Sex Hormones in Postmenopausal Women with Primary Biliary Cirrhosis

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To evaluate serum sex hormone profiles in nonalcoholic postmenopausal women with liver disease, 25 women with primary biliary cirrhosis (11 in cirrhotic stage) and 46 healthy controls were studied.

The patients had significantly ($p < 0.05$) elevated serum concentrations of estrone and androstenedione and significantly ($p < 0.05$) lower concentrations of estrone sulfate, dehydroepiandrosterone sulfate and 5α-dihydrotestosterone compared with the 46 controls. Serum concentrations of sex hormone binding globulin, testosterone, non-sex hormone binding globulin–bound testosterone and non-protein-bound testosterone did not differ significantly ($p > 0.05$) between primary biliary cirrhosis patients and controls. Patients in the cirrhotic stage had significantly ($p < 0.05$) higher concentrations of sex hormone binding globulin than did controls.

Patients in the cirrhotic stage had significantly ($p < 0.05$) higher sex hormone binding globulin and estrone sulfate levels compared with noncirrhotic patients with primary biliary cirrhosis. Otherwise, no significant differences were observed between cirrhotic and noncirrhotic patients.

The observed changes in steroid concentrations may be a consequence of hepatic dysfunction. (HEPATOLOGY 1991;13:865-869.)

The liver is a sex hormone–responsive organ, and significant changes in hepatic functions are observed during pregnancy and treatment with sex hormones (1-3).

Endocrine disturbances are frequent in patients with chronic liver disease (4-9). In women with alcoholic liver disease, one clinical disturbance is early menopause (8, 10). Changes in sex hormone concentrations are seen in postmenopausal women with chronic alcoholic (11, 12) and nonalcoholic liver disease (13-16). Whether these changes are induced by the liver disease *per se* or by a concomitant ethanol-induced hypothalamic/pituitary/gonadal/adrenal defect is, however, still debated because it has been difficult to estimate the relative contributions of liver disease and ethanol to the observed hormone alterations.

PBC is a chronic nonalcoholic liver disease seen mainly in postmenopausal women. The aim of this study was to study changes in sex hormone concentrations in postmenopausal women with this disease and to study whether these changes are dependent on the degree of liver dysfunction.

SUBJECTS AND METHODS

Patients and Controls. Female patients older than 55 yr with duration of amenorrhea longer than 1 yr, alcohol consumption less than 30 gm daily and clinical and histological findings compatible with PBC according to accepted histological criteria (17) were included. Excluded from the study were patients with HBsAg positivity, hepatic encephalopathy, severe obesity (>100 kg), malignant or systemic diseases, earlier oophorectomy and/or hysterectomy, alcohol consumption in the week before the study and medication with histamine-2-receptor antagonists (14 days before study), spironolactone (14 days before study), glucocorticoids (6 mo before study), sex hormones (1 yr before study) and cytostatics (at any time).

Clinical, menstrual and biochemical data on 25 patients fulfilling the above criteria are given in Table 1. Further, disease stages according to histological classification (17) are given for the PBC patients: stage I, chronic destructive cholangitis; stage II, portal inflammation with ductular proliferation and destruction; stage III, scarring (fibrosis); and stage IV, cirrhosis.

Healthy female volunteers ($n = 46$) older than 55 yr and with amenorrhea for more than 1 yr were included as controls. All had alcohol consumption of less than 30 gm daily, and none had signs or history of liver disease. The same exclusion criteria were used for patients and controls. Age and data on menstrual history are shown in Table 1.

The Helsinki II declaration was adhered to throughout, and the protocol was approved by the regional ethical committees.

