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Coinfection with hepatitis C virus (HCV), oxidative stress and antioxidant status in HIV-positive drug users in Miami,[1,2]

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Abstract

Background—The pathogenesis of HIV/HCV-coinfection is poorly understood. We examined markers of oxidative stress, plasma antioxidants and liver disease in HIV/HCV-coinfected and HIV-monoinfected adults.

Methods—Demographics, medical history, and proof of HIV, hepatitis A, B and C were obtained. HIV-viral load, CD4-count, CBC, chemistries, plasma zinc, selenium, vitamins A and E were determined. Malondialdehyde (MDA) and glutathione-peroxidase were obtained as measures of oxidative stress. APRI and FIB-4 markers were calculated.

Results—Significant differences were found between HIV/HCV-coinfected and HIV-monoinfected participants in levels of ALT (51.4±50.6 vs. 31.9±43.1U/L, p=0.014), AST (56.2±40.9 vs. 34.4±30.2U/L; p<0.001), APRI (0.52±0.37 vs. 0.255±0.145, p=0.0001), FIB-4 (1.64±.0.91 vs. 1.03±0.11, p=0.0015) and plasma albumin (3.74±0.65 vs. 3.94±0.52g/dL, p=0.038). There were no significant differences in CD4-count, HIV-viral load or ART between groups. Mean MDA was significantly higher (1.897±0.835 vs. 1.344±0.223nmol/mL, p=0.006), and plasma antioxidants were lower, (vitamin A [39.5±14.1 vs. 52.4±16.2μg/dL, p=0.0004], vitamin E [8.29±2.1 vs. 9.89±4.5μg/mL, p=0.043] and zinc [0.61±0.14 vs. 0.67±0.15mg/L, p=0.016]) in the HIV/HCV-coinfected compared to the HIV-monoinfected participants, which remained significant after adjusting for age, gender, CD4-count, HIV-viral load, injection drug-use and race. There were no significant differences in glutathione-peroxidase, selenium, BMI, and alcohol, and tobacco between groups. Glutathione-peroxidase significantly increased as liver disease advanced, as measured by APRI (β= 0.00118, p=0.0082) and FIB-4 (β=0.0029, p=0.0177). Vitamin A significantly decreased (β=−0.00581, p=0.0417) as APRI increased.

Conclusion—HIV/HCV-coinfection is associated with increased oxidative stress and decreased plasma antioxidants when compared to HIV-monoinfection. Research is needed to determine whether antioxidant supplementation delays liver disease in HIV/HCV-coinfection.

Keywords

HCV; HIV; antioxidants; oxidative stress

[1]Appropriate informed consent was obtained and clinical research was conducted in accordance with guidelines for human experimentation as specified by the U.S. Department of Health and Human Services and/or authors' institutions.

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INTRODUCTION

About one quarter to half of the persons infected with the human immunodeficiency virus (HIV) in the United States are also infected with hepatitis C (HCV) [1]. As antiretroviral treatment (ART) has dramatically reduced HIV-1 related mortality for other causes, HIV/HCV-coinfection is becoming the main cause of death among these patients [2]. Increased mortality related to liver conditions as well as a compromised response to HIV therapy among HIV/HCV-coinfected persons have been identified as contributors to this trend [1]. The most important sequelae of chronic HCV-infection are progressive liver fibrosis leading to cirrhosis, end-stage liver disease, and hepatocarcinoma [3]. The factors that promote liver disease progression include older age at time of infection, male gender, immunosuppressed state such as that associated with HIV infection, concurrent hepatitis B, alcohol use, iron overload, hepatotoxic medications [4], obesity [5], and oxidative stress [6].

The pathogenesis of HCV and the subsequent liver injury is poorly understood. The damage results from combination of the immune response and direct effects of the HCV on the hepatocytes, including chronic inflammation, and stellate cell activation ensuing in formation of abnormal extracellular matrix [4]. The expression of HCV in hepatocytes also causes inhibition of electron transport, production of reactive oxygen species, and decreased mitochondrial glutathione [7]. The resulting elevated oxidative stress in conjunction with decreased antioxidant defenses are thought to be responsible for events on cellular and tissue levels that lead to the progression of liver fibrosis [8].

