

# Pituitary glycoprotein hormone $\alpha$ -subunit secretion by cirrhotic patients

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## Abstract

Secretion of the  $\alpha$ -subunit of pituitary glycoprotein hormones usually follows the secretion of intact gonadotropins and is increased in gonadal failure and decreased in isolated gonadotropin deficiency. The aim of the present study was to determine the levels of the  $\alpha$ -subunit in the serum of patients with cirrhosis of the liver and to compare the results obtained for eugonadal cirrhotic patients with those obtained for cirrhotic patients with hypogonadotropic hypogonadism. Forty-seven of 63 patients with cirrhosis (74.6%) presented hypogonadism (which was central in 45 cases and primary in 2), 7 were eugonadal, and 9 women were in normal menopause. The serum  $\alpha$ -subunit was measured by the fluorimetric method using monoclonal antibodies. Cross-reactivity with LH, TSH, FSH and hCG was 6.5, 1.2, 4.3 and 1.1%, respectively, with an intra-assay coefficient of variation (CV) of less than 5% and an interassay CV of 5%, and sensitivity limit of 4 ng/l. The serum  $\alpha$ -subunit concentration ranged from 36 to 6253 ng/l, with a median of 273 ng/l. The median was 251 ng/l for patients with central hypogonadism and 198 ng/l for eugonadal patients. The correlation between the  $\alpha$ -subunit and basal LH levels was significant both in the total sample ( $r = 0.48$ ,  $P < 0.01$ ) and in the cirrhotic patients with central hypogonadism ( $r = 0.33$ ,  $P = 0.02$ ). Among men with central hypogonadism there was a negative correlation between  $\alpha$ -subunit levels and total testosterone levels ( $r = -0.54$ ,  $P < 0.01$ ) as well as free testosterone levels ( $r = -0.53$ ,  $P < 0.01$ ). In conclusion, although the  $\alpha$ -subunit levels are correlated with LH levels, at present they cannot be used as markers for hypogonadism in patients with cirrhosis of the liver.

## Key words

- $\alpha$ -Subunit
- Pituitary
- Gonadotropins
- Hypogonadism
- Cirrhosis

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## Introduction

The  $\alpha$ -subunit of glycoprotein hormones, usually detected in the serum of normal individuals (1-3), has been used as a tumor marker for gonadotropin- or TSH-secreting pituitary adenomas (4), for GH-secreting tumors (4,5) and for nonsecreting pituitary tumors (3,4). The  $\alpha$ -subunit is secreted in a pulsatile manner by the thyrotropes and by

the gonadotropes (6), but especially by the latter (7). Its production is stimulated by TRH (7) and by GnRH (8) and is suppressed with the use of thyroid hormone (9) or estradiol (1,10) in the absence of nonfunctioning tumors. Regulation by GnRH is the most important one, with 90% concordance between the pulses of the  $\alpha$ -subunit and of LH (11). This pulsatility decreases after blockage of GnRH receptors induced by their

antagonist (11) and is normalized in individuals with hypogonadotropic hypogonadism treated with GnRH (12).

In nonphysiological situations, most of the times the  $\alpha$ -subunit oscillates according to the respective intact heterodimeric hormone, increasing in primary hypothyroidism (9,13) and in primary gonadal failure (6,10), and decreasing in hypogonadotropic hypogonadism (14,15). Dissociation between the secretion of the  $\alpha$ -subunit and secretion of the complete hormone may occasionally occur, as observed during the use of GnRH agonists in men with glycoprotein hormone-secreting pituitary tumors, when LH is suppressed and high subunit levels are maintained (16). In patients with cirrhosis of the liver, hypogonadism is a frequent finding regardless of the etiology of the disease (17). Most of the time, despite the generalized advanced gonadal failure, gonadotropin levels are low, indicating involvement of the hypothalamus-pituitary axis. The site of this disorder is the hypothalamus since these patients do not present the usual LH pulsatility (18), since exogenous GnRH stimulates LH and FSH (19-21) and since the picture of hormonal deficiency is reversed after liver transplant (21-23). The secretory status of the  $\alpha$ -subunit has not been established in this form of hypogonadism.

The aim of the present study was to determine the  $\alpha$ -subunit levels in the serum of patients with cirrhosis of the liver and to compare the results obtained for hypogonadal and non-hypogonadal patients.

