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Lower levels of dehydroepiandrosterone sulfate are associated with more advanced liver fibrosis in chronic hepatitis C

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Summary

Chronic infection with the hepatitis C virus induces liver fibrosis, but it is unknown why some patients progress to advanced fibrosis while others remain with mild disease. Recently, an inverse association between serum levels of dehydroepiandrosterone sulphate (DHEA-S) and liver fibrosis in patients with nonalcoholic fatty liver disease was described, and it was postulated that dehydroepiandrosterone (DHEA) has antifibrotic effects. Our aim was to compare serum DHEA-S levels with liver fibrosis in hepatitis C patients. We collected serum samples from hepatitis C patients at the same day they underwent a liver biopsy. S-DHEA was compared to different stages of fibrosis. Binary logistic regression models were applied to evaluate independent variables associated to fibrosis. We included 287 patients (43.9% male). According to fibrosis stages 0, 1, 2, 3 and 4, median serum DHEA-S levels were 103 (26-462), 73 (5-391), 46 (4-425), 35 (6-292) and 28 (2-115) μg/dL, respectively ($P < .001$). Median serum DHEA-S levels were 74 (5-462) vs 36 (2-425) μg/dL for mild (F0-1) vs significant (F2-4) fibrosis, respectively ($P < .001$). Median serum DHEA-S levels were 64 (4-462) vs 31 (2-292) μg/dL for non advanced (F0-2) vs advanced fibrosis (F3-4), respectively ($P < .001$). The same association was found when the subgroup of HCV patients with and without steatosis or steatohepatitis was analysed. The association between lower DHEA-S levels and advanced fibrosis was independent of age, gender, diabetes mellitus, obesity and steatosis. Lower circulating DHEA-S levels are associated with more advanced stages of liver fibrosis in hepatitis C patients.

KEYWORDS
dehydroepiandrosterone sulphate, fibrogenesis, hepatitis C, liver fibrosis, S-DHEA

1 | INTRODUCTION

Hepatitis C chronic infection affects over 170 million people worldwide and is the most common cause of death from liver disease in developed countries.[1,2] A great number of different factors affect the clinical course of chronic hepatitis C and the rate of liver fibrosis progression.[3] Nevertheless, the physiophatologic mechanism that leads to interpersonal heterogeneity of fibrosis evolution is still incompletely understood.[4]

Endocrine hormones control cell functioning and play important interactions with liver metabolism.[5] In the past decades, there

Abbreviations: BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulphate; mRNA, messenger ribonucleic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PDGF, platelet-derived growth factor; TGF-ß, transforming growth factor beta.

has been a growing interest in the hepatoadrenal axis.[6-10] Recently, it was reported that lower levels of dehydroepiandrosterone sulphate (DHEA-S) are associated with more advanced liver fibrosis in patients with nonalcoholic fatty liver disease (NAFLD).[11-13] Initially, it was postulated that insulin resistance mediated this association.[11] Nevertheless, further data suggested that this relationship was independent of insulin levels.[12] So, it remains unclear why low levels of DHEA-S are found in more advanced NAFLD. It is possible that a relative deficiency of this hormone may play a role in fibrosis progression, but with the current available data, it is impossible to extrapolate if the hormone levels are cause or consequence of liver fibrosis.[11-13]

Dehydroepiandrosterone (DHEA) is a hormone produced by human adrenal glands in response to adrenocorticotrophin stimulation (ACTH).[14] Dehydroepiandrosterone sulfotransferase converts DHEA to DHEA-S, which is the most abundant circulating adrenal steroid hormone in humans.[15,16] The serum levels of DHEA-S reach a peak during the third decade of life[17] and thereafter decline gradually, so that by the age of 70, the concentrations are less than 20%-30% of corresponding values in young adults.[18-22] DHEA-S has a longer half-life and lesser circadian variability than DHEA.[19,21] In the past, DHEA-S was thought to function only as a precursor to other active androgens and estrogens.[23] However, new data suggest that DHEA and DHEA-S bind specific cellular receptors exerting itself hormone activity.[24-28]

The recent data about the association between DHEA-S levels and liver fibrosis in NASH patients brings novel pathways of hepatic fibrogenesis to light.[11-13] As fibrosis is the final outcome of a liver injury process independent of disease aetiology,[29] we hypothesize that the association between DHEA-S and fibrosis may be also present in other liver diseases as hepatitis C. The main objective of this study was to compare the serum levels of DHEA-S with liver fibrosis in chronic hepatitis C patients.

