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HEPATOLOGY

Androgen profiles among Egyptian adults considering liver status

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Abstract

Background and aim: Hepatitis C virus (HCV) and environmental hepatotoxins may have an indirect influence on health by altering the synthesis and function of hormones, particularly reproductive hormones. We aimed to evaluate liver diseases and sex steroid hormones in Egypt, which has the highest prevalence of HCV worldwide.

Methods: We measured markers of hepatitis B virus (HBV), HCV and schistosomiasis infection as well as liver function in 159 apparently healthy subjects. We measured total testosterone (T), sex-hormone binding globulin (SHBG) and albumin, and calculated the free androgen index.

Results: Anti-HCV antibodies were detected in 51% of men and 42% of women. Based on HCV reverse transcription PCR (RT-PCR) of 44 men and 33 women, 11% of men and 21% of women showed HCV viremia. There was schistosomiasis in 25% of men and 9% of women, and mixed HCV viremia and schistosomiasis in 57% of men and 52% of women. Compared with men with schistosomiasis only (mean 593.3 ± 73.4 ng/dL), T was higher in men with mixed HCV viremia and schistosomiasis (mean 854.5 ± 47.9 ng/dL; $P = 0.006$) and men with mixed chronic HCV and schistosomiasis (mean 812.1 ± 43.3 ng/dL; $P = 0.001$). Men with mixed chronic HCV and schistosomiasis had also significantly higher SHBG (mean 57.7 ± 3.9 ng/dL) than males with schistosomiasis only (mean 34.8 ± SE 4.5 ng/dL; $P = 0.0003$).

Conclusion: Future investigations should consider that a high prevalence of asymptomatic liver disease may alter associations between hormone concentrations and chronic disease etiology.

Introduction

Liver insults may have an underappreciated effect on the health of populations by compromising hormone status, particularly sex steroid hormones. In the liver, cholesterol, the precursor for sex steroid hormones, undergoes a series of cytochrome P450 enzyme-mediated conversions to testosterone (T) and estradiol (E2). Also synthesized in the liver are albumin and sex-hormone binding globulin (SHBG),[1,2] the carrier proteins which bind to the sex steroid hormones and mediate the amount of bioavailable hormone. Though androgens can reduce hepatic synthesis of SHBG and estrogens stimulate SHBG synthesis,[2] other factors also alter binding protein status. Hepatitis infections and aging increase circulating SHBG concentrations, and obesity, hypothyroidism and hyperinsulinemic states decrease circulating SHBG levels.[2]

Chronic disease patterns, especially diseases that are hormone dependent, may differ because of infection exposures that affect the liver. Gynecomastia has been reported in men with chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC).[1–4] While E2 and estrone (E1) concentrations are typically normal or even mildly elevated in affected men, T and dihydrotestosterone (DHT) levels are decreased, leading to elevated estrogen : testosterone ratios.

Chronic disease patterns may be particularly altered in people in Egypt and the Middle East because of compromised liver status (and, by extension, altered sex steroid production). Two viral conditions contribute to impaired liver status in Egypt. El-Khoby[5] and colleagues estimated the prevalence of the liver disease-causing parasite *Schistosoma mansoni* in five northern Egyptian governorates was 36% and the prevalence of the urinary disease-causing agent, *S. hematobium*, was 8% in four southern Egyptian governorates. Further, the majority of the world's 350–400 million hepatitis B carriers live in the Middle East, Africa and Asia.[6] Prior to the introduction of HBV vaccination,[7] the HBV carrier rate in Egypt ranged from 18 to 60%.[8] Egypt also has the highest

prevalence of hepatitis C virus (HCV) worldwide, due in part, although not limited to, the intravenous tartar emetic administration that had been incorporated into the nationwide anti-schistosomal campaign in the 1960s to 1980s.[5,9] As a result, approximately 8–10 million Egyptians have been exposed to the virus and, of these, 5–7 million currently display active viral infection.[10] In studies assessing the prevalence of HCV among 'apparently healthy' Egyptian blood donors, the estimated prevalence of HCV infection is 15%[8] compared with 3% worldwide.[11]

This pattern of liver compromise may contribute to the unique patterns of high incidence of young-onset hormone-related cancers among apparently healthy Egyptian populations. Our research over the past 10 years has documented the unusually high incidence of young-onset colorectal and pancreatic cancers in Egypt.[12–15] This high incidence of young-onset cancers has not been linked to specific environmental exposures or hereditary factors.[16,17] Furthermore, recent population-based cancer registration studies from Egypt have also documented that liver cancer rates have increased significantly in Egypt and have become the second most common cancer subsequent to the increasing rates of HCV infection.[18,19] Colorectal, pancreatic and liver cancers are hormone-dependent cancers, and the increasing and unusual age distributions of those cancers were the impetus for our conducting this study.