Elevated levels of malondialdehyde (MDA), a product of lipid peroxidation used as a marker of oxidative stress, have been found both in liver and in blood of patients who are monoinfected with HCV [8–10], or with HIV [11]. In addition, MDA levels decreased while antioxidant enzymes increased after treatment with pegylated-interferon alfa-2b plus ribavirin combination therapy associated with reduction of HCV viral load and reduction of inflammation [12,13]. The antioxidant micronutrients are also severely depleted both in plasma and liver biopsy specimens of patients with chronic HCV infection [14]. Both the oxidative stress and the decreased levels of hepatic micronutrient antioxidants correlate with increased liver fibrosis, and are thought to promote the chronicity of the HCV infection [14]. Conversely, administration of antioxidants reduces oxidative stress and toxicity induced by HIV and HCV *in-vitro* [15]. Thus, oxidative injury appears to occur as a direct result of HCV infection of hepatocytes. In addition, the number of mitochondrial DNA copies is reduced in HIV/HCV-coinfection compared to either HIV or HCV monoinfection, reflecting the consequences of oxidative stress [16]. Disease progression is attributable, at least in part, to cumulative oxidative stress and antioxidant depletion [17] and provides the basis for one of the mechanisms for hepatic disease progression.

Infection with HIV is also characterized by increased oxidative stress [11,18–20], and depletion of antioxidant nutrients, including vitamins A and E, zinc, and selenium [17,21,22]. Both HIV [11] and HCV-monoinfections have been recognized as conditions that elevate oxidative stress, which in turn contributes to liver fibrosis [9,10,13]. However, the information on measures of oxidative stress and antioxidant status in HIV/HCV coinfection is sparse. The objective of our study was to determine oxidative stress and antioxidant status in a cohort of HIV/HCV-coinfected and HIV-monoinfected drug users in Miami in order to provide basis for potential future adjuvant therapies for patients with HIV/HCV-coinfection.

MATERIALS AND METHODS

1. Population

From March 2002 to February 2006, 212 HIV-infected drug users were recruited for this study in Miami. Participants needed to be older than 18 years of age, confirmed with HIV seropositivity, and active drug users (determined by urine toxicology). This study was approved by the Florida International University Institutional Review Board. All participants gave written informed consent.

2. Study Design and Methods

After being screened for eligibility, participants underwent an assessment interview that included demographic, medical, nutritional, and recreational drug related questionnaires. Physical examination was completed and anthropometries were measured. After overnight fasting, blood samples were obtained to confirm HIV and HCV and HBV status, and determine CD4+ cell count, HIV viral load, complete blood cell count and blood chemistry, including the plasma concentration of antioxidant nutrients (vitamins A and E, zinc, and selenium), and markers of oxidative stress (plasma malondialdehyde [MDA], and a major antioxidant enzyme, glutathione peroxidase). Lymphocyte phenotype was determined with four-color immunophenotyping panel of monoclonal antibodies. Differential counts were determined using a Coulter MaxM hematology instrument and corroborated with cytocentrifuge smears. HIV viral load was obtained by the reverse transcriptase polymerase chain reaction using the Roche Amplicor reagents and protocol. History of HIV-infection and hepatitis A, B or C was obtained from the interview and confirmed serologically and by medical charts. Serological proof of coinfection with HCV and HIV was obtained using Procleix HIV-1/HCV nucleic acid testing kit (Gen-Probe, CA, US) [23].

Plasma malondialdehyde (MDA) was tested as the marker of oxidative stress using TBAR kit (ZeptoMetrix, NY, US). In this test, Thiobarbituric acid was reacted with MDA, and the concentration of MDA in plasma determined by a fluorometry at an excitation wave length of 530 nm and emission of 550 nm. Plasma glutathione peroxidase (GPx) activity was determined using Total Glutathione Peroxidase assay kit (ZeptoMetrix, NY, US). Plasma levels of zinc (Zn) and selenium (Se) levels were determined by flame atomic absorption spectrophotometry. Plasma vitamin A and vitamin E levels were determined by high performance liquid chromatography (HPLC).

Weight and height were obtained in participants wearing light clothing and no shoes utilizing a standard scale calibrated prior to each measurement. The height was measured with the participant's heels touching the base of the vertical board of the stadiometer. The moveable headboard was brought to the most superior point on the head with sufficient pressure to compress the hair. Body Mass Index (BMI) was calculated using the standard formula that divides the weight in Kg by the square of the Height in meters (Kg/m^2).