2 | MATERIALS AND METHODS

2.1 | Design and overview

This is a single centre (Federal University of Rio de Janeiro, Rio de Janeiro, Brazil) and cross-sectional research. This study received IRB approval at the Independent Ethics Committee in Research from the University Hospital of the Federal University of Rio de Janeiro. It is in conformance with the 1975 Helsinki Declaration. All participants gave written informed consent.

2.2 | Study population

During a period of 36 months, all consecutive patients with chronic hepatitis C scheduled for liver biopsy at the Gastroenterology Department of Federal University of Rio de Janeiro were invited for enrolment in the study. The inclusion criteria were detection of hepatitis C virus RNA in blood serum and age between 18 and 75 years old. The exclusion criteria were concomitant endocrine disease (except compensated type 2 diabetes mellitus and treated hypothyroidism), others chronic liver diseases (including hepatitis B virus infection), history of hormone replacement (except contraceptive pills, levothyroxine and insulin), chronic renal disease on haemodialysis or creatinine clearance below 30 mL/min, transplant recipients and human immunodeficiency virus (HIV) infection. A detailed alcohol consumption history was obtained from all participants, and only patients with ethanol use of less than 20 g/day were enrolled.

Demographic (age, gender, race) and clinical (diabetes mellitus, weight, height, body mass index (BMI) and waist circumference) variables were obtained from anamnesis, physical examination and medical records. Body mass index was calculated using the following formula: weight (in kilograms)/height^2 (in metres). Obesity was defined as BMI ≥ 30 kg/m^2. Waist circumference was measured midway between the lowest rib and the superior border of the iliac crest at the end of normal expiration with an inelastic measuring tape. Abdominal obesity was defined in men as waist circumference of 102 cm or more and in women as 88 cm or more.

2.3 | Clinical laboratory parameters

Venous blood was drawn after a 12 hours fasting at the same day of the liver biopsy (blood was collected before the biopsy). Laboratory evaluation included platelets count and measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ-glutamyltransferase (γGT), total bilirubin, albumin, fasting glucose, creatinine and thyroid-stimulating hormone. These parameters were measured using the standard laboratory techniques of clinical biochemistry laboratories.

All patients underwent the determination of DHEA-S levels performed by electrochemiluminescence immunoassay (ECLIA—Roche Diagnostics®, Rotkreuz, Switzerland) at the same laboratory. A duplicate analysis was performed in the first 125 patients to validate the quality of laboratory technique.

2.4 | Histology

Ultrasound-guided percutaneous liver biopsy was performed in all patients using a 15 cm × 14 G or 15 cm × 16 G trucut needle. The liver specimens were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin, Masson trichrome and reticulin silver stain.

All histological analyses were performed by 2 experiment hepatopathologists of the Pathology Department from Federal University of Rio de Janeiro. Both were blinded to all the other variables of the study, except the presence of comorbidities. Only liver specimen with at least 1 cm length and 6 or more portal tracts were considered adequate for histological evaluation.

The liver fibrosis and necroinflammatory activity were evaluated semiquantitatively according to the METAVIR scoring system.[30,31] Fibrosis was staged on a 5-point scale ranging from 0 to 4 according to METAVIR scoring system. Furthermore, for statistical analyses, the patients were categorized according to amount of liver fibrosis: mild fibrosis (F0 or F1) vs significant fibrosis (F2-F4) and nonadvanced fibrosis (F0-F2) vs advanced fibrosis (F3 or F4).

The presence of steatosis was also evaluated as percentage of fat in the hepatocytes and classified as absent if there is less than 5% (S0), as grade 1 if it is between 5 and 33% (S1), as grade 2 if it is between 34 and 66% (S2) and as grade 3 it is more than 66% (S3) following Brunt classification. The diagnosis of nonalcoholic steatohepatitis (NASH) was defined by the presence of steatosis and hepatocyte injury (typically ballooning degeneration) and lobular inflammation (typically in acinar zone 3) with or without fibrosis.

