Serum Serotonin Levels are Associated with Antiviral Therapy Outcomes in Patients with Chronic Hepatitis C

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Abstract: The role of serotonin in contributing to viral clearance has not been investigated in patients with chronic hepatitis C (HCV). In this prospective study we collected blood samples from 39 patients prior to and during antiviral therapy. Participants completed mood rating scales to monitor psychiatric symptoms, and serum serotonin and tryptophan levels were measured. Significant differences in serotonin levels were found between patients who achieved sustained viral responses (SVRs) and those who did not. Regression analysis revealed that serotonin was the only variable with a statistically significant relationship with antiviral therapy outcomes, even after controlling for other variables known to be associated with outcomes. Baseline serum serotonin levels, in combination with other variables such as degree of liver fibrosis, may be clinically useful for identifying patients in whom HCV can be cleared by antiviral therapy. Additional clinical predictors that could forecast treatment success are needed so that interventions to improve SVR rates, and reduce side effects, can be developed.

INTRODUCTION

The World Health Organization estimates that about 3% of the world population has chronic hepatitis C viral infection (HCV), which leads frequently to fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Although HCV is treatable in some patients, there is no vaccine and the current treatment [*i.e.*, combination therapy with pegylated interferon-alpha (IFN-α) and ribavirin] has only limited efficacy. Sustained viral response (SVR) is typically achieved in only about 40-50% of those with HCV genotype 1 and 75-80% of those with HCV genotypes 2/3[2]. Thus, considerable effort has been devoted to identifying predictors of viral clearance for patients with HCV.

Both viral (*e.g.*, HCV genotype) and host (*e.g.*, age [3], gender [4], body weight [5], and ethnicity [6, 7]) factors have been investigated for their roles as predictors of treatment efficacy. Among these factors, it is generally accepted that the HCV genotype is one variable that significantly influences HCV course and treatment prognosis. Specifically, infection with HCV genotype 1 is associated with more severe liver disease and a poorer response to antiviral therapy than is infection with other HCV genotypes [8, 9]. Recently a genetic polymorphism near the interleukin (IL) 28B gene was identified and may account for some of these differences in response to treatment [10]. However, the accuracy of predicting antiviral response based on these host factors has generally been poor [11].

In addition to limited efficacy, HCV treatment is also associated with significant neuropsychiatric side effects [12]. The symptoms of IFN-α-induced depression are similar to those seen in major depressive disorder, and may include: depressed mood, irritability, emotional lability, agitation, fatigue, apathy, anhedonia, anorexia, psychomotor retardation, sleep disturbance, sexual dysfunction, memory impairment, and diminished ability to concentrate. Rates of IFN-α-induced depression range from 0% to 44% [13, 14]. Although changes in the formulation of IFN-α have improved response efficacies for some patients, side effects of depression persist and occur in at least 20% of patients with HCV who receive peglylated IFN-α [15]. Early symptom detection and intervention can frequently prevent an adverse outcome. The depressive symptoms associated with IFN-α therapy are generally transient and remit following termination of therapy; however, a handful of cases have reported a worsening of depression following withdrawal from IFN-α therapy. In a case series of five patients treated with IFN-α for chronic HCV, suicide was attempted in four cases after IFN-α withdrawal and was responsible for two deaths [16]. In another report, two attempted suicides and one successful suicide during or

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shortly after IFN-α therapy for chronic HCV were described [17] (Janssen *et al.*, 1994). Thus, given the low response rates and high rates of neuropsychiatric side effects [12], identification of the factors associated with a favorable response to treatment is a high priority.

The role of serotonin in contributing to viral clearance has not been investigated in patients with chronic HCV infection. Peripherally, serotonin mediates vascular contraction and relaxation, gastrointestinal motility, cell proliferation, apoptosis and platelet aggregation--functions that play critical roles in maintaining liver health [18, 19]. Serotonin is present in high concentrations in platelets, and in their dense bodies, platelets store and release serotonin. Serotonin additionally acts as a growth factor [20, 21]. *In vitro* and *in vivo* studies show that serotonin has regenerative properties in both mouse and rat livers [22-25]. However, little attention has been paid to serotonin's mechanisms of action in the human liver. Peripheral levels of serotonin and changes in tryptophan have been studied and were hypothesized to play a role in the etiology of IFN-α-induced depression [26, 27], but serotonin has not been investigated in regards to viral clearance or liver function in patients with chronic HCV infection. Recent reports including our own findings do not support consistent correlations between IFN-α-induced depression and serotonin or tryptophan levels [28-31]. Thus, given serotonin's role in immunoregulation the goals of this study were: 1) to determine the relationship between serotonin and viral clearance in patients with chronic HCV undergoing antiviral therapy, and 2) to generate hypotheses for future testing regarding the role of serotonin on liver health and regeneration.