To estimate liver disease stage, we calculated the APRI and FIB-4 Indexes that include routine tests to predict liver fibrosis in patients with HIV/HCV coinfection [24]. The objectives were (1) to determine whether there was a significant difference in the proportion and degree of liver damage between the HIV/HCV-coinfected and the HIV-monoinfected groups and (2) whether there was a relationship between the stage of liver disease and oxidative stress and plasma antioxidants, regardless of the etiology of liver damage and HCV status. The APRI was calculated according to the formula: AST (× upper limit of normal range) × 100 / platelet count (10*9/L). Upper limit of normal for the present study was 0.45. The FIB-4 formula uses age and the relatively inexpensive test of transaminases (AST and ALT) and platelet counts (PLT): age ([yr] × AST [U/L]) / ((PLT [10(9)/L]) × (ALT [U/L])$^{(1/2)}$). At a cutoff of <1.45, the negative predictive value to exclude advanced

fibrosis (stage 4–6 of Ishak scale) was 90% with a sensitivity of 70%. A cutoff of >3.25 had a positive predictive value of 65% and a specificity of 97% to predict advanced disease [24].

Animal and human studies have associated obesity, type 2 diabetes and hypertriglyceridemia with increased oxidative stress and non-alcoholic liver disease [25,26]. For this reason, only the values for participants without diabetes, whose BMI was <28 kg/m^3, and plasma triglycerides <150 mg/dL were used in the final analysis. Descriptive statistics were expressed as mean±SD or as percent of population. Significance (α-value) was defined as $p<0.05$. The primary statistical objective was to compare the difference in population characteristics including oxidative stress and antioxidant status between the HIV/HCV-coinfected and the HIV-monoinfected groups. Independent samples t-test, Mann Whitney U test, adjusted linear regressions and MANOVA modeling were performed. SAS 9.0 and SPSS 14.0 were used for the analyses.

RESULTS

Of the 212 HIV+ adults who completed the assessment, 20 participants were excluded because of coinfection with hepatitis B (HBV). The comparison analyses included 192 participants, 57 HIV/HCV-coinfected and 135 HIV-monoinfected. As Table 1 shows, age is significantly different between the HIV/HCV-coinfected and the HIV-monoinfected participants, with the coinfected group being significantly older (45.2±6.5 years, $p=0.001$) than the HIV-monoinfected group (40.7±7.5 years). For this reason all subsequent analyses were controlled for age. In addition, there was a significant difference in race; the number of black participants in the HIV-monoinfected group was higher than that in the HIV/HCV-coinfected group (66.7% vs. 83.0%, $p=0.013$). All subsequent analyses were also controlled for race. While the mean BMI was not different between the two groups, the proportion of individuals whose BMI≥28 kg/m^2 was significantly higher among the HIV/HCV-coinfected participants than those who were HIV-monoinfected (16.6% vs 31.8%, $p=0.05$). As obesity may influence oxidative stress, we excluded participants with BMI≥28 kg/m^2 from the analyses.

As Table 2 shows, CD4+ cell counts and HIV viral loads were not significantly different between the HIV/HCV-coinfected and the HIV-monoinfected participants (413.9±276 CD4+ cells/μL vs. 335±256 CD4 cells/μL, respectively, $p<0.063$, and viral load 9.8± 2.6 $\log_{10}$RNA copies/mL vs. 9.4±2.4 $\log_{10}$RNA copies/mL). There were statistically significant differences, however, between the HIV/HCV-coinfected and HIV-monoinfected participants in their levels of ALT (51.4±50.6 U/L vs. 31.9±43.1 U/L, respectively, $p=0.014$), AST (56.2±40.9 U/L vs. 31.9±43.177 U/L, respectively; $p< 0.001$), APRI (0.52±0.37 vs. 0.255±0.145, $p=0.0001$), FIB-4 (1.64±.0.91 vs. 1.03±0.11, $p=0.0015$),and WBC (4.85±1.5 U/L vs. 4.22±1.6 IU/L respectively; $p=0.01$). The proportion of those identified by FIB-4 with liver disease (FIB-4 >1.45), was significantly higher in the HIV/HCV coinfected group (41% vs. 10.8% HIV monoinfected, $p=0.0023$). Only one (2.7%) participant in the HIV-monoinfected and three (7.7%) in the HIV/HCV-coinfected group had advanced liver disease (FIB-4 >3.25). Plasma albumin was significantly lower among the HIV/HCV-coinfected patients (3.74±0.65 g/dL, $p<0.038$) than those that were HIV-monoinfected (3.94±0.52 g/dL). The rate of BMI≥28 kg/m^2 was significantly higher in the HIV monoinfected than in the HIV/HCV coinfected group (21% vs. 4.48%, $p=0.05$). All statistical differences between the groups remained significant after controlling for age, gender, CD4+ count, viral load, injecting illicit drugs and race, using multivariate analysis of variance. There were no significant differences in the use of ART, BMI, hemoglobin, hematocrit, and bilirubin between these two groups.

