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The association between serological and dietary vitamin d levels and hepatitis c-related liver disease risk differs in african american and white males

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Abstract

Background—Vitamin D may affect the severity of HCV-related liver disease. We examined the association between serum vitamin D levels and advanced liver disease in a multiethnic U.S. cohort of HCV patients, and accounted for dietary and supplemental intake.

Methods—We measured serum 25-hydroxyvitamin D levels and FibroSURE-ActiTest to assess hepatic pathology in a cohort of HCV-infected male veterans. We estimated and adjusted for daily intake of vitamin D from diet using a Dietary History Questionnaire, and dispensed prescriptions prior to study enrollment. We used race-stratified logistic regression analyses to evaluate the relationship between serum vitamin D levels and risk of advanced fibrosis (F3/F4-F4) and advanced inflammation (A2/A3-A3).

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Authorship Contributions:

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Results—A total of 163 African American (AA) and 126 White non-Hispanics were studied. Overall, ~44% of AAs and 15% of Whites were vitamin D deficient or insufficient (<20 ng/mL); 4% of AAs and 9% of Whites had an elevated level (>50 ng/mL). Among AAs, patients with elevated serum vitamin D levels had significantly higher odds of advanced fibrosis (OR=12.91, p=0.03) than those with normal levels. In contrast, AAs with insufficient or deficient levels had > two-fold excess risk of advanced inflammation (p=0.06). Among Whites, there was no association between vitamin D levels and advanced fibrosis (F3/F4-F4) or inflammation (A2/A3-A3) risk.

Conclusions—We observed potential differences in the association between vitamin D levels and degree of HCV-related hepatic fibrosis between White and AA males. Additional research is necessary to confirm that high serum vitamin D levels may be associated with advanced fibrosis risk in African American males, and to evaluate racial differences exist in HCV-infected females.

Background

Vitamin D is an essential fat-soluble micronutrient in mammals. In addition to playing a key role in maintenance of skeletal health, there is increasing recognition that vitamin D is involved in multiple other important physiologic functions including immune response and wound healing.[1] The liver plays a critical role in vitamin D metabolism by hydroxylating vitamin D synthesized endogenously secondary to sun exposure (as Vitamin D3) or else obtained directly from dietary and supplemental sources (as Vitamin D2 or D3) into its key metabolic intermediate, 25-hydroxyvitamin D or 25(OH)D. Exported from the liver bound to Vitamin D Binding Protein, 25-hydroxyvitamin D is then further hydroxylated (primarily by the kidneys) to its bioactive form, 1, $25(OH)_2D$ which is the target ligand for the nuclear hormone vitamin D receptor.

Vitamin D may affect the severity of HCV-related liver disease. The vitamin D3 intermediate hydroxyvitamin-D3 has been shown to reduce HCV core antigen levels in a dose-dependent manner in HCV-infected HuH-7 cell lines.[2] Several[3–6] though not all[7,8] epidemiological studies performed in HCV-infected populations undergoing treatment have shown that lower serum hydroxyvitamin D levels are associated with reduced likelihood of achieving a sustained virological response (SVR) with interferon and ribavirin combination antiviral treatment. Findings of improved SVR were also reported in some[9,10] though not all[11] studies of patients receiving Vitamin D supplementation.

An association between lower serum vitamin D levels and increased risk of advanced HCV-related liver fibrosis has also been shown in several[3,5,12] though not all[7,8] case-control and cross-sectional studies, many performed in European populations. The only nested case-control study was performed among prospectively recruited participants from the multicenter HALT-C treatment trial in the U.S.[13] Although no association was found between fibrosis progression and serum hydroxyvitamin D levels overall, a potentially increased fibrosis progression risk was observed with higher baseline levels in certain sub-groups, including diabetics and also possibly African Americans (AA) and individuals using vitamin D supplements.

