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SHORT COMMUNICATION

Viral load is a negative predictor of antioxidant levels in hepatitis C patients

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Abstract

The pathogenesis of chronic hepatitis C (HCV) infection is not fully known, but oxidative stress may play a role. The aim of this study was to assess the relationship between HCV load and antioxidant status among patients with chronic HCV infection. Among 23 patients, HCV load, as well as plasma β-carotene, retinol, ascorbic acid and α-tocopherol were measured. Plasma retinol, ascorbic acid and α-tocopherol were low in 17%, 26% and 4% of the patients, respectively. Plasma ascorbic acid and α-tocopherol declined 9.7 μmol/l (95% CI 3.3–16.2) and 4.5 μmol/l (95% CI 2.1–7.0), respectively, and plasma β-carotene declined by a factor of 0.60 (95% CI 0.37–0.98) per log increase in viral load. Smoking was independently associated with 8.9 μmol/l (95% CI 4.1–13.7) lower levels of plasma α-tocopherol and with 0.27 (95% CI 0.11–0.71) times lower plasma β-carotene. The effect on plasma ascorbic acid was not significant (−9.2 μmol/l, 95% CI −21.9–3.5). The association may reflect consumption of antioxidants due to HCV, although effects of low antioxidant status on viral replication cannot be excluded.

Introduction

New antiviral therapy has considerably improved the cure rate of chronic hepatitis C (HCV) infection, but may be contraindicated or unaffordable to some patients, while others do not respond or relapse. In chronic viral infections, production of reactive oxygen species (ROS) may increase antioxidant requirements. If the intake is inadequate, oxidative stress develops which may increase viral replication and mutation rate [1–3]. ROS and impaired antioxidant status may also play a role in the pathogenesis of HCV infection [4–8].

We aimed to assess the role of viral load as a predictor of antioxidant status in chronic HCV patients.

Materials and methods

Chronic HCV-infected adults with elevated plasma alanine aminotransferase (ALT) within 6 months were included. Exclusion criteria were: other liver disease or serious diseases, antiviral treatment or antioxidant supplementation (>1 RDA) within 3 months and active drug or alcohol abuse. Fasting blood samples were taken: serum concentrations of ALT, cholesterol and ferritin, and plasma concentrations of α_1-antichymotrypsin (ACT), retinol, β-carotene, ascorbic acid, α-tocopherol and HCV-RNA were measured [9–11].

Data on β-carotene and ferritin were $\log_{10}$-transformed. The Mann-Whitney *U*-test and χ^2 test were used to test for differences in medians and proportions, respectively. Multiple linear regression analyses were used to assess the relationship between HCV load and antioxidant levels, while adjusting for possible confounding by smoking, age and gender. The number of explanatory variables allowed was square root of n, and $p < 0.05$ was significant.

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The study was registered at the Danish Data Protection Agency, conducted in accordance with the Declaration of Helsinki and approved by Danish medical ethics committees and the Danish Medicines Agency. Written informed consent was obtained from the participants.

Results

Of 23 patients, 8 (35%) had previously been on antiviral treatment, 2 (9%) had cirrhosis, and 15 (65%) were current smokers (Table I). The HCV load ranged from 6 to 10 $\log_{10}$ eqv/l. Median plasma ALT was 81 U/l (range 23–348). None had elevated plasma ACT. Plasma retinol, ascorbic acid and α-tocopherol were low in 17%, 26% and 4% of patients, respectively.

HCV load was a negative predictor of plasma β-carotene, ascorbic acid and α-tocopherol, while smoking was a negative predictor of plasma β-carotene and α-tocopherol (Table II). With plasma β-carotene $\log_{10}$-transformed, the regression coefficient of HCV load ($B = -0.22$) corresponds to a decline in plasma β-carotene level by a factor $10^{-0.22}$ or 0.60 (95% CI 0.37–0.98) per log increase in viral load. Plasma ascorbic acid and α-tocopherol declined 9.7 μmol/l (95% CI 3.3–16.2) and 4.5 μmol/l (95% CI 2.1–7.0), respectively, per log increase in viral load. Viral load was not a significant predictor of α-tocopherol if expressed as plasma α-tocopherol:serum cholesterol ratio, since serum cholosterol also declined with increasing viral load. Neither plasma retinol nor serum ferritin were predicted by viral load (data not shown).

Among smokers, plasma β-carotene was 0.27 (95% CI 0.11–0.71) times that of non-smokers, and smoking was associated with 8.9 μmol/l (95% CI 4.1–13.7) lower plasma α-tocopherol and with 1.2 μmol/mmol (95% CI 0.4–2.0) lower plasma α-tocopherol:serum cholesterol ratio compared to non-smokers. In addition, smoking was associated with 9.2 μmol/l (95% CI −3.5–21.9) lower plasma ascorbic acid, although not significantly. Smoking had neither influence on plasma retinol nor serum ferritin (data not shown). Age and gender had negligible effects on the regression coefficients of HCV load and smoking, and were therefore not included in the final models.

