Sex hormone changes in post-menopausal women with primary biliary cirrhosis (PBC) and with cryptogenic chronic liver disease

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Summary: Sex hormones and sex hormone binding globulin (SHBG) have been studied in 32 female post-menopausal patients (16 with Primary Biliary Cirrhosis (PBC) and 16 with cryptogenic chronic liver disease (CLD).

Dehydroepiandrosterone-sulfate (DHEA-S) serum levels were significantly higher in PBC compared to CLD subjects ($p<0.005$). In PBC DHEA-S concentration was higher in precirrhotic than in cirrhotic patients ($p<0.02$). SHBG was raised in both PBC and CLD patients but higher in CLD compared to PBC subjects ($p<0.002$).

PBC reveals a sex hormone pattern similar to post-menopausal subjects with breast cancer.

These results suggest that sex hormone alteration is present in females with different types of liver disease, but the metabolic pattern is not due to liver disease per se.

Key words: Cirrhosis; Liver diseases; Sex hormones.

INTRODUCTION

Abnormalities in gonadal function are common features of cirrhosis in males. They are explained by changes in sex hormone concentration such as low testosterone levels and high levels of oestrone and oestradiol ([1, 2]).

Sex hormones have been also assayed in 24 post-menopausal women with non alcoholic liver cirrhosis ([3]). In this group of liver disease patients plasma oestradiol and sex hormone binding globulin (SHBG) have been found to be significantly elevated in respect to sex and age-matched controls. On the contrary, plasma dehydroepiandrosterone sulphate and plasma androstenedione levels have been found to be very low.

No reports have dealt with concentration of sex hormone in primary biliary cirrhosis (PBC), a chronic liver disease predominantly affecting the females around menopause. There are only indirect demonstrations regarding an endocrine dysfunction in PBC. Stellon *et al.* found an increased incidence of menstrual dysfunctions and of hysterectomies and curettages before clinical appraisal ([4]). Furthermore, the incidence of breast cancer in women with PBC was found to be significantly higher than in an age and sex matched control population from the same geographical area ([5, 6]). Finally in PBC a very high incidence of osteoporosis is reported ([7, 8]): in this respect oestrogen de-

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ficiency is generally recognized to play a role in the pathogenesis of post-menopausal bone loss.

The aim of this study has been to investigate the peripheral sex hormone concentration in post-menopausal females with PBC and with cryptogenic chronic liver disease.

MATERIAL AND METHODS

Patients

– PBC subjects: Sixteen post-menopausal female patients (mean age 58 years, range 46-68 years) entered the study. The mean±SD of body weight was 59±8 kg. The diagnosis of PBC was established by biochemical, immunological (positive test for anti-mitochondrial antibody) tests and compatible liver histology. Four subjects had histological Stage I-II, 7 Stage III and 6 Stage IV according to Scheuer's classification (9).

– Cryptogenic CLD: Sixteen post-menopausal female patients (mean age 60 years, range 52-63 years) were also studied. The mean±SD of body weight was 64±9 kg. The diagnosis of CLD was obtained using biochemical, immunological and histological criteria. They presented a cryptogenetic chronic active hepatitis (CAH) (12 with associated cirrhosis). None had decompensated cirrhosis nor was taking steroid therapy.

Methods

Informed consent was obtained from all patients prior to venesection.

Venous blood was sampled, quickly separately and stored at —20° C until analysis.

In each case the following sex hormones: dehydroepiandrosterone sulphate (DHEA-S), androstenedione, testosterone, 17 β-estradiol, and sex hormone binding globulin (SHBG) were determined.

Serum DHEA-S was assayed by RIA (RADIM S.p.A. Pomezia, Italy). The normal range in post-menopausal females was 0.27-2.1 μmol/L.

Serum androstenedione was assayed by RIA (Diagnostic Systems Laboratories inc. Webster. Texas). The normal range was 0.5-1.3 nmol/L.

Serum testosterone was assayed by RIA (Clone Systems). The normal range was in post-menopausal females was 0.3-1.4 nmol/L. Serum levels of testosterone obtained by this method represent the total unconjugated form of sex steroid, because it is impossible to distinguish the free and protein-bound fractions.

Table 1. – *Interassay and intra-assay coefficients of variations of sensitivities.*

	Intra-assay coefficient of variation	Interassay coefficient of variation	sensitivity
DHEA-S	4.9%	7.2%	0.05 umol/L
androstenedione	4.1%	9.7%	0.34 nmol/L
testosterone	6.9%		–
17β oestradiol	4.3%	6.8%	29.3 pmol/L
SHBG	5%	4-7%	0.8 nmol/L

Serum oestradiol was assayed by RIA (Diagnostic Products Corporation, Los Angeles, CA). The normal range in post-menopausal females was 20-90 pmol/L.

Serum SHBG was assayed by direct immunofluorescence technique (LKB Wallach, Finland). The normal range in females was 30-90 nmol/L. Inter-assay and intra-assay coefficients of variation of sensitivities are shown on Table 1.

Statistical Analysis

Results have been analyzed by linear regression test, chisquare and Student's t test for unpaired data as appropriate.