Few studies have been performed in ethnically-diverse general clinic populations. Another smaller U.S. cross-sectional study in HCV patients who received antiviral therapy reported decreased risk of advanced fibrosis with higher serum D levels only in Caucasians.[14] Therefore, the association between serum vitamin D levels and the risk of hepatic fibrosis in the general HCV patient population, many of whom are contraindicated for antiviral therapy, remains unclear. Furthermore, none of these studies evaluated customary dietary intake as a possible explanatory co-factor. Some recent research suggests vitamin D2 which is obtained in many fortified foods and supplements may not be as bioavailable or effective as vitamin D3 which is obtained endogenously or from a limited number of foods like cod

liver oil or milk.[15] Finally, the association between serum D levels and hepatic inflammatory activity risk is unclear.

To address the gaps in the HCV– vitamin D literature, and with a working hypothesis that low serum vitamin D is associated with more advanced liver disease in HCV-infected patients, we performed a cross-sectional study to determine if there were potential racial differences in the association between serologically-determined total vitamin D levels and advanced liver disease in a large and multiethnic HCV patient cohort not on antiviral therapy in the U.S. Our study also included a novel exploratory evaluation of how customary dietary and also supplemental intake of vitamin D influence observed associations between total serum vitamin D levels and advanced liver disease risk.

Methods

Study Design and Population

We performed a cross-sectional study in chronically hepatitis C-infected veterans who were seen at the Michael E. DeBakey VA Medical Center (MEDVAMC) in Houston, Texas. We prospectively recruited consecutive veterans with a confirmed or possible HCV diagnosis at the dedicated Hepatitis C clinic at MEDVAMC prior to a routine clinic appointment between (5/1/2009–4/1/2012). All patients with a possible HCV are scheduled for a mandatory education and clinical evaluation in the HCV clinic in accordance with VA policy. To help assure the internal validity and reliability of our findings, veterans were eligible for our current study analysis if they met the following criteria: 1) they were self-identified African American (AA) or White non-Hispanic males ages 18 to 70 years old at recruitment as they comprised almost 90% of the underlying patient population; 2) had neither self- nor medical record-reported history of liver transplant, decompensated liver disease, hepatocellular carcinoma, or gastric bypass surgery; 3) had serologically-confirmed HCV viremia and concomitantly tested negative for both HIV and hepatitis B virus (HBV) surface antigen; and 4) were not currently receiving anti-viral therapy as <0.5% were receiving therapy at the time of study recruitment. This study was approved jointly by the Institutional Review Boards for Baylor College of Medicine and the Michael E. DeBakey VA Medical Center.

Data Collection and Study Measures

Veterans completed a research assistant administered comprehensive risk factor and dietary survey, had anthropometric measures taken, completed a fasting blood draw for serology, and consented to a comprehensive VA electronic medical record and pharmacy utilization at recruitment.

Serological Measures—A fasting blood draw was performed at recruitment and was analyzed by the MEDVAMC's CLIA-certified Central Laboratory Service for: HCV antibodies, genotype and quantitative viral load.

Baseline serum vitamin D level was assessed by measuring serum 25-hydroxyvitamin D levels (combined 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 levels) as it is recognized as the only clinically useful measure[16] as well as being a stable long-term predictor of vitamin D status.[17] We used DiaSorin's validated LIAISON automated immunochemiluminometric assay (ICMA),[18] with levels were considered clinically "normal" if between 20 and 50 ng/mL, "deficient" if between 12 and 19 ng/ml, "insufficient" if below 12 ng/mL, and "elevated" if above 50 ng/ml based on Institute of Medicine (IOM) guidelines.[19] We created a dichotomous variable for season as a proxy variable for potential direct sunlight exposure classified as Winter if the blood was drawn

during the 4 months with the shortest daylight hours (November-February), or otherwise as Non-Winter.

Surveys—Trained research assistants (RAs) administered a computerized sociodemographic, clinical, and risk factor survey which interrogated self-reported race/ethnicity, lifetime history of alcohol use and presence of comorbid diagnoses including diabetes.

Dietary and Nutritional Supplemental Vitamin D Intake Measurement—We estimated average annual daily intake of vitamin D from diet and from nutritional supplements (combined dietary oral intake of Vitamin D2 and D3) using participant's self-reported responses to the National Cancer Institute's (NCI) validated Dietary History Questionnaire (NCI-DHQ).[20] All dietary analyses were restricted to the ~77% of participants who returned surveys with average daily caloric intake between 500 and 5,000 kcal/day.