MATERIALS AND METHODOLOGY

Research Participants

Participants were recruited through the hepatology clinics of two Veterans Affairs (VA) Medical Centers, the Portland VA Medical Center and the Seattle VA Medical Center of the Puget-Sound Health Care System between January 2003 and February 2006, for a study examining the efficacy of antidepressant therapy to prevent the development of IFN-α-induced depression. Participants were randomly assigned to receive citalopram or placebo two weeks prior to the initiation of antiviral therapy. Participants were included in this study if they were 18 years or older, had a diagnosis of HCV, were eligible for antiviral therapy, and agreed to undergo combination therapy with pegylated IFN-α and ribavirin. Participants were excluded from this study if they had ongoing depression or active psychotic symptoms during the three months prior to study start, substance abuse in the prior six months, and current use of antidepressant medication, or medical co-morbidities that could interfere with treatment. Total participant enrollment was 39 (Table **1**). All of the participants completed informed consent prior to their participation in this research study. The protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Institutional Review Boards at both institutions. Additional details regarding the methods and outcomes from this randomized, double-blind, placebo-controlled trial can be found in Morasco *et al.* [32].

Procedures

Participants were administered study measures two weeks prior to the initiation of antiviral therapy and weekly throughout treatment. At the baseline assessment, a trained research assistant administered the Structured Clinical Interview for DSM-IV (SCID), in order to assess for current psychiatric disorders. Participants also completed the Beck Depression Inventory, version II (BDI-II), a 21-item self-report inventory of depression severity shown to have a high degree of reliability and validity [33]. Blood samples were collected at baseline (two weeks prior to the start of antiviral therapy and on the day of citalopram or placebo treatment: Week-2) and at the first day of antiviral therapy (which was two weeks after citalopram or placebo disbursement: Week 0). Once participants started antiviral therapy, blood was drawn every two weeks for the first three months and every four weeks for the second three months. Research study visits were coordinated with liver clinic appointments and blood samples were generally collected within a 3-hour timeframe (11am-2pm). Blood was drawn by venipuncture into serum separation tubes (SST) for serum determinations of serotonin and tryptophan (BD Vacutainer Systems, Franklin Lakes, NJ). The blood was centrifuged at 1500 RCF for 20 min at room temperature (22-25° C). Serum was collected and immediately aliquoted in polypropylene tubes (Phenix Research Products, Hayward, CA) and frozen at −80 ◦C until assayed.

Antiviral Therapy

Antiviral therapy included weekly self-administered injections of pegylated interferon-α (IFN-α) along with twice daily oral doses of ribavirin. The doses of IFN-α and ribavirin were determined according to standard antiviral therapy guidelines and were monitored by a hepatology clinic provider. The intended duration of antiviral therapy was a minimum of 24 weeks (genotypes 2 and 3), although some patients continued with treatment for 48 weeks (genotype 1). Patients were given the option to discontinue treatment at any time.

Administration and Monitoring of Citalopram or Placebo

A double-blind, randomized block design was used to assign participants to an experimental condition: citalopram [selective serotonin reuptake inhibitor antidepressant (SSRI)] or placebo. Stratification was based on the geographical location of the study. Upon study entry, 20 mg tablets of citalopram or identical appearing tablets of the placebo were blindly dispensed to participants in the respective study arms. Participants were asked to self-administer one of the oral tablets on a daily basis. Depressive symptoms were monitored using rating scales at each clinical visit and the severity of depression, as measured by BDI-II scores, determined whether dose increases were necessary. After 24 weeks, the code for treatment group assignment was broken for all participants and those who continued with antiviral therapy for 48 weeks were offered the option of open-label citalopram for the duration of antiviral therapy. Four patients in the placebo group and three patients in the SSRI group developed moderate depression and entered the rescue arm of the study. For these seven patients, the code for treatment

Table 1.   Comparison of Demographic Characteristics

	Full Sample (n=39)	**Achieved SVR (n=17)**	**No SVR (n=22)**	*p*-**Value**
Age, M (SD)	53.0 (7.3)	50.7 (7.5)	54.8 (6.8)	0.08
Gender				1.00
Male	36 (92.3%)	16 (94.1%)	20 (90.9%)	
Female	3 (7.7%)	1 (5.9%)	2 (9.1%)	
Race/Ethnicity				0.68
Caucasian	33 (84.6%)	15 (88.2%)	18 (81.8%)	
Other	6 (15.4%)	2 (11.8%)	4 (18.2%)	
Education				0.04
$\leq$ High School Diploma	13 (33.3%)	9 (52.9%)	4 (18.2%)	
$\geq$ 1 year of College	26 (66.7%)	8 (47.1%)	18 (81.8%)	
Employment Status				0.18
Employed	20 (51.3%)	6 (35.3%)	14 (63.6%)	
Retired	8 (20.5%)	4 (23.5%)	4 (18.2%)	
Other	11 (28.2%)	7 (41.2%)	4 (18.2%)	
Genotype				0.53
1	21 (53.8%)	8 (47.1%)	13 (59.1%)	
2 or 3	18 (46.2%)	9 (52.9%)	9 (40.9%)	
Baseline HCV Load				0.55
Low	15 (40.5%)	5 (13.5%)	10 (27.0%)	
Moderate	11 (29.7%)	5 (13.5%)	6 (16.2%)	
High	11 (29.7%)	6 (16.2%)	5 (13.5%)	
Cirrhosis of the Liver				0.59
Yes	6 (16.2%)	3 (8.1%)	3 (8.1%)	
No	31 (83.8%)	14 (37.8%)	17 (45.9%)	
Body Mass Index, M (SD)	30.9 (4.9)	31.3 (4.6)	30.6 (5.4)	0.68
Current Depression Severity, M (SD)	4.3 (4.3)	3.7 (3.5)	4.7 (4.9)	0.47

group assignment was broken prior to week 24 and they either started on citalopram (placebo group) or received medication adjustments (SSRI group).