Table 1 shows the proportion of alcohol, cigarettes, and illicit drugs, including injected drugs, used by the two groups. Alcohol was habitually consumed by 54.7% of participants, however, there were no significant differences between the two groups in the proportion of participants who used alcohol, whether obtained as a "yes" and "no" question (57.9% in the coinfected vs. 54.7% in the HIV-monoinfected group, p=0.562), or alcohol used more than 2 drinks daily (17.5% among co-infected vs. 12.6% among HIV mono-infected, p=0.367). Cigarette smoking was reported by 83.3% of the participants, with frequent cigarette smoking (>1 package daily) reported by 70.2%; there was also no difference between the HIV/HCV-coinfected and the HIV-monoinfected groups in the proportion of participants smoking cigarettes. There were no significant differences in use of illicit drugs between the two groups with the exception of injected drugs. There was a small number of injecting drug users (N=4), and all of them were in the HIV/HCV-coinfected group (p=0.045). We adjusted for this variable in the regression models.

A random sub-sample of the two groups was selected, one including 40 HIV/HCV-coinfected and the other 38 HIV-monoinfected participants for more detailed studies. Oxidative stress was represented by the plasma level of MDA. MDA levels were significantly elevated in those with triglycerides ≥150 mg/dL (β=0.47, p=0.0029) compared to those with normal triglycerides levels, and showed a strong trend, although not significant, in those who were obese (BMI≥28 kg/m^2, β=0.28, p=0.07) compared to those with BMI<28 kg/m^2. As shown in Table 3, the mean MDA in both the HIV/HCV-coinfected and the HIV-monoinfected groups were higher than the normal reference value of <1.3 nmol/mL. MDA was significantly higher in HIV/HCV-coinfected participants (1.897±0.835 nmol/mL, p=0.006) compared to those who were HIV-monoinfected (1.344±0.223 nmol/mL). The HIV/HCV-coinfected group had also significantly lower levels of plasma antioxidants, including vitamin A (39.5±14.1μg/dL vs. 52.4±16.2μg/dL, p=0.0004), vitamin E (8.29±2.1μg/mL vs. 9.89±4.5μg/mL, p=0.043) and plasma zinc (0.61±0.14 mg/L vs. 0.67±0.15 mg/L, p=0.016) than the HIV-monoinfected group. The differences between the two groups remained significant after adjusting for age, gender, race, CD4+ cell count, injecting drugs of abuse and viral load at p<0.029 for MDA, p<0.003 for vitamin A, p<0.012 for zinc, and p<0.05 for vitamin E (Table 4). There were no differences, however, in glutathione-peroxidase levels (779±79 IU/L vs. 788±94 IU/L, p=0.710); plasma selenium levels for all participants were adequate (selenium >0.085 mg/dL) with no significant differences (0.12±0.02 mg/dL vs. 0.12±0.01 mg/dL, p=0.901) between the two groups.

Glutathione-peroxidase, an enzymatic antioxidant, was significantly increased in liver disease, as measured by APRI (β= 0.00118, p=0.0082) and FIB-4 (β=0.0029, p=0.0177), or FIB-4 >1.45 (β=0.00178, p=0.0287), regardless of HCV status. Vitamin A significantly decreased (β=−0.00581, p=0.0417) as APRI increased. As shown in Table 5, all antioxidants showed a tendency to be decreased as the indexes of the liver disease increased, and for those identified by FIB-4 (>1.45) with liver disease.