Hormone Analyses. Serum samples were drawn from a peripheral vein between 9 AM and 1 PM. Serum concentra-

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TABLE 1. Clinical and biochemical characteristics of controls and postmenopausal women with noncirrhotic and cirrhotic PBC

Characteristics	Controls (n = 46)	PBC (n = 25)	Noncirrhotic PBC (n = 14)	Cirrhotic PBC (n = 11)
Age (yr)	63[a]	61	61	61
	(59-66)	(58-66)	(57-65)	(58-67)
Duration of menopause (yr)	12	11	10	12
	(5-18)	(7-17)	(7-14)	(7-26)
Age at menopause (yr)	50	50	50	49
	(48-53)	(48-51)	(49-51)	(48-51)
Serum bilirubin (μmol/L)		12	9[b]	23
	(5-17)[c]	(7-24)	(6-14)	(12-39)
Serum AST (U/L)		69	48	70
	(10-40)[c]	(35-96)	(35-95)	(44-104)
Serum alkaline phosphatase (U/L)		836	751	1213
	(80-275)[c]	(576-1,914)	(530-1,719)	(626-1,926)
Serum albumin (μmol/L)		597	619	600
	(540-800)[c]	(563-626)	(589-648)	(539-612)
Plasma-coagulation factors 2, 7		1.20	1.25	1.16
and 10 (arb. units)	(0.7-1.3)[c]	(0.98-1.47)	(1.15-1.61)	(0.86-1.38)
Mitochondrial antibody	ND	17 (68%)	7 (50%)[d]	10 (91%)

ND = not done.
[a]Values are medians (with interquartile ranges in parentheses) or numbers (with percentages in parentheses).
[b]$p < 0.05$ (Wilcoxin rank test).
[c]Normal reference limits.
[d]$p < 0.05$ (Fisher's exact test).

tions of steroid hormones were measured as described previously (18, 19). Estrone, estradiol, testosterone, 5α-dihydrotestosterone (DHT) and androstenedione were measured by specific RIAs after ether extraction and Sephadex LH-20/celite column chromatography (20, 21). Non-protein-bound estradiol was measured by centrifugal ultrafiltration–dialysis (22) and non-sex hormone binding globulin (SHBG)–bound estradiol was determined by a modification of the technique described by Tremblay and Dube (23). Binding capacity of SHBG was determined from the binding of ^{3}H-dihydrotestosterone, and the molar concentration of SHBG was calculated from the assumption of one binding site for DHT per SHBG molecule (24). Serum non-protein-bound and non-SHBG-bound testosterone were calculated using the concentrations of testosterone and SHBG and a fixed albumin concentration of 500 μmol/L by means of the law of mass action as described earlier (24). Dehydroepiandrosterone sulfate (DHAS) and estrone sulfate were also analyzed by specific RIAs after further ethyl acetate extraction, solvolysis/hydrolysis and column chromatography (25, 26). The individual interassay variations for SHBG, testosterone, androstenedione, DHT, estrone, estradiol and estrone sulfate were 7.5%, 13.8%, 11.4%, 11.0%, 9.6%, 10.5% and 10.5%, respectively, and the intraassay variations were 5.2%, 8.2%, 9.4%, 9.1%, 7.0%, 7.4% and 7.0%, respectively.

Statistical Methods. Nonparametric statistical methods were used because of the small groups and the unequal variances between groups. For comparisons of continuous variables between groups the Wilcoxon rank test was used. Hormonal data were compared using distribution-free multivariate hypothesis tests using Wilcoxon ranks, stratifying on the nuisance variables of chronological and menopausal age (M-rank procedure, SAS [Statistical Analysis System]) (27). This test is a stratified and generalized version of the Wilcoxon test. Two-tailed significance level of the type I error was fixed at 5%.

RESULTS

No significant differences were observed between patients and controls in age, duration of menopause or age at menopause (Table 1). Of the 25 patients with PBC, 11 (44%) were in stage IV (cirrhosis) (Table 1). Cirrhotic PBC patients had significantly higher bilirubin levels than did noncirrhotic patients ($p < 0.05$; Table 1). The prevalence of mitochondrial antibodies was 68% (95% confidence interval = 47% to 85%) (Table 1). More than 90% of the cirrhotic patients had mitochondrial antibodies, compared with 50% of noncirrhotic patients ($p < 0.05$; Table 1).

