these patients to alleviate oxidative stress caused by viral infection.

In conclusion, this study provided novel clinical findings regarding B vitamins status and oxidative features in HBV- and HCV-infected patients. Both HBV and HCV infection enhanced oxidative injury. HBV infection promoted lipid metabolism disorder, and HCV infection accelerated B vitamins depletion. In order to avoid other healthy risk, nutrition status should be monitored and limitation or supplementation of certain nutrients might be helpful for HBV- or HCV-infected patients.

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Conflict of interest None of the authors reports a conflict of interest.

References

1. Tan A, Yeh SH, Liu CJ, Cheung C, Chen PJ (2008) Viral hepatocarcinogenesis: from infection to cancer. Liver Int 28: 175–188
2. Fung J, Lai CL, Yuen MF (2009) Hepatitis B and C virus-related carcinogenesis. Clin Microbiol Infect 15:964–970
3. Lim YS, Kim WR (2008) The global impact of hepatic fibrosis and end-stage liver disease. Clin Liver Dis 12:733–746
4. Wu JT, Kral JG (2004) Ghrelin: integrative neuroendocrine peptide in health and disease. Ann Surg 239:464–474
5. Lin CC, Yin MC (2007) B vitamins deficiency and decreased anti-oxidant state in patients with liver cancer. Eur J Nutr 46:293–299
6. Nair J, Srivatanakul P, Haas C, Jedpiyawongse A, Khuhaprema T, Seitz HK, Bartsch H (2010) High urinary excretion of lipid peroxidation-derived DNA damage in patients with cancer-prone liver diseases. Mutat Res 683:23–28
7. Swietek K, Juszczyk J (1997) Reduced glutathione concentration in erythrocytes of patients with acute and chronic viral hepatitis. J Viral Hepat 4:139–141
8. Chrobot AM, Szaflarska-Szczepanik A, Drewa G (2000) Antioxidant defense in children with chronic viral hepatitis B and C. Med Sci Monit 6:713–718
9. Fujita N, Sugimoto R, Ma N, Tanaka H, Iwasa M, Kobayashi Y, Kawanishi S, Watanabe S, Kaito M, Takei Y (2008) Comparison of hepatic oxidative DNA damage in patients with chronic hepatitis B and C. J Viral Hepat 15:498–507
10. Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL,

HALT-C Trial Investigators (2008) Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med 359:2429–2441
11. Department of Health (1998) Taiwan nutrient databases 1998. Department of Health, Executive Yuan, Taipei
12. Talwar D, Ha T, Cooney J, Cooney J, Brownlee C, O'Reilly DS (1998) A routine method for the simultaneous measurement of retinol, α-tocopherol and five carotenoids in human plasma by reverse phase HPLC. Clin Chim Acta 270:85–100
13. Karlsen A, Blomhoff R, Gundersen TE (2005) High-throughput analysis of vitamin C in human plasma with the use of HPLC with monolithic column and UV-detection. J Chromatogr B Analyt Technol Biomed Life Sci 824:132–138
14. Prajda N, Weber G (1975) Malignant transformation-linked imbalance: decreased XO activity in hepatomas. FEBS Lett 59: 245–249
15. Talwar D, Davidson H, Cooney J, St JO'Reilly D (2000) Vitamin B (1) status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: comparison with erythrocyte transketolase activation assay. Clin Chem 46: 704–710
16. Speek AJ, van Schaik F, Schrijver J, Schreurs WH (1982) Determination of the B2 vitamer flavin—adenine dinucleotide in whole blood by high-performance liquid chromatography with fluorometric detection. J Chromatogr 228:311–316
17. Talwar D, Quasim T, McMillan DC, Kinsella J, Williamson C, O'Reilly DS (2003) Optimisation and validation of a sensitive high-performance liquid chromatography assay for routine measurement of pyridoxal 5-phosphate in human plasma and red cells using pre-column semicarbazide derivatisation. J Chromatogr B Analyt Technol Biomed 792:333–343
18. Hong YC, Lee KH, Yi CH, Ha EH, Christiani DC (2002) Genetic susceptibility of term pregnant women to oxidative damage. Toxicol Lett 129:255–262
19. Konishi M, Iwasa M, Araki J, Kobayashi Y, Katsuki A, Sumida Y, Nakagawa N, Kojima Y, Watanabe S, Adachi Y, Kaito M (2006) Increased lipid peroxidation in patients with non-alcoholic fatty liver disease and chronic hepatitis C as measured by the plasma level of 8-isoprostane. J Gastroenterol Hepatol 21:1821–1825
20. Pellicano R, Puglisi G, Ciancio A, Balzola F, Saracco G, Ciccone G, Baldi I, Abate ML, Smedile A, Rizzetto M (2008) Is serum uric acid a predictive factor of response to IFN-treatment in patients with chronic hepatitis C infection? J Med Virol 80: 628–631
21. Moriya K, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Yotsuyanagi H, Iino S, Kimura S, Koike K (2003) Serum lipid profile of patients with genotype 1b hepatitis C viral infection in Japan. Hepatol Res 25:371–376
22. Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, Kim HH, Yang US, Yu DY, Cheong J (2007) Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma. Gastroenterology 132:1955–1967
23. Russell AP, Schrauwen P, Somm E, Gastaldi G, Hesselink MK, Schaart G, Kornips E, Lo SK, Bufano D, Giacobino JP, Muzzin P, Ceccon M, Angelini C, Vergani L (2003) Decreased fatty acid beta-oxidation in riboflavin-responsive, multiple acylcoenzyme A dehydrogenase-deficient patients is associated with an increase in uncoupling protein-3. J Clin Endocrinol Metab 88:5921–5926
24. Morris AA, Turnbull DM (1998) Fatty acid oxidation defects in muscle. Curr Opin Neurol 11:485–490
25. Hosoda H, Kojima M, Kangawa K (2006) Biological, physiological, and pharmacological aspects of ghrelin. J Pharmacol Sci 100:398–410
26. Legakis I, Stathopoulos J, Matzouridis T, Stathopoulos GP (2009) Decreased plasma ghrelin levels in patients with advanced cancer and weight loss in comparison to healthy individuals. Anticancer Res 29:3949–3952
27. Gori AM, Sofi F, Marcucci R, Giusti B, Franco Gensini G, Abbate R (2007) Association between homocysteine, vitamin B(6) concentrations and inflammation. Clin Chem Lab Med 45:1728–1736
28. Ullegaddi R, Powers HJ, Gariballa SE (2004) B-group vitamin supplementation mitigates oxidative damage after acute ischaemic stroke. Clin Sci (Lond) 107:477–484
29. Rivlin RS (1986) Riboflavin. Adv Exp Med Biol 206:349–355
30. McGowan C (1989) Influence of vitamin B6 status on aspects of lead poisoning in rats. Toxicol Lett 47:87–93
31. Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O (2005) Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. BMC Infect Dis 5:95–102
32. Choi J, Ou JH (2006) Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. Am J Physiol Gastrointest Liver Physiol 290:847–851