## Material and Methods

The sample consisted of 63 patients, 33 men and 30 women, average age 53 years (range: 19 to 76 years), with a clinical and/or histological diagnosis of cirrhosis of liver of different etiologies such as alcohol (N = 13), hepatitis C virus (N = 22), alcohol plus hepatitis C virus (N = 15), hepatitis B virus (N = 1), hepatitis B and C viruses (N = 2), hepatitis C

virus plus sclerosing cholangitis (N = 1), primary biliary cirrhosis (N = 1), and cryptogenic cirrhosis (N = 8). The clinical diagnosis was based on a previous history or present manifestations of cirrhosis, such as portal hypertension, bleeding esophageal and gastric varices, ascites, spontaneous bacterial peritonitis, encephalopathy and coagulopathy. In 37 cases the liver biopsy confirmed hepatic cirrhosis. The severity of liver damage was evaluated in terms of Child-Pugh score, which takes into account the degree of encephalopathy and ascites, the bilirubin and albumin levels and the prothrombin time (24). All patients were euthyroid, as determined by TSH measurement. None of the patients was using hormones or drugs which could alter the gonadotropin levels.

Gonadal function was evaluated on the basis of clinical and laboratory parameters. Total (normal range = 2.8-9.8 ng/ml) and free (normal range = 12.4-40.0 pg/ml) plasma testosterone were determined by RIA and estradiol (normal range for males = up to 40 and for females = 30-150 pg/ml), LH (normal range for males = 1.3-19.8 and for females = 0.7-35.2 mIU/ml) and FSH (normal range for males = 2.0-12.8 and for females = 3.1-15.7 mIU/ml) were determined by chemiluminescence using commercial kits in our laboratory. The patients were divided into 4 groups: group 1 consisted of eugonadal patients, group 2 of amenorrheic women older than 50 years with increased basal gonadotropins (normal menopause), and group 3 and group 4 consisted of women in the fertile age with hypogonadism, e.g., presenting amenorrhea or absence of vaginal bleeding for at least 6 months and men with hypogonadism, e.g., presenting low levels of free testosterone. Hypogonadism was considered to be primary when one or both gonadotropins were increased (group 3). Group 4 was composed of patients with hypogonadism of central origin, e.g., no increase in basal gonadotropins.

The free  $\alpha$ -subunit was determined by the fluorimetric method, using monoclonal

antibodies. Cross-reactivity with LH, TSH, FSH and hCG was 6.5, 1.2, 4.3 and 1.1%, respectively, with an intra-assay coefficient of variation (CV) of less than 5% and an interassay CV of 5%, and sensitivity limit of 4 ng/l. The values considered normal for the method range from 120 to 790 ng/l (median: 250) for men, from 88 to 604 ng/l (median: 291) for women, and from 341 to 4071 ng/l (median 1856) for menopausal women (25).

For statistical analysis, the results are reported as median, minimum and maximum values of distribution, with the exception of serum albumin levels which are reported as means  $\pm$  SD. The nonparametric Mann-Whitney or Kruskal-Wallis test was used according to the number of groups evaluated. The chi-square association test and the Spearman correlation coefficient test were calculated. The level of significance was considered to be  $P < 0.05$ .

This study was approved by the Ethics Committee of the institution and informed consent was obtained from the subjects.

## Results

Hypogonadism was diagnosed in 47 individuals, with 45 cases being of central etiology and 2 of primary etiology. Of the remaining patients, 7 were eugonadal and 9 were menopausal women. Total testosterone levels ranged from 3.0 to 5.8 ng/ml for eugonadal men and from 0.1 to 14.0 ng/ml for central hypogonadal men, while free testosterone ranged from 15 to 21 and from 0.3 to 13.0 pg/ml for these same groups. Amongst men, 5 presented increased estradiol while among women, 19 presented estradiol levels which were lower than the minimum expected for the follicular phase. The range of estradiol values was 26.0 to 225 pg/ml in eugonadal women, less than 10 to 95 pg/ml in menopausal women and less than 10 to 75 pg/ml in women with central hypogonadism. Regarding gonadotropins, the median, minimum and maximum values for LH were 3.9 (0.3-55.9),

Table 1 – Severity of hepatic disease and gonadal function in the cirrhotic patients who participated in the study.

Group	N	Child-Pugh A	Child-Pugh B	Child-Pugh C
Eugonadal	7	5	2	-
Menopausal	9	3	6	-
Primary hypogonadism	2	-	1	1
Central hypogonadism	45	6	23	16

34.5 (6.7-102.5) and 4.1 mIU/ml (0.1-20.4), respectively, for eugonadal and menopausal subjects and for subjects with central hypogonadism. For FSH, these values were 4.3 (2.4-14.0), 60.7 (26.8-129.0) and 5.5 (0.1-19.5) mIU/ml. There was no significant difference in LH or FSH levels between eugonadal and central hypogonadal patients.