Electronic Medical Record (EMR) review—A single physician (HA) performed structured EMR review to determine the dispension of either out- or in- patient prescriptions for vitamin D by the VA pharmacy, including for multivitamins or ergocalciferol (Vitamin D2) prescribed alone or in conjunction with calcium. Information on dose, duration, and timing, including whether the patient had received a VA-dispensed vitamin D prescription within the year prior to study enrollment, was also collected.

FibroSURE-ActiTest—We estimated degree of hepatic fibrosis and inflammation using the FibrosSURE(FibroTest)-ActiTest (FS-AT). The FS-AT has been validated against hepatic biopsy in multiple study populations including in HCV+ populations.[21–24] It provides METAVIR biopsy-based equivalent hepatic fibrosis (F0, no fibrosis present – F4, cirrhosis) and inflammatory activity (A0, no inflammatory activity – A3, severe inflammatory activity). We classified study participants as advanced fibrosis cases if the FS-AT is F3/F4-F4 (cirrhosis), or else as mild fibrosis controls if F0-F3; and as advanced inflammatory activity cases if A2/A3-A3 (severe activity), or else as mild activity controls if A0-A2.

Statistical Analyses

All analyses were race-stratified. We compared serum hydroxyvitamin D levels (as well as sociodemographic, dietary, and clinical characteristics) in advanced fibrosis cases (F3/F4-F4) vs. mild fibrosis controls (F0-F3), and in advanced inflammation cases (A2/A3-A3) vs. mild inflammation controls (A0-A2) using T-tests for means and the Brown-Mood test for medians, respectively, and the χ^2 and Fisher's exact test for large and small sample categorical variables, respectively.

We performed binary logistic regression to evaluate the association between serum hydroxyvitamin D levels and risk of both advanced hepatic fibrosis (F3/F4-F4 vs. F0-F3) and advanced inflammatory activity (A2/A3-A3 vs. A0-A2). Our baseline multivariate models included adjustment for: age, history of alcohol abuse, presence of overweight or obesity, viral load, and receipt of a VA-dispensed vitamin D therapy during the year prior to baseline blood draw. Season of blood draw (Winter/non-Winter) was not included in multivariate analyses because it was not associated with either serum vitamin D levels or advanced inflammatory activity or fibrosis risk in Whites or AAs in any analyses.

To assess how self-reported vitamin D intake influenced observed associations between serum hydroxyvitamin D and risk of advanced liver disease, we performed two sets of

exploratory multiple logistic regression analyses that included additional adjustment of our baseline multivariate model for either DHQ-determined average daily dietary intake of vitamin D or for average annual total vitamin D intake combining both dietary and nutritional supplement intake. We also performed sensitivity analyses where we recalculated risk estimates after removing all participants on VA-prescribed vitamin D therapy.

All model-based parameter estimates are reported as odds ratios with associated 95% confidence intervals, with all analyses conducted using SPSS 18.

Results

A total of 289 male veterans with chronic HCV (163 African American (AA) and 126 White non-Hispanic) fulfilled our study eligibility criteria (overall participation rate=82%). The mean age of AAs was almost two years older than that of Whites (57.4 vs. 55.7 years, p=0.005). (Table 1) The proportions of advanced fibrosis (F3/F4-F4) and advanced inflammatory activity (A2/A3-A3) were both non-significantly higher in Whites (43% vs. 39% advanced fibrosis prevalence and 39% vs. 26% advanced activity prevalence in White and AA HCV+ males, respectively). Median serum 25(OH)D levels were higher in Whites (28.9 ng/mL vs. 21.6 ng/mL in Whites vs. AAs, p<0.001), though median values for both sub-groups were in the normal range (20–50 ng/mL). (Table 2) Overall, ~44% of AAs and ~15% of White HCV+ males were either vitamin D deficient or insufficient, with 4% of AAs and 9% of Whites elevated.