DISCUSSION

Both HIV and HCV-monoinfections have been recognized as conditions that elevate oxidative stress, which in turn contributes to liver fibrosis, and may be one of the mechanisms involved in the pathogenesis of HCV. There is limited information in the literature, however, on oxidative stress and antioxidant status in HIV/HCV-coinfection. Our study shows that the HIV/HCV-coinfected participants had evidence of liver damage as substantiated by significantly increased transaminases, significantly lower levels of plasma albumin, and elevated APRI and FIB-4 indexes. The HIV/HCV-coinfected participants had significantly higher levels of oxidative stress demonstrated by elevated plasma levels of MDA, a marker of oxidative stress, and significantly lower levels of plasma antioxidants

(vitamins A and E, zinc) than the HIV-monoinfected group. These relationships remained after adjusting for age, gender, CD4+ cell count, HIV RNA viral load, and race and were not related to ART. In addition, glutathione-peroxidase was significantly increased as the markers of liver disease, APRI and FIB-4, increased, and in those with FIB-4 >1.45.

HIV infection increases oxidative stress [11,27,28], which is also accompanied by decreased levels in plasma antioxidant micronutrients, including vitamins A, E, zinc and selenium [29,30]. It is also well documented that HCV produces oxidative stress that is more severe than that observed in other inflammatory liver diseases [10,31–33] and is accompanied by reduced hepatic and plasma levels of antioxidants [34]. Increased levels of oxidative stress were demonstrated in patients who were monoinfected with HCV [10,33,35,36]. Moreover, the oxidative stress in the form of increased MDA levels has been shown to correlate with severity of HCV [14,37,38]. We have shown that the plasma levels of MDA were significantly higher in HIV-monoinfected patients than the norm, and even higher in HIV/HCV-coinfection. In addition, glutathione-peroxidase was increased in those with liver disease as measured by APRI and FIB-4, in response to increased oxidative stress, a finding that is consistent with other studies that show elevation of glutathione-peroxidase in mild to moderate liver disease [39]. *In-vitro* and animal studies have demonstrated that oxidative stress generated in hepatocytes is one of the important factors that stimulates the hepatic stellate cell proliferation and accumulation of collagen, initiating and facilitating the fibrogenic process [40]. Thus, in addition to the immunosuppression and antioxidant deficiencies caused by HIV and HCV, the elevated oxidative stress observed in HIV/HCV-coinfection may contribute to a more rapid progression of liver fibrosis by stimulating HCV replication and increasing production of reactive oxygen species in hepatocytes [6,10,37,38].

Oxidative stress is a nonspecific pathogenic state of imbalance in the prooxidant-antioxidant balance produced by infected hepatocytes during traumatic and inflammatory lesions [8]. Oxidative stress, exacerbated by immunosuppression, concomitant exposure to viral infections, and depletion of antioxidants, causes hepatic cell damage [41]. Our results show that HIV/HCV-coinfection, which is a condition characterized by immunosuppression due to HIV infection and concomitant exposure to HCV, is also accompanied by significantly lower plasma levels of vitamin A, E, and zinc that are significantly lower than those found either in HIV- or HCV-monoinfections [42,43]. In addition, more advanced liver disease, as estimated by the APRI index, was significantly associated with lower Vitamin A, regardless of HCV status. Other antioxidants decreased with higher indexes of liver disease, but did not reach significance; potentially due to small sample sizes. Addictive drugs produce significant differences in markers of HIV disease progression [44], and altered nutritional indices [45], as shown in our earlier studies, manifesting multiple deficiencies of antioxidant micronutrients, including vitamins A, E, C, zinc and selenium [46]. While a relatively large percentage of the present study participants consumed alcohol, cigarettes and illicit drugs, the proportions of substance abuse did not differ between the groups, and thus were not likely to cause the differences in oxidative stress and plasma antioxidant micronutrient levels found between the HIV/HCV-coinfected and HIV-monoinfected groups.

Vitamins A, E and zinc are a part of the wide array of enzymatic and non-enzymatic antioxidant defenses that have been found in reduced amounts both in plasma [14,42,47] and in liver biopsies of patients with chronic HCV [14]. In our study, the HIV/HCV-coinfected group showed significantly greater depletion of both the vitamins A and E, and zinc, in conjunction with greater oxidative stress. Moreover, in the regression model, as the index of liver disease APRI increased, Vitamin A significantly decreased. These results are in line with findings of loss of vitamin A storage capacity in the liver due to the hepatic cells undergoing transformation in the process of liver fibrosis [42,48].