2.5 | Statistical methods

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Continuous (quantitative) variables were summarized with means and standard deviations or median with maximum and minimum values. Categorical (qualitative) variables were reported as absolute and relative frequency.

Statistical differences among continuous data were determined using Kruskal-Wallis or Mann-Whitney tests. Statistical differences among categorical data were determined using chi-square test or Fisher's exact probability test when necessary.

Binary logistic regression models were applied to evaluate the variables independently associated with advanced fibrosis. A level of significance of 0.05 was adopted.

3 | RESULTS

3.1 | Patient characteristics

A total of 287 patients were included of which 43.9% were male, 44.3% were white, 80.8% were genotype 1, and the mean age was 51 ± 11 years. Table 1 summarizes the clinical, laboratory and histological data of the population.

The mean length of the liver specimen obtained by biopsy was 15 ± 3 mm, and the mean number of portal tracts in the sample was 15.2 ± 5.9.

Regarding the METAVIR score, the most frequent fibrosis stage was F1 (51%), followed by F2 (23%), F3 (16%), F4 (7%) and F0 (3%). In fibrosis categorization mild (F0-1) vs significant (F2-4), 54% were mild, and 46% were significant. In fibrosis categorization nonadvanced (F0-2) vs advanced (F3-4), 77% were nonadvanced, and 23% were mild. In this sample, older age, presence of DM, obesity based on BMI and abdominal obesity were associated with more advanced fibrosis. Higher means of ALT, AST, γGT, alkaline phosphatase and total bilirubin were seen more frequent in more severe fibrosis groups. The same was shown to lower means of platelets and albumin (Table 1).

Steatosis was present in 43.1% of patients and NASH in 7.7%. Both steatosis and NASH were associated with more severe fibrosis (Table 1).

3.2 | DHEA-S analysis

A duplicate DHEA-S dosage was performed in the first 125 patients. The intraclass correlation coefficient was 0.998 with IC 95% 0.995-0.999, ($P < .001$).

The median DHEA-S was lower in female (39.4 μg/dL) than in male (90.1 μg/dL; $P < .001$). In the study population, 35.2% of patients had DHEA-S levels below normal range for age and gender.

3.3 | Comparative analysis of serum DHEA-S and fibrosis

The comparative analysis between median DHEA-S levels and fibrosis is shown in Table 1. There was an important inverse association of DHEA-S levels and fibrosis stage. Lower median DHEA-S levels were found as more advanced was the fibrosis stage as illustrated in Figure 1. The median of serum DHEA-S was 103 (26-462), 73 (5-391), 46 (4-425), 35 (6-292) and 28 (2-115) μg/dL for fibrosis stages 0, 1, 2, 3 and 4, respectively ($P < .001$). In F4 subgroup, 60% of the patients presented serum DHEA-S below normal range compared to 45.7% in F3, 40.9% in F2, 27.2% in F1 and 12.5% in F0 ($P < .001$; Figure 2).

When fibrosis was evaluated according to both categorizations, the statistically significant inverse correlation was maintained between the median of DHEA-S and fibrosis (Table 1). Median serum DHEA-S levels were 74 (5-462) and 36 (2-425) μg/dL for mild (F0-1) and significant (F2-4) fibrosis, respectively ($P < .001$). Median serum DHEA-S levels were 64 (4-462) and 31 (4-462) μg/dL for nonadvanced (F0-2) and advanced fibrosis (F3-4), respectively ($P < .001$). In the significant fibrosis subgroup (F2-4), 45.4% of patients presented serum DHEA-S below normal range compared to 26.5% in mild fibrosis (F0-1; $P < .001$). In the advanced fibrosis subgroup (F3-4), 50% of patients had low DHEA-S compared to 26.5% in nonadvanced subgroup (F0-2; $P = .004$).

To exclude the impact of steatosis or NASH on the association between serum DHEA-S and fibrosis, a subanalysis was performed only in the subgroup of patients without steatosis or steatohepatitis (Table 2). Median serum DHEA-S levels were 120 (59-462), 76 (5-391), 68 (7-425), 36 (6-292), 25 [4-31] μg/dL for fibrosis stages 0, 1, 2, 3 and 4, respectively ($P = .005$). Lower serum DHEA-S was also associated with more severe fibrosis in both fibrosis significant and advanced fibrosis groups (Table 2).