Median serum 25(OH)D levels were minimally lower in the advanced fibrosis cases (F3/F4-F4) and advanced inflammatory activity cases (A2/A3-A3) compared to their respective mild disease controls in bivariate analysis (20.7 vs. 21.8 ng/mL for advanced vs. mild fibrosis and 19.4 vs. 22.7 ng/mL for advanced vs. mild activity in AAs, and 28.1 vs. 29.3 ng/mL for advanced vs. mild fibrosis and 29.2 vs. 28.6 ng/mL for advanced vs. mild activity in Whites). (Table 2) There were no significant associations between serum vitamin D levels and season of blood draw, diabetes status or HCV viral load in either AA or White HCV+ males.

Medical record review indicated that 27 participants (n=14 AA and n=13 White) had received a prescription containing vitamin D that was dispensed by the VA pharmacy of up to 15 years. (Table 2) The proportion of *ever receiving* a vitamin D prescription was modestly higher in Whites than AA (10.3% vs. 8.6% in Whites and. AAs, respectively), while that of *current* users defined as on prescribed vitamin D therapy during the one year prior to blood draw was substantially higher in Whites (9.5% vs. 4.3% in Whites and AAs, respectively, p=0.08). Vitamin D prescriptions dispensed by the VA pharmacy were typically (~92% of time) for a daily multivitamin/mineral supplement which contained 400 IU of ergocalciferol (D2) along with 66 mg of calcium. The median duration for vitamin D therapy was 30 months in Whites and 60 months in African Americans, though this difference was not significant. (Table 2) The proportion of HCV+ males receiving a VA prescribed vitamin D prescription in the prior year was similar among those with high (>50 ng/mL) vs. non-high (<=50 ng/mL) serum D in both sub-groups (8% vs. 9% in Whites males with high vs. non-high serum D levels, respectively, and 0% vs. 5% in AA males with high vs. non-high serum D levels, respectively). (data not shown)

Most participants (n=223, ~77%) returned completed NCI-DHQ dietary surveys with average dietary intake between 500 and 5000 kcal/day. The DHQ-estimated median daily dietary intake of vitamin D (i.e., from food and beverage intake only) was modestly lower in AAs than Whites (3.64 vs. 4.01 μg/day in AAs and Whites, respectively, p=0.20). AAs were also significantly less likely to self-report obtaining additional vitamin D via use of

nutritional supplements (36.6% vs. 49.0% in AAs and Whites, respectively, p=0.02). (Table 2) However, AA who had high serum D levels were significantly more likely than those with non-high levels (<=50 ng/mL) to report using supplements that contained vitamin D in the prior year (83% vs. 34%, p=0.01). In contrast, in Whites there were neither strong nor significant differences (55% reported use of over-the-counter supplements with vitamin D in the prior year vs. only 48% in those with non-high serum levels, N.S.). (data not shown)

Race-stratified logistic regression models for the association between serum hydroxyvitamin D levels and advanced hepatic fibrosis risk (F3/F4-F4) are reported in Table 3. In our baseline multivariate analysis, compared to AA HCV+ males with normal levels, those with elevated serum vitamin D levels had highly elevated odds of advanced fibrosis (OR=12.91, p=0.03). AA HCV+ males who were hydroxyvitamin D insufficient or deficient (<20 ng/mL) had a non-significant elevation in relative risk of advanced fibrosis compared to AA HCV+ males with normal serum levels (OR=1.76, p=0.12). The associations observed in Whites differed with no excess advanced fibrosis risk with elevated serum vitamin D levels (OR=0.84, p=0.82) and only a slight non-significant excess advanced fibrosis risk with insufficient or deficient levels (OR=1.18, p=0.78).

Race-stratified logistic regression models evaluating the association between serum hydroxyvitamin D levels and advanced hepatic inflammatory activity risk (A2/A3-A3) are reported in Table 4. In our baseline multivariate analysis, AA HCV+ males who were hydroxyvitamin D insufficient or deficient (<20 ng/mL) had greater than two-fold excess risk that approached significance (p=0.06) compared to AA HCV+ males with normal levels. AA with high serum D levels had only slightly elevated risk, though this association was not significant (OR=1.43, p=0.76). The results again differed in Whites; compared to Whites males with normal serum D levels, those with either insufficient or deficient levels or with elevated levels had no evidence of excess risk (OR=0.70, p=0.54 and OR=0.73, p=0.66 for White males with insufficient/deficient and elevated serum D levels, respectively).