Vitamin E prevents lipid peroxidation and is the principal lipid-soluble antioxidant in mitochondria, microsomes, and lipoproteins [49]. Zinc levels in plasma and in liver of patients with HCV infection are lower than in healthy volunteers, potentially due to pronounced hyperzincuria in HCV [17]. A high prevalence of zinc deficiency that is associated with faster disease progression was also noted in HIV infection [37,38]. Moreover, zinc deficiency in both viral infections may account for the associated anorexia and a loss of taste and smell that further aggravate nutritional deficiencies [17]. The importance of zinc in HCV is also indicated by a study showing that zinc supplementation in combination with the standard therapy enhances the response to interferon therapy in patients with intractable chronic HCV [50].

Glutathione-peroxidase is part of the enzymatic antioxidant defenses; patients with mild to moderate liver damage, comparable to those in the present study, had increased glutathione-peroxidase levels in response to increased oxidative stress [39]. Although we did not observe a difference in glutathione-peroxidase levels between the HIV-monoinfected and HIV/HCV-coinfected groups, as the severity of liver disease increased, regardless of its etiology or HCV status, glutathione-peroxidase levels significantly increased (Table 5). This is consistent with the studies that show systemic increase in glutathione-peroxidase in response to increased oxidative stress [39,51].

While previous studies of antioxidant therapy have been inconclusive, several small clinical trials of antioxidant supplementation in conjunction with interferon-ribavirin therapy reported that antioxidants have been effective in reducing oxidative stress in a portion of HCV-monoinfected patients [52–54] and in decreasing HCV viral burden [55]. The administration of antioxidants appeared to be effective even in patients who have failed to respond to previous anti-HCV therapy [56]. While the use of antioxidants may not eliminate the virus, it may reduce hepatic inflammation and fibrosis and slow disease progression. Optimal therapy with a spectrum of antioxidants may slow progression of liver disease, while interferon alpha and ribavirin treatment eliminates HCV [42].

The findings of this study show that in the HIV/HCV-coinfection, MDA, a marker of oxidative stress, was significantly higher, and the plasma levels of antioxidants (vitamins A and E, zinc) were significantly lower, than in the HIV-monoinfected group. These relationships remained after adjusting for age, gender, CD4 cell count, viral load, injection drug use and race, and were not related to the use of ART. Moreover, glutathione-peroxidase increased in liver disease, as measured by APRI and FIB-4, compared to those without liver disease or in the early stages of liver disease, regardless of HIV status. This evidence suggests that there is an increased metabolic requirement for antioxidants in HIV/HCV-coinfection, particularly when the liver is compromised. Since the most effective therapy for HCV is currently successful only in a modest percentage of patients, particularly if they are HIV/HCV-coinfected [57], alternative treatments are needed. Although antioxidants are not likely to be the most important etiological determinants, they alter immune function, their deficiency facilitates HIV disease progression, modulates oxidative stress, and has a significant impact upon disease processes and related morbidity and mortality [42,58]. More research is needed on the optimal levels of antioxidant supplementation, and the potential role of non-nutritive antioxidants in controlling oxidative damage in the doubly compromised defense systems of HIV/HCV-coinfected persons. In addition, longitudinal studies with adequate sample size are needed to establish cause and effect, and to elucidate the complex relationships between increased oxidative stress, antioxidant defenses, immune failure and progression of liver fibrosis in HIV/HCV-coinfection.

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Table 1

Characteristics of the Population

Characteristics	Overall N=192	HIV/HCV N=57	HIV only N=135	p- value
Male	75.0%(144)	71.9% (41)	76.3% (103)	0.523
Age	42.0 ± 7.5	45.2 ± 6.5	40.7 ± 7.5	<0.001*
Monthly Income (dollars)	308.8 ± 386	275.6 ± 360	322.6 ± 397	0.450
Highest grade completed	11.2 ± 2.8	11.3 ± 2.9	11.1 ± 2.8	0.734
Black	78.1% (150)	66.7% (38)	83.0% (112)	0.013*
Receiving HAART	64.6% (124)	56.1% (32)	68.2% (92)	0.112
BMI kg/m^2	25.03±4.9	24.9 ± 4.7	25.1±5.1	0.779
BMI≥28 kg/m^2 in %	27% (52)	16.6% (9)	31.8%(43)	0.05*
Frequent alcohol use >2 drinks daily	14.1%(27)	17.5(10)	12.6(17)	0.367
Frequent cigarette use ≥ 1 pkg. daily	70.2%(134)	64.9(37)	72.4(97)	0.302
Frequent marijuana use > daily	4.3%(8)	0(0)	6.02(8)	0.107
Frequent Cocaine/Crack use > daily	12.5%(24)	14.04(8)	11.85(16)	0.676
Injecting Drug Use	2%(4)	7% (4)	0% (0)	0.045*