Several multivariate logistic regression models were run to evaluate if the association of DHEA-S levels with advanced fibrosis (F3-4) was confounded by age, gender or clinical comorbidities (DM and obesity). As shown in Table 3, the unadjusted (model 1) association of DHEA-S levels with advanced fibrosis remained highly significant when adjusted by age (model 2) and gender (model 3) and, thereafter, for clinical comorbidities (model 4-7).

4 | DISCUSSION

Hepatitis C chronic infection induces liver fibrosis, nevertheless it still remains unknown why some patients progress to advanced fibrosis and other stay stable with only mild fibrosis level. The heterogeneity of liver fibrosis evolution is also observed in other liver diseases as NAFLD, suggesting that a common pathway may exist independent of the insulting aetiology.

TABLE 1 Clinical, laboratory and histological characteristics of the study population

Variable	Population	F0	F1	F2	F3	F4	*P*-value	F0-1	F2-4	*P*-value	F0-2	F3-4	*P*-value
N	287	8 (3%)	147 (51%)	66 (23%)	46 (16%)	20 (7%)		155 (54%)	132 (46%)		221 (77%)	66 (23%)	
Gender													
Male	43.9%	1.0%	21.3%	11.5%	7.3%	2.8%	.79	22.3%	21.6%	.19	33.8%	10.1%	.55
Female	56.1%	1.7%	30.0%	11.5%	8.7%	4.2%		31.7%	24.4%		43.2%	12.9%	
Age (years)	51 ± 11	49 ± 11	48 ± 12	54 ± 10	53 ± 9	56 ± 7	.001	48 ± 12	54 ± 9	<.001	50 ± 12	54 ± 8	.01
Comorbidities													
DM	16.7%	0%	9.5%	25.8%	32.6%	10%	.001	9.0%	25.8%	<.001	14.0%	25.8%	.02
BMI ≥ 30 kg/m^2	20.9%	14.3%	17.3%	19.7%	28.6%	35%	.26	17.1%	25.2%	.07	17.9%	30.6%	.02
Abdominal obesity	52.6%	57.1%	46.4%	55%	67.4%	55%	.18	46.9%	59.3%	.02	49.3%	63.5%	.03
Laboratory data													
AST (IU/L)	66 ± 50	29 ± 8	46 ± 29	70 ± 39	94 ± 61	154 ± 65	<.001	45 ± 28	91 ± 59	<.001	52 ± 34	112 ± 68	<.001
ALT (IU/L)	97 ± 67	53 ± 18	73 ± 46	110 ± 63	128 ± 79	185 ± 78	<.001	72 ± 46	128 ± 75	<.001	83 ± 54	146 ± 83	<.001
γGT (mg/dL)	120 ± 37	76 ± 71	88 ± 106	126 ± 146	191 ± 186	194±91	<.001	87 ± 105	159 ± 158	<.001	99 ± 120	192 ± 163	<.001
AP (IU/l)	101 ± 37	97 ± 27	95 ± 35	102 ± 34	112 ± 31	128 ± 53	.001	95 ± 35	109 ± 37	.001	97 ± 35	117 ± 39	<.001
Platelets (x10^3/mm^3)	204 ± 64	271 ± 76	223 ± 61	193 ± 56	170 ± 45	145 ± 60	<.001	225 ± 62	178 ± 55	<.001	216 ± 62	163 ± 51	<.001
Albumin (g/dL)	4.0 ± 0.37	4.1 ± 0.2	4.1 ± 0.3	3.9 ± 0.4	3.9 ± 0.3	3.7 ± 0.3	<.001	4.1 ± 0.3	3.9 ± 0.4	<.001	4.0 ± 0.3	3.9 ± 0.3	.001
Total bilirubin (mg/dL)	0.6 ± 0.3	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.2	0.9 ± 0.5	<.001	0.5 ± 0.2	0.7 ± 0.3	.001	0.6 ± 0.2	0.7 ± 0.3	.005
DHEA-S	57 (2-462)	103 (26-462)	73 (5-391)	46 (4-425)	35 (6-292)	28 (2-115)	<.001	74 (5-462)	36 (2-425)	<.001	64 (4-462)	31 (2-462)	<.001
Histological													
Steatosis	43.1%	25%	32.6%	52.3%	50%	83.3%	<.001	32.2%	55%	<.001	38.2%	59.4%	.002
Steatohepatitis	7.7%	0%	4.1%	7.6%	15.2%	22.2%	.01	3.9%	12.3%	.008	5%	17.2%	.003