In anticipation of studies of hormone-dependent chronic diseases, including cancers and heart disease, we evaluated the frequency of impaired liver function in relation to serum and urinary hormone levels in Egyptians who are 'apparently healthy'.

Methods

Research site and population

This study was conducted in Fakkous, Egypt (a town with 150 000 individuals) and eight surrounding villages, for a total population of 450 000 individuals. At the time of our recruitment, the community medical center was initiating a health education program and, as a result, had updated the census of the population; this provided the sampling frame for recruiting our study participants.

This study was conducted in 225 'apparently healthy' males and females who were randomly selected from a listing of healthy adult residents aged 18–80 years of age without a diagnosis of liver disease. Women were excluded for pregnancy, breastfeeding or use of exogenous hormone therapy (including tamoxifen therapy and fertility therapy) at the time of recruitment ($n = 60$), as well as refusal to venipuncture, leading to a study enrollment of 159 participants (78 males and 81 females). Written or oral consent was obtained from all subjects prior to enrollment using a protocol was approved by the University of Michigan Institutional Review Board and the Cairo University National Cancer Institute (NCI-Cairo) and Fakkous Cancer Center human subject review committees.

Data collection

Standardized questions from National Health and Nutrition Examination Survey (NHANES) family questionnaires[20] were used to gather information about sociodemographic characteristics; smoking and work history; environmental and occupational exposures; medical history; and menstrual and female reproductive history. The Arabic translation has been tested for reliability in previous Egyptian studies.[21–23] Following the interview, weight and height were measured and data used to calculate body mass index (BMI; kg/m^2).

Laboratory methodology

Venipuncture was performed following a minimum 6-h fast and blood collected in cyrovials with and without preservative. Blood was taken to the Fakkous Cancer Center, processed, frozen and transferred to NCI-Cairo where the storage temperature was reduced to −80°C. The biological specimens were hand-carried on dry ice to the University of Michigan School of Public Health's CLASS laboratory in the USA.

Hormone analysis

Hormone assays were conducted using an ACS-180 automated analyzer (Bayer Diagnostics, Norwood, MA, USA). Serum T concentrations were measured using a modified ACS-180 total testosterone assay with increased precision in the lower ranges. The assay involves competitive binding of a DMAE-labeled testosterone derivative to a rabbit polyclonal antitestosterone antibody premixed with monoclonal antirabbit IgG antibody immobilized on solid-phase paramagnetic particles. Inter- and intra-assay coefficients of variation were 8.7% and 6.8%, respectively. To assess the activity of non-bound T, the free androgen index was calculated: FAI = (total T [ng/dL] ÷ SHBG [nmol/L]). Serum E2 concentrations were measured with a modified ACS-180 (E2-6) immunoassay (Bayer Diagnostics). Inter- and intra-assay coefficients of variation were 10.2% and 6.4%, respectively, over the assay range. Estrone (E1) concentrations were measured with an estrone enzyme immunosorbent assay (RSL/ICN Diagnostics, Carson, CA, USA). Inter- and intra-assay coefficients were 5.0% and 3.1%, respectively.

Liver status

Quantitative colorimetric determination was used to assess albumin levels (Stanbio Albumin LiquiColor Procedure no. 0285; Stanbio Laboratory, Boerne, TX, USA). Total bilirubin (TBR) and direct bilirubin (DBR) levels were colorimetrically determined (Diamond Diagnostics, Holliston, MA, USA). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were assessed by standard methods (Randox Laboratories, Antrim, UK). Alkaline phosphatase (AP) was quantitatively determined by spectrophotometry (Stanbio Alkaline Phosphatase LiquiColor® Procedure no. 2900; Stanbio Laboratory). Manufacturer-established inter- and intra-assay coefficients of variation on three commercial controls for 5 consecutive days for these assays were 1.7%, 1.2% and 0.9%, and 1.8%, 1.0% and 1.3%, respectively.