Mean serum albumin levels were 3.1 g/dl (normal values = 3.5-5.5; range 2.0-4.5). The severity of hepatic disease in each group, according to the CHILD score, is presented in Table 1; 22.9% of the patients were classified as Child-Pugh A, 50.8% as Child-Pugh B and 27% as Child-Pugh C. The analysis of a possible correlation between gonadal function and Child-Pugh score was significant, suggesting a correlation between these variables.

The concentration of the  $\alpha$ -subunit ranged from 36 to 6253 ng/l (median: 273 ng/l). In the two patients with primary hypogonadism, the  $\alpha$ -subunit levels were 1568 and 1005 ng/l. The median and distribution of the  $\alpha$ -subunit levels for the remaining hypogonadal, eugonadal and menopausal subjects are listed in Table 2. No statistically significant difference in  $\alpha$ -subunit levels was detected among these groups. The  $\alpha$ -subunit levels of patients with cirrhosis associated with alcohol (N = 28, median 222.5 ng/l) were not statistically different from the levels observed in the patients with cirrhosis of other etiologies (N = 35, median 313 ng/l).

The correlation between  $\alpha$ -subunit and basal LH levels was significant both for the total sample ( $r = 0.48$ ,  $P < 0.01$ ) and for cirrhotic patients with central hypogonadism ( $r$

Table 2 – Serum  $\alpha$ -subunit concentrations of cirrhotic patients.

Group	N	Median (ng/l)	Distribution (ng/l)
Eugonadal	7	198	41-699
Menopausal	9	549	163-3778
Central hypogonadism	45	251	36-6253

= 0.33,  $P = 0.02$ ). A significant negative correlation between  $\alpha$ -subunit levels and total testosterone ( $r = -0.54$ ,  $P < 0.01$ ) and free testosterone ( $r = -0.53$ ,  $P < 0.01$ ) was observed in men with central hypogonadism. No association was observed between  $\alpha$ -subunit levels or serum albumin levels and severity of hepatic disease according to the Child-Pugh score.

## Discussion

The high prevalence of hypogonadism of central etiology in the population of cirrhotic patients evaluated here is related to the severity of liver disease, which triggers a hypothalamic disorder secondary to an adverse environment (21). This diagnosis, however, does not invalidate the presence of gonadal lesion in some patients from this group.

The production of the  $\alpha$ -subunit is parallel to LH secretion during the different phases of life, increasing from prepuberty to adolescence (26). Thus, our finding of a positive correlation between  $\alpha$ -subunit levels and LH levels agrees with data reported elsewhere (6,12,26). On the other hand, no statistically significant difference in  $\alpha$ -subunit levels was detected between patients with central hypogonadism, eugonadism and menopausal women. Classically,  $\alpha$ -subunit levels are elevated in menopause (1-3,13), a fact that was observed here only as a tendency. Our findings are probably related to the small sample of menopausal women studied (9 patients), since their gonadotropin levels were as expected.

Studies on gonadotropin deficiency alone in hypogonadotropic hypogonadism have

reported low  $\alpha$ -subunit values (12,14). Spratt and colleagues (12) studying 6 hypogonadal men with normal endogenous TSH secretion detected circulating  $\alpha$ -subunit levels close to or below assay detection limits. Winters and Troen (14) studying 4 hypogonadal men observed low but usually detectable levels of  $\alpha$ -subunit. Several possibilities may justify the absence of this finding among the hypogonadal patients in the present series. The first is that our sample is not homogeneous in terms of number of patients in each group and that there is dispersion of the results for hypogonadal patients, two of whom had the highest  $\alpha$ -subunit value in the sample, including menopausal women. The second possibility is that, although a hypothalamic disorder exists both in gonadotropin deficiency alone and in hypogonadism associated with cirrhosis, the basic mechanism of the diseases is different: in the first condition endogenous GnRH secretion is undetectable, whereas in the second GnRH deficiency is transitory. This factor may be a determinant of the differential behavior of the  $\alpha$ -subunit. Another explanation is that in hypogonadism associated with cirrhosis, basal LH levels are higher than those observed in individuals with gonadotropin deficiency alone, a possibility that was not investigated here. The possibilities of alteration in the hepatic clearance of the  $\alpha$ -subunit or of a toxic factor associated with cirrhosis which may interfere with the pituitary gland dynamics should also be considered.

Also important was our finding of a negative correlation between the levels of  $\alpha$ -subunit and testosterone, contrary to what would be expected for central hypogonadism.

In conclusion, it seems clear that there is a parallelism between hepatic and gonadal dysfunction, and that in hypogonadism secondary to cirrhosis of liver,  $\alpha$ -subunit levels are correlated with LH levels but cannot be used as an additional diagnostic criterion of hypogonadism in these patients.

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