Our sensitivity analyses where we removed participants on vitamin D therapy in the prior year (current users) indicated that our observed associations between serum vitamin D levels and risk of advanced hepatic fibrosis (F3/F4-F4) and of advanced inflammatory activity (A2/A3-A3) were robust. In further exploratory analyses, we observed that additional adjustment for NCI-DHQ determined daily intake of vitamin D (whether dietary only or total including from supplements) only modestly attenuated observed associations between serum D levels and risk of advanced fibrosis or inflammatory activity.(data not shown)

Discussion

We evaluated the association between serum vitamin D levels and risk of advanced hepatic fibrosis (F3/F4-F4) and inflammatory activity (A2/A3-A3) in a large and multiethnic population mono-infected with chronic HCV. We observed racial differences in the association between vitamin D levels and degree of HCV-related hepatic fibrosis in our cohort of HCV-infected male veterans.

We found AA males with clinically elevated serum levels of vitamin D (>50 ng/mL) have a higher risk of advanced fibrosis (F3/F4-F4) than those with normal vitamin D levels. These results are consistent with results of a study performed in the HALT-C cohort which found that AA whose liver fibrosis clinically progressed over a 4-year period had higher baseline vitamin D levels compared to AA whose fibrosis had not progressed (32.7 vs. 25.2 ng/mL serum vitamin D in AA fibrosis progressors vs. non-progressors, respectively, p=0.08).[13] Also consistent with the HALT-C study was our finding of no association between serum

vitamin D levels and degree of hepatic fibrosis in White males with chronic HCV. In contrast, our results differ from those from a smaller cross-sectional study performed in Memphis, Tennessee that found no association between serum vitamin D levels and degree of fibrosis in AA (n=106), and also reported significantly increased risk of advanced fibrosis with low vitamin D levels in Whites (n=65).[14] However, given known gender-based differences in vitamin D metabolism, the divergence of our findings and those of the HALT-C study from those of the smaller Memphis, Tennessee HCV study may be attributable to differences in gender ratio among studies (100% vs. 73% vs. 27% male in our cohort vs. the HALT-C and Tennessee HCV study cohorts, respectively). Notably, all three studies reported inter-racial differences in association between serum D levels and HCV-related liver disease. Together, these three studies are consistent with a growing number of reports suggestive of variation in vitamin D metabolism and its association with health outcomes in populations of Caucasian and African ancestry.[25–27] However, given differences among these three studies in population and design, additional research in larger prospectively followed cohorts in necessary to confirm the presence of racial differences in the association between serum D and advanced HCV-related liver disease risk.

We also found an association between serum vitamin D levels and excess advanced inflammatory activity risk (A2/A3-A3) only in African American males who were vitamin D deficient or insufficient (<20 ng/mL); they had over 2-fold excess risk that closely approached significance in multivariate analyses (p=0.06). In contrast, we found neither strong nor significant associations between serum vitamin D levels and advanced inflammatory activity risk in Whites. Few studies have examined the association between serum vitamin D levels and degree of hepatic inflammatory activity in the background of chronic HCV;[6,7,28] none were performed in U.S. populations and none specifically examined for interethnic differences. Our findings are consistent with those from the Swiss Hepatitis C Cohort Study (n=496) which reported no association between serum vitamin D levels and necroinflammatory activity in the unadjusted analysis (p=0.9).[28] However, our findings differ from reports from two other groups: a multicenter Italian cohort study which found a strong and significant association between pre-treatment hepatic inflammatory grade and low serum vitamin D levels in 144 HCV+ biopsied patients (ORunadjusted=3.42, p=0.004);[6] and a study performed in the Australian and New Zealand CHARIOT cohort (n=274) which found higher prevalence of vitamin D deficiency in those with higher hepatic activity grade (21% vs. 11% serum levels <50 nmol/L for grade A0/A1 vs. A2/A3, respectively, p=0.03).[7] Although we cannot readily explain this discordance in findings among Whites, explanatory factors likely include differences in underlying study populations (e.g., the other studies included participants eligible to receive antiviral therapy vs. our study in a general HCV clinic population that included many individuals with contraindications for treatment; all other studies included both genders vs. ours which was restricted to males only, and only our study adjusted for potential confounders of serum D levels like BMI).