Hepatitis B surface antigen (HBsAg) was qualitatively assessed by chromographic assay (QUALITEST HBsAg One Step Hepatitis B Surface Antigen Test Device [Serum/Plasma]; QUALITAS Bioscience GmbH, Germany) with a relative sensitivity >99.0%, a relative specificity >96.9% and an accuracy of 98.3%. The qualitative HCV Spot Test (ClinPro International LLC, Union City, CA,

USA) incorporates a reaction between serum anti-HCV antibodies and membrane-embedded viral proteins in a color response to the application of a horseradish peroxidase conjugate.

Schistosomal seropositivity was qualitatively assessed by indirect hemagglutination (Laboratoires Fumouze, France), which does not selectively differentiate between species of *S. mansoni*, *S. hematobium* or *S. intercalatum*. To test for HBV viremia, PCR amplification was performed on extracted and purified serum[24,25] using 1× reaction buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl at pH 9.0, 1.5 mol/L $MgCl_2$), 0.1% Triton ×100, 0.2 mmol/L dNTPs, 50 pmol of each primer (primer RB-6A, TTGCCTTCTGAACT TCTTTCC; primer RB-6B, TCTGCGAGGCGAGGGAGTTCT) and 2.5 U *Taq* polymerase (Promega Biotech, Madison, WI, USA). Thermocycling with a Thermal Cycle 480 (Perkin Elmer, Waltham, MA, USA) included an initial denaturation at 95°C for 5 min, followed by 35 amplification cycles at 95°C with 1 min for denaturation, 1 min at 55°C for annealing and 2 min at 72°C for extension. PCR product (10 μL) was subjected to electrophoresis on a 2% agarose gel in Tris-acetate EDTA (pH 8.0) stained with ethidium bromide and visualized under UV transillumination.

For reverse transcription,[25–27] a 25 μL reaction mixture containing 67 mmol/L Tris-HCl (pH 8.8), 17 mmol/L ammonium sulfate, 1 μmol β-mercaptoethanol, 6 mmol/L EDTA (pH 8.0), 0.2 mg bovine serum albumin (Boehringer, Columbus, OH, USA), 6 mmol/L $MgCl_2$, 25 ng HCV-6 primer, 0.6 μL dNTPs, 11.5 μL nucleic acid eluate, 200 U Superscript-II RNAase-H reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA) and 20 U RNAase inhibitor (Promega Biotech) was subjected to incubation at room temperature for 5 min, 60 min at 42°C and 5 min at 95°C for RT-II denaturation. The amplification reaction mixture (100 μL) contained 50 mmol/L Tris-HCl (pH 8.3), 20 mmol/L KCI, 1.5 mmol/L $MgCl_2$, 1 mg/mL bovine serum albumin, 200 mmol/L dNTPs, 12.5 μL RT reaction mixture, 100 ng of each primer (RB-6A, RB-6B) and 2.5 U *Taq* polymerase (Perkin Elmer Cetus). After denaturation for 5 min at 95°C, the following sequence was used for 30 cycles: denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min. PCR product (10 μL) was electrophoresed in Tris-acetate EDTA (pH 8.0) on a 2% agarose gel containing ethidium bromide. DNA was transferred to a nitrocellulose membrane in 4 N NaOH buffer, incubated at 80°C for 2–3 h and hybridized with an internal probe.[28]

Ultrasonography

Ultrasound examination was undertaken only on subjects who were serologically positive for HCV or HBV and on the two men who had elevated SGOT and SGPT levels but were negative for both HCV and HBV on serological testing. There were 74 HBsAg and HCV seropositive participants as well as four HCV seronegative enrollees referred for abdominal ultrasound and 67 (86.0%) presented for examination. Increased hepatic size, periportal thickening, hepatic parenchyma changes, dilated portal vein (>12 mm) and irregular hepatic contours were used in the sonographic assessment.

Statistical analysis

Data were entered into the BioDBX version 5 database system and analyzed using the SAS statistical package.[29] Age and BMI data were log transformed to meet the assumptions of normal residuals in statistical modeling. Categorical variables (residence and occupation frequencies between men and women) were evaluated using χ^2 tests of homogeneity. Student's *t*-test was used to test for sex differences in the transformed hormone and liver biomarkers, and Fisher's exact test was used to evaluate HBsAg differences because of small cell sizes. Linear regression analyses with dummy variables were used to relate dependent and independent variables. Odds ratios were used to assess the magnitude of risk of schistosomal infection, chronic HCV and HCV viremia according to gender.

