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MAJOR ARTICLE

Testosterone in Men With Chronic Hepatitis C Infection and After Hepatitis C Viral Clearance

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Background. Hepatitis C virus (HCV) and hepatic dysfunction are associated with low total and free testosterone (TT and FT) and high sex hormone-binding globulin (SHBG). However, little is known about changes in testosterone following successful HCV treatment.

Methods. We evaluated testosterone levels and the prevalence of low testosterone in a cohort of 327 men with chronic HCV infection (human immunodeficiency virus [HIV] coinfection = 150) and in a subset of 85 men with testosterone levels obtained pre-HCV treatment and after sustained virologic response (SVR). Median follow-up was 36 months.

Results. Participants with active HCV at baseline had higher TT ($P < .0001$) and SHBG ($P < .0001$) compared with participants who had achieved SVR, whereas FT did not differ. Low TT (<10.4 nmol/L) was more prevalent in participants with SVR compared with active HCV ($P = .002$); however, low FT (<0.1735 nmol/L) was common (50% active HCV, 43% SVR) and did not different between groups. For participants with longitudinal determinations, TT and SHBG decreased significantly ($P < .0001$) while FT remained unchanged post-SVR. Low FT persisted after SVR (pre-treatment 58%, post-SVR 54%, $P = .72$). HIV status and change in aspartate aminotrasferase-to-platelet ratio were significant independent predictors of change in FT following SVR.

Conclusions. During active HCV infection, testosterone deficiency may be masked due to elevated SHBG. Despite improvements in SHBG following SVR, low FT was common and persisted after HCV clearance, indicating the need for enhanced awareness and screening using estimates of FT following successful treatment of chronic HCV.

Clinical Trials Registration. NCT01350648.

Keywords. testosterone; HCV; SVR; SHBG; hypogonadism.

Male hypogonadism, characterized by low testosterone, is seen in association with liver disease and human immunodeficiency virus (HIV) infection [1, 2]. Previous research has shown that low total testosterone (TT), low free testosterone (FT), and elevated sex hormone-binding globulin (SHBG) are extrahepatic manifestations of chronic hepatitis C virus (HCV) when compared with healthy controls [3–5]. In general, the existing literature has focused on single time point testosterone determinations of chronically infected patients. However, with the advent of potent directly acting antivirals (DAAs), the overwhelming majority of HCV-infected patients who are treated clear their HCV infection and achieve sustained virologic response (SVR) [6]. There is now a need for studies to examine the effect of HCV and HCV viral clearance on long-term testosterone levels and hypogonadal status. Because HIV is associated with hypogonadism [7], it may compound the observed low levels of testosterone in HCV/HIV-coinfected patients. To date, there have been limited observations on the effect of HIV coinfection on the serum testosterone levels of chronic HCV-infected patients. Therefore, in this study, we aimed to use prospective data from a large cohort of HCV-infected participants with and without HIV coinfection in order to characterize TT and FT status in this population and to address testosterone deficiencies, including biochemical hypogonadism, related to HCV. In addition, we examined longitudinal testosterone levels pre-treatment and after SVR to determine the influence of HCV viral clearance on testosterone.

METHODS

We evaluated testosterone levels in men with chronic HCV infection as part of a prospective, longitudinal cohort study of long-term health outcomes in adults with viral hepatitis. There were 327 participants identified with a history of chronic HCV infection (150 [46%] with HIV coinfection) with at least 1 determination of TT. A subset of 85 participants had testosterone determination completed pre- and post-HCV treatment and clearance and were evaluated longitudinally for changes after SVR. We excluded participants for a missing TT value (n = 4) or an active hormonal replacement

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therapy prescription (n = 3). HCV genotypes 1A and 1B (94%), 2 (3%), and 4 (3%) were included in the analysis. All participants provided written informed consent. In accordance with the guidelines from 1975 Declaration of Helsinki, the protocol was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board. Data were collected from September 2011 to June 2018.