Significantly higher concentrations of estrone (Table 2) and androstenedione (Table 3) were observed in PBC patients compared with controls. Serum concentrations of estrone sulfate (Table 2), DHAS (Table 3) and DHT (Table 3) were significantly lower in PBC patients compared with controls. Serum concentrations of testosterone, non-SHBG-bound testosterone, non-protein-bound testosterone and SHBG did not differ significantly between PBC patients and controls ($p > 0.05$; Tables 2 and 3).

Only one of the controls had estradiol levels above the detection limit of the assay (40 pmol/L), compared with none of the PBC patients.

To study the variation in hormone concentrations with disease stage, cirrhotic patients (stage IV) were compared with noncirrhotic patients (stages I, II and

TABLE 2. Estrone, estrone sulfate and SHBG concentrations in postmenopausal patients with PBC and controls

Hormone	Controls vs. PBC		Noncirrhotic vs. cirrhotic	
	(n = 46)	(n = 25)	(n = 14)	(n = 11)
Estrone (pmol/L)	155[a, b]	255[b]	260[c]	205[c]
	(108-203)	(183-298)	(208-335)	(170-290)
Estrone sulfate (pmol/L)	1,700[b]	1,200[b]	1,100[c, d]	1,400[d]
	(1,375-2,125)	(1,025-1,500)	(975-1,350)	(1,175-1,775)
SHBG (nmol/L)	98	124	88[d]	197[c, d]
	(79-142)	(83-199)	(72-129)	(107-284)

Distribution-free multivariate hypothesis tests using Wilcoxon ranks stratifying on chronological and menopausal age were applied.
[a]Values are medians (with interquartile ranges in parentheses).
[b]$p < 0.05$, comparisons between PBC patients and controls.
[c]$p < 0.05$, comparisons between noncirrhotic or cirrhotic patients and controls.
[d]$p < 0.05$, comparison between cirrhotic and noncirrhotic patients.

TABLE 3. Testosterone, androstenedione and DHAS concentrations in cirrhotic and noncirrhotic postmenopausal women with PBC and controls

Hormone	Controls vs. PBC		Noncirrhotic vs. cirrhotic	
	(n = 46)	(n = 25)	(n = 14)	(n = 11)
Testosterone (nmol/L)	0.83[a]	0.87	0.92	0.84
	(0.52-1.03)	(0.64-1.55)	(0.60-1.63)	(0.64-1.50)
Non-SHBG-bound testosterone (nmol/L)	0.17	0.17	0.21	0.13
	(0.08-0.23)	(0.11-0.25)	(0.11-0.28)	(0.08-0.19)
Non-protein-bound testosterone (pmol/L)	8	8	10	6
	(4-11)	(5-12)	(5-13)	(4-9)
DHT (nmol/L)	0.48[b]	0.44[b]	0.43	0.44
	(0.37-0.64)	(0.20-0.53)	(0.20-0.53)	(0.24-0.54)
Androstenedione (nmol/L)	3.1[b]	5.2[b]	5.0[c]	6.4[c]
	(2.2-4.0)	(4.2-6.9)	(3.8-6.6)	(4.7-7.0)
DHAS (nmol/L)	2,500[b]	980[b]	1,200[c]	860[c]
	(1,550-3,850)	(180-2,200)	(173-3,525)	(180-1,400)

Distribution-free multivariate hypothesis tests using Wilcoxin ranks stratifying on chronological and menopausal age were applied.
[a]Values are medians (with interquartile ranges in parentheses).
[b]$p < 0.05$, comparisons between PBC patients and controls.
[c]$p < 0.05$, comparisons between noncirrhotic or cirrhotic patients and controls.

III). Cirrhotic patients had significantly higher SHBG and estrone sulfate levels compared with noncirrhotic patients with PBC, as shown in Table 2. Otherwise, no significant differences were observed between cirrhotic and noncirrhotic patients.

Patients in the cirrhotic stage had significantly higher SHBG levels than did controls ($p = 0.01$; Table 2). Estrone sulfate levels were significantly lower in noncirrhotic patients but not in cirrhotic patients compared with controls (Table 2).