Analysis of covariance with orthogonal contrasts was used to compare mean T, FAI and SHBG differences between three groups: (i) mixed active or chronic HCV/schistosomal infection to no infection; (ii) schistosomiasis only to no infection; (iii) and mixed active or chronic HCV/schistosomal infection to schistosomiasis only, while controlling for age and BMI. Type I error was defined with a P-value < 0.05.

Results

As shown in Table 1, men had greater BMI than women (26.8 ± 3.0 *vs* 25.6 ± 3.9; $P = 0.01$), higher T concentrations than women (706 ± 251 *vs* 52 ± 1.2 ng/dL; $P < 0.0001$), higher FAI (60 ± 24 *vs* 3.7 ± 2.9; $P < 0.0001$) and lower SHBG than women (48 ± 26 *vs* 63 ± 34 nmol/L; $P = 0.0008$).

Serology

Although the population was selected to be apparently healthy and without a diagnosis of liver disease, there was substantial evidence of liver disease, shown in Table 2. In apparently healthy men, potentially impaired liver status could be from mixed anti-HCV and anti-schistosomal antibodies ($n = 32$, 41%), schistosomiasis ($n = 24$, 31%) or HCV ($n = 8$, 10%). There were 14 men (18%) with no evidence of liver impairment ($n = 14$, 18%). In apparently healthy women, potentially impaired liver status could be from mixed infection ($n = 20$, 25%), schistosomiasis ($n = 26$, 32%) or

Table 1 Comparison of study characteristics in men and women

	Men	Women	*P*
Gender	$n = 78$	$n = 81$	0.81
Age (years)	39.4 ± 10.7	41.5 ± 11.6	0.35
BMI (kg/m^2)	26.8 ± 3.0	25.6 ± 3.9	0.01
Testosterone (ng/dL)	706.2 ± 250.5	51.9 ± 1.2	<0.0001
Free androgen index	59.9 ± 23.7	3.7 ± 2.9	<0.0001
Sex-hormone binding protein (nmol/l)	47.7 ± 26.1	63.0 ± 33.6	0.0008
Residence			
Rural	39 (50.0)	32 (39.5)	0.18
Semi-urban	39 (50.0)	49 (60.5)	
Occupation			
Professional	42 (53.9)	28 (34.6)	<0.0001
Nonprofessional	30 (38.5)	15 (18.5)	
Retired or unemployed	6 (7.7)	38 (46.9)	

Data are expressed as mean ± SD, or number (percentage) of participants, as indicated.

Table 2 Comparison of markers of liver status by gender

Measure number (percentage)	Men $n = 78$	Women $n = 81$	P	OR (95% CI)
Chronic infection				
Anti-HCV antibody only	8 (10)	14 (17)	0.12	–
Mixed infection	32 (41)	20 (25)		
Schistosomiasis only	24 (31)	26 (32)		
No infection	14 (18)	21 (26)		
Active infection (viremia)				
HCV viremia only	5 (11)	7 (21)	0.11	–
Mixed infection	25 (57)	17 (52)		
Schistosomiasis only	11 (25)	3 (9)		
No infection	3 (6)	6 (18)		
Anti-HCV antibody				
Positive	40 (51)	34 (42)	–	1.5 (0.78–2.7)
Negative	38 (49)	47 (58)		
HBsAg				
Positive	1 (1)	0 (0)	0.49	–
Negative	77 (99)	81 (100)		
Anti-schistosomal antibody				
Positive	56 (72)	46 (57)	–	1.9 (1.0–3.7)
Negative	22 (28)	35 (43)		
HCV viremia†‡				
Positive	30 (68)	24 (73)	–	0.80 (0.30–2.2)
Negative	14 (32)	9 (27)		
HBV viremia				
Positive	0 (0)	0 (0)	NA	
Negative	1 (100)	0 (0)		

†Missing RT-PCR report for one anti-HCV antibody positive female.
‡Includes four subjects who, while negative on HCV antibody screening, were evaluated for viremia based on elevated liver transaminases ($n = 2$), self-reported HCV ($n = 1$) and HBsAg on HBV screening ($n = 1$).
NA, not applicable.