*Significant

Table 2

Mean differences in laboratory values and estimated liver disease indexes between HIV/HCV-coinfected and HIV-Monoinfected Groups

Laboratory Values	HIV/HCV N=57	HIV only N=135	p- values
Viral load (log)	9.0 ± 2.6	9.4±2.4	0.326
CD4 cells/mm^3	413.9 ± 276	335.9±256	0.063
AST, IU/L	56.2 ± 40.9	34.4±30.2	<0.001*
ALT, IU/L	51.4 ± 50.6	31.9±43.1	0.014*
VLDL mg/dL	26.8±14.4	22.1±16.4	0.060
Albumin g/dL	3.74±0.65	3.94±0.52	0.038*
Bilirubin mg/dL	0.52±0.41	0.48±0.35	0.600
APRI	0.52±0.37	0.255±0.145	0.0001*
FIB-4	1.64±.0.91	1.03±0.11	0.0015*
% FIB-4 > 1.45	41%	10.8%	0.023*
Hgb g/dL	14.1±4.2	13.5±1.7	0.337
Total cholesterol mg/dL	166±34.8	165±52.7	0.881
Trig mg/dL	134±72.7	147.2±145.0	0.418
HDL mg/dL	47.8±20.8	46.6±20.8	0.719
LDL mg/dL	91.4±25.6	85.7±46.0	0.285
WBC IU/L	4.85±1.5	4.22±1.6	0.010*
RBC $\times 10^{-6}$	4.35±0.59	4.36±0.58	0.907
HCT %	40.9±4.7	40.6±4.9	0.734
LDH, U/L	206.5±52.1	199.7±46.9	0.384

*Significant

Table 3

Differences in oxidative stress and plasma antioxidants between the HIV-monoinfected and HIV/HCV-coinfected groups.

Blood Values	HIV/HCV coinfected	HIV monoinfected	p-values
MDA (TBARS), nmol/mL[a]	1.897±0.835	1.344±0.223	0.006*
GPX, IU/L [a]	782.5±95.8	803.8±64.9	0.811
Zinc, mg/L	0.61 ± 0.14	0.67 ± 0.15	0.016*
Selenium, mg/dL	0.122 ± 0.052	0.121 ± 0.029	0.889
Vitamin A, μg/dL	39.5 ± 14.1	52.4 ± 16.2	0.0004*
Vitamin E, μg/mL	8.29 ± 2.1	9.89 ± 4.5	0.043*

[a] After excluding those with BMI>25.9 kg/m^2, diabetes and triglycerides >150 mg/dL

* Significant

Table 4

Estimated effect of HIV/HCV-coinfection on blood values after adjusting for gender, age, CD4 cell count, viral load, injecting drugs and race in Linear Regression Models.

Laboratory Value	Estimated effect (β)	95% CI	p-value
MDA (T-bars), nmol/mL	0.54	0.09, 0.99	0.026*
AST, IU/L	19.0	7.85, 30.2	0.001*
ALT, IU/L	16.7	1.86, 31.5	0.029*
Albumin, g//dL	−0.19	−0.35, −0.02	0.032*
WBC-p2, IU/L	0.47	0.01, 0.92	0.045*
Plasma vitamin A, μg/dL	−12.4	−20.4, −4.4	0.004*
Plasma zinc mg/L	−0.15	−0.094, −0.0015	0.043*
Plasma vitamin E, μg/mL	−1.45	−3.33, 0.43	0.136

* Significant

Table 5

Effect of liver disease progression as estimated by the APRI and FIB-4 indexes on micronutrient and enzymatic antioxidants.

Antioxidants	APRI	p-value	FIB-4	p-value-	FIB-4 > 1.45	p-value
Vitamin A	β=−0.00581	0.0417*	β= −0.012	0.074	β=−0.00875	0.076
Vitamin E	β=−0.012	0.16	β=−0.028	0.1761	β= −0.02	0.1804
Zinc	β=−0.416	0.29	β=−1.31	0.2313	β= −0.937	0.2
Selenium	β=−0.00043	0.86	β= −0.0016	0.8091	β=−0.0047	0.29
Glutathione-peroxidase	β= 0.00118	0.0082*	β=0.0029	0.0177*	β=0.00178	0.0287*

*Significant