Liver Health Laboratory Measures

For all research participants liver health indices [i.e., platelet counts, SVRs, and <u>a</u>spartate aminotransferase (AST) values] were collected from each veteran's electronic medical record. HCV viral load was determined using the Ortho VITROS chemiluminescence assay (CIA) method for detection of antibodies to HCV. Sustained viral response (SVR) was defined as undetectable HCV at 6 months following the completing of antiviral therapy. APRI scores were calculated based on <u>A</u>ST to <u>P</u>latelet <u>R</u>atio <u>I</u>ndex and were used as an assessment of liver fibrosis.

Serotonin Assays

High performance liquid chromatography (HPLC) was used to measure serum serotonin levels. A modified Waters Corp Oasis MCX method was used to extract serotonin from human serum. Both samples and standards selected for analysis were thawed at 4°C prior to Solid Phase Exchange (SPE) workup. Prior to SPE workup a 200µL aliquot of sample serum was prepared with 10% ascorbic acid [w/v], 5-hydroxytryptophan (5-HTP) internal standard (150 ng/mL), and 1% formic acid (aq). Endogenous levels of serum serotonin (5-HT) were calculated using a linear range of 5-HT standards consisting of 7.5, 15, 50, 150, 500 and 2500 ng/mL. All standards were prepared and treated identically to samples; however, standards used 200µL of 5-HT-free serum (Valley Biomedical, Winchester, VA, USA). The 30mg MXC cartridges (Waters, Milford, MA, USA) were equilibrated and conditioned with 1mL of both methanol and 1% formic acid (aq), respectively. After sample application the cartridges were washed with 1mL of 0.1N HCl (aq) followed by 1mL of methanol. Serotonin was removed from the SPE cartridge with 1.5mL of 7.5% NH_4OH in methanol. The eluent was collected and desiccated under nitrogen and reconstituted in a 7:1 solution of mobile phase and an

aqueous antioxidant mixture containing 1.0mM oxalic acid, 3.0mM L-cysteine, and 0.1M acetic acid [34].

The HPLC system consisted of an ESA Biosciences model 580 single channel pump, 540 autosampler, and an ESA Coulochem II electrochemical detector (ESA Biosciences, Milford, MA, USA). The mobile phase buffer was prepared by filtering $NaH_2PO_4 \cdot H_2O$ (30mM) and sodium citrate (14.7mM) with a 0.45µm nylon filter in 18MΩ Milli-Q water. Both Na_2EDTA (2.7mm) and 1-octanesulfonic acid (2.23mM) were subsequently added whereupon the mobile phase pH was adjusted to 3.5 with o-phosphoric acid; the addition of the organic modifiers 8% acetonitrile and 1% tetrahydrofuran, immediately preceded followed by degassing (Supelco, Bellefonte, PA, USA) using a 1.5µm Teflon membrane under vacuum [35]. Injections of 20µl were isocratically chromatographed at 0.6 mL/min over a 3.2µm, 3.0x150-mm reverse phased C_{18} column with 4.0x10-mm guard (ESA Biosciences, Milford, MA, USA) at 25°C for twenty minutes. The electrochemical 5020 ESA guard cell potential was +350mV while both ESA 5011A analytical electrodes E1 and E2 (ESA Biosciences, Milford, MA, USA) potentials were experimentally determined by Hydrodynamic Voltammogram (data not shown) at-50mV, Range (1µA), and +200mV, Range (100nA), respectively. All cued samples were incubated at 4°C and injected in duplicate by the 540 autosampler.

Chromatographic isolation of both 5-HTP and 5-HT from other potential interfering compounds was achieved as evidenced by their average retention times of 2.8 and 11.7-minutes, respectively. External calibration curve intra-precision indices of linearity averaged $R^2 = 0.9996 \pm 0.3E\text{-}3$ (SD) yielding a coefficient of variation (CV) of 0.03%. The population average percent recovery of 5-HTP was 90.0% ± 6.8 (SD) and yielded a CV of 7.6% (n = 32). Limits of detection were ≥ 7.5 ng/mL at operating potentials with a signal-to-noise ratio of 47.8.

Tryptophan Assay

Tryptophan concentrations were measured by HPLC on reversed phase C18 material using 3-nitro-L-tyrosine as internal standard, as previously described. Briefly, serum protein precipitated with 2mmol/L trichloroacetic acid and tryptophan was monitored by its natural fluorescence at 285 nm excitation and 350 nm emission wavelengths [36, 37].