HCV ($n = 14$, 17%). There were 21 women with no evidence of liver impairment (26%). The levels of aminotransferase, albumin and bilirubin did not differ between the groups, and the observed differences within the groups were not associated with disease severity (Table 3).

Ultrasound findings

There were 66 of 77 individuals who presented for abdominal ultrasound; 24 (15%) displayed hepatomegaly and 15 (9%) splenomegaly. Steatosis, or fatty liver, was observed in 11 (7%) subjects. Periportal thickening, ranging from 'fine' to 'mild', was detected in 27 (17%) individuals. Bright and coarse echotexture was noted in 22 (14%) and 12 (8%), respectively. Evidence of cirrhosis was seen in six (4%) subjects.

Hormone studies in men

Testosterone, FAI and SHBG values in men are shown in Table 4. Based on orthogonal contrasts seen in Table 5, men with mixed chronic HCV and schistosomal infection had statistically significantly higher serum T levels (mean 812.1 ± 43.3 ng/dL) than men with schistosomiasis only (mean 585.1 ± 49.5 ng/dL; $P = 0.001$). Further, men with mixed HCV viremia/schistosomal infection had statistically higher mean T levels compared with men with schistosomiasis (mean 854.5 ± 47.9 *vs* 593.3 ± 73.4 ng/dL; $P = 0.006$).

Men with mixed chronic HCV and schistosomal infection had statistically significantly higher SHBG levels (mean 57.7 ± 3.9 ng/dL) than men with schistosomiasis only (mean 34.8 ± 4.5 ng/dL; $P = 0.0003$), although FAI values were not statistically different (Table 4). Testosterone levels in men with abnormal ultrasound characteristics were not significantly different from levels in men without abnormalities.

Hormone studies in women

Testosterone, FAI and SHBG for women are shown in Table 4. As seen in Table 5, there were no statistically significant mean differences in T or FAI levels in women according to infection subtype. There was a statistically significant difference in SHBG concentrations in women with mixed chronic infection (mean 80.5 ± 7.7 ng/dL) compared with women with schistosomiasis (mean 58.1 ± 6.7 ng/dL; $P = 0.03$).

Mean testosterone levels in women with liver cirrhosis (101 ± 81 pg/mL), coarse echotexture (83 ± 52 pg/mL) or splenomegaly (77 ± 49 pg/mL) by ultrasound were significantly higher than levels in women without ultrasound abnormalities.

Discussion

There are three primary findings in this evaluation. There was consistent evidence of frequent liver infections (past and present) among men and women who were asymptomatic and apparently healthy at the time of recruitment to the study. Second, hormone concentrations as well as SHBG levels were different based on the presence or absence of potential liver impairment. Having HCV and schistosomiasis constituted a distinct group of participants in terms of their biochemical and hormonal profiles, particularly higher testosterone levels. Hepatitis C viral infection may act as a cofactor modulating the effect of schistosomiasis, which leads to chronic liver disease, liver fibrosis and appears to have relatively greater impact on hormone metabolism than viral infection.[30] Men with HCV and schistosomiasis coinfection displayed significantly higher T and SHBG levels compared with men with either condition alone. Women with mixed chronic infection had elevated mean SHBG levels compared with women with schistosomiasis only.

There are few studies that have characterized hormone values in liver disease in men and women. A study of Egyptian men with schistosomiasis with and without cirrhosis reported significantly reduced serum T and luteinizing hormone (LH) concentrations in men characterized as 'infertile' cirrhotics with *S. mansoni* and *S. hematobium* coinfection compared with 'infertile' males with either concomitant infection or *S. mansoni* or *S. hematobium* infection. Significant differences were not observed when they were compared with normal controls or 'fertile' men with mixed *S. mansoni* and *S. hematobium* coinfection.[31] In contrast, a study observed no significant difference in serum T concentration in 38 Brazilian men singly infected with *S. mansoni* compared with controls.[32]

High testosterone levels in participants with concomitant HCV and schistosomiasis is consistent with studies from China and