Results are presented as percentages for qualitative data or as means with standard deviation for quantitative data.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; AP, alkaline phosphatase; TSH, thyroid stimulating hormone; BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate.

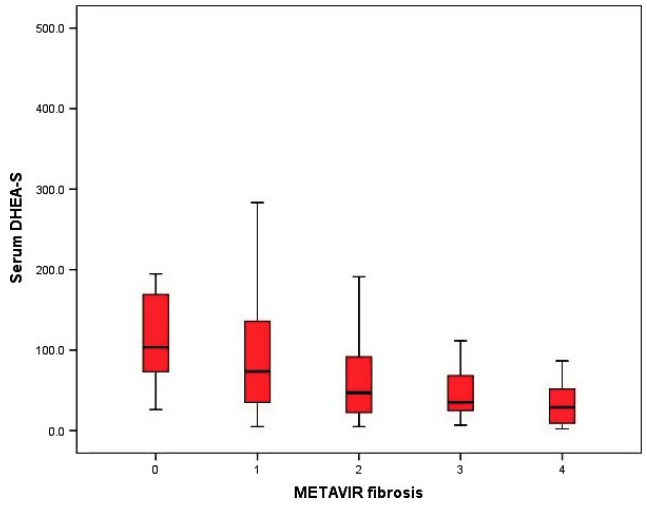

FIGURE 1 Variations in DHEA-S leves with fibrosis stage. A "dose-effect" of lower DHEA-S and severe fibrosis was observed with median of DHEA-S of 103 μg/dL (26-462), 73 μg/dL (5-391), 46 μg/dL (4-425), 35 μg/dL (6-292) and 28 μg/dL (2-115) for fibrosis stages 0,1,2,3 and 4, respectively (n = 287)

between DHEA-S and liver fibrosis in patients with chronic hepatitis C, in order to investigate if this association is disease specific or part of a broader fibrotic mechanism. We analysed the DHEA-S levels in 287 patients with chronic hepatitis C. Liver fibrosis was assessed by liver biopsy in all subjects. Significant lower levels of DHEA-S were found in more severe liver fibrosis (Table 1). There was a stepwise effect of lower median hormone levels in more advanced fibrosis stages (Figure 1). Furthermore, 60% of patients with METAVIR fibrosis F4 had low DHEA-S levels in the hypoadrenal range. When fibrosis was categorized in both mild vs significant and nonadvanced vs advanced, the association with low DHEA-S levels remained highly significant. This is the first time that association is described in a large cohort of chronic HCV patients.

As age and gender can influence DHEA-S levels. We used regression models adjusted for these variables to exclude that they were confounders. As illustrated in Table 3, the unadjusted association of DHEA-S levels with severity of liver fibrosis remained highly significant when adjusted for age (model 2) and gender (model 3).

To investigate if the association described in our study was mediated by steatosis (43%) or NASH (7.7%), we also evaluated