Changes in estrone, androstenedione and DHAS concentrations compared with those in controls were observed in cirrhotic and noncirrhotic patients (Tables 2 and 3).

DISCUSSION

This study shows that nonalcoholic postmenopausal women with PBC have significantly elevated serum concentrations of estrone and androstenedione and significantly lower concentrations of estrone sulfate, DHAS and DHT compared with a group of healthy controls. Furthermore, cirrhotic PBC patients have significantly elevated SHBG concentrations compared with noncirrhotic patients and controls.

The prevalence of positive mitochondrial antibodies was 68% (95% confidence interval = 47% to 85%); this is lower than usually reported, but in accordance with the 80% (95% confidence interval = 70% to 89%) prevalence of mitochondrial antibodies reported in 95 patients with PBC from our group (28). One reason may be the inclusion of a high percentage of patients in the noncirrhotic stage.

Because onset of PBC occurs in middle-aged women, the duration of disease may not be sufficient to induce earlier occurrence of menopause as seen in women with chronic alcoholic liver disease (8, 10). In agreement with our results, SHBG levels have been reported to be elevated in patients with nonalcoholic liver diseases, including PBC (13, 15, 16), although the pathogenesis is still obscure.

The significantly elevated estrone and decreased estrone sulfate concentrations reflect redistribution between unconjugated estrone and estrone sulfate because unchanged total estrone concentration (estrone sulfate + unconjugated estrone) has been found in PBC patients (16). Varying degrees of portosystemic shunting exist in patients with chronic liver disease, resulting in enhanced peripheral aromatization of androstenedione and DHAS to estrogens (29). Another contributing factor may be feminization of the hepatic steroid metabolism through altered pituitary growth hormone release, as seen in rats after portal vein ligation (29, 30).

Decreased DHT concentrations in women with PBC could be precipitated by reduced 5-α-reductase activity, as demonstrated in men and women with alcoholic liver disease (31). No association was found in that study between 5α-reductase activity and liver structure; however, this could be due to a type II error because the patient group was small (31).

Grün, Günther and Kaffarnik (32) have shown that compensated cirrhotic patients have lower-than-normal androstenedione levels, whereas decompensated patients have elevated androstenedione levels compared with normal controls. We found significantly higher androstenedione levels in the PBC patients compared with controls in accordance with earlier studies (14, 16), in contrast to the study by Bannister, Sheridan and Losowsky (13). Increased production of androstenedione and increased interconversion rate from testosterone have earlier been demonstrated in men with alcoholic cirrhosis (33, 34). Additional sources could be increased hepatic conversion of DHA to androstenedione (35) and decreased hepatic metabolism of androstenedione (36).

In keeping with the results of Bannister, Sheridan and Losowsky (13), while studying women with nonalcoholic cirrhosis (including five PBC patients) we found significantly lower concentrations of steroid sulfates (DHAS and estrone sulfate), indicating decreased sulfotransferase activity as demonstrated earlier (26, 37) or reduced substrate concentrations. Previous studies have demonstrated reduced sulfoxidation in patients with PBC (38), inducing a reduced supply of endogenous sulfate. However, the significantly higher levels of estrone sulfate in cirrhotic patients compared with noncirrhotic patients does not favor this hypothesis.

The changes we saw in sex hormones are similar to those found in men with chronic alcoholic liver disease consisting of low levels of DHT (18, 26), DHAS (26) and estrone sulfate (26); increased concentrations of estradiol (18, 39) and SHBG (7); high (18) or normal (39) androstenedione concentrations; and often elevated estrone concentrations (7, 18).

In conclusion, we found significantly elevated serum concentrations of estrone, androstenedione and SHBG (cirrhotic patients) and significantly lower concentrations of estrone sulfate, DHAS and DHT in a group of nonalcoholic postmenopausal women with PBC compared with a group of healthy controls. These changes may well be a consequence of hepatic dysfunction and related factors such as portosystemic shunting.

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