Statistical Analysis

Baseline demographic differences between the two groups (participants who achieved SVR versus those who did not achieve SVR) were compared using chi-square tests for categorical variables and analysis of variance (ANOVA) for continuous variables. The effects of antiviral therapy on peripheral serotonin and tryptophan levels were assessed with analyses of covariance (ANCOVAs). In these analyses, education level was a covariate, as it significantly differed between the two groups at the baseline assessment. Separate analyses were conducted for serotonin and for tryptophan. ANOVAs were also conducted to evaluate whether randomization to citalopram or placebo had an impact on serotonin or tryptophan levels, at different time points. Finally, hierarchical logistic regression was used to evaluate variables associated with antiviral treatment outcome. Body

mass index (BMI) and level of education were entered in Step 1. In Step 2, HCV genotype, baseline viral load counts (defined as low 0-1,569,999; moderate 1,570,000-4,129,999; or high 4,130,000 or more copies/mL), and whether or not patients had cirrhosis (yes or no) were entered into the equation. *Note*: viral loads are reported in copies per mL as opposed to International Units per mL (IU/mL). Given that these lab values were collected prior to the study onset using differing clinical laboratory assays, applying a standard conversion factor to the values was not appropriate [38]. Viral load was placed into categorical data as the original data were not normally distributed. For Step 3, baseline serotonin and tryptophan levels were entered. Genotype, viral load, cirrhosis status and education were categorical variables, while BMI and serotonin and tryptophan levels were continuous. Results were considered statistically significant if $P \le 0.05$, two-tailed. This study had power greater than 80% to detect a 10-point difference in scores on the BDI-II in 39 patients. Calculations were performed using SPSS software.

RESULTS

Patient demographics are reported in Table **1**, including HCV genotype, BMI, viral load at baseline, and other variables putatively associated with antiviral therapy outcome. Significant differences between patients who achieved SVR and those who did not were found only for level of education (Table **1**), as the SVR group had less education.

Association Between Serum Serotonin Levels and Antiviral Therapy Response

Higher peripheral serotonin levels were significantly associated with improved treatment outcomes in patients undergoing antiviral therapy before and during treatment. At week-2 [$F (2, 26) = 6.15$, $P = 0.02$], week 12 [$F (2, 17) = 4.45$, $P = 0.05$] and week 24 [$F (2, 20) = 6.65$, $P = 0.02$], the patients who had undetectable HCV at six months following the completion of treatment (SVR group) had significantly higher serum serotonin levels as compared with patients who didn't achieve an SVR (no SVR group) (Fig. **1A**). To determine whether the differences in serotonin and SVR rates were associated with changes in the bioavailability of serotonin's precursor, tryptophan levels were also measured. Patients who achieved viral clearance did not have significantly different levels of tryptophan than patients who did not achieve an SVR (Fig. **1B**).