RESULTS

No correlation was found between serum concentration of DHEA-S, androstenedione, testosterone, 17-β oestradiol, SHBG and body weight of either PBC and CLD patients.

Serum levels of DHEA-S were within the normal range in 7 out of 16 PBC subjects (44%). Only one patient had DHEA-S below normal, while 7 out of 16 (44%) subjects presented serum DHEA-S above the normal range. Ten out of 16 (63%) CLD patients showed DHEA-S serum levels within the normal: 6 subjects had values slightly below the normal range.

The mean±SD value of DHEA-S was significantly higher in PBC patients (2.5±0.6 μmol/L) compared to CLD patients (0.5±0.1 μmol/L) ($p<0.005$) (Fig. 1).

Androstenedione values showed a wide variability in both PBC and CLD patients.

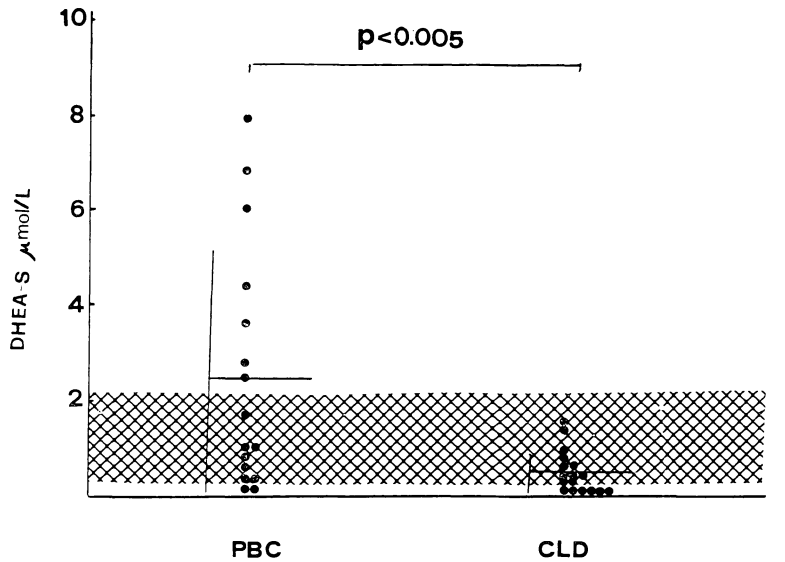

Fig. 1. – Serum values of DHEA-S in PBC and cryptogenic CLD patients.

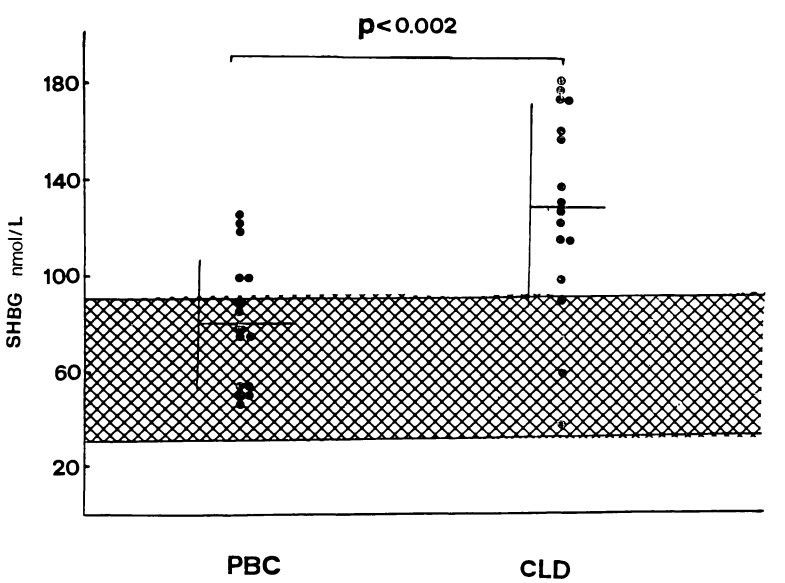

Fig. 2. – Serum values of SHBG in PBC and cryptogenic CLD patients.

The mean ±SD values were within the normal range in both groups, without significant difference (0.7±0.1 nmol/L in PBC patients vs 0.7±0.1 nmol/L in CLD patients).

Serum testosterone levels were within the normal range in both PBC and CLD patients (the mean±SD was 0.9±0.5 in PBC patients and 0.9±0.6 in CLD patients).

No alterations were observed in 17 β-oestradiol levels in both PBC and in CLD subjects. The mean±SD was 62.6±8.9 pmol/L in PBC subjects and 61.7±9.0 pmol/L in CLD subjects respectively.

Serum values of SHBG were higher than normal in 5 out of 16 patients with PBC (31%) and in 13 out of 16 patients with CLD (81%). The difference between the two groups was statistically significant ($p<0.01$). Serum concentration of SHBG was significantly lower in PBC patients (82.8±31.2 nmol/L) compared to CLD patients (129.7±42.4 nmol/L) ($p<0.002$) (Fig. 2).