Our study has multiple strengths including its focus on inter-ethnic differences; our prospective consecutive recruitment; our larger sample size particularly for AA; and adjustment for known confounders of vitamin D status like BMI. Another was our novel evaluation of vitamin D intake from dietary and from nutritional supplement intake as potential confounders. Although our findings suggest adjustment for oral vitamin D intake is of marginal benefit as associations between serum D levels and advanced liver disease risk were only minimally altered, future research is needed to confirm this finding given reductions reduced power for our dietary analyses. Finally, we measured and controlled for VA-dispensed vitamin D prescriptions over a 15-year time period and demonstrated that our observed associations were robust to removal of individuals who received vitamin D prescriptions.

Our study also has several limitations that bear discussion. Our primary limitation is our cross-sectional design which precludes causal inferences. We also had a limited number of AA males with high serum vitamin D levels resulting in reduced power, particularly in multivariate analyses. However, the consistency in our results with those reported for the prospectively followed HALT-C study cohort that found potentially substantial excess fibrosis risk for AA with high vitamin D levels is supportive of our study findings. We had only a single (not repeated) measure for our primary exposure, DiaSorin ICMA-determined serum hydroxyvitamin D levels, and outcomes, FibroSURE-ActiTest determined fibrosis and inflammatory activity levels. However, these measurements were obtained for all study participants using validated tests in certified laboratories with personnel blinded to participant characteristics helping to assure the internal validity and reliability of our findings. We do not have molecular marker measures for vitamin D binding protein or the vitamin D receptor that also have been potentially implicated in risk of HCV-related liver pathology,[4,29,30] either in conjunction with or instead of serum vitamin D levels. There were some clinical differences between our AA and White HCV male patients including a higher prevalence of genotype 1 in AA (95% vs. 68% in Whites) that may contribute to observed differences in the relationship with vitamin D and advanced liver disease risk. Additionally, our study was performed in a single clinic population in a sub-tropical humid climate where strong seasonal variations in serum vitamin D levels were not observed. Finally, as our study was limited to White and AA males, it is unclear if our findings are generalizable to HCV+ males from other race/ethnic groups or to HCV+ females.

There has been considerable recent interest in the role of vitamin D supplementation as a preventive or palliative measure for HCV-related liver disease. Our preliminary findings in a general HCV clinic population not on antiviral therapy suggested an increased advanced fibrosis risk among African American males with chronic HCV who had elevated serum vitamin D levels. Our results in conjunction with those from the HALT-C trial which suggested there may be excess fibrosis risk in AA males and also among individuals using vitamin D supplements underscore the need for larger prospective studies to confirm these findings in other multiethnic HCV-positive male cohorts, and to assess if similar differences exist in HCV-positive females.

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Abbreviations

AA	African American
DHQ	Dietary History Questionnaire
HCV	hepatitis C virus
OR	odds ratio
RA	research assistants
VA	Department of Veterans Affairs

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Table 1

Demographic and clinical characteristics of 289 male veterans with chronic Hepatitis C according to race/ethnicity.

Characteristic	African American n=163	White n=126	p-value
Age in years - mean (sd)	57.4 (4.2)	55.7 (5.5)	0.005
Chronic alcohol abuse^- n (%)			0.004
Negative	101 (62.0)	55 (43.7)	
Positive	61 (37.4)	70 (55.6)	
Missing	1 (0.6)	1 (0.7)	
HCV viral genotype- n (%)			
1	155 (95.1)	86 (68.3)	<0.001
2	1 (0.6)	19 (15.1)	
3	0 (0)	14 (11.0)	
4	2 (1.2)	0 (0)	
Missing	5 (3.1)	7 (5.6)	
HCV Viral Load (log 10 in eq/mL) -mean (sd)	6.53 (0.73)	6.41 (0.93)	0.23
Diabetes mellitus- n (%)			
Absent	124 (76.1)	94 (74.6)	0.77
Present	39 (23.9)	32 (25.4)	
BMI- mean (sd)	28.3 (5.04)	28.8 (5.7)	0.48
Fibrosis Stage^^			
Mild Fibrosis (0–3)	100 (61.3)	72 (57.1)	0.55
Advanced Fibrosis (3/4–4)	63 (38.7)	54 (42.9)	
Inflammatory Activity Grade^^			
Mild Activity (0–2)	121 (74.2)	77 (61.1)	0.02
Advanced Activity (2/3–3)	42 (25.8)	49 (38.9)	