Table 3 Markers of hepatobiliary functions by gender and type of infection

Measure	Men (Intergender) ($\alpha = 0.05$) Mean ± SD	Women (Intragender) ($\alpha = 0.05$) Mean ± SD	(Intergender) *P*	(Intragender) *P*
Chronic infection				
Albumin				
Mixed infection	4.2 ± 0.45	4.1 ± 0.28	0.1043	M: 0.6115
Schistosomiasis only	4.3 ± 0.37	4.4 ± 0.32		W: 0.0572
No infection	4.3 ± 0.24	4.2 ± 0.30		
Direct bilirubin				
Mixed infection	0.18 ± 0.060	0.14 ± 0.029	0.3903	M: 0.7445
Schistosomiasis only	0.15 ± 0.055	0.15 ± 0.12		W: 0.3430
No infection	0.14 ± 0.033	0.18 ± 0.16		
Total bilirubin				
Mixed infection	0.74 ± 0.15	0.60 ± 0.13	0.2728	M: 0.6354
Schistosomiasis only	0.64 ± 0.16	0.58 ± 0.16		W: 0.2122
No infection	0.63 ± 0.12	0.67 ± 0.31		
SGPT				
Mixed infection	17.0 ± 10.6	12.2 ± 4.6	0.7323	M: 0.0757
Schistosomiasis only	16.6 ± 11.5	8.4 ± 5.3		W: 0.0745
No infection	11.6 ± 8.9	10.0 ± 5.1		
SGOT				
Mixed infection	15.9 ± 7.9	12.9 ± 4.9	0.8921	M: 0.7034
Schistosomiasis only	14.2 ± 7.1	9.9 ± 3.4		W: 0.6416
No infection	12.8 ± 6.5	10.0 ± 3.2		
Alkaline phosphatase				
Mixed infection	12.7 ± 4.4	11.2 ± 3.2	0.6530	M: 0.4821
Schistosomiasis only	12.0 ± 2.6	9.1 ± 2.4		W: 0.8063
No infection	11.0 ± 2.8	8.9 ± 1.4		
Active infection				
Albumin				
Mixed infection	4.2 ± 0.42	4.1 ± 0.28	0.9870	M: 0.1934
Schistosomiasis only	4.0 ± 0.52	4.4 ± 0.00		W: 0.0167
No infection	4.3 ± 0.49	3.9 ± 0.24		
Direct bilirubin				
Mixed infection	0.17 ± 0.047	0.14 ± 0.029	0.4396	M: 0.5284
Schistosomiasis only	0.15 ± 0.055	0.14 ± 0.044		W: 0.2814
No infection	0.19 ± 0.023	0.16 ± 0.022		
Total bilirubin				
Mixed infection	0.72 ± 0.14	0.60 ± 0.13	0.7475	M: 0.7102
Schistosomiasis only	0.78 ± 0.1	0.58 ± 0.16		W: 0.1408
No infection	0.78 ± 0.19	0.68 ± 0.059		
SGPT				
Mixed infection	16.6 ± 8.7	12.5 ± 4.5	0.4946	M: 0.2851
Schistosomiasis only	21.7 ± 17.4	8.0 ± 2.0		W: 0.0930
No infection	14.0 ± 7.2	13.7 ± 5.2		
SGOT				
Mixed infection	15.4 ± 6.4	13.1 ± 4.8	0.8100	M: 0.3616
Schistosomiasis only	18.5 ± 12.0	9.0 ± 1.0		W: 0.0877
No infection	13.3 ± 3.1	14.8 ± 5.4		
Alkaline phosphatase				
Mixed infection	12.3 ± 3.1	11.5 ± 3.6	0.2455	M: 0.3214
Schistosomiasis only	14.2 ± 6.7	10.6 ± 2.2		W: 0.9940
No infection	11.9 ± 2.0	10.7 ± 1.2		

Chronic infection: mixed infection (40 M, 34 W), schistosomiasis only (24 M, 26 W) and no infection (14 M, 21 W); Active infection: mixed infection (30 M, 24 W), schistosomiasis only (11 M, 3 W) and no infection (3 M, 6 W). Mixed infection: HCV infection only plus mixed HCV/schistosomiasis infection; Schistosomiasis only: schistosomiasis infection only; No infection, no HCV and no schistosomiasis infection.