Participants completed annual visits with nonfasting/random laboratory tests, demographic, and clinical data collected each year. For the cross-sectional analysis, only the first protocol visit (month 0) was used for each participant, regardless of SVR status at the time of the visit. For the longitudinal analysis on the subset of participants with pre-treatment and post-viral clearance testosterone values, the pre-treatment time point was defined as laboratory determination immediately prior to start of HCV therapy, and the post-treatment time point was defined as the last visit following SVR. SVR was defined as absence of HCV RNA ≥12 weeks after termination of treatment and confirmed at each subsequent visit. The threshold for low TT was <10.4 nmol/L and for low FT it was <0.1735 nmol/L [8]. Albumin, TT, and SHBG values were measured directly from the colorimetric assay, the competitive chemiluminescent enzyme immunoassay, and 2-site chemiluminescent immunometric assay, respectively (Roche Holding AG, Basel, Switzerland and Siemens Healthcare GmbH, Munich, Germany). FT was calculated using the Vermeulen formula at each time point [9, 10].

We examined TT, FT, and SHBG values as well as the prevalence of low TT and low FT for all participants at the month 0 visit and compared clinical characteristics based on whether the participant had active HCV or had achieved SVR at the time of month 0 (baseline). We calculated the change in TT, FT, and SHBG between pre-treatment and last available follow-up post-HCV clearance to evaluate the influence of SVR and various clinical characteristics (eg, HIV status) on the degree of change in testosterone parameters. We also determined the change in prevalence of low TT and low FT in the cohort following HCV viral clearance. Statistical comparisons between and within the groups were completed using 2-tailed Student *t* test, χ^2, Fisher exact test, paired *t* test, and McNemar test where appropriate. Characteristics found to be significantly associated with change in FT after SVR by univariate analyses were included in a multivariable regression to identify independent associations for change in FT. Sensitivity analyses that excluded participants with nonmorning testosterone determinations were performed; all statistical comparisons and analyses were replicated with this data subset. All analyses were completed using JMP software (version 13.0, SAS Institute Inc., Cary, NC).

RESULTS

Cross-sectional

Participants (n = 327) had a mean age of 57 ± 7 years at baseline. The majority of participants were black (83%). In general, participants were overweight, with mean body mass index (BMI) of 28 ± 6 kg/m^2, and had elevated transaminases at baseline. Fifteen percent of participants had diagnosed cirrhosis. The majority (74%) of participants had HCV for longer than 10 years based on self-reported risk behaviors and had a history of injection drug use (67%). Additionally, of the 313 participants with self-reported data on the past year's alcohol intake, 70% reported consuming alcohol never or only 1–2 times per year, 12% reported 1–3 times per month, 12% 1–5 times per week, and 6% daily.

A subset of the cohort had achieved HCV clearance (n = 138, 42%) at the time of the first visit. Participants who were treated for HCV and achieved SVR received a variety of regimens, including interferon and ribavirin (6%); interferon, ribavirin, and DAAs (18%); ribavirin and DAAs (14%); and DAAs only (62%). DAA regimens included a wide range of drugs, including asunaprevir, beclabuvir, daclatasvir, ledipasvir, sofosbuvir, and telaprevir. A subset of the cohort was coinfected with HIV (n = 150, 46%), in which 134 (89%) participants were on antiretroviral therapy and 118 (86%) had HIV viral loads below the limit of detection. Participants with HIV coinfection were more likely to have active HCV at the baseline visit (P = .0004), most likely due to the early exclusion of participants with HIV coinfection from the initial HCV DAA treatment trials.

Participants had a mean TT of 15.6 ± 9.8 nmol/L, mean FT of 0.20 ± 0.20 nmol/L, and mean SHBG of 76.6 ± 44.7 nmol/L at the baseline visit. When participants were compared by active HCV status, those with active HCV had significantly higher TT and SHBG compared with those who had achieved SVR (both P < .0001; Table 1). There was not, however, a significant difference in FT of participants according to SVR status. A significantly higher percentage of participants had low TT with SVR (35%) compared with those with active HCV (20%, P = .002). While the frequency of low FT was slightly lower in the group with SVR (43% vs 50% in active HCV), this difference was not significant (P = .21). In a subanalysis that excluded participants with nonmorning testosterone determinations, no differences were observed in the levels or frequency of low TT and FT and there was no change in statistically significant observations. There were no significant differences between the active HCV and the SVR groups in terms of age or BMI; however, the groups did differ significantly in terms of transaminase levels and racial identity. TT and FT values post-SVR did not differ (P > .2) between participants who were treated with interferon-containing regimens (n = 33; TT 13.7 ± 6.8 nmol/L, FT 0.18 ± 0.09 nmol/L) and those treated with interferon-free regimens (n = 105; TT 12.3 ± 4.4 nmol/L, FT 0.19 ± 0.07 nmol/L).