^ Chronic alcohol abuse defined as greater than 3 drinks a day for at least 10 years.

^^ Determined by FibroSURE-ActiTest (FS-AT).

Table 2

Vitamin D measures based on serology, prescriptions and dietary and supplement intake in 289 male veterans with chronic Hepatitis C according to race/ethnicity.

	African American	White	
	n=163	n=126	p-value
Serology-based			
Serum vitamin D – median (IQR)	21.6 (13.4)	28.9 (13.0)	<0.001
Serum vitamin D category- n (%)			
Deficient (< 12 ng/ml)	16 (9.8)	2 (1.6)	<0.001
Insufficient (12 - <20 ng/ml)	55 (33.7)	17 (13.5)	
Normal (20 – 50 ng/ml)	86 (52.8)	96 (76.2)	
Elevated (>50 ng/ml)	6 (3.7)	11 (8.7)	
Season blood drawn- n (%)			
Non-Winter (March-October)	100 (61.3)	79 (62.7)	0.82
Winter (November-February)	63 (38.7)	47 (37.3)	
Serum vitamin D – median (IQR)			0.46 (White), 0.56 (African American)
Non-Winter (March-October)	22.0 (13.6)	30.0 (13.6)	
Winter (November-February)	20.5 (13.5)	28.3 (12.3)	
Correlation viral load and vitamin D- Spearman rho	0.03	0.08	0.18 (White), 0.72 (African American)
Serum vitamin D by diabetes diagnosis history– median (IQR)			
Negative	21.7 (14.1)	28.6 (13.5)	0.54 (White), 0.97 (African American)
Positive	21.6 (12.4)	29.9 (11.2)	
Serum vitamin D by fibrosis category-median (IQR)			0.59 (White), 0.80 (African American)
Mild fibrosis control (F0-F3)	21.8 (12.4)	29.3 (12.1)	
Advanced fibrosis case (F3/F4-F4)	20.7 (16.1)	28.1 (12.3)	
Serum vitamin D by activity category- median (IQR)			1.0 (White), 0.12 (African American)
Mild activity control (A0-A2)	22.7 (13.7)	29.2 (12.0)	
***Advanced activity case*(A2/A3-A3)**	19.4 (12.8)	28.6 (14.1)	
***Prescriptions from electronic medical record review*^^**			0.46
Vitamin D prescription ever in past 15 years by type- n (%)			
Multivitamin with Vitamin D2	14 (8.6)	11 (8.7)	
Calcium/Vitamin D2 combination	0 (0)	1 (0.8)	
Vitamin D2 alone	0 (0)	1 (0.8)	
None	149 (91.4)	113 (89.7)	
Vitamin D prescription in year pre-blood draw- n (%)			0.08
Yes	7 (4.3)	12 (9.5)	
No	156 (95.7)	114 (90.5)	
Duration vitamin D therapy months-median (IQR)	30 (63)	60 (48)	0.71
***Dietary and supplement intake prior year based on NCI -DHQ*^^^**			

	African American	White	
	n=163	n=126	p-value
Daily vitamin D intake (μg/day) dietary sources only– median (IQR)	3.64 (3.78)	4.01 (4.09)	0.20
Daily dietary vitamin D intake (μg/day) - n (%)			0.78
Tertile 1 (<= 2.9)	42 (34.1)	33 (33.0)	
Tertile 2 (> 2.9–5.3)	42 (34.1)	31 (31.0)	
Tertile 3 (> 5.3)	39 (31.8)	36 (36.0)	
Daily vitamin D intake from nutritional supplements only (μg/day) – n (%)			0.02
None	78 (63.4)	51 (51.0)	
Low supplement use (< 10 mcg vitamin D per day)	36 (29.3)	30 (30.0)	
High supplement use (≥10 mcg vitamin D per day)	9 (7.3)	19 (19.0)	

^ Based on Institute of Medicine (IOM) guidelines.