Table 4 Serum testosterone, free androgen index and SHBG levels according to type of infection and gender

Measure	Mean ± SD men	Mean ± SD women	Men	*P* (M)†	Women	*P* (W)‡
Chronic infection						
Testosterone (ng/dL)						
Anti-HCV antibody only	727.5 ± 192.0	64.0 ± 31.1	8	0.3113	14	0.5200
Mixed infection	806.6 ± 251.8	48.3 ± 21.5	32		20	
Schistosomiasis only	586.9 ± 216.9	46.5 ± 19.	24		26	
No infection	668.9 ± 254.0	54.0 ± 29.3	14		21	
Free androgen index						
Anti-HCV antibody only	52.7 ± 21.8	4.3 ± 4.3	8	0.3490	14	0.9123
Mixed infection	53.5 ± 21.2	2.7 ± 1.9	32		20	
Schistosomiasis only	64.1 ± 24.1	3.5 ± 2.1	24		26	
No infection	71.4 ± 26.1	4.4 ± 3.5	14		21	
SHBG (nmol/L)						
Anti-HCV antibody only	56.0 ± 28.8	68.2 ± 26.1	8	0.8176	14	0.3313
Mixed infection	59.5 ± 27.7	79.1 ± 42.6	32		20	
Schistosomiasis only	34.8 ± 14.6	58.7 ± 33.3	24		26	
No infection	38.4 ± 24.4	49.7 ± 22.0	14		21	
Active infection						
Testosterone						
Anti-HCV antibody only	734.6 ± 237.8	64.3 ± 16.4	5	0.1255	7	0.9452
Mixed infection	850.4 ± 232.0	51.6 ± 21.4	25		17	
Schistosomiasis only	602.8 ± 243.8	29.4 ± 9.9	11		3	
No infection	715.7 ± 124.6	65.9 ± 46.3	3		6	
Free androgen index						
Anti-HCV antibody only	50.3 ± 17.6	5.1 ± 5.4	5	0.9030	7	0.3863
Mixed infection	53.1 ± 15.7	2.9 ± 1.9	25		17	
Schistosomiasis only	56.9 ± 28.6	1.9 ± 1.8	11		3	
No infection	56.6 ± 31.7	3.8 ± 3.2	3		6	
SHBG (nmol/L)						
Anti-HCV antibody only	56.6 ± 31.2	64.2 ± 25.2	5	0.4200	7	0.7024
Mixed infection	61.1 ± 26.3	78.1 ± 41.3	25		17	
Schistosomiasis only	44.8 ± 29.2	84.6 ± 59.8	11		3	
No infection	55.0 ± 30.9	69.9 ± 30.4	3		6	

Chronic infection: mixed infection (40 M, 34 W), schistosomiasis only (24 M, 26 W) and no infection (14 M, 21 W); Active infection: mixed infection (30 M, 24 W), schistosomiasis only (11 M, 3 W) and no infection (3 M, 6 W). ; †Comparison between the four values of men in the category using ANOVA; ‡Comparison between the four values of women in the category using ANOVA. SHBG, sex-hormone binding globulin.

Taiwan. In those studies, high testosterone levels were identified in follow-up studies of chronic HCV and HBV patients who subsequently developed HCC.[33,34]

Previous studies reported high testosterone levels in follow-up studies of chronic HCV patients who subsequently developed hepatocellular carcinoma (HCC). For example, a nested case–control study of HCC cases based on a follow-up of a cohort of 18 244 men in Shanghai, China,[33] revealed higher testosterone levels in HCC cases than controls. After an average follow up of 5.3 years, HCC cases had significantly higher mean level of testosterone than controls (570 ng/dL *vs* 485 ng/dL in cases and controls, respectively; $P = 0.0005$). A study in Taiwan of 9691 male adults reported that elevated testosterone levels were associated with increased risk of HCC after adjustment for HBV, HCV and other risk factors. Men with testosterone levels in the upper tertile had more than four times higher risk for HCC than men with testosterone levels in the middle and lower tertiles (OR = 4.1; 95% CI, 1.3–13.2; $P = 0.016$). High testosterone levels were associated with HCC in men in China through androgen signaling mechanisms.[34,35]

These study results suggest that testosterone levels could be used as a marker in screening; future studies could capitalize on the results of this study and consider using hormone profile as a screening marker in this population.