Patients randomly assigned to the SSRI group displayed significantly lower serotonin levels than patients randomized to the placebo group at week 0 [$F (1, 24) = 24.6$, $P < 0.001$], week 2 [$F (1, 21) = 30.2$, $P < 0.001$], week 8 [$F (1, 14) = 16.5$, $P = 0.001$], week 12 [$F (1, 19) = 12.9$, $P = 0.002$], week 16 [$F (1, 13) = 10.6$, $P = 0.007$] and week 24 [$F (1, 22) = 14.6$, $P = 0.001$] (Fig. **2A**). Patients in the SSRI group were not statistically less likely to achieve SVRs, as compared to patients in the placebo group, but the SVR rate in the SSRI group was notably lower when compared with the placebo group [36.8% (7/19) *vs* 50% (10/20), respectively][32]. No significant differences in tryptophan levels were detected during the course of antiviral therapy between patients in the SSRI and placebo groups (all P-values ≥ 0.05; Fig. **2B**).
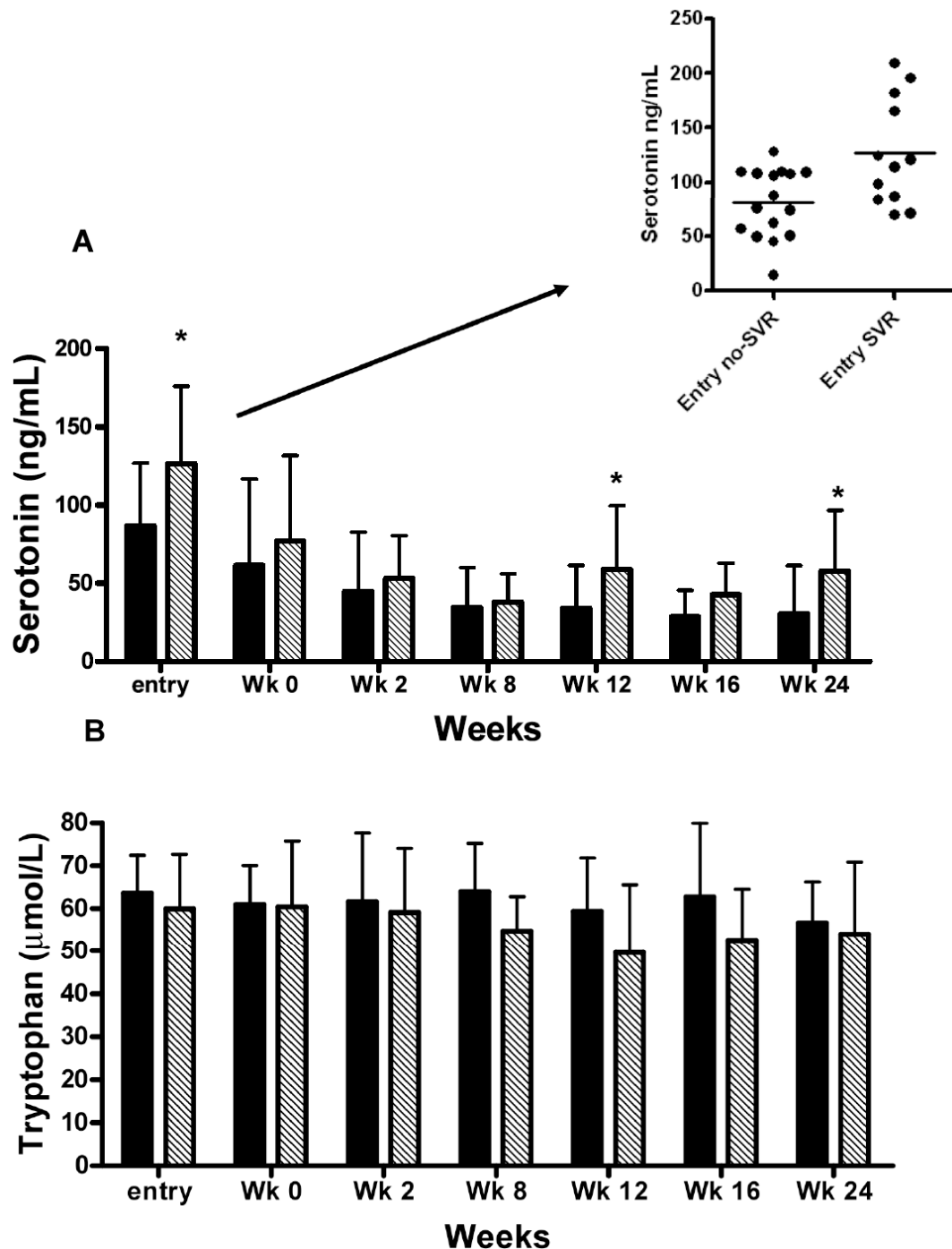
Fig. (1). Higher peripheral serotonin levels are associated with improved antiviral therapy outcomes in patients undergoing treatment for HCV. Serum samples were analyzed for serotonin and tryptophan levels in patients who did and did not achieve sustained viral responses six months after the completion of therapy (SVR, n = 17 and no SVR, n = 22). For serotonin levels, significant differences were found between the groups at study entry (P = 0.02), week 12 (P = 0.05) and week 24 (P = 0.02) (**A**). The same relationship was not evident for tryptophan levels and antiviral therapy outcomes (**B**). Solid bars represent the no SVR group and cross-hatched bars represent the SVR group. ARUP Laboratories, a National Reference Laboratory (Salt Lake City, UT), report that the average reference interval for serotonin levels in serum is 50-220 ng/mL. Data are expressed as means ± standard deviations (SD).