Table 1. Clinical Characteristics of the Cross-sectional Cohort at Baseline

Baseline Characteristic	Total (n = 327)	Active HCV (n = 189)	HCV Clearance (Sustained Virologic Response) (n = 138)	*P* Value
Age (years)	57.4 ± 6.8	57.3 ± 6.6	57.4 ± 7.1	.92
Race, n (%)				
Black	271 (83)	165 (88)	105 (77)	…
White	51 (15)	19 (10)	31 (23)	.004
Mixed/Other	5 (2)	4 (2)	1 (1)	…
HIV coinfection, n (%)	150 (46)	103 (68)	47 (34)	.0003
Aspartate aminotransferase (U/L)	45.7 ± 35.7	59.4 ± 40.2	26.9 ± 14.0	<.0001
Alanine aminotransferase (U/L)	48.6 ± 42.4	66.5 ± 44.9	23.7 ± 20.8	<.0001
Body mass index (kg/m^2)	28.1 ± 5.6	27.7 ± 5.3	28.8 ± 6.0	.07
TT (nmol/L)	15.6 ± 9.8	17.7 ± 11.7	12.6 ± 5.1	<.0001
FT (nmol/L)	0.20 ± 0.20	0.20 ± 0.25	0.19 ± 0.08	.63
Sex hormone-binding globulin (nmol/L)	76.6 ± 44.7	92.7 ± 48.6	54.4 ± 25.7	<.0001
Low TT, n (%)	85 (26)	37 (20)	48 (35)	.002
Low FT, n (%)	151 (47)	93 (50)	58 (43)	.21
HIV Infected (n = 150)	Total n = 150	Active HCV (n = 103)	HCV Clearance (n = 47)	*P* Value
Low TT HIV positive, n (%)	39 (26)	25 (24)	14 (30)	.48
Low FT HIV positive, n (%)	71 (48)	51 (50)	20 (44)	.46

All values are mean ± standard deviation, unless noted. Low TT defined as <10.4 nmol/L. Low FT defined as <0.1735 nmol/L.

Abbreviations: FT, free testosterone; HCV, hepatitis C virus; HIV, human immunodeficiency virus; TT, total testosterone.

In the subset of participants with HIV coinfection, frequencies of low TT and FT were similar to those in HCV-monoinfected participants. There were no significant differences between the frequency of low TT or low FT between the HIV-coinfected group with either active HCV or SVR (Table 1). Further, no differences in TT, FT, SHBG levels or prevalence of low TT and FT were observed among participants coinfected with HIV when comparing those with detectable HIV virus to those with HIV suppression (data not shown).

Longitudinal

In the subgroup of participants for whom data were collected longitudinally pre-treatment and post-SVR (n = 85), median duration of follow-up was 36 months (interquartile range, 24–50 months). There were significant differences in aminotransferase levels pre- and post-SVR (aspartate aminotransferase, $P < .0001$; alanine aminotransferase, $P < .0001$; Table 2). There was also a significant decrease in fibrosis as estimated by the aspartate aminotransferase-to-platelet ratio index (APRI) [11], with a mean score of 0.96 ± 1.15 before treatment and a score of 0.35 ± 0.40 after SVR ($P < .0001$). There was no significant change in BMI after SVR ($P = .28$).

Mean TT and SHBG decreased significantly after SVR, and this decrease was significant in both HIV-coinfected and HCV-monoinfected groups (all comparison, $P < .0001$). In contrast, while there was no change in FT after SVR in the study group overall ($P = .99$), among the HIV-infected group, FT increased significantly post-SVR (0.16 to 0.19 nmol/L, $P = .01$), whereas FT decreased among the HCV-monoinfected group (0.18 to 0.15 nmol/L, $P = .001$). These comparisons were replicated in the subset of

Table 2. Testosterone and Clinical Characteristics Pretreatment and Post–sustained Virologic Response