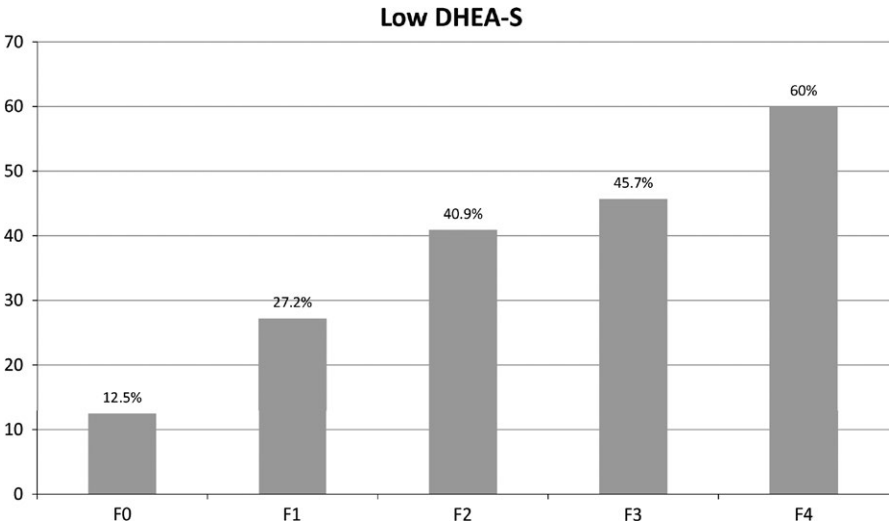

FIGURE 2 Frequency of DHEA-S below normal range adjusted for age and gender (n = 287; *P* = <.001)

Charlton et al[11] were pioneers describing lower DHEA-S levels in advanced NAFLD. The study also analysed a control group with cholestatic liver disease and described that the association was not reproducible in this population. This led to a conclusion that the relationship between DHEA-S and NAFLD severity was disease-specific, possibly mediated by insulin resistance. Two years later, Sumida et al[12] validated the association between DHEA-S and NAFLD in Japanese patients and went further studying models of insulin resistance (HOMA index and QUICKI model). Their findings suggested that insulin resistance did not mediate that association. Moreover, Koehler et al studied a population of obese patients and confirmed both Charlton et al. and Sumida et al findings.[13]

As the physiopathology of liver fibrogenesis is different in NAFLD and cholestatic diseases, we sought to evaluate the association

TABLE 2 Comparative analysis between serum DHEA-S and fibrosis in patients without steatosis or Nonalcoholic steatohepatitis (n = 287)

	Median	Min	Max	*P*-value
F 0	120	59	462	
F 1	76	5	391	
F 2	68	7	425	.005
F 3	36	6	292	
F 4	25	4	31	
F0-F1	81	5	462	
F2-F4	48	4	425	.005
F0-F2	77	5	462	
F3-F4	33	4	292	.002

TABLE 3 Logistic regression models of the association between DHEA-S levels and advanced fibrosis (F3-4)

Variables	OR	95% IC	*P*-value
Model 1			
DHEA-S	0.989	0.983-0.995	<.001
Model 2			
DHEA-S	0.989	0.983-0.996	.002
Age	1.008	0.977-1.039	.619
Model 3			
DHEA-S	0.987	0.980-0.994	<.001
Age	1.004	0.974-1.036	.787
Gender (male)	1.779	0.952-3.323	.071
Model 4			
DHEA-S	0.987	0.980-0.995	.001
Age	1.001	0.969-1.033	.970
Gender (male)	1.733	0.925-3.344	.086
DM	1.628	0.805-3.292	.175
Model 5			
DHEA-S	0.988	0.981-0.995	.001
Age	0.996	0.964-1.029	.798
Gender (male)	2.017	1.034-3.937	.040
DM	1.662	0.801-3.450	.173
BMI ≥ 30 Kg/m^2	0.525	0.258-1.065	.074
Model 6			
DHEA-S	0.988	0.980-0.995	.001
Age	0.999	0.966-1.033	.948
Gender (male)	2.421	1.161-5.047	.018
DM	1.689	0.804-3.545	.166
BMI ≥ 30 Kg/m^2	0.541	0.249-1.175	.120
Abdominal obesity	1.375	0.658-2.871	.397
Model 7			
DHEA-S	0.989	0.981-0.997	.004
Age	0.995	0.961-1.029	.762
Gender (male)	2.480	1.160-5.301	.019
DM	1.861	0.872-3.970	.108
BMI (≥30 Kg/m^2)	0.555	0.249-1.237	.150
Abdominal obesity	1.264	0.587-2.721	.549
Steatosis	1.987	1.028-3.840	.041

DM, diabetes mellitus; DHEA-S, dehydroepiandrosterone sulphate; BMI, body mass index.

the subgroup of hepatitis C patients without steatosis or NASH. The association still remained highly significant, showing that the relationship between DHEA-S and fibrosis is independent of NAFLD.