^^ Based on physician-performed electronic medical review for dispensed vitamin D prescriptions including multivitamins for period up to 15 years.

^^^ Restricted to the 77% of participants (n=100 White and n=123 African Americans) who returned validly completed NCI -DHQ.

Abbreviations: IQR, interquartile range; NCI-DHQ; National Cancer Institute Food Frequency Questionnaire

Table 3

Race-stratified logistic regression models for association between total serum Vitamin D and other measures of Vitamin D status and risk of FibroSURE-ActiTest-determined advanced hepatic fibrosis (F3/F4 and F4) in HCV+ males.

	Univariate Models			Multivariate Model-Baseline^		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
AFRICAN-AMERICAN (N=163)						
Total Serum Vitamin D Category^^						
Deficient (< 12 ng/ml) or Insufficient (12–<20 ng/ml)	1.36	(0.78–2.60)	0.36	1.76	(0.86–3.56)	0.12
Normal (20 – 50 ng/ml)	1.0 (Ref)	-	-	1.0 (Ref)	-	-
High(>50 ng/ml)	9.83	(1.10–88.08)	0.04	12.91	(1.30–128.16)	0.03
WHITE (N=126)						
Total Serum Vitamin D Category^^						
Deficient (< 12 ng/ml) or Insufficient (12–<20 ng/ml)	1.21	(0.45–3.24)	0.71	1.18	(0.37–3.69)	0.78
Normal (20 – 50 ng/ml)	1.0 (Ref)	-	-	1.0 (Ref)	-	-
High(>50 ng/ml)	0.77	(0.21–2.79)	0.69	0.84	(0.20–3.59)	0.82

^All multivariate models including baseline model adjust for age, diabetic status, HCV viral load, chronic alcohol abuse, adiposity (BMI>25) and whether on prescribed vitamin D therapy on year prior to study enrollment.

^^Based on Institute of Medicine guidelines.

Abbreviation: CI, confidence interval; NCI-DHQ, National Cancer Institute Dietary History Questionnaire; OR, odds ratio.

Table 4

Association between total serum Vitamin D and other measures of Vitamin D status and risk of FibroSURE-ActiTest-determined advanced hepatic inflammatory activity (A2/A3 and A3) in male veterans with chronic hepatitis C infection.

	Univariate Models			Multivariate Model-Baseline^		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
AFRICAN-AMERICAN (N=163)						
Total Serum Vitamin D Category^						
Deficient (< 12 ng/ml) or Insufficient (12–<20 ng/ml)	2.07	(1.01–4.27)	0.048	2.16	(0.98–4.79)	0.06
Normal (20 – 50 ng/ml)	1.0 (Ref)	-	-	1.0 (Ref)	-	-
High(>50 ng/ml)	0.81	(0.09–7.41)	0.85	1.43	(0.15–14.11)	0.76
WHITE (N=126)						
Total Serum Vitamin D Category^						
Deficient (< 12 ng/ml) or Insufficient (12–<20 ng/ml)	0.70	(25–2.01)	0.51	0.70	(0.23–2.18)	0.54
Normal (20 – 50 ng/ml)	1.0 (Ref)	-	-	1.0 (Ref)	-	-
High(>50 ng/ml)	1.27	(0.36–4.46)	0.71	0.73	(0.18–2.96)	0.66

^All multivariate models including baseline model adjust for age, diabetic status, HCV viral load, chronic alcohol abuse, adiposity (BMI>25) and whether on prescribed vitamin D therapy on year prior to study enrollment.

^^^Based on Institute of Medicine guidelines.

Abbreviation: CI, confidence interval; NCI-DHQ, National Cancer Institute Dietary History Questionnaire; OR, odds ratio.