Table I. Characteristics of 23 patients with chronic hepatitis C.[a]

Background characteristics	
Female gender	12 (52%)
Age (y)	45 (23–55)
Smokers	15 (65%)
Disease characteristics	
Previous antiviral treatment	8 (35%)
Plasma α_1-antichymotrypsine (g/l)	0.26 (0.16–0.32)
Serum cholesterol (mmol/l)	4.8 (3.0–7.0)
Plasma ALT (U/l)	81 (23–348)
Plasma HCV-RNA ($\log_{10}$ eqv/l)	8.83 (6.30–10.0)
Genotype 1	17 (74%)
Cirrhosis[b]	2 (9%)
Markers of micronutrient status	
Plasma β-carotene (μmol/l)	0.18 (0.00–2.18)
Plasma retinol (μmol/l)	1.31 (0.43–2.76)
<1.05 μmol/l	4 (17%)
Plasma ascorbic acid, total (μmol/l)	37.8 (11.8–62.5)
<28 μmol/l	6 (26%)
Plasma α-tocopherol (μmol/l)	25.8 (10.5–36.3)
<12 μmol/l	1 (4%)
Plasma α-tocopherol:s-cholesterol (μmol:mmol)	5.3 (2.9–7.3)
<2.5 μmol:mmol	0 (0%)
Serum ferritin (μg/l)	111 (12-699)
<12 μg/l	0 (0%)

[a] Values are given as median (range) or number (percentage).
[b] A liver biopsy was previously performed in 22 patients.

Discussion

Although low antioxidant status was common, the study participants may have had better nutritional status than HCV patients in general. This is likely, as socially unstable patients were excluded. Moreover, only 2 had cirrhosis, with no history or actual signs of decompensation. Finally, some of the participants had improved their diet in response to the HCV diagnosis.

Comparison to healthy controls was considered inherently invalid, since any difference would probably reflect differences in diet and other life style factors, as HCV infection does not occur at random. The finding of an inverse association between HCV load and plasma antioxidant level has greater internal validity. We cannot make inferences about the direction of a possible cause-effect relationship, due to the cross-sectional design. While a low antioxidant status may increase viral replication, as has been shown in HIV infection [12], we consider this less likely, since antioxidant levels were not severely impaired among the study participants. It seems more likely that increasing viral load, like smoking, may have led to increased antioxidant consumption.

Only a few studies of micronutrient status among chronic HCV patients have been published. In a study of 24 HCV patients, significantly lower serum α-tocopherol was found compared to controls [13]. Others found that serum α-tocopherol, but also retinol and ascorbic acid were lower among 42 HCV patients compared to controls, with even lower values of serum α-tocopherol and retinol in cirrhotics compared to non-cirrhotics [7]. Finally, serum α-tocopherol and β-carotene were significantly lower

Table II. Predictors of plasma levels of antioxidant vitamins among 23 patients with chronic HCV infection.[a]

	B (95% CI)	10^B (95% CI)	*p*-value
P-β-carotene (μmol/l)[b]			
Smoking		0.27 (0.11; 0.71)	0.01
HCV load (log_{10}(eqv/l))		0.60 (0.37; 0.98)	0.04
P-ascorbic acid (total) (μmol/l)			
Smoking	−9.2 (−21.9; 3.5)		0.15
HCV load (log_{10}(eqv/l))	−9.7 (−16.2; −3.3)		0.005
P-α-tocopherol (μmol/l)			
Smoking	−8.9 (−13.7; −4.1)		0.001
HCV load (log_{10}(eqv/l))	−4.5 (−7.0; −2.1)		0.001
P-α-tocopherol:s-cholesterol (μmol:mmol)			
Smoking	−1.2 (−2,0; −0.4)		0.005
HCV load (log_{10}(eqv/l))	−0.4 (−0.8; 0.1)		0.09

[a] HCV load (log_{10}(eqv/l)) and smoking (coded 1 for yes and 0 for no) were forced into all models.
[b] β-carotene was log_{10}-transformed, and the regression coefficient anti-logged.

in 20 HCV patients compared to controls despite a similar dietary intake [8]. While these studies suggest that chronic HCV patients have impaired micronutrient status, this cannot be attributed to HCV per se, since nutritional and other environmental factors, including tobacco and alcohol, may have differed between HCV patients and healthy controls. Furthermore, none of the studies controlled for the acute phase response, known to invalidate most markers of micronutrient status. Thus, acute phase responses among the HCV infected patients would lead to overestimation of the prevalence of low micronutrient status and the magnitude of the inverse association between HCV and micronutrient status.

HCV load is believed not to influence the clinical course of HCV infection, although low levels predict treatment response [14]. However, our data demonstrate that viral load was an independent negative predictor of levels of the antioxidants β-carotene, ascorbic acid and α-tocopherol, but not retinol and ferritin. The associations were neither due to confounding by age, gender, acute phase response nor smoking.

The finding that current smoking was an independent negative predictor of plasma antioxidants, could be due to oxidative stress induced by smoking per se, or through increased necro-inflammatory activity in the liver, as previously seen among HCV patients [15].

HCV infection, as well as smoking, may independently increase antioxidant requirements. If the requirements are not met, oxidative stress may develop and contribute to disease progression. Randomized trials are needed to assess the effect of antioxidant supplementation in patients with chronic HCV infection.

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