Baseline Serotonin Level as a Predictor of Viral Response in HCV Patients

Table **2** shows results from the hierarchical logistic regression examining variables putatively associated with antiviral therapy outcome. Step 1, with education levels and BMI included, was not significant, χ^2 (*df*=2, *n*=24) = 0.70, *P* = 0.70. For Step 2, liver health (i.e., presence or absence of cirrhosis and HCV load) and viral (i.e., HCV genotype) factors were included and were not significant, χ^2 (*df*=8, *n*=24) = 7.08, *P* = 0.53. The inclusion of serotonin and tryptophan levels in Step 3 evidenced a trend toward
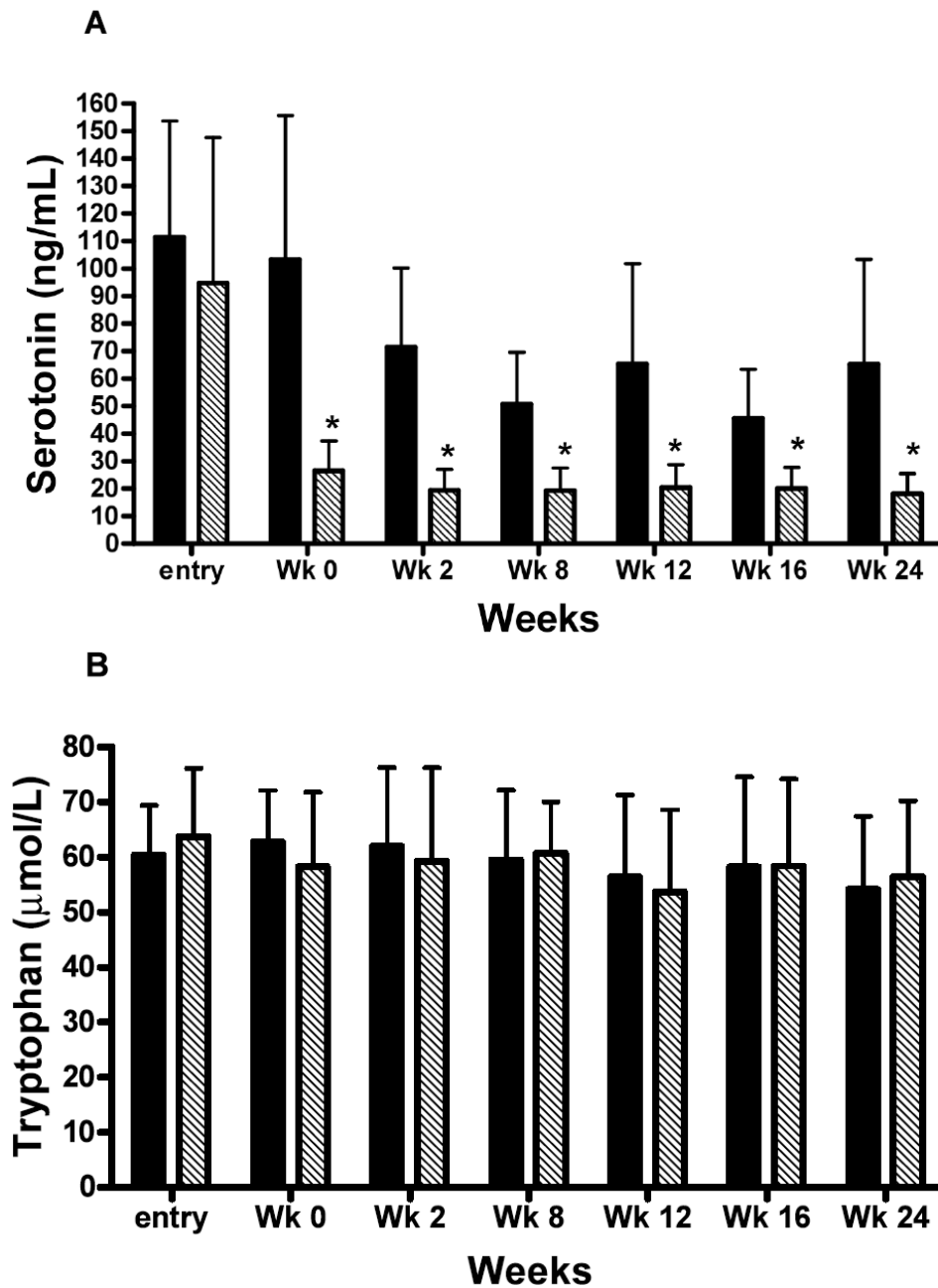
A

B

Fig. (2). Serotonin levels are significantly lower in patients on citalopram as compared to placebo. Patients with HCV were randomly assigned to SSRI (citalopram, n = 14) or placebo (n = 15) two weeks prior to starting antiviral therapy. Significant differences in serotonin levels were found between SSRI and placebo groups at week 0 ($P < 0.001$), week 2 ($P < 0.001$), week 8 ($P = 0.001$), week 12 ($P = 0.002$), week 16 ($P = 0.007$) and week 24 ($P = 0.001$) (**A**). However, patients in the SSRI group were not less likely to achieve sustained viral response rates, as compared to patients in the placebo group. The SVR rate in the SSRI group was 36.8% (7/19) compared to 50% (10/20) in the placebo group ($P = 0.41$). No significant differences in tryptophan levels were detected (**B**). Solid bars represent the placebo group and cross-hatched bars represent the SSRI group. Data are expressed as means ± SD.

significance, χ^2 (*df*=2, *n*=24) = 5.62, *P* = 0.06, but the overall model was not significant, χ^2 (*df*=8, *n*=24) = 9.87, *P* = 0.27. Baseline serotonin level was the only variable with a statistically significant relationship with antiviral therapy outcome (*P* = 0.05).

Blood Platelet Numbers are Slightly Higher in Patients with SVRs

Baseline platelet numbers were compared between patients who did and did not achieve SVR. Patients who achieved an SVR had higher numbers of platelets as compared with patients who did not; however, this difference was not statistically significant (*P* = 0.55; means ± SD: 192.89 ±74.55 K/cmm *vs* 174.71 ± 67.58 K/cmm, respectively). Because platelet counts are lower than normal in patients with liver cirrhosis, we tested the possibility that differences in degree of liver damage also influenced SVR. Unlike serotonin levels, the presence or absence of cirrhosis was not a statistically significant predictor of SVR (Tables **1** and **2**).