No correlation was found between serum concentration of DHEA-S, androstenedione, testosterone, 17 β-oestradiol, SHBG and indices of cholestasis (alkaline phosphatase and GGT) in PBC patients. In the same group of patients there were no differences in serum concentrations of testosterone and 17 β-oestradiol according to the presence of cirrhosis.

Nevertheless serum DHEA-S and serum androstenedione levels were higher in pre-cirrhotic PBC patients (3.1±2.7 μmol/L) compared to cirrhotic patients. Serum DHEA-S was respectively 3.1±2.7 μmol/L in pre-cirrhotic and 1.02±0.8 μmol/L in cirrhotic PBC patients, ($p<0.05$). Serum androstenedione concentration was higher in pre-cirrhotic PBC group (0.4±0.2 μmol/L) compared to cirrhotic group (1.3±0.9 μmol/L) ($p<0.05$).

Among CLD group no differences were found in serum concentration of DHEA-S, androstenedione, testosterone, 17 β-oestradiol and SHBG in relation to the presence or not of cirrhosis.

DISCUSSION

The results of this study show that alteration in sex hormones and SHBG are present in post-menopausal patients with PBC and CLD. These alterations are characterized by some differences between the two groups of subjects.

We observed significantly higher serum levels of DHEA-S in post-menopausal PBC patients compared to cryptogenic CLD subjects. This indicates that liver disease per se is not associated with sex hormone changes.

Bannister *et al.* ([3]) observed a lower plasma DHEA-S levels in 24 women with non-alcoholic liver cirrhosis than matched controls. In this series only 5 patients with PBC were included, so they could not find any difference between PBC and non-PBC chronic liver disease.

Wilkinson *et al.* ([10]) evaluated sex hormones (oestradiol, testosterone, dihydrotestosterone) and SHBG in 29 PBC subjects (including 8 men), but in this study DHEA-S were not assayed.

DHEA-S represents an important product of surrenalic secretion and it is considered a good index of androgenic metabolism. Raised serum DHEA-S levels are due to changes in their production or catabolic rate. Probably the metabolic conversion of DHEA-S to androstenedione is slowed in PBC patients. High plasma concentrations of DHEA and DHEA-S have also been found in post-menopausal women with breast cancer ([11]). The latter condition was found to have an increased incidence in PBC patients ([5, 6]).

Nevertheless the interpretation of these results has not been clarified so far. However several workers ([12, 13]) have suggested a «two-disease» theory of breast can-

cer, namely that there are two different diseases in pre-menopausal and post-menopausal patients. The pre-menopausal patients have subnormal plasma DHEA and DHEA-S levels, while the post-menopausal patients have supranormal levels. Our findings suggest an analogy between DHEA-S concentration in post-menopausal PBC and in post-menopausal women with breast cancer. This interesting finding could explain at least in part the higher incidence of breast cancer in PBC ([5, 6]).

DHEA-S serum levels are significantly higher in precirrhotic PBC patients compared to those with histological Stage IV. This observation strengthens the idea that sex hormone alterations are not correlated to liver disease per se. In fact, in cryptogenic CLD there were no differences in peripheral sex hormone and SHBG concentrations between precirrhotic and cirrhotic patients.

In post-menopausal patients SHBG were significantly higher in PBC compared to CLD patients, SHBG is a plasma glycoprotein which specifically binds sex hormones and it is synthetized by the liver. It has a high affinity for testosterone, and less affinity for oestrogens. Raised levels of SHBG have been observed in both men and women with cirrhosis from different aetiology ([3, 14, 15, 16]).

Raised levels of SHBG in liver disease can be explained by increased rate of active metabolites from oestradiol. However this mechanism can be due to increase in 16 α-hydroxilation occurring in liver disease ([17]).

High levels of SHBG can also be due to an increased production rate due to testosterone deficiency ([18, 19]). However in our study serum levels of testosterone and oestradiol were within the normal range, in both PBC and CLD, thus this does not explain our findings.

Serum androstenedione showed a wide variability but the mean ± SD was within the normal in both PBC and CLD patients. Bannister *et al.* ([3, 20]) found raised levels of oestradiol in females with non-alcoholic liver disease and in men with alcoholic liver disease, but normal levels in men with non-alcoholic liver disease. Shaaban *et al.* found similar plasma oestradiol levels in 18 women with liver cirrhosis compared with controls ([16]). So oestradiol level in liver disease seems to be a controversial point. In the liver the two main steps of oestradiol metabolism include the alpha hydroxylation (with formation of estriol and 16 α-hydroxyestrone) and 2-hydroxylation (with formation of 2-hydroxyestrone and 2-methoxyestrone). It has been demonstrated that in liver cirrhosis the 2-hydroxylation is reduced while 16 a-hydroxylation is raised ([17]). One possibility is that alcohol may directly alter oestrogen metabolism ([21]). So raised concentration of oestradiol in alcoholic liver disease may play a role in the clinical signs of feminization such as gynaecomastia and loss of body hair. However no obvious explanation exists for these discrepancies.

In conclusion, changes in sex hormone and SHBG are present in post-menopausal females with PBC and CLD. Liver disease per se does not alter the endocrinological pattern.

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