Characteristic	Pretreatment	Post–sustained Virologic Response	*P* Value
Aspartate aminotransferase (U/L)	59.3 ± 50.0	23.7 ± 11.5	<.0001
Alanine aminotransferase (U/L)	73.4 ± 59.3	21.8 ± 17.3	<.0001
Aspartate aminotransferase-to-platelet ratio index score	0.96 ± 1.15	0.35 ± 0.40	<.0001
Body mass index (kg/m^2)	27.9 ± 5.5	27.6 ± 5.0	.28
Total testosterone (nmol/L)	16.1 ± 7.1	11.2 ± 5.6	<.0001
Free testosterone (nmol/L)	0.17 ± 0.07	0.17 ± 0.08	.99
Sex hormone-binding globulin (nmol/L)	89.8 ± 42.8	50.1 ± 23.8	<.0001
Low total testosterone, n (%)	18 (21.2)	47 (55.3)	<.0001
Low free testosterone, n (%)	49 (57.6)	45 (53.6)	.72

Values reported are mean ± standard deviation, unless noted. *P* values represent paired *t* test for continuous variables and McNemar test for dichotomous variables for pretreatment and post–sustained virologic response group comparisons. n = 85.

participants with confirmed morning testosterone determinations, and there was no change in the findings, except in the change in FT in the HCV-monoinfected group (0.19 to 0.16 nmol/L, $P = .08$).

In a multivariate regression analysis that adjusted for age, BMI, duration of follow-up, and type of HCV therapy, HIV status ($P = .0002$) and change in APRI score ($P = .02$) were the only significant, independent predictors of change in FT. In order to account for the potential contribution of change in SHBG following SVR, change in SHBG was added to the regression model with similar results; HIV status ($P = .0003$) and change in APRI ($P = .04$) remained significant predictors. Neither alcohol intake nor level of pre-treatment HCV viremia was associated with change in FT. Only 8 of the 47 participants coinfected with HIV in this subset had detectable HIV viral loads, so no conclusive observations were made regarding the effect of HIV viremia on changes in testosterone.

The frequency of low TT increased from 21% pre-treatment to 55% post-SVR ($P < .0001$), whereas the frequency of biochemical hypogonadism based on low FT was relatively stable (58% pre-treatment to 54% post-SVR, $P = .72$). The frequency of low FT at last follow-up was not significantly different in those with HIV infection (48%) compared with those without HIV infection (61%, $P = .24$). However, frequency of low FT tended to decrease in the HIV group from pre-treatment to post-SVR (62% to 48%, $P = .24$) and tended to increase among those without HIV infection (53% to 61%, $P = .58$). These observations remained unchanged when analyzed in the subset of participants with verified morning testosterone determinations.

DISCUSSION

In the present study, we prospectively examined testosterone values among men with a history of chronic HCV infection in order to determine the effect of the virus and its clearance on testosterone and SHBG. Active HCV infection was characterized by higher TT and increased levels of SHBG compared with participants who achieved HCV viral clearance. In participants who had pre-treatment and post-SVR determinations, increases in FT following HCV clearance were associated with HIV status and improvements in fibrosis as measured by APRI score. However, biochemical hypogonadism defined by low FT remained common and persisted in nearly half of the cohort post-SVR regardless of HIV status.

The relationships among testosterone, liver function impairment, and chronic viral infection are complex. Active HCV infection and accompanying impaired liver function are recognized as risk factors for hypogonadism as well as altered semen production [3, 4, 12, 13]. Hypogonadism is also a recognized complication of end stage liver disease, which was observed to improve after orthotopic liver transplant [14]. SHBG is elevated in chronic liver disease and increases further with advanced disease progression [15–18]. The majority of testosterone circulates bound to SHBG, with a smaller proportion bound to albumin and only a small fraction circulating unbound ("free"). FT levels, either directly measured or indirectly estimated using TT and SHBG measurements, as was done in this study, are an accurate reflection of gonadal status. In contrast, TT levels are largely reflective of SHBG levels, with higher SHBG resulting in higher serum TT measurements regardless of "true" gonadal status. This relationship complicates the interpretation of TT levels in patients with liver disease, such that use of TT levels in isolation may be misleading. For example, multiple studies in the literature report an association between higher testosterone values and higher risk of development of hepatocellular carcinoma and advanced liver disease [19, 20], but these associations are likely to be due primarily to increased SHBG [18]. One study to date has shown high serum TT values in conjunction with chronic HCV. El-Serafi et al observed significantly higher TT values in HCV-infected patients compared with healthy controls, although this finding was limited by the lack of reporting on FT and SHBG levels in participants [13].