Additional studies of Egyptian people with chronic HCV infection and schistosomiasis who exhibit high testosterone levels are needed to see if these higher hormone values present at early-stage hepatic carcinogenesis and if they impact disease progression. Several studies from Egypt have shown that concomitant chronic HCV infection and schistosomiasis present a greater risk for HCC than the independent risk of HCV, HBV or schistosoma alone.[36–38]

This study has strengths, including our access to a representative sample of an apparently healthy population in one of the countries with the highest prevalence of HCV. To our knowledge, this is the first report to address biochemical and hormonal profiles in apparently healthy asymptomatic participants where multiple causes of

Table 5 Orthogonal contrasts to show differences between testosterone, free androgen index and SHBG values in subtypes of infection by gender

Gender	Outcome	Covariate	β	SE	*P* (2 digits)
Chronic infection					
Men					
Testosterone	Chronic	Chronic			
	0	1	–152.4	80.4	0.06
	0	2	74.6	82.7	0.37
	1	2	227.0	65.9	0.00
Free androgen index	Chronic	Chronic			
	0	1	9.5	6.7	0.16
	0	2	1.7	6.9	0.81
	1	2	–7.8	5.5	0.16
SHBG	Chronic	Chronic			
	0	1	–15.2	7.3	0.16
	0	2	7.8	7.5	0.30
	1	2	22.9	6.0	0.00
Women					
Testosterone	Chronic	Chronic			
	0	1	2.8	7.1	0.70
	0	2	8.3	6.7	0.22
	1	2	5.5	6.9	0.43
Free androgen index	Chronic	Chronic			
	0	1	1.6	0.82	0.06
	0	2	0.96	0.76	0.22
	1	2	–0.60	0.79	0.45
SHBG	Chronic	Chronic			
	0	1	–31.2	10.6	0.16
	0	2	–8.9	9.9	0.37
	1	2	22.4	6.0	0.03
Viremia					
Men					
Testosterone	Viremia	Viremia			
	0	1	–138.2	147.9	0.36
	0	2	123.0	158.7	0.44
	1	2	261.2	88.9	0.01
Free androgen index	Viremia	Viremia			
	0	1	10.8	12.1	0.38
	0	2	4.1	13.0	0.75
	1	2	–6.7	7.3	0.36
SHBG	Viremia	Viremia			
	0	1	–13.5	16.4	0.41
	0	2	6.0	17.6	0.74
	1	2	19.5	9.9	0.06
Women					
Testosterone	Viremia	Viremia			
	0	1	16.7	14.1	0.25
	0	2	39.9	20.7	0.07
	1	2	23.2	18.3	0.22
Free androgen index	Viremia	Viremia			
	0	1	0.97	1.1	0.40
	0	2	2.1	1.7	0.21
	1	2	1.2	1.5	0.44
SHBG	Viremia	Viremia			
	0	1	–9.5	21.1	0.66
	0	2	–14.5	30.9	0.64
	1	2	–5.0	27.4	0.86

Chronic infection: mixed infection (40 M, 34 W), schistosomiasis only (24 M, 26 W) and no infection (14 M, 21 W); Active infection: mixed infection (30 M, 24 W), schistosomiasis only (11 M, 3 W) and no infection (3 M, 6 W). SHBG, sex-hormone binding globulin.
Chronic: 0, no infection; 1, mixed chronic HCV and schistosomiasis infection; 2, schistosomiasis only.
Viremia: 0, no infection; 1, mixed HCV viremia and schistosomiasis infection; 2, schistosomiasis only.

liver disease exist. However, we could not explore the true capacity of the binding of testosterone to its receptor. The relatively small sample size in this population with multiple subgroups limited the power to detect some associations. Neither a causal relationship between HBV, HCV, schistosomiasis and liver disease nor a temporality was assessed, as a limitation of the cross-sectional nature of the study; however, the important results of this study would set the stage for a future large-scale study to test the hypothesis of testosterone level modulation in chronic diseases by HCV and schistosomal infection.

In summary, we observed marked biochemical and hormonal changes of androgen profile among HCV–schistosoma-infected participants who were asymptomatic and apparently healthy at the time of recruitment in the study in Egypt. Men with chronic HCV and schistosomiasis coinfection displayed significantly higher testosterone and SHBG levels compared with men with either condition. Future investigations should explore the association between chronic disease etiology and hormonal imbalance in populations with a high prevalence of asymptomatic liver disease.

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