Table 2.   Logistic Regression Analysis Predicting Antiviral Therapy Outcome

	Beta (SD)	Wald	Significance	Odds Ratio 95% (Confidence Interval)
Step 1				
BMI	0.01 (0.09)	0.01	0.92	1.0 (0.9-1.2)
Education	0.72 (0.88)	0.67	0.42	2.1 (0.4-11.6)
Step 2				
Liver cirrhosis	0.96 (1.35)	0.50	0.48	2.6 (0.2-37.0)
HCV genotype	-0.60 (0.92)	0.43	0.51	0.6 (0.1-3.3)
Viral load	0.13 (0.67)	0.04	0.85	1.1 (0.3-4.2)
Step 3				
Serotonin	0.03 (0.01)	3.90	0.05	1.1 (1.0-1.1)
Tryptophan	-0.05 (0.06)	0.59	0.44	0.9 (0.8-1.1)

Serotonin Levels Correlate with Fibrosis Scores

Fig. (**3**) illustrates a significant negative correlation between serotonin levels and APRI scores [an assessment of liver fibrosis; aspartate aminotransferase (AST) to platelet ratio index], such that higher serotonin levels at entry were associated with lower APRI scores and putatively less liver fibrosis. Serotonin levels were significantly correlated with APRI values suggesting that higher serotonin levels might be a surrogate marker for less advanced liver disease.

Depressive Symptoms During Antiviral Therapy are Not Directly Correlated with Serotonin and Tryptophan Levels

To test the hypothesis that IFN-α-induced decreases in serotonin or tryptophan are associated with an increase in depressive symptoms, BDI-II scores were correlated with serotonin and tryptophan levels before and during antiviral therapy--at week-2, week 0, week 2, week 8, week 12, week 16 and week 24 (Table **3**). Pearson correlations were conducted, and tryptophan was significantly correlated with

BDI-II scores at week 8 ($r = 0.55$, $P = 0.04$), while serotonin was significantly correlated with BDI-II scores at week 12 ($r = 0.50$, $P = 0.02$). Statistically significant negative correlations were not found between BDI-II scores and tryptophan or serotonin levels.

DISCUSSION

During a prophylactic antidepressant study that was conducted to evaluate the safety and efficacy of pre-treating patients with citalopram, an SSRI antidepressant, prior to and during antiviral therapy [32], a novel role for serotonin was discovered. The concept that serotonin may play a role in hepatic wound healing is growing in interest and several recent empirical studies show the benefits of serotonin in rodent liver regeneration [19, 39]. The liver regenerative properties of serotonin may be of particular importance in chronic HCV, where the virus attacks the liver through the lytic cycle destroying hepatocytes, which could be regenerated *via* platelet-derived serotonin or specific serotonin agonists [22, 23]. Proinflammatory cytokines may increase the availability of hepatic serotonin [40], but
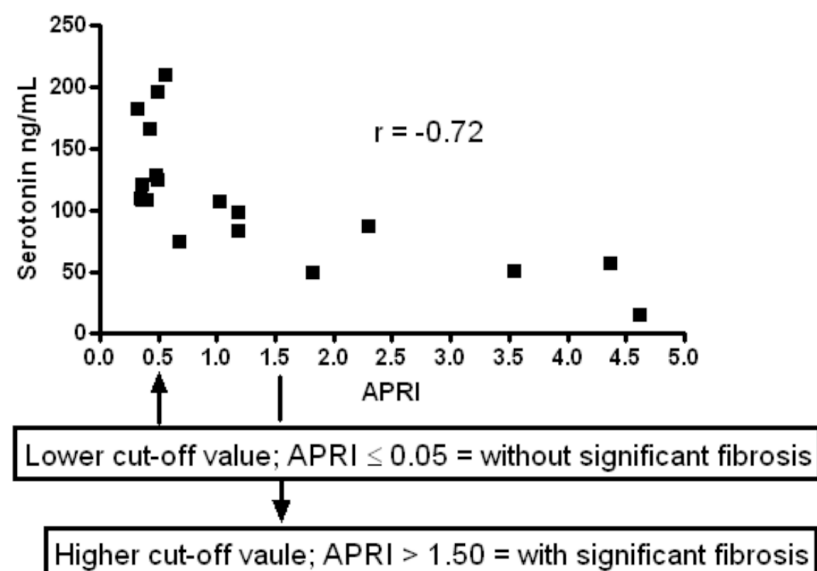

Fig. (3). Correlation between serotonin levels and APRI values (Pearson r =-0.72, P = 0.0005).

Table 3.   Pearson Correlations Between BDI-II Scores and Serum Biomarkers in Patients with HCV Undergoing Antiviral Therapy

Week	BDI-II Score Mean (SD)	Serotonin (ng/mL) Mean (SD)	Tryptophan (µmol/L) Mean (SD)	Serotonin (*r*)	Tryptophan (*r*)
-2	4.28 (4.35)	95.15(55.51)	62.04 (10.69)	-0.06	0.25
0	3.54 (3.35)	66.47 (54.40)	60.83(11.26)	-0.02	0.36
2	6.23 (5.32)	47.83 (34.07)	60.69 (15.33)	-0.16	0.12
8	7.18 (6.10)	36.11(21.69)	60.08 (10.73)	0.26	0.55*
12	6.72 (6.18)	45.16 (35.46)	55.00 (14.60)	0.50*	0.11
16	7.36 (6.43)	34.67 (19.14)	58.28 (15.59)	0.15	-0.03
24	5.33 (4.79)	44.83(37.20)	55.40 (13.24)	-0.13	-0.41

*Indicates statistical significance of $P \leq 0.05$.

serotonin may also contribute to improved viral clearance by enhancing the release of cytokines at the site of liver injury [41, 42]. The molecular mechanisms regarding how serotonin aids in HCV clearance are yet to be determined.