In agreement with the findings of El-Serafi et al [13], in the present study, we demonstrated higher TT and SHBG levels in participants with active HCV compared with those participants who achieved SVR. Further, we observed decreases in TT and SHBG after HCV clearance in the subset of participants who had evaluable testosterone determinations both pre-treatment and post-SVR. The reduction in TT following SVR is likely attributable to the reduction in SHBG. Testosterone is primarily metabolized by the liver, and the metabolic clearance of testosterone is reduced in severe liver disease [21]. Following HCV clearance, testosterone metabolism may recover to some degree, altering the level of testosterone measured in the blood.

In the current cohort, HIV coinfection was not related to increases in biochemical hypogonadism. Conversely, HIV coinfection was associated with a decrease in biochemical hypogonadism after SVR in the longitudinal cohort. HIV/HCV-coinfected patients have been shown to have higher prevalence of biochemical hypogonadism than HIV-monoinfected patients and may therefore experience greater increases in testosterone following HCV clearance [22]. In the context of HIV-associated chronic immune activation, elimination of HCV virus may also support enhanced recovery of testosterone in this subgroup. In both the cross-sectional and longitudinal analyses, only approximately 15% of participants had detectable HIV viremia. Controlling for HIV viremia in the cross-sectional data had no impact on TT, FT, or SHBG values, suggesting active HIV viremia was not influencing the observations. The relatively small number of participants with active HIV viremia, however, may have limited the ability to detect differences in testosterone levels based on HIV replication status.

Regression of hepatic fibrosis is an important potential benefit of HCV viral clearance that has been demonstrated using noninvasive measures of liver stiffness, such as elastography and serum biomarkers of fibrosis including fibrosis-4 and APRI

[23, 24]. In the current study, a change in APRI score that indicated a reduction in hepatic fibrosis was associated with greater increases in FT following SVR. This observation was statistically significant after adjusting for HIV status as well as change in SHBG. Prior cross-sectional investigations identified an inverse relationship between levels of testosterone and hepatic fibrosis in both chronic HCV and non-alcoholic fatty liver disease in men with type 2 diabetes [5, 25]; however, here we demonstrate that regression of fibrosis accompanies improvements in FT following successful HCV treatment.

Our study comprehensively analyzed the relationship between HCV and levels of TT, FT, and SHBG but did not measure other key hormones related to testosterone production. Namely, participants' luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol, and estrone levels were not measured. The hypogonadism that typically accompanies liver failure is thought to be due primarily to hypothalamic pituitary dysfunction, with a possible component of testicular dysfunction (ie, primary hypogonadism) as well [2, 26]. Prolactin is elevated in advanced liver disease and suppresses gonadotropin-releasing hormone [27]. Additionally, serum estrogens are increased in advanced liver disease due, at least in part, to increased conversion from androgens; estrogen also directly suppresses gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone [21]. Cytokines, undernutrition, and other aspects of chronic disease may also have suppressive effects on gonadotropin-releasing hormone. In the present study, we were unable to draw conclusions about how changes in prolactin and estrogens following SVR affect changes in TT and FT, and we do not have data on whether luteinizing hormone or follicle-stimulating hormone changes with SVR.

Testosterone values were not consistently measured in the morning, which is a limitation, as testosterone has a circadian rhythm with a peak in the morning. There should be no systematic difference in when values were measured between active HCV and SVR groups or between pre- and post-SVR. Consequently, testosterone measurement at random times during the day should not be a source of systematic bias in our comparisons. Further, when analyses were repeated excluding nonmorning samples, all the major observations in the study remained significant. We used an immunoassay to quantify TT and present calculated FT here because it is the measure most widely available to clinicians and correlates very well with gold-standard assays of FT by ultrafiltration or equilibrium dialysis [28, 29]. Finally, symptoms of hypogonadism were not directly assessed in the current study; therefore, our data represent biochemical hypogonadism rather than a clinical diagnosis.

The association between chronic viral infection of the liver as well as hepatic dysfunction and testosterone metabolism and hypogonadism is well documented, though not fully understood. Our study provides important additional information that characterizes the effects of HCV clearance on changes in testosterone. We demonstrate that increased SHBG may mask hypogonadism in active HCV, particularly if TT is the only measure assessed. The persistence of hypogonadism following successful viral treatment demonstrates the need for enhanced awareness and potential screening in the growing number of HCV-treated patients. Additional research is required to establish the mechanism responsible for hypogonadism in both patients with chronic HCV infection and in patients post-SVR and to develop strategies to optimize the recognition and management of testosterone deficiency in this setting.

Notes

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