To determine if the significant association of DHEA-S levels and advanced fibrosis was confounded by the presence of clinical comorbidities as obesity and DM, several multivariated logistic regression models were run (Table 3; models 4-6). The unadjusted association of DHEA-S levels with advanced fibrosis (model 1) remained highly significant when adjusted for all this variables (model 4-6). Finally, steatosis was also included in the last model and did not alter the primary association (model 7).

The present study shows that the relationship between serum S-DHEA and fibrosis is not disease specific. This finding suggests that there might exist an unknown link between adrenal function and liver fibrogenesis.

Based on these data our hypothesis is that lower DHEA-S levels exert an interspecific permissive effect on liver fibrosis because of the antifibrotic properties of this hormone. There is consistent data in literature suggesting that DHEA-S has antifibrotic effect. It was described that DHEA-S levels were decreased both in serum and bronchoalveolar lavage of patients with idiopathic pulmonary fibrosis (IPF) compared to healthy controls.[32] The antifibrotic effect of DHEA in lungs is associated with decrease in fibroblast proliferation, increase in fibroblast apoptosis, decrease in PDGF-induced fibroblast migration, decrease in TGFß-induced fibroblast to myofibroblast differentiation and decrease in collagen production.[32] There is also evidence that DHEA downregulates cardiac fibrosis by attenuating procollagen type I mRNA expression and, consequently, procollagen type I synthesis in the cellular matrix.[33] Furthermore, in rats, DHEA oral supplementation was able to inhibit cardiac fibrosis.[33] Finally, in animal models of induced obstructive jaundice, DHEA-treated rabbits had no hepatocellular fibrosis or necrosis, milder mononuclear inflammation in the portal region and less hepatocyte degeneration when compared to control animals, suggesting that DHEA may have both anti-inflammatory and antifibrotic activity in liver.[34]

So far, there is no other report of the association between DHEA-S levels and severity of liver fibrosis in a large cohort of patients with hepatitis C. Until now, only 2 small studies evaluated the levels of DHEA-S in hepatitis C patients but did not compare with liver fibrosis stages.[35,36]

There is still a long way to go through until we understand the importance of adrenal function on liver fibrosis. As the association between DHEA-S and fibrosis is proved to exist in 2 different liver diseases, and as there is enough data suggesting an antifibrotic effect of DHEA, we hypothesize that DHEA-S is a hormone that plays a role in modulating liver fibrogenesis process. Our findings strength further studies to evaluate the use of DHEA replacement as a new therapeutic target to refrain liver fibrosis.

Another application of this data to medical practice is the use of DHEA-S measurement as part of noninvasive laboratory models to predict liver fibrosis stages. Recently, Tokushige et al[37] proposed this use of S-DHEA and other metabolomics in NAFLD patients. The present study brings into light the possibility of the use of S-DHEA for this purpose also in patients with chronic hepatitis C. The main limitation of this study is that its design did not allow evaluating if lower levels of DHEA-S are cause or consequence of advanced

fibrosis. In contrast, there are strong positive points to highlight about the methodology: large amount of patients, fibrosis score analysed by liver biopsy, excellent quality of liver specimens, same day S-DHEA dosing and liver biopsy, mutivariated models, among others.

In conclusion, lower levels of DHEA-S are present in hepatitis C advanced liver fibrosis patients, and this association is independent of age, gender, DM, obesity and steatosis.

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DISCLOSURES

All authors have nothing to disclosure.

AUTHOR CONTRIBUTIONS

João Marcello de Araujo Neto involved in study design; acquisition of data; analysis and interpretation of data; drafting of the manuscript. Cristiane Alves Villela-Nogueira performed critical revision of the manuscript for important intellectual content. Renata de Mello Perez performed critical revision of the manuscript for important intellectual content. Henrique Sérgio Moraes Coelho involved in study concept and design. Maria Chiara Chindamo performed acquisition of data. Guilherme Ferreira da Motta Rezende performed acquisition of liver biopsy data. Adriana Marques Caroli de Freitas Bottino involved in analysis and interpretation of pathology data. Vera Lucia Nunnes Pannain carried out analysis and interpretation of pathology data. Luiz Fernando Bruzzi Porto performed analysis and interpretation of laboratory data. Ronir Luiz involved in statistical analysis.

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