Not all research supports the theory that serotonin aids in hepatic wound healing, as serotonin has been shown in some animal studies to contribute to liver injury following intestinal and hepatic ischemia-reperfusion [43, 44]. Further, Lang *et al.* recently reported that serotonin contributes to viral persistence in mice infected with the noncytopathic lymphocytic choriomeningitis (LCMV) [45]. More specifically, CD8^{+} T cell antiviral response was delayed by serotonin treatment and replication of LCMV was enhanced in serotonin treated mice [45]. In contrast to the effects of serotonin in their mouse model of LCMV-induced hepatitis, we found that higher serotonin levels were significantly associated with improved antiviral therapy outcomes in patients with HCV undergoing antiviral therapy (Fig. **1A**). Our findings are in agreement with a recent study by Manéglier *et al.* who used human cells to show that serotonin may also help in human immunodeficiency viral (HIV) infections [46]. The authors found that serotonin decreases HIV-1 infection most likely *via* a decrease in the expression of CCR5 [HIV-1/Ba-L receptor (CD4) coreceptor] and/or an increase in the expression of CCR5's ligand, macrophage inflammatory protein-1alpha (MIP-1α) [46]. Although serotonin's mechanisms of action in aiding viral clearance are likely different for HCV and HIV infections, collectively these findings support a vital role for platelet serotonin in contributing to these processes in humans [18].

Peripheral levels of serotonin and changes in tryptophan have been studied and were hypothesized to play a role in the etiology of the neuropsychiatric side effects associated with antiviral therapy, particularly depression [27, 28], but more recent reports including our own findings do not support consistent correlations between IFN-α-induced depression and serotonin or tryptophan levels [28-31, 47]. In agreement with Fontana *et al.*, we also found significant IFN-α-induced decreases in peripheral serotonin levels (most notably in the SSRI group), which did not negatively correlate with depression rating scale scores (Table **3**) [31]. Given that both proinflammatory cytokines (*e.g.*, interferons) and HCV are associated with an upregulation of indolamine 2,3 dioxygenase (an enzyme which converts tryptophan into

kynurenine) [48], further research is needed to determine more sensitive markers of serotonin metabolism such as serotonin gene polymorphisms [49, 50] which could be used to predict or influence IFN-α-induced depression.

Robaeys *et al.* recently asked an important question about whether or not the use of antidepressant medications reduces the efficacy of interferon (IFN)-based therapies in patients with hepatitis C (HCV) undergoing antiviral treatment [51]. Kraus *et al.* aptly responded by emphasizing the role for serotonin: 1) in the hypothesized etiology of IFN-induced depression and 2) in the use of selective serotonin reuptake inhibitor (SSRI) antidepressants to treat IFN-induced depression [52]. Serotonin's role in liver health and regeneration is an additional consideration. We report that patients with HCV who achieved sustained viral responses (SVRs) six months following the completion of therapy had significantly higher serotonin levels prior to and during IFN therapy, as compared to patients who didn't achieve SVRs (Table **2**; Fig. **1A**). Further, patients who were prophylactically prescribed SSRIs prior to IFN therapy displayed significantly lower serotonin levels than patients in the placebo group (Fig. **2A**). Although patients in the SSRI group were not statistically less likely to achieve SVRs, as compared to patients in the placebo group, the SVR rate in the SSRI group was notably lower when compared with the placebo group [7/19 (36.8%) and 10/20 (50%), respectively]. Serotonin levels were also significantly correlated with the estimated degree of liver fibrosis (Fig. **3**), suggesting that higher serotonin levels might be a surrogate marker for less advanced liver disease, and consequently improved HCV treatment outcomes. Our findings do not provide an answer to the questions raised by Robaeys *et al.* and Kraus *et al.* [51, 52], but the data do support the need for more research regarding the effects of serotonin on liver health and regeneration in humans.

There exist several limitations to the current results. In general, the majority of patients in this research study experienced only modest increases in depressive symptoms during the course of antiviral therapy. A greater degree of psychopathology may be needed in order to identify and test causal relationships between IFN-α-induced changes in serotonin and tryptophan levels and depression. A concern also exists regarding power, as the sample size was low to detect significant effects. In addition, our study used the APRI as a measure of disease severity, which has been

validated in HCV patients [53, 54] but has limited sensitivity and specificity for values of 0.5 [55]. The APRI is a less reliable estimate of liver disease severity than liver biopsies or measures of portal vein hypertension, which were beyond the resources of this study to obtain. Finally, we recruited our sample of patients from two VA hospitals, and it was composed almost exclusively of men. Thus, the results may not generalize to other clinical populations. Despite these concerns, there are also several strengths of the present study, including the prospective design, randomization scheme, and collection of blood and psychological measures at multiple time points.

CONCLUSIONS

In conclusion, these preliminary findings suggest that higher peripheral serotonin levels are associated with improved antiviral treatment outcomes in patients with HCV. These results should be replicated, as they are based on secondary analysis from the original randomized clinical trial, but may have clinical implications regarding the use of SSRIs in patients with HCV